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Contents

01 1. Division of Bioconvergence Technology
   - BioNanotechnology Research Center
   - Aging Research Center
   - Brain Research Center
   - Integrative Omics Research Center
   - BioMonitoring Research Center

21 2. Division of Translational Research
   - Medical Genomics Research Center
   - Development and Differentiation Research Center
   - Medical Proteomics Research Center

47 3. Division of Biosystems Research
   - Industrial Biotechnology & Bioenergy Research Center
   - Plant Systems Engineering Research Center
   - Bioinformatics Research Center
   - Industrial Bio-materials Research Center
   - Environmental Biotechnology Research Center

79 4. Division of Bio R&D Infrastructure
   - Microbial Resource Center (Korea Biological Resource Center)
   - Plant Resource Center
   - Human Derived Material Center
   - Genome Resource Center
   - Animal Model Resource Center

97 5. Division of Reading R&D
   - Korean Bioinformation Center
   - Viral Infectious Disease Research Center
   - DAEJEON-KRIBB-FHCRC Research Cooperation Center
   - International Biological Material Research Center
6. Bio-Therapeutics Research Institute
- Therapeutic Antibody Research Center
- Stem Cell Research Center
- Immune Modulator Research Center
- Molecular Cancer Research Center
- Chemical Biology Research Center

7. Division of Bio-Infra Structure
- Bio-Evaluation Center
- Korea National Primate Research Center
- Biomedical Mouse Resource Center
- Biotechnology Process Engineering Center

8. Jeonbuk Branch Institute
- Microbe-based Fusion Technology Research Center
- Eco-Friendly Biomaterial Research Center
- Bioindustrial Process Center

9. Cooperating and Supporting the Other Institution

Indexes
- Author Index
- Journal Index
- Keyword Index
1. Division of Bioconvergence Technology

- BioNanotechnology Research Center
- Aging Research Center
- Brain Research Center
- Integrative Omics Research Center
- BioMonitoring Research Center
An operationally simple colorimetric assay of hyaluronidase activity using cationic gold nanoparticles

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An operationally simple colorimetric way for measuring hyaluronidase activity was developed using cysteamine-bound gold nanoparticles. The addition of gold nanoparticles into hyaluronidase-containing solutions resulted in color changes, which could easily be observed with the naked eye or a UV/Vis spectrophotometer.

ANALYST, 134(7): 1291-1293.

Keyword : bladder-cancer; ultrasensitive detection; plasmon resonance; DNA detection; morganelson

Photoactivable antibody binding protein: site-selective and covalent coupling of antibody

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Here we report new photoactivable antibody binding proteins, which site-selectively capture antibodies and form covalent conjugates with captured antibodies upon irradiation. The proteins allow the site-selective tagging and/or immobilization of antibodies with a highly preferred orientation and omit the need for prior antibody modifications. The minimal Fc-binding domain of protein G, a widely used antibody binding protein, was genetically and chemically engineered to contain a site-specific photo cross-linker, benzophenone. In addition, the domain was further mutated to have an enhanced Fc-targeting ability. This small engineered protein was successfully crosslinked only to the Fc region of the antibody without any nonspecific reactivity. SPR analysis indicated that antibodies can be site-selectively biotinylated through the present photoactivable protein. Furthermore, the system enabled light-induced covalent immobilization of antibodies directly on various solid surfaces, such as those of glass slides, gold chips, and small particles. Antibody coupling via photoactivable antibody binding proteins overcomes several limitations of conventional approaches, such as random chemical reactions or reversible protein binding, and offers a versatile tool for the field of immunosensors.

ANALYTICAL CHEMISTRY, 81(3): 936-942.

Keyword : immobilization methods; solid supports; gold surface; IGG
Synthesis and characterization of a photoluminescent nanoparticle based on fullerene-silica hybridization

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Fullerene-silica hybrid nanoparticles have bright photoluminescence, high photostability, and low cytotoxicity, which are assets for bioimaging agents. The origin of the photoluminescence of the nanoparticle is the C-O-Si bon.


\textbf{Keyword} : fullerenes; imaging agents; nanoparticles; photoluminescence; silica; sol-gel

High-throughput quantitative analysis of plant N-glycan using a DNA sequencer

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High-throughput quantitative analytical method for plant N-glycan has been developed. All steps, including peptide N-glycosidase (PNGase) A treatment, glycan preparation, and exoglycosidase digestion, were optimized for high-throughput applications using 96-well format procedures and automatic analysis on a DNA sequencer. The glycans of horseradish peroxidase with plant-specific core alpha(1,3)-fucose can be distinguished by the comparison of the glycan profiles obtained via PNGase A and F treatments. The peaks of the glycans with (91%) and without (1.2%) alpha(1,3)-fucose could be readily quantified and shown to harbor bisecting beta(1,2)-xylose via simultaneous treatment with alpha(1,3)-mannosidase and beta(1,2)-xylosidase. This optimized method was successfully applied to analyze N-glycans of plant-expressed recombinant antibody, which was engineered to contain a minor amount of glycan harboring beta(1,2)-xylose. These results indicate that our DNA sequencer-based method provides quantitative information for plant-specific N-glycan analysis in a high-throughput manner, which has not previously been achieved by glycan profiling based on mass spectrometry.


\textbf{Keyword} : plant N-glycan; DNA sequencer; glycan analysis; alpha(1,3)-fucose; beta(1,2)-xylose
Detection of conformationally changed MBP using intramolecular FRET

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The principal objective of this study was to explore protein conformational changes using fluorescence resonance energy transfer (FRET) technology. Maltose binding protein (MBP) was adopted as a target model, due to its well-characterized structure and ligand specificity. To the best of our knowledge, this is the first report to provide information regarding the biological distance between the two lobes of MBP upon maltose binding. For the FRET pair, ECFP and EYFP were used as the donor and the acceptor, and were linked genetically to the C-terminal and N-terminal regions of MBP (ECFP:MBP:EYFP), respectively. After the FRET reaction, maltose-treated MBP was shown to exhibit a considerable energy transfer (FRET efficiency (E) = ~0.11, Distance (D) = ~6.93 nm) at the ensemble level, which was regarded as reflective of the increase in donor quenching and the upshift in acceptor emission intensity, thereby suggesting that the donor and the acceptor had been brought close together as the result of structural alterations in MBP. However, upon glucose treatment, no FRET phenomenon was detected, thereby implying the specificity of interaction between MBP and maltose. The in vitro FRET results were also confirmed via the acceptor photobleaching method. Therefore, our data showed that maltose-stimulated conformational changes of MBP could be measured by FRET, thereby providing biological information, including the FRET efficiency and the intramolecular distance.

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 388(3): 560-564.

Keyword : fluorescence resonance energy transfer; FRET; maltose binding protein, MBP; conformational change

Simultaneous intracellular delivery of targeting antibodies and functional nanoparticles with engineered protein G system

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Cellular internalization of functional nanoparticles that have optical and magnetic properties is very important in the cellular imaging and manipulation of specifically targeted biomolecules. In this study, a robust method to deliver functional nanoparticles and targeting antibodies into cells was suggested. The engineered protein G system, which contains an affinity tag and a cell penetration peptide in the N- and C-terminals, respectively, can capture surface-modified nanoparticles and antibodies without chemical reaction, and then non-invasively deliver them into the cells. Finally, gold-coated iron oxide nanoparticle(engineered protein G hybrid systems were successfully employed as multifunctional cargo systems for the targeting, imaging, and manipulation of mitochondria.

BIOMATERIALS, 30(6): 1197-1204.

Keyword : nanoparticle; gold; engineered protein; intracellular delivery; surface modification; cell imaging
Novel application of surface plasmon resonance biosensor chips for measurement of advanced glycation end products in serum of Zucker diabetic fatty rats

Young Sook Kim, So Yeon Yi, Junghyun Kim, Moonil Kim, Chan-Sik Kim, Bong Hyun Chung, and Jin Sook Kim

Advanced glycation end products (AGEs) have been implicated in diabetic complications. To measure AGEs, especially N-epsilon-(carboxymethyl)lysine (CML), in sera from Zucker diabetic fatty rats (ZDF) and Zucker lean rats (ZL), we used a novel method of protein chip and surface plasmon resonance imaging (SPRI). Serum samples were obtained from male ZDF and ZL rats at 20 weeks of age. Antibodies to AGEs or CIVIL were immobilized on a gold surface, which was modified by cysteine-tagged, protein-G constructs. The Fold chip upon which the serum was spotted was optically coupled with a prism coupler. The reflected images from the gold chip were obtained using a charge-coupled device (CCD) camera. The direct analysis of the glycated proteins and products using SPRI showed that AGEs and CML levels were elevated in ZDF serum, compared with ZL serum. The lowest detection limit of AGEs was 10 ng/ml, with a working range covering the physiological range. These results indicate that the protein chip and SPRI system is very suitable for the measurement of glycated proteins and end products in serum samples. This system offers high sensitivity without any fluorescent or other labeling of the components and saves a substantial amount of time, resources, and labor. Our results suggest that SPRI systems can be used as a tool to diagnose diabetic complications.

Cascade enzyme-linked immunosorbent assay (CELISA)

Young-mi Lee, Yujin Jeong, Hyo Jin Kang, Sang J. Chung, and Bong Hyun Chung

Immunoassays are representative biochemical detection methods. Among them, sandwich-type immunoassays, typified by sandwich ELISA, have used in disease diagnosis or biochemical detection with high target selectivity. Horseradish peroxidase and alkaline phosphatase have been typically used for signal amplification in ELISA. Recently developed sandwich-type immunoassays such as biobarcode immunoassays, immuno-PCR, and immuno-RCA have improved sensitivity by changing mainly the signal amplification method. To develop a novel amplification method in ELISA, an enzyme-cascading system was incorporated into an ELISA, and the new assay is termed a cascading enzyme-linked immunosorbent assay (CELISA). This CELISA includes a trypsinogen-enterokinase combination as the cascading enzyme system, and was used to detect alpha-fetoprotein (AFP), which is a liver cancer marker, and prostate-specific antigen (PSA). Using a colorimetric reagent for signal generation, CELISA had 0.1-10 pM limits-of-detection for AFP and PSA in whole human serum and assay buffers, depending on the platform, well plate, or microbead type used. This study represents the first example that incorporated an enzyme cascading step in an ELISA system, resulting in successful signal amplification with sensitive detection of pathogenic antigens in serum.

[Keyword]: advanced glycated end products (AGEs); diabetic complications; N-epsilon-(carboxymethyl)lysine (CML); surface plasmon resonance imaging (SPRI); Zucker diabetic fatty rats (ZDF)
Proteomic analysis of porcine pancreas development

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Porcine pancreas development is not well studied at the molecular level despite being a therapeutic resource for diabetic patients. In this study, we investigated expression of lineage markers and performed proteomic analysis. Expression of the early lineage markers Pdx1 and Ptf1a was developmentally conserved between mice and pigs, whereas expression of the islet differentiation marker Pax4 was delayed in porcine compared with murine pancreas development. Proteomic analysis found that expression levels of chymotrypsinogen were down-regulated during porcine pancreas development while those of digestive enzymes like lipases, elastase and serine protease were up-regulated. In addition, specific isoforms of protein folding assistants such as protein disulfide isomerase and prefoldin were expressed at specific stages during the maturation of digestive enzymes. Taken together, these results show that development of the porcine pancreas is regulated by a concerted interplay of pancreas lineage marker proteins and other specified proteins, resulting in a functional endocrine and exocrine organ.

BMB REPORTS, 42(10): 661-666.

Keyword: development; lineage markers; porcine pancreas; proteomics

Chronic glutamate toxicity in mouse cortical neuron culture

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Two pathways for glutamate toxicity have been described, receptor-mediated excitotoxicity and non-receptor mediated oxidative glutamate toxicity. Here, we show that two distinct forms of receptor-mediated primary cortical neuronal death exist, chronic and acute glutamate toxicity, and that these depend on exposure time. In vitro, neuronal sensitivity to chronic glutamate exposure increased as neurons matured and the initial plating medium contributed as well. In immature neurons, high concentrations of glutamate induced neuronal death. The chronic glutamate toxicity was independent of neuronal density, whereas increased density potentiated acute glutamate toxicity. Activation of ionotropic glutamate receptors (iGluRs) contributed to induction of chronic and acute glutamate toxicity at similar rates at DIV14. Inactivation of the metabotropic glutamate receptors (mGluRs) by AIDA increased neuronal sensitivity to chronic glutamate exposure but not to acute exposure. Neuronal death by acute toxicity was much faster than by chronic toxicity in which activation of mGluRs was involved. These results suggest that acute glutamate toxicity is quite different from chronic toxicity, in which activation of mGluRs is associated with resistance to glutamate toxicity.

BRAIN RESEARCH, 1273: 138-143.

Keyword: glutamate toxicity; acute exposure; chronic exposure; ionotropic glutamate receptor; metabotropic glutamate receptor
**Article 11**

**Functionalization of fullerene nanowhiskers using pyrenebutanoic acid, succinimidyl ester in an aqueous solution**

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The functionalization of fullerene nanowhiskers was accomplished by non-covalent π-π interaction using pyrenebutanoic acid, succinimidyl ester to immobilize target molecules on the nanowhiskers. Fourier-transform infrared spectroscopy and fluorescence microscopy proved that the fullerene nanowhiskers were functionalized without damaging the nanostructure.

*CARBON*, 47(8): 2124-2127.

**Keyword**: nanotubes; fullerene nanowhiskers

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**Article 12**

**p21<sup>Cip1</sup> regulation by ERK2: a post-translational mechanism that relays a proliferation signal**

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In these studies, TGFβ1-induced colon cancer cell proliferation was associated with K-Ras-mediated post-transcriptional downregulation of p21<sup>Cip1</sup> and myotube differentiation was associated with p21<sup>Cip1</sup> upregulation caused by inhibition of the Raf-MEK-ERK pathway. In this report, we found that activated ERK2 regulates p21<sup>Cip1</sup> by directly interacting with and phosphorylating p21<sup>Cip1</sup>, and showed that this modification induces p21<sup>Cip1</sup> cytoplasmic translocation and degradation by the ubiquitin-dependent proteasome pathway. It would appear that one mitogen might simultaneously generate opposing signals. Thus, it is important to understand how these signals are integrated to determine outcome and how one signal predominates over the other signal at the right place and time. p21<sup>Cip1</sup> regulation at the post-translational level, demonstrated by us, provides new insight into the mechanisms by which the ERK cascade drives cell cycle progression upon mitogenic stimulation.

*CELL CYCLE*, 8(22): 3625-3626.

**Keyword**: protein stability; cytoplasmic localization; phosphorylation; ERK cascade
Shape auxiliary approach for carboxylate-functionalized gold nanocrystals

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A shape auxiliary approach for the synthesis of shape-controlled gold nanocrystals with functional moieties is established; carboxylate-functionalized gold polyhedra were successfully synthesized in a one-pot reaction in the presence of poly(dimethylaminoethyl methacrylate), which contains the dimethylaminoethyl group as a shape auxiliary. CHEMICAL COMMUNICATIONS, (10): 1276-1278.

Keyword: surface-plasmon resonance; nanoparticles; nanostructures; nanorods

A highly selective fluorescent ESIPT probe for the dual specificity phosphatase MKP-6

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A highly selective fluorescent probe for a protein tyrosine phosphatase (PTP) was designed by a simple phosphorylation of the 2-(2'-hydroxyphenyl)benzothiazole (HBT) chromophore: upon selective enzymatic hydrolysis, an excited-state intramolecular proton transfer (ESIPT) occurs, resulting in a large Stokes shift. CHEMICAL COMMUNICATIONS, (39): 5895-5897.

Keyword: protein-tyrosine phosphatases; phosphorylation; PTP1b; ESIPT
**Article 15**

Multifunctional perfluorocarbon nanoemulsions for $^{19}$F-based magnetic resonance and near-infrared optical imaging of dendritic cells

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A novel type of bimodal imaging nanoprobe based on $^{19}$F-based magnetic resonance imaging and near-infrared optical imaging has been synthesized and applied for the labeling and imaging of dendritic cells both in vitro and in vivo.

CHEMICAL COMMUNICATIONS, (45): 6952-6954.

**Keyword**: MRI contrast agents; melanoma patients; quantum dots; lymph-nodes; nanoparticles

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**Article 16**

A facile one-pot synthesis of hydroxyl-functionalized gold polyhedrons by a surface regulating copolymer

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We report a simple, one-pot process for hydroxyl-functionalized gold nanocrystals with precise morphology control by a surface regulating copolymer. The copolymer, poly(vinyl pyrrolidone-ran-vinyl acetate) (PVP-PVAc), has two distinct moieties including pyrrolidone for nanoparticle formation and acetate for additional functionalization. The particle sizes are readily controlled in the range of 35-80 nm by changing the solvent volume, and the shapes are tuned from octahedral to cuboctahedral and cubic by the addition of AgNO₃. Concomitantly, hydrolysis of the acetate groups during the reaction generates multiple hydroxyl groups on the particle surface, which can readily conjugate with versatile functionalities through esterification. This copolymer-based process achieves the synthesis, morphology control, and functionalization of gold nanocrystals via a one-step reaction, as this is superior to the other multistep synthetic procedures.


**Keyword**: shape-controlled synthesis; platinum nanoparticles; cluster molecules; polyol process; nanorods; nanocrystals
Synapse formation regulated by protein tyrosine phosphatase receptor T through interaction with cell adhesion molecules and Fyn

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The receptor-type protein tyrosine phosphatases (RPTPs) have been linked to signal transduction, cell adhesion, and neurite extension. PTPRT/RPTPq is exclusively expressed in the central nervous system and regulates synapse formation by interacting with cell adhesion molecules and Fyn protein tyrosine kinase. Overexpression of PTPRT in cultured neurons increased the number of excitatory and inhibitory synapses by recruiting neuroligins that interact with PTPRT through their ecto-domains. In contrast, knockdown of PTPRT inhibited synapse formation and withered dendrites. Incubation of cultured neurons with recombinant proteins containing the extracellular region of PTPRT reduced the number of synapses by inhibiting the interaction between ecto-domains. Synapse formation by PTPRT was inhibited by phosphorylation of tyrosine 912 within the membrane-proximal catalytic domain of PTPRT by Fyn. This tyrosine phosphorylation reduced phosphatase activity of PTPRT and reinforced homophilic interactions of PTPRT, thereby preventing the heterophilic interaction between PTPRT and neuroligins. These results suggest that brain-specific PTPRT regulates synapse formation through interaction with cell adhesion molecules, and this function and the phosphatase activity are attenuated through tyrosine phosphorylation by the synaptic tyrosine kinase Fyn.

EMBO JOURNAL, 28(22): 3564-3578.

Keyword : cell adhesion molecule; Fyn; PTPRT; synapse formation; tyrosine phosphorylation

An ISFET biosensor for the monitoring of maltose-induced conformational changes in MBP

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Here we describe an ion sensitive field effect transistor (ISFET) biosensor, which was designed to monitor directly the surface charge of structurally altered maltose binding protein (MBP) upon stimulation with maltose. This study is the first report of the application of a FET biosensor to the monitoring of conformationally changed proteins. Consequently, a significant drop in current on the basis of the charge-dependent capacitance measurement has been clearly observed in response to maltose, but not for the glucose control, thereby indicating that the substrate-specific conformational properties of the target protein could be successfully monitored using the ISFET. Collectively, our results clearly suggest that ISFET provide a high fidelity system for the detection of maltose-induced structural alterations in MBP.


Keyword : ISFET; biosensor; surface charge; conformational change; MBP
Processed short neuropeptide F peptides regulate growth through the ERK-insulin pathway in Drosophila melanogaster

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The Drosophila sNPF gene regulates growth through the ERK-insulin pathway. sNPF encodes a precursor protein that is processed and produces biologically active sNPF peptides. However, the functions of these peptides are not known. In Drosophila neuronal cells in culture and in flies in vivo, sNPF1 and sNPF2 activated the ERK-insulin pathway and regulated body growth. In addition, the sNPF precursor and the processed sNPF peptide were co-localized in the neurons of the central nervous system. These results indicate that sNPF1 and sNPF2 peptides processed from the sNPF precursor are sufficient for regulating body growth through the ERK-insulin pathway in Drosophila.

FEBS LETTERS, 583(15): 2573-2577.

Keyword : short neuropeptide F; processed peptide; growth; ERK-insulin pathway; drosophila

Multiplexed imaging of therapeutic cells with multispectrally encoded magnetofluorescent nanocomposite emulsions

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Here, we describe the fabrication of multispectrally encoded nanoprobes, perfluorocarbon (PFC)/quantum dots (QDs) nanocomposite emulsions, which could provide both multispectral MR and multicolor optical imaging modalities. Our strategy exploited the combination of the multispectral MR properties of four different PFC materials and the multicolor emission properties of three different colored CdSe/ZnS QDs. The PFC/QDs nanocomposite emulsions were fabricated by exchanging hydrophobic ligands coated onto CdSe/ZnS QDs using 1H,1H,2H,2H-perfluorooctanethiol, which renders the QDs to be dispersible in the PFC liquids. To provide biocompatibility, the PFC liquids containing QDs were emulsified into aqueous solutions with the aid of phospholipids. The distinct 19F-based MR images of PFC/QDs nanocomposite emulsions were obtained by selective excitation of the nanocomposite emulsions with magnetic resonance frequency of each PFC, while a specific fluorescence image of them could be selected using appropriate optical filters. The uptake of PFC/QDs nanocomposite emulsions was high in phagocytic cells such as macrophages (90.55%) and dendritic cells (85.34%), while it was low in nonphagocytic T cells (33%). We have also shown that the nanocomposite emulsions were successfully applied to differentially visualize immunotherapeutic cells (macrophages, dendritic cells, and T cells) in vivo. The PFC/QDs nanocomposite emulsions are expected to be a promising multimodality nanoprobe for the multiplexed detection and imaging of therapeutic cells both in vitro and in vivo.


Keyword : core/shell quantum dots; MRI contrast agents; in-vivo; magnetic-resonance; drug-delivery; nanoparticles
Characterization of the *Streptococcus pneumoniae* BgaC protein as a novel surface beta-galactosidase with specific hydrolysis activity for the Gal beta 1-3GlcNAc moiety of oligosaccharides

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*Streptococcus pneumoniae* is a causative agent of high morbidity and mortality. Although sugar moieties have been recognized as ligands for initial contact with the host, only a few exoglycosidases have been reported to occur in *S. pneumoniae*. In this study, a putative beta-galactosidase, encoded by the bgaC gene of *S. pneumoniae*, was characterized for its enzymatic activity and virulence. The recombinant BgaC protein, expressed and purified from *Escherichia coli*, was found to have a highly regiospecific and sugar-specific hydrolysis activity for the Gal beta 1-3GlcNAc moiety of oligosaccharides. Interestingly, the BgaC hydrolysis activity was localized at the cell surface of *S. pneumoniae*, indicating that BgaC is expressed as a surface protein although it does not have a typical signal sequence or membrane anchorage motif. The surface localization of BgaC was further supported by immunofluorescence microscopy analysis using an antibody raised against BgaC and by a reassociation assay with fluorescein isothiocyanate-labeled BgaC. Although the bgaC deletion mutation did not significantly attenuate the virulence of *S. pneumoniae* in vivo, the bgaC mutant strain showed relatively low numbers of viable cells compared to the wild type after 24 h of infection in vivo, whereas the mutant showed higher colonization levels at 6 and 24 h postinfection in vivo. Our data strongly indicate for the first time that *S. pneumoniae* bgaC encodes a surface beta-galactosidase with high substrate specificity that is significantly associated with the infection activity of pneumococci.

*JOURNAL OF BACTERIOLOGY*, 191(9): 3011-3023.

**Keyword**: chinchilla tracheal epithelium; virulence factors; bacillus-circulans; escherichia-coli; genome sequence; strain r6

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**Article 22**

**JNK/FOXO-mediated neuronal expression of fly homologue of Peroxiredoxin II reduces oxidative stress and extends life span**

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Activation of c-Jun N-terminal kinase (JNK) signaling in neurons increases stress resistance and extends life span, in part through FOXO-mediated transcription in *Drosophila*. However, the JNK/FOXO target genes are unknown. Here, we identified *Jafrac1*, a *Drosophila* homolog of human *Peroxiredoxin II* (*hPrxII*), as a downstream effector of JNK/FOXO signaling in neurons that enhances stress resistance and extends life span. We found that *Jafrac1* was expressed in the adult brain and induced by paraquat, a reactive oxygen species-generating chemical. RNA interference-mediated neuronal knockdown of *Jafrac1* enhanced, while neuronal overexpression of *Jafrac1* and *hPrxII* suppressed, paraquat-induced lethality in flies. Neuronal expression of *Jafrac1* also significantly reduced ROS levels, restored mitochondrial function, and attenuated JNK activation caused by paraquat. Activation of JNK/FOXO signaling in neurons increases the *Jafrac1* expression level under both normal and oxidative stressed conditions. Moreover, neuronal knockdown of *Jafrac1* shortened, while overexpression of *Jafrac1* and *hPrxII* extended, the life span in flies. These results support the hypothesis that JNK/FOXO signaling extends life span via amelioration of oxidative damage and mitochondrial dysfunction in neurons.

*JOURNAL OF BIOLOGICAL CHEMISTRY*, 284(43): 29454-29461.

**Keyword**: hydrogen-peroxide; mitochondrial dysfunction; gene family; *drosophila*
**Article 23**

**Purification and characterization of recombinant human erythropoietin from milk of transgenic pigs**

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**BACKGROUND:** Human erythropoietin (hEPO), a hydrophobic acidic glycoprotein responsible for the regulation of red blood cell production in mammals, is used for the treatment of anemia. In general, the purification of transgenic animal-derived therapeutic proteins is not easy due to their low titer concentrations and abundant contaminant proteins. For the first time, here the purification and characterization of rhEPO from the milk of transgenic pigs are described.

**RESULTS:** The rhEPO was purified by heparin chromatography, reverse-phase chromatography, and gel filtration chromatography, resulting in a 16.5% yield and >98% purity. The rhEPO purified from the milk of transgenic pigs contained less acidic isoforms and was underglycosylated in contrast to CHO-derived rhEPO. Cell proliferation of the F-36/EPO-dependent cell line was proportional to the dose of transgenic pig-derived rhEPO.

**CONCLUSION:** Transgenic pig-derived rhEPO with high purity was achieved after three-step chromatography following two-step precipitation. The transgenic pig-derived rhEPO was demonstrated to have comparable potency with CHO-derived rhEPO. Transgenic pig-derived rhEPO may not be therapeutically feasible because of different glycosylation, and thus further studies are required to elucidate the effect of this aberrant glycosylation on the biological activity and stability in vivo.


**Keyword:** transgenic pig; recombinant human erythropoietin (rhEPO); purification; glycosylation

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**Article 24**

**A highly effective and facile way to prepare cellular labelling quantum dots with cetyltrimethylammonium bromide**

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We report a facile and effective way to prepare cell labelling quantum dots (QDs) using cetyltrimethylammonium bromide (CTAB) and to enhance biocompatibility of the QD labels. There are several reports on the use of this ligand to encapsulate nanoparticles including QDs. However, due to its high cellular toxicity, CTAB has still not been employed to prepare QDs for cellular labelling. After removing the free ligand by dialysis, we could successfully use CdSe/CdS/ZnS (core/shell/shell) QDs for cellular labelling. In addition, we found that the simple introduction of a sonication step to cause the emulsion of the QDs in the aqueous surfactant solution could lead to a five-times higher encapsulation of the QDs as compared to other methods. Fluorescent microscopy images of HeLa cells revealed that the QDs were evenly dispersed inside them. Furthermore, fluorescent morphological images of the QD labelled cells were more distinct than bright field images.

*JOURNAL OF EXPERIMENTAL NANOSCIENCE*, 4(2): 105-112.

**Keyword:** quantum dot; cellular labelling; cellular morphology; cancer; surfactant
Evidence against the physiological role of acetyl phosphate in the phosphorylation of the ArcA response regulator in *Escherichia coli*

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The Arc two-component signal transduction system of *Escherichia coli* comprises the ArcB sensor kinase and the ArcA response regulator. Under anoxic growth conditions, ArcB autophosphorylates and transphosphorylates ArcA, which, in turn, represses or activates its target operons. ArcA has been shown to be able to autophosphorylate *in vitro* at the expense of acetyl-P. Here, the *in vivo* effect of acetyl phosphate on the redox signal transduction by the Arc system was assessed. Our results indicate that acetyl phosphate can modulate the expression of ArcA-P target genes only in the absence of ArcB. Therefore, the acetyl phosphate dependent ArcA phosphorylation route does not seem to play a significant role under physiological conditions. *JOURNAL OF MICROBIOLOGY*, 47(5): 657-662.

**Keyword**: acetyl phosphate; Arc two-component signal transduction; ArcA response regulator; *E. coli*

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Monitoring of cleavage preference for caspase-3 using recombinant protein substrates

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The apoptotic caspases have been classified in accordance with their substrate specificities, as the optimal tetrapeptide recognition motifs for a variety of caspases have been determined via positional scanning substrate combinatorial library technology. Here, we focused on two proteolytic recognition motifs, DEVD and IETD, owing to their extensive use in cell death assay. Although DEVE and IETD have been generally considered to be selective for caspase-3 and -8, respectively, the proteolytic cleavage of these substrates does not display absolute specificity for a particular caspase. Thus, we attempted to monitor the cleavage preference for caspase-3, particularly using the recombinant protein substrates. For this aim, the chimeric GST:DEVD:EGFP and GST:IETD:EGFP proteins were genetically constructed by linking GST and EGFP with the linkers harboring DEVD and IETD. To our best knowledge, this work constitutes the first application for the monitoring of cleavage preference employing the recombinant protein substrates that simultaneously allow for mass and fluorescence analyses. Consequently, GST: IETD:EGFP was cleaved partially in response to caspase-3, whereas GST:DEVD:EGFP was completely proteolyzed, indicating that GST:DEVD:EGFP is a better substrate than GST:IETD:EGFP for caspase-3. Collectively, using these chimeric protein substrates, we have successfully evaluated the feasibility of the recombinant protein substrate for applicability to the monitoring of cleavage preference for caspase-3. *JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY*, 19(9): 911-917.

**Keyword**: tetrapeptide; proteolytic indicator; caspase substrate; protease activity
Non-structural 5A protein of hepatitis C virus induces a range of liver pathologies in transgenic mice

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Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC). However, the mechanism of HCV pathogenesis is not well understood. Our previous in vitro studies suggested that non-structural 5A (NS5A) protein may play an important role in liver pathogenesis. To elucidate the mechanism of HCV-induced liver pathogenesis, we investigated the histopathological changes of liver in transgenic mice harbouring the NS5A gene. We generated transgenic mice harbouring HCV NS5A gene under the control of hepatitis B virus (HBV) enhancer. Pathological changes were analysed by immunohistochemical staining and western blot analysis. Lipid composition and reactive oxygen species (ROS) production in NS5A transgenic mice were analysed. HCV NS5A transgenic mice developed extraordinary steatosis over 6 months old and induced HCC in some mice. NS5A was co-localized with apolipoprotein A-1 in fatty hepatocytes. In addition, the extraordinarily high levels of ROS, NF-kappa B and STAT3 were detected in hepatocytes of NS5A transgenic mice. These data suggest that NS5A, independent of other HCV viral proteins, may play an important role in the development of hepatic pathologies, including steatosis and hepatocellular carcinoma in transgenic mice.


Keyword: hepatitis C virus; liver pathogenesis; NS5A protein; steatosis; transgenic mice

Extracellular signal-regulated Kinase 2-dependent phosphorylation induces cytoplasmic localization and degradation of p21Cip1

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p21Cip1 is an inhibitor of cell cycle progression that promotes G1-phase arrest by direct binding to cyclin-dependent kinase and proliferating cell nuclear antigen. Here we demonstrate that mitogenic stimuli, such as epidermal growth factor treatment and oncogenic Ras transformation, induce p21Cip1 downregulation at the posttranslational level. This downregulation requires the sustained activation of extracellular signal-regulated kinase 2 (ERK2), which directly interacts with and phosphorylates p21Cip1, promoting p21Cip1 nucleocytoplasmic translocation and ubiquitin-dependent degradation, thereby resulting in cell cycle progression. ERK1 is not likely involved in this process. Phosphopeptide analysis of in vitro ERK2-phosphorylated p21Cip1 revealed two phosphorylation sites, Thr57 and Ser130. Double mutation of these sites abolished ERK2-mediated p21Cip1 translocation and degradation, thereby impairing ERK2-dependent cell cycle progression at the G1/S transition. These results indicate that ERK2 activation transduces mitogenic signals, at least in part, by downregulating the cell cycle inhibitory protein p21Cip1.

MOLECULAR AND CELLULAR BIOLOGY, 29(12): 3379-3389.

Keyword: activated protein-kinase; cyclin-dependent kinases; CDK inhibitors; uv-irradiation; PCNA binding; map kinase
Nanogap biosensors for electrical and label-free detection of biomolecular interactions

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We demonstrate nanogap biosensors for electrical and label-free detection of biomolecular interactions. Parallel fabrication of nanometer distance gaps has been achieved using a silicon anisotropic wet etching technique on a silicon-on-insulator (SOI) wafer with a finely controllable silicon device layer. Since silicon anisotropic wet etching resulted in a trapezoid-shaped structure whose end became narrower during the etching, the nanogap structure was simply fabricated on the device layer of a SOI wafer. The nanogap devices were individually addressable and a gap size of less than 60 nm was obtained. We demonstrate that the nanogap biosensors can electrically detect biomolecular interactions such as biotin/streptavidin and antigen/antibody pairs. The nanogap devices show a current increase when the proteins are bound to the surface. The current increases proportionally depending upon the concentrations of the molecules in the range of 100 fg ml⁻¹ -100 ng ml⁻¹ at 1 V bias. It is expected that the nanogap developed here could be a highly sensitive biosensor platform for label-free detection of biomolecular interactions.


Keyword : prostate-specific antigen; electrodes; nanoparticles

Near-infrared emitting fluorescent nanocrystals-labeled natural killer cells as a platform technology for the optical imaging of immunotherapeutic cells-based cancer therapy

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This study describes the development of near-infrared optical imaging technology for the monitoring of immunotherapeutic cell-based cancer therapy using natural killer (NK) cells labeled with fluorescent nanocrystals. Although NK cell-based immunotherapeutic strategies have drawn interest as potent preclinical or clinical methods of cancer therapy, there are few reports documenting the molecular imaging of NK cell-based cancer therapy, primarily due to the difficulty of labeling of NK cells with imaging probes. Human natural killer cells (NK92MI) were labeled with anti-human CD56 antibody-coated quantum dots (QD705) for fluorescence imaging. FACS analysis showed that the NK92MI cells labeled with anti-human CD56 antibody-coated QD705 have no effect on the cell viability. The effect of anti-human CD56 antibody-coated QD705 labeling on the NK92MI cell function was investigated by measuring interferon gamma (IFN-gamma) production and cytolytic activity. Finally, the NK92MI cells labeled with anti-human CD56 antibody-coated QD705 showed a therapeutic effect similar to that of unlabeled NK92MI cells. Images of intratumorally injected NK92MI cells labeled with anti-human CD56 antibody-coated QD705 were acquired using near-infrared optical imaging both in vivo and in vitro. This result demonstrates that the immunotherapeutic cells labeled with fluorescent nanocrystals can be a versatile platform for the effective tracking of injected therapeutic cells using optical imaging technology, which is very important in cell-based cancer therapies.


Keyword : positron-emission-tomography; quantum dots; cytotoxicity
Detection of biomolecular binding through enhancement of localized surface plasmon resonance (LSPR) by gold nanoparticles

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To amplify the difference in localized surface plasmon resonance (LSPR) spectra of gold nano-islands due to intermolecular binding events, gold nanoparticles were used. LSPR-based optical biosensors consisting of gold nano-islands were readily made on glass substrates using evaporation and heat treatment. Streptavidin (STA) and biotinylated bovine serum albumin (Bio-BSA) were chosen as the model receptor and the model analyte, respectively, to demonstrate the effectiveness of this detection method. Using this model system, we were able to enhance the sensitivity in monitoring the binding of Bio-BSA to gold nano-island surfaces functionalized with STA through the addition of gold nanoparticle-STA conjugates. In addition, SU-8 well chips with gold nano-island surfaces were fabricated through a conventional UV patterning method and were then utilized for image detection using the attenuated total reflection mode. These results suggest that the gold nano-island well chip may have the potential to be used for multiple and simultaneous detection of various bio-substances.

SENSORS, 9(4): 2334-2344.

Keyword : localized surface plasmon resonance (LSPR); gold nano-island; gold nanoparticle; attenuated total reflection (ATR); well chip

Ion-sensitive field-effect transistor for biological sensing

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In recent years there has been great progress in applying FET-type biosensors for highly sensitive biological detection. Among them, the ISFET (ion-sensitive field-effect transistor) is one of the most intriguing approaches in electrical biosensing technology. Here, we review some of the main advances in this field over the past few years, explore its application prospects, and discuss the main issues, approaches, and challenges, with the aim of stimulating a broader interest in developing ISFET-based biosensors and extending their applications for reliable and sensitive analysis of various biomolecules such as DNA, proteins, enzymes, and cells.

SENSORS, 9(9): 7111-7131.

Keyword : ISFET; ion-sensitive field-effect transistor; biosensor; biomolecules
Multifunctional silica nanocapsule with a single surface hole

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Multifunctional silica nanocapsules containing magnetic nanoparticles and fluorescent quantum dots with a single surface hole fabricated by a single-step emulsion-mediated process are described. The silica nanocapsules are easily internalized by phagocytic dendritic cells and show a high potential as bimodal imaging contrast agents (for fluorescence and magnetic resonance imaging) in vivo as well as in vitro. SMALL, 5(3): 324-328.

Keyword : magnetic materials; nanoparticles; silica; hollow polymer microspheres; quantum dots; magnetic nanoparticles; dendritic cells

Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles

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In general, gold nanoparticles are recognized as being as nontoxic. Still, there have been some reports on their toxicity, which has been shown to depend on the physical dimension, surface chemistry, and shape of the nanoparticles. In this study, we carry out an in vivo toxicity study using 13 nm-sized gold nanoparticles coated with PEG (MW 5000). In our findings the 13 nm sized PEG-coated gold nanoparticles were seen to induce acute inflammation and apoptosis in the liver. These nanoparticles were found to accumulate in the liver and spleen for up to 7 days after injection and to have long blood circulation times. In addition, transmission electron microscopy showed that numerous cytoplasmic vesicles and lysosomes of liver Kupffer cells and spleen macrophages contained the PEG-coated gold nanoparticles. These findings of toxicity and kinetics of PEG-coated gold nanoparticles may have important clinical implications regarding the safety issue as PEG-coated gold nanoparticles are widely used in biomedical applications. TOXICOLOGY AND APPLIED PHARMACOLOGY, 236(1): 16-24.

Keyword : gold nanoparticles; pharmacokinetics; intravenous injection; inflammation; accumulation
Type II transmembrane serine proteases in cancer and viral infections

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Regulated proteolysis of cellular factors is pivotal to tissue development and homeostasis, whereas uncontrolled proteolytic activity is linked to disease. Type II transmembrane serine proteases (TTSPs) are expressed at the cell surface and are thus ideally located to regulate cell-cell and cell-matrix interactions. Increasing evidence demonstrates that aberrant expression of TTSPs is a hallmark of several cancers and recent studies have defined molecular mechanisms underlying TTSP-promoted carcinogenesis. In addition, new findings suggest that influenza and other respiratory viruses could exploit TTSPs to promote their spread, making these proteases potential targets for intervention in cancer and viral infections. Here, we review the role of TTSPs in tumorigenesis and viral infection and discuss potential approaches to therapy.

TRENDS IN MOLECULAR MEDICINE, 15(7): 303-312.

Keyword : trypsin-like protease; surface proteolytic enzymes; tissue microarray analysis; hepatocyte growth-factor; squamous-cell carcinoma; influenza-virus entry
2. Division of Translational Research

- Medical Genomics Research Center
- Development and Differentiation Research Center
- Medical Proteomics Research Center
Purification, crystallization and data collection of the apoptotic nuclease endonuclease G

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Endonuclease G (EndoG) is a mitochondrial enzyme that responds to apoptotic stimuli by translocating to the nucleus and cleaving chromosomal DNA. EndoG is the main apoptotic endonuclease in the caspase-independent pathway. Mouse EndoG without the mitochondrial localization signal (amino-acid residues 1-43) was successfully overexpressed, purified and crystallized using a microbatch method under oil. The initial crystal (type I) was grown in the presence of the detergent CTAB and diffracted to 2.8 angstrom resolution, with unit-cell parameters \(a = 72.20\), \(b = 81.88\), \(c = 88.66\) angstrom, \(\beta = 97.59\) degrees in a monoclinic space group. The crystal contained two monomers in the asymmetric unit, with a predicted solvent content of 46.6%. Subsequent mutation of Cys110 improved the stability of the protein significantly and produced further crystals of types II, III and IV with space groups \(C_2\), \(P_4_2_2_2\) (or \(P_4_2_2_2\)) and \(P_2_1_2_1\), respectively, in various conditions. This suggests the critical involvement of this conserved cysteine residue in the crystallization process.


**Keyword**: active-site; mitochondrial protein; periplasmic nuclease; DNA-binding

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Farnesyl protein transferase inhibitory components of Polygonum multiflorum

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The methanolic extract of the roots of Polygonum multiflorum (Polygonaceae) was found to show inhibitory activity towards farnesyl protein transferase (FPTase). Bioassay-guided fractionation of the methanolic extract resulted in the isolation of two anthraquinone glycosides, as inhibitors of FPTase. These compounds inhibited the FPTase activity in a dose-dependent manner.


**Keyword**: Polygonum multiflorum; polygonaceae; anthraquinone glycosides; FPTase
Reduced formation of advanced glycation endproducts via interactions between glutathione peroxidase 3 and dihydroxyacetone kinase 1

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Dihydroxyacetone (DHA) induces the formation of advanced glycation endproducts (AGEs), which are involved in several diseases. Earlier, we identified dihydroxyacetone kinase 1 (Dak1) as a candidate glutathione peroxidase 3 (Gpx3)-interacting protein in Saccharomyces cerevisiae. This finding is noteworthy, as no clear evidence on the involvement of oxidative stress systems in DHA-induced AGE formation has been found to date. Here, we demonstrate that Gpx3 interacts with Dak1, alleviates DHA-mediated stress by upregulating Dak activity, and consequently suppresses AGE formation. Based on these results, we propose that defense systems against oxidative stress and DHA-induced AGE formation are related via interactions between Gpx3 and Dak1. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 389(1): 177-180.

Keyword: advanced glycation endproduct; dihydroxyacetone; dihydroxyacetone kinase 1; glutathione peroxidase 3; oxidative stress

Involvement of PTP-RQ in differentiation during adipogenesis of human mesenchymal stem cells

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Mesenchymal stem cells (MSCs) are self-renewable multipotent progenitor cells with the capacity to differentiate into several distinct mesenchymal lineages. While MSCs display significant potential in tissue engineering and therapeutic applications, the regulatory mechanisms underlying the differentiation of these cells are yet to be established. Phosphorylation is a post-translational modification that plays a significant role in diverse biological phenomena. In this study, to mine the protein tyrosine phosphatases (PTPs) involved in adipogenesis of human MSCs, differential expression of human PTPs was examined using RT-PCR analysis. Among the 107 human PTPs, PTP-RQ was dramatically downregulated during the early phase of adipogenesis. PFP-RQ was classified as a receptor-type III PTP with phosphatidylinositol phosphatase (PIPase) activity. Overexpression of PTP-RQ consistently led to reduced differentiation of MSCs into adipocytes via decreasing the phosphatidyl inositol phosphate level in cells, and consequently downregulating Akt/PKB phosphorylation. Our results collectively suggest that PFP-RQ is a useful target protein for regulating the differentiation of MSCs into adipocytes, and may be used to develop novel drugs for the treatment of obesity. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 383(2): 252-257.

Keyword: adipocyte; adipogenesis; differentiation; human mesenchymal stem cells; protein tyrosine phosphatase RQ
Activation of autophagy during glutamate-induced HT22 cell death

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Recent evidence suggests that autophagy plays a role in oxidative injury-induced cell death. Here we examined whether glutamate-mediated oxidative toxicity induces autophagy in murine hippocampal HT22 cells and if autophagy induction affects the molecular events associated with cell death. Markers for autophagy induction including LC3 conversion, suppression of mTOR pathway, and GFP-LC3 dot formation were enhanced by glutamate treatment. By contrast, autophagy inhibition blocked glutamate-induced LC3 conversion and consequently reduced cell death. Activation of ERK1/2, a hallmark of glutamate-induced cytotoxicity, was also decreased by autophagy inhibition. Interestingly, autophagy inhibition also affected the expression of chaperones including Hsp60 and Hsp70, which are differentially regulated during HT22 cell death. Activation of ERK1/2, a hallmark of glutamate-induced cytotoxicity, was also decreased by autophagy inhibition. Together these results suggest that glutamate-induced cytotoxicity involves autophagic cell death and chaperones may play a role in this process.


Keyword : HT22; glutamate toxicity; autophagy; erk1/2; molecular chaperones; hsp60

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PTP inhibitor IV protects JNK kinase activity by inhibiting dual-specificity phosphatase 14 (DUSP14)

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Protein phosphorylation plays critical roles in the regulation of protein activity and cell signaling. The level of protein phosphorylation is controlled by protein kinases and protein tyrosine phosphatases (PTPs). Disturbance of the equilibrium between protein kinase and PTP activities results in abnormal protein phosphorylation, which has been linked to the etiology of several diseases, including cancer. In this study, we screened protein tyrosine phosphatases (PTPs) by in vitro phosphatase assays to identify PTPs that are inhibited by bis (4-trifluoromethyl-sulfonamidophenyl, TFMS)-1,4-diisopropylbenzene (PTP inhibitor IV). PTP inhibitor IV inhibited DUSP14 phosphatase activity. Kinetic studies with PTP inhibitor IV and DUSP14 revealed a competitive inhibition, suggesting that PTP inhibitor IV binds to the catalytic site of DUSP14. PTP inhibitor IV effectively and specifically inhibited DUSP14-mediated dephosphorylation of JNK, a member of the mitogen-activated protein kinase (MAPK) family.


Keyword : DUSP14; PTP inhibitor IV; JNK; phosphatase assay
Structure-based de novo design and biochemical evaluation of novel Cdc25 phosphatase inhibitors

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Cdc25 phosphatases have been considered as attractive drug targets for anticancer therapy due to the correlation of their overexpression with a wide variety of cancers. We have been able to identify 32 novel Cdc25 phosphatase inhibitors with micromolar activity by means of a structure-based de novo design method with the two known inhibitor scaffolds. Because the newly discovered inhibitors are structurally diverse and have desirable physicochemical properties as a drug candidate, they deserve further investigation as anticancer drugs. The differences in binding modes of the identified inhibitors in the active sites of Cdc25A and B are addressed in detail.


Keyword: Cdc25 phosphatase; de novo design; docking; anticancer agents

Effects of sample size on robustness and prediction accuracy of a prognostic gene signature

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Background: Few overlap between independently developed gene signatures and poor inter-study applicability of gene signatures are two of major concerns raised in the development of microarray-based prognostic gene signatures. One recent study suggested that thousands of samples are needed to generate a robust prognostic gene signature.

Results: A data set of 1,372 samples was generated by combining eight breast cancer gene expression data sets produced using the same microarray platform and, using the data set, effects of varying samples sizes on a few performances of a prognostic gene signature were investigated. The overlap between independently developed gene signatures was increased linearly with more samples, attaining an average overlap of 16.56% with 600 samples. The concordance between predicted outcomes by different gene signatures also was increased with more samples up to 94.61% with 300 samples. The accuracy of outcome prediction also increased with more samples. Finally, analysis using only Estrogen Receptor-positive (ER+) patients attained higher prediction accuracy than using both patients, suggesting that sub-type specific analysis can lead to the development of better prognostic gene signatures.

Conclusion: Increasing sample sizes generated a gene signature with better stability, better concordance in outcome prediction, and better prediction accuracy. However, the degree of performance improvement by the increased sample size was different between the degree of overlap and the degree of concordance in outcome prediction, suggesting that the sample size required for a study should be determined according to the specific aims of the study.

BMC BIOINFORMATICS, 10: Art. No. 147.

Keyword: negative breast-cancer; microarray data; expression data; mindact trial
Article 44

Nucleologenesis and embryonic genome activation are defective in interspecies cloned embryos between bovine ooplasm and rhesus monkey somatic cells

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Interspecies somatic cell nuclear transfer (iSCNT) has been proposed as a tool to address basic developmental questions and to improve the feasibility of cell therapy. However, the low efficiency of iSCNT embryonic development is a crucial problem when compared to in vitro fertilization (IVF) and intraspecies SCNT. Thus, we examined the effect of donor cell species on the early development of SCNT embryos after reconstruction with bovine ooplasm. No apparent difference in cleavage rate was found among IVF, monkey-bovine (MB)-iSCNT, and bovine-bovine (BB)-SCNT embryos. However, MB-iSCNT embryos failed to develop beyond the 8- or 16-cell stages and lacked expression of the genes involved in embryonic genome activation (EGA) at the 8-cell stage. From ultrastructural observations made during the peri-EGA period using transmission electron microscopy (TEM), we found that the nucleoli of MB-iSCNT embryos were morphologically abnormal or arrested at the primary stage of nucleologenesis. Consistent with the TEM analysis, nuclear component proteins, such as upstream binding transcription factor, fibrillarin, nucleolin, and nucleophosmin, showed decreased expression and were structurally disorganized in MB-iSCNT embryos compared to IVF and BB-SCNT embryos, as revealed by real-time PCR and immunofluorescence confocal laser scanning microscopy, respectively. The down-regulation of housekeeping and imprinting genes, abnormal nucleolar morphology, and aberrant patterns of nucleolar proteins during EGA resulted in developmental failure in MB-iSCNT embryos. These results provide insight into the unresolved problems of early embryonic development in iSCNT embryos.

BMC DEVELOPMENTAL BIOLOGY, 9: Art. No. 44.

Keyword: nuclear transfer embryos; cytoplasm supports development; preimplantation mouse embryos; small nucleolar RNA; pre-ribosomal-RNA

Article 45

Notch signaling is required for maintaining stem-cell features of neuroprogenitor cells derived from human embryonic stem cells

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Background: Studies have provided important findings about the roles of Notch signaling in neural development. Unfortunately, however, most of these studies have investigated the neural stem cells (NSCs) of mice or other laboratory animals rather than humans, mainly owing to the difficulties associated with obtaining human brain samples. It prompted us to focus on neuroectodermal spheres (NESs) which are derived from human embryonic stem cell (hESC) and densely inhabited by NSCs. We here investigated the role of Notch signaling with the hESC-derived NESs.

Results: From hESCs, we derived NESs, the in-vitro version of brain-derived neurospheres. NES formation was confirmed by increased levels of various NSC marker genes and the emergence of rosette structures in which neuroprogenitors are known to reside. We found that Notch signaling, which maintains stem cell characteristics of in-vivo-derived neuroprogenitors, is active in these hESC-derived NESs, similar to their in-vivo counterpart. Expression levels of Notch signaling molecules such as NICD, DLLs, JAG1, HES1 and HES5 were increased in the NESs. Inhibition of the Notch signaling by a gamma-secretase inhibitor reduced rosette structures, expression levels of NSC marker genes and proliferation potential in the NESs, and, if combined with withdrawal of growth factors, triggered differentiation toward neurons.

Conclusion: Our results indicate that the hESC-derived NESs, which share biochemical features with brain-derived neurospheres, maintain stem cell characteristics mainly through Notch signaling, which suggests that the hESC-derived NESs could be an in-vitro model for in-vivo neurogenesis.

BMC NEUROSCIENCE, 10: Art. No. 97.

Keyword: in-vitro differentiation; neural precursors; neuronal differentiation; progenitor cells
Discovery of novel and potent Cdc25 phosphatase inhibitors based on the structure-based de novo design

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Cdc25 phosphatases have been considered as attractive drug targets for anticancer therapy due to the correlation of their overexpression with a wide variety of cancers. We have been able to identify five novel Cdc25 phosphatase inhibitors with micromolar activity by means of a structure-based de novo design method with a known inhibitor scaffold. Because the newly discovered inhibitors are structurally diverse and have desirable physicochemical properties as a drug candidate, they deserve further investigation as anticancer drugs. The differences in binding modes of the identified inhibitors in the active sites of Cdc25A and B are addressed in detail.


Keyword : Cdc25 phosphatase; de novo design; inhibitor; anticancer agents

Molecular interaction between a Bcl-2 homolog from kaposi sarcoma virus and p53

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In this report, we show the first case where the direct interaction between viral Bcl-2 homolog and p53 is observed. Considering the structural similarity with KSHV Bcl-2, the other viral Bcl-2 homologs such as M11L from myxoma virus may also be involved in the direct interaction with p53 TAD. For cellular Bcl-2 family proteins, in vivo interaction of Bcl-2 and Bcl-XL with p53 was observed at the cellular level9 and the interactions were shown to be mediated via the DNA-binding domain of p53. Recently, Bcl-XL was observed to interact with the N-terminal domain of p53. Therefore, it is possible that KSHV Bcl-2 prevents the transcription-independent apoptosis of p53 by interfering with the direct interactions between p53 and cellular anti-apoptotic Bcl-2 family proteins. Dysregulation of the host apoptosis mechanism by viral Bcl-2 homolog may enhance the survival of the virusinfected host cells. The information on the dysregulated pathway and the binding site should contribute to the development of antiviral therapies. In conclusion, we demonstrated direct interaction between KSHV Bcl-2 and p53 TAD using NMR binding experiments. Our chemical shift perturbation data determined the KSHV Bcl-2-binding sites on p53 TAD at the atomic level, indicating that the binding sites coincide with those for mdm2, p300, or hTAFII31. Our observation suggests that the other viral Bcl-2 homologs may be involved in the interaction with p53 as cellular Bcl-2 family proteins.

BULLETIN OF THE KOREAN CHEMICAL SOCIETY, 30(7): 1655-1657.

Keyword : KSHV Bcl-2; p53; NMR; HSQC; molecular interaction
Cryptotanshinone inhibits constitutive signal transducer and activator of transcription 3 function through blocking the dimerization in DU145 prostate cancer cells

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Because signal transducer and activator of transcription 3 (STAT3) is constitutively activated in most human solid tumors and is involved in the proliferation, angiogenesis, immune evasion, and antiapoptosis of cancer cells, researchers have focused on STAT3 as a target for cancer therapy. We screened for natural compounds that inhibit the activity of STAT3 using a dual-luciferase assay. Cryptotanshinone was identified as a potent STAT3 inhibitor. Cryptotanshinone rapidly inhibited STAT3 Tyr705 phosphorylation in DU145 Prostate cancer cells and the growth of the cells through 96 hours of the treatment. Inhibition of STAT3 Tyr705 phosphorylation in DU145 cells decreased the expression of STAT3 downstream target proteins such as cyclin D1, survivin, and Bcl-xL. To investigate the cryptotanshinone inhibitory mechanism in DU145 cells, we analyzed proteins upstream of STAT3. Although phosphorylation of Janus-activated kinase (JAK) 2 was inhibited by 7 μmol/L cryptotanshinone at 24 hours, inhibition of STAT3 Tyr705 phosphorylation occurred within 30 minutes and the activity of the other proteins was not affected. These results suggest that inhibition of STAT3 phosphorylation is caused by a JAK2-independent mechanism, with suppression of JAK2 phosphorylation as a secondary effect of cryptotanshinone treatment. Continuing experiments revealed the possibility that cryptotanshinone might directly bind to STAT3 molecules. Cryptotanshinone was colocalized with STAT3 molecules in the cytoplasm and inhibited the formation of STAT3 dimers. Computational modeling showed that cryptotanshinone could bind to the SH2 domain of STAT3. These results suggest that cryptotanshinone is a potent anticancer agent targeting the activation STAT3 protein. It is the first report that cryptotanshinone has antitumor activity through the inhibition of STAT3.


Keyword: stat3 serine phosphorylation; salvia-miltiorrhiza bunge; growth-factor receptor; tanshinone-iia; breast-cancer; SRC oncoprotein

Human ZNF312b promotes the progression of gastric cancer by transcriptional activation of the K-ras gene

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Gastric cancer ranks second among the most common causes of cancer deaths worldwide. Recent studies reported target molecules that are candidates for new therapeutic interventions; however, their molecular mechanism has not been clearly defined. In this study, we found that ZNF312b plays a role in tumor progression and metastasis in gastric cancer via transcriptional activation of the K-ras oncogene. ZNF312b seems to be specifically overexpressed in gastric cancer tissues and cell lines. The overexpression of ZNF312b induces cancer-like phenotypes, including accelerated proliferation and increased tumor masses in nude mice, which are completely reversed by its knockdown in gastric cancer cell lines, implying direct involvement in gastric tumor progression. From analyses using deletion mutants of ZNF312b and K-ras promoter-driven luciferase reporters, we found that it translocates into the nucleus via the proline-rich domain of its COOH terminus to activate transcription of the K-ras gene, resulting in an enhancement of the extracellular signal-regulated kinase signaling pathway that governs cell proliferation. Taken together, these results suggest that ZNF312b contributes to the promotion of gastric cancer by triggering K-ras oncogene expression. The current study is the first to report that ZNF312b, a novel transcription factor, was associated with tumorigenicity of gastric cancer. This might be a valuable target that could provide new insight into the development of new therapeutic modalities for patients with gastric cancer.

CANCER RESEARCH, 69(7): 3131-3139.

Keyword: zinc-finger gene; polymerase chain-reaction; expressed sequence tags; fez-like
NDRG2 expression decreases with tumor stages and regulates TCF/beta-catenin signaling in human colon carcinoma

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NDRG (N-Myc downstream-regulated gene)-2 is a member of the NDRG family. Although it has been suggested that NDRG2 is involved in cellular differentiation and tumor suppression, its intracellular signal and regulatory mechanism are not well known. Here, we show the differential expression of NDRG2 in human colon carcinoma cell lines and tissues by reverse transcription-polymerase chain reaction and immunohistochemical analyses with monoclonal antibody against NDRG2. NDRG2 was strongly expressed in normal colonic mucosa and colonic adenomatous tissues (25 of 25) but not in all invasive cancer tissues [44 of 99 (44%)]. Most distinctive results indicated that the high expression level of NDRG2 has a positive correlation with tumor differentiation and inverse correlation with tumor invasion depth and Dukes' stage of colon adenocarcinoma. To investigate the roles of NDRG2 in tumorigenesis, we used in vitro cell culture system. SW620 colon cancer cell line with a low level of intrinsic NDRG2 protein was transfected with NDRG2-expressing plasmid. TOPflash luciferase reporter assay showed that the transcriptional activity of T-cell factor (TCF)/lymphoid enhancer factor (LEF) was reduced by NDRG2 introduction, but not by the introduction of mutant NDRG2 generated by deletion or site-directed mutagenesis. Intracellular beta-catenin levels were slightly reduced in the NDRG2-transfected SW620 cells and this regulation of beta-catenin stability and TCF/LEF activity were mediated through the modulation of glycogen synthase kinase-3beta activity by NDRG2 function. Our results suggest that NDRG2 might play a pivotal role as a potent tumor suppressor by the attenuation of TCF/beta-catenin signaling for the maintenance of healthy colon tissues.

Carcinogenesis, 30(4): 598-605.

Keyword: glycogen-synthase kinase-3; beta-catenin; cell differentiation; suppressor gene

Peroxisiredoxin I contributes to TRAIL resistance through suppression of redox-sensitive caspase activation in human hepatoma cells

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Reactive oxygen species (ROS) have been implicated in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance of many cancers. We evaluated the role of peroxiredoxin (Prx) I in TRAIL resistance governed by coupling of nicotinamide adenine dinucleotide phosphate oxidase (Nox)-derived ROS signaling with the p38 mitogen-activated protein kinase (MAPK)/caspase-signaling cascade in liver cancer cells. Uregulated Prx I expression was found in neoplastic regions of human patient liver, and Prx I knockdown resulted in accelerated TRAIL-induced cell death in SK-Hep-1 human hepatoma cells. The TRAIL cytotoxicity by Prx I knockdown was dependent on activation of caspase-8/3 cascades, which was ablated by addition of inhibitors for p38 MAPK, ROS or Nox, suggesting the association with Nox-driven redox signaling. Furthermore, we found that Nox4 was constitutively expressed in both SK-Hep-1 cells and tumor regions of patient livers, knockdown of Nox4 expression could alleviate ROS generation and TRAIL-mediated cytotoxicity. In accordance with previous findings, increased activation of both p38 MAPK and caspase cascades by Prx I knockdown was inhibited by either Nox4 knockdown or SB203580 addition. Collectively, these data suggest that Prx I functions to block propagation of Nox-derived ROS signaling to the p38 MAPK/caspase/cell death cascade during TRAIL treatment and also provides a molecular mechanism by which Prx I contributes to TRAIL resistance in liver cancers.

Carcinogenesis, 30(7): 1106-1114.

Keyword: mediated up-regulation; necrosis-factor-alpha; rat mesangial cells; lung-cancer cells; n-terminal kinase; reactive oxygen; hydrogen-peroxide
PPAR gamma partial agonist, KR-62776, inhibits adipocyte differentiation via activation of ERK

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Indenone KR-62776 acts as an agonist of PPAR gamma without inducing obesity in animal models and cells. X-ray crystallography reveals that the indenone occupies the binding pocket in a different manner than rosiglitazone. 2-Dimensional gel-electrophoresis showed that the expression of 42 proteins was altered more than 2.0-fold between KR-62776- or rosiglitazone-treated adipocyte cells and control cells. Rosiglitazone down-regulated the expression of ERK1/2 and suppressed the phosphorylation of ERK1/2 in these cells. However, the expression of ERK1/2 was up-regulated in KR-62776-treated cells. Phosphorylated ERK1/2, activated by indenone, affects the localization of PPAR gamma, suggesting a mechanism for indenone-inhibition of adipogenesis in 3T3-L1 preadipocyte cells. The preadipocyte cells are treated with ERK1/2 inhibitor PD98059, a large amount of the cells are converted to adipocyte cells. These results support the conclusion that the localization of PPAR gamma is one of the key factors explaining the biological responses of the ligands. CELLULAR AND MOLECULAR LIFE SCIENCES, 66(10): 1766-1781.

Keyword : peroxisome proliferators-activated receptor gamma; rosiglitazone; adipocyte; lipolysis; erk; indenone

Upregulation of the cysteine protease inhibitor, cystatin SN, contributes to cell proliferation and cathepsin inhibition in gastric cancer

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Background: Cysteine proteases like cathepsins are widely distributed proteolytic enzymes and form tight equimolar complexes with cystatins at their active sites. Among cystatins, CST1, encoding cystatin SN, is a member of the type 2 salivary cystatin family found in a variety of fluids and secretions, including plasma, tears, and saliva. CST1 was identified as an upregulated gene in gastric cancer tissues compared to noncancerous regions using our Affymetrix GeneChip microarray.

Methods: The upregulation of CST1 in gastric cancer was analyzed using RT-PCR (n = 15), immunochemistry, and clinicopathological (n = 77) analysis. CST1-siRNA was used for the suppression of CST1 gene expression and cathepsin proteolytic activity was assayed.

Results: CST1 was upregulated in cancerous lesions of gastric cancer tissues compared to noncancerous regions and clinicopathological analysis showed a significant correlation between high expression of CST1 and pTNM stage (p = 0.044). In CST1-siRNA transfected cells, cell proliferation was reduced and the proteolytic activity of cathepsins was increased.

Conclusions: CST1 might be highly involved in gastric tumorigenesis and regulate the proteolytic activity of cysteine proteases. CLINICA CHIMICA ACTA, 406(1-2): 45-51.

Keyword : CST1; cystatin sn; cathepsin; proliferation; gastric cancer
Upregulation and secretion of macrophage inhibitory cytokine-1 (MIC-1) in gastric cancers

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Background: Macrophage inhibitory cytokine-1 (MIC-1), a distant member of the transforming growth factor (TGF)-beta superfamily, has been reported to be upregulated and secreted from several cancers. We examined MIC-1 expression and secretion in gastric cancers.

Methods: MIC-1 mRNA and protein levels in cancer tissues and cell lines were analyzed by RT-PCR and Western blot. MIC-1 expression in cancer tissues and its secretion in serum were analyzed using immunohistochemistry and ELISA.

Results: MIC-1 was significantly upregulated in gastric cancer tissues and cell lines. MIC-1 was secreted from gastric SNU620 cells and its levels in the serum of cancer patients were 10-fold higher than those of healthy controls. In addition, the staining of MIC-1 expression was strongly increased in metastatic gastric cancers.

Conclusions: MIC-1 was obviously overexpressed in gastric cancers and MICA secretion into blood may be useful for the prediction of gastric cancer progression.

CLINICA CHIMICA ACTA, 401(1-2): 128-133.

Keyword: MIC-1; macrophage-inhibitory cytokine-1; GDF-15; growth differentiation factor-15; gastric cancer

Cytoplasmic localization of oocyte-specific variant of porcine DNA methyltransferase-1 during early development

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DNA methyltransferase-1 (Dnmt1) is involved in the maintenance of genomic methylation patterns. Rather than full-length Dnmt1, mouse oocytes have a truncated variant called Dnmt1o. Immunofluorescence data showed that Dnmt1o localized to the cytoplasm, but this has not been confirmed using more direct methods. The cytoplasmic localization of Dnmt1o has been assigned to the main cause of global DNA demethylation in early mouse embryos. We studied localization of Dnmt1o in mouse and pig embryos.

We identified pig Dnmt1o protein and its transcript with unique 5'-end sequence. Physically separating mouse and pig 2-cell embryos into their nuclear and cytoplasmic components demonstrated that Dnmt1o of both species localized to the cytoplasm. Cloned pig embryos had Dnmt1o as the main form, with no indication of somatic Dnmt1. These findings indicate that Dnmt1o is cytoplasmic during early development; its presence in both pig and mouse embryos further suggests that Dnmt1o is conserved in mammals.

DEVELOPMENTAL DYNAMICS, 238(7): 1666-1673.

Keyword: DNA methyltransferase-1 (Dnmt1); Dnmt1o; Dnmt1s; oocyte-specific; pig cloned embryos; preimplantation development
Regulation of glucose metabolism-related genes and VEGF by HIF-1 alpha and HIF-1 beta, but not HIF-2 alpha, in gastric cancer

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Hypoxia-inducible factors (HIFs) are transcription factors that activate the transcription of target genes involved in crucial aspects of cancer development. This study investigated the expression of HIFs and their contribution to the regulation of target genes related to angiogenesis and glucose metabolism in gastric cancer. The data showed that HIFs were over-expressed in gastric cancer and that activation of the target genes was observed mainly in the early stages. Moreover, the results of the present study revealed that only HIF-1 alpha, but not HIF-2 alpha dimerizes with HIF-1 beta and then regulates expression of target genes in response to hypoxia. The results of the present study demonstrate that HIF-1 alpha and HIF-1 beta enhances expression of VEGF and glucose metabolism-related genes in response to hypoxia in gastric cancer. These data offer important information regarding HIF pathways in the development of gastric cancer.


Keyword: glucose metabolism disorders; hypoxia-inducible factor 1; neovascularization; pathologic; stomach neoplasms; vascular endothelial growth factor A

Generation of expression clone set for functional proteomics of human gastric and liver cancers

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Two thousand sixty-eight multi-purpose expression clones for the 326 candidate genes related to gastric or liver cancers were constructed using the Gateway system. These clones can be expressed as His, Glutathione-S-transferase (GST) or Enhanced version of the green fluorescent protein (EGFP) fusion proteins in E. coli, insect cells or mammalian cells. For the 246 E. coli expression clones, the GST fusion proteins had greater expression efficiency and solubility than the His fusion proteins. Approximately 20% of the expressed proteins had unexpected molecular weights. A detailed sequence analysis of these clones revealed frameshift mutations resulting from insertion, deletion or substitution of nucleotides. The results indicate that these changes in the candidate genes may affect the occurrence of gastric or liver cancers. In addition, when 105 proteins, which were expressed in E. coli at very low or undetectable levels, were expressed in insect cells, 76% of the proteins were expressed very well and most were soluble. We also found that most of the 30 proteins prepared using EGFP mammalian expression clones were localized to cellular compartments expected by Gene ontology (GO) and this localization was unaffected if the EGFP-fusion was at the N-terminal or C-terminal region of the protein. Antibody production and subcellular localization analysis of the candidate genes as well as a screen of genes involved in carcinogenesis pathways are currently in progress using these expression clones. These studies provide a valuable resource for developing a better understanding of the molecular mechanism of carcinogenesis in both gastric and liver cancer and would be very helpful in diagnosis and therapeutic predictions.

EXPERIMENTAL BIOLOGY AND MEDICINE, 234(10): 1220-1229.

Keyword: high-throughput expression; gastric cancer; liver cancer; gateway system; functional study
Broadly neutralizing anti-HBV antibody binds to non-epitope regions of preS1

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Broadly neutralizing anti-hepatitis B virus (HBV) antibody HzKR127 undergoes a fairly large conformational change of CDR H3 loop upon binding to HBV preS1 epitope peptide. In this study, we identified low-affinity antibody-binding sites in the largely unstructured preS1 region by nuclear magnetic resonance and biochemical studies, indicating that the antibody binds to the preS1 region outside the major immune epitope with low affinity. Surface plasma resonance experiments showed that the full-length preS1 has approximately three fold higher affinity for HzKR127 Fab than the preS1 epitope peptide, suggesting that the presence of low-affinity sites in the preS1 region increases the antibody-binding affinity. Therefore, the low-affinity binding of the antibody to non-epitope regions of preS1 may contribute to effective neutralization.

FEBS LETTERS, 583(18): 3095-3100.

Keyword: hepatitis B virus; neutralizing antibody; nuclear magnetic resonance; epitope; preS1

Association between catechol-o-methyltransferase gene polymorphism and attention-deficit hyperactivity disorder in Korean population

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Recently, the relationship between allele frequency distribution and attention-deficit hyperactivity disorder (ADHD) has been actively studied. In Korea, the relationship between the genetic type and alleles for catechol-O-methyltransferase (COMT) gene has been studied in ADHD patients. ADHD was diagnosed in 60 patients according to the Diagnostic and Statistical Manual of Mental Disorders Version IV (DSM-IV) diagnostic criteria and Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL), and they were selected for the study. For the control group, normal volunteers were chosen. Blood samples were taken from the 160 subjects. DNA was extracted from blood lymphocytes, and PCR was performed for COMT NlaI VNTR polymorphism. For the case-control analyses, allele and genotype frequencies were compared using the \( \chi^2 \) method. When the ADHD group and the normal control group were compared, significant difference was seen on the COMT genetic type, but was not seen on the allele distribution. As a result, it is viewed that there is no relationship between ADHD and the COMT gene, but final decision is indefinite.

GENETIC TESTING AND MOLECULAR BIOMARKERS, 13(2): 233-236.

Keyword: cardio-facial syndrome; turkish children; no association; ADHD
**Article 60**

NDRG2 suppresses cell proliferation through down-regulation of AP-1 activity in human colon carcinoma cells

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Recently, the anti-tumor activity of N-myc downstream-regulated gene 2 (NDRG2) was elucidated, but the molecular mechanism of how NDRG2 works as a tumor suppressor is not well known. To determine the function of NDRG2 as a tumor suppressor, we established stable cell lines expressing NDRG2 protein or its mutant forms, and studied their effects on tumor cell growth. Interestingly, constitutive expression of wild-type NDRG2 induced the growth retardation of SW620 colon carcinoma cells. Introduction of NDRG2 into SW620 cells induced the decrease of c-Jun phosphorylation at Ser63, followed by the attenuation of activator protein-1 (AP-1) function as a transcriptional activator. Subsequently, the down-regulation of cyclin D1, which is known as a major target for AP-1 transcription activator, resulted in cell cycle arrest at G1/S phase. Additionally, treatment of NDRG2-siRNA on NDRG2-expressing cells has induced the recovery of c-Jun phosphorylation and cyclin D1 expression. Cell proliferation of those cells was also increased compared with untreated cells. NDRG2 mutants of which the phosphorylation sites at C-terminal region were removed by deletion or site-directed mutagenesis have shown no effect on cyclin D1 expression and could not induce cell growth retardation. In conclusion, NBRG2 modulates intracellular signals to control cell cycle through the regulation of cyclin D1 expression via phosphorylation pathway.


**Keyword**: growth retardation; cyclin D1/p21; c-Jun phosphorylation; NDRG2

**Article 61**

RhoB induces apoptosis via direct interaction with TNFAIP1 in HeLa cells

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RhoB, a tumor suppressor, has emerged as an interesting cancer target, and extensive studies aimed at understanding its role in apoptosis have been performed. In our study, we investigated the involvement of RhoB-interacting molecules in apoptosis. To identify RhoB-interacting proteins, we performed yeast-two hybrid screening assays using RhoB as a bait and isolated TNFAIP1, a TNF alpha-induced protein containing the BTB/POZ domain. The interaction between RhoB and TNFAIP1 was demonstrated in vivo through coimmunoprecipitation studies and in vitro binding assays. RFP-TNFAIP1 was found to be partially colocalized with EGFP-RhoB. The partial colocalization of RhoB and TNFAIP1 in endosomes suggests that RhoB-TNFAIP1 interactions may have a functional role in apoptosis. TNFAIP1 elicited proapoptotic activity, while simultaneous expression of RhoB and TNFAIP1 resulted in a dramatic increase in apoptosis in HeLa cells. Furthermore, knockdown of RhoB using siRNA clearly rescued cells from apoptosis induced by TNFAIP1. This finding suggests that interactions between RhoB and TNFAIP1 are crucial for induction of apoptosis in HeLa cells. The observation of increased SAPK/JNK phosphorylation in apoptotic cells and the finding that a JNK inhibitor suppressed apoptosis indicates that SAPK/JNK signaling may be involved in apoptosis induced by RhoB-TNFAIP1 interactions. In conclusion, we found that RhoB interacts with TNFAIP1 to regulate apoptosis via a SAPK/JNK-mediated signal transduction mechanism.


**Keyword**: RhoB; TNFAIP1; apoptosis; SAPK/JNK
Utility of reaction intermediate monitoring with photodissociation multi-stage (MS²) time-of-flight mass spectrometry for mechanistic and structural studies: phosphopeptides

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In tandem mass spectra of phosphopeptides, intact sequence ions are often missing or appear weakly. Instead, dephosphorylated sequence ions appear prominently. In this work, we used photodissociation (PD) multi-stage (MS²) time-of-flight mass spectrometry that can monitor reaction intermediates with lifetime as short as 100 ns to study the formation of dephosphorylated sequence ions such as y₃-H₃PO₄, y₅-H₅PO₄, y₆-H₆PO₄, etc. It was found to be formed mainly by H₃PO₄ loss from y₅. For doubly phosphorylated peptides, y₅ seemed to lose H₃PO₄ stepwise and form y₅-H₃PO₄, y₆-H₅PO₄, etc. Even when y₅ was absent in PD-MS² spectrum, its m/z could be predicted from those of y₅-H₃PO₄ and/or y₆-2H₅PO₄. Complete sequence coverage was possible when the data from PD-MS² and PD-MS³ were combined, demonstrating the utility of transient ion detection by PD-MS³ for structure analysis.


Keyword : reaction intermediate monitoring; photodissociation; MS²; phosphopeptide

Evaluation of annexin II as a potential serum marker for hepatocellular carcinoma using a developed sandwich ELISA method

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Annexin 11 (Annexin A2, ANXA2) is a 36 kDa calcium-dependent phospholipid-binding protein that is located on the surface of most eukaryotic cells. ANXA2 is involved in several biological processes, including anti-inflammatory effects, Ca²⁺-dependent exocytosis, immune responses, Ca²⁺ transport and phospholipase A2 regulation. In our previous study, ANXA2 was identified as an up-regulated gene in hepatocellular carcinoma (HCC) tissue by cDNA microarray. In the present study, we have evaluated ANXA2 as a tumor-associated marker of HCC. We determined the ANXA2 levels in human liver tissues with HCC using real-time RT-PCR and Western blot analysis. For quantitative analysis of the ANXA2 protein in body fluids, we developed a sandwich ELISA system in which a polyclonal antibody and a monoclonal antibody specific to ANXA2 were employed as a capture antibody and a probe antibody, respectively. We detected the ANXA2 concentration in human serum using our newly developed system and evaluated its usefulness as a tumor marker. Overexpression of ANXA2 in human liver tissue was confirmed by real-time RT-PCR and Western blot analysis. The sandwich ELISA system for ANXA2 was developed for the detection of ANXA2 in human samples. The dose-response relationship between ANXA2 and optical density was linear in the range of 0-10 μg/ml and the sensitivity was 0.02 μg/ml. We determined the ANXA2 concentration in serum specimens using the newly developed sandwich ELISA. The serum ANXA2 concentrations of the patients with HCC (53.38 +/- 36.23 μg/ml) were significantly elevated when compared with those of normal individuals (28.81 +/- 24.94 μg/ml). These results suggest that expression of ANXA2 may be increased in HCC patients and may play an important role in liver cancer progression. This new ELISA method can be used as a tool for the detection of ANXA2 in human serum, particularly for cancer diagnostics.


Keyword : annexin II; hepatocellular carcinoma; ELISA; serum; tissue
Dephosphorylation of the C-terminal tyrosyl residue of the DNA damage-related histone H2A.X is mediated by the protein phosphatase eyes absent

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In mammalian cells, the DNA damage-related histone H2A variant H2A.X is characterized by a C-terminal tyrosyl residue, Tyr-142, which is phosphorylated by an atypical kinase, WSTF. The phosphorylation status of Tyr-142 in H2A.X has been shown to be an important regulator of the DNA damage response by controlling the formation of gamma H2A.X foci, which are platforms for recruiting molecules involved in DNA damage repair and signaling. In this work, we present evidence to support the identification of the Eyes Absent (EYA) phosphatases, protein-tyrosine phosphatases of the haloacid dehalogenase superfamily, as being responsible for dephosphorylating the C-terminal tyrosyl residue of histone H2A.X. We demonstrate that EYA2 and EYA3 displayed specificity for Tyr-142 of H2A.X in assays in vitro. Suppression ofeya3 by RNA interference resulted in elevated basal phosphorylation and inhibited DNA damage-induced dephosphorylation ofTyr-142 ofH2A.X in vivo. This study provides the first indication of a physiological substrate for the EYA phosphatases and suggests a novel role for these enzymes in regulation of the DNA damage response. JOURNAL OF BIOLOGICAL CHEMISTRY, 284(24): 16066-16070.

Keyword : gamma-H2AX; chromatin; eye absent(EYA)

Upregulation of the cycline kinase subunit CKS2 increases cell proliferation rate in gastric cancer

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Purpose: CKS2 was identified as an upregulated gene in gastric cancer via our DNA microarray. This study was to verify the upregulation of CKS2 in many gastric cancer patients and to examine the CKS2-mediated cellular response.

Methods: CKS2 upregulation was analyzed using reverse transcriptase PCR, real-time PCR, and immunohistochemical and clinicopathological analyses. GFP-CKS2 or CKS2-siRNA was used to analyze the cellular localization and proliferation.

Results: The strong upregulation of mRNA and protein levels of CKS2 was identified. In CKS2-overexpressing cells, tumor suppressor p53 and p21cip1 were downregulated and cell growth was increased. In contrast, CKS2-siRNA-transfected cells showed an increased tumor suppressor expression and decreased cell growth.

Conclusions: We showed that CKS2 was significantly upregulated in gastric cancers and a high level of CKS2 was highly correlated with histologic tumor differentiation and pathological grade of the tumor size, lymph node, and metastasis stage. We suggest that the cell cycle regulator CKS2 might be deeply involved in gastric cancer progression. JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY, 135(6): 761-769.

Keyword : CKS2; CDK1; p53; gastric cancer
Regulation of adipogenic differentiation by LAR tyrosine phosphatase in human mesenchymal stem cells and 3T3-L1 preadipocytes

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Mesenchymal stem cells (MSCs) are multipotent adult stem cells that can differentiate into a variety of mesodermal-lineage cells. MSCs have significant potential in tissue engineering and therapeutic applications; however, the low differentiation and proliferation efficiencies of these cells in the laboratory are fundamental obstacles to their therapeutic use, mainly owing to the lack of information on the detailed signal-transduction mechanisms of differentiation into distinct lineages. With the aid of protein-tyrosine-phosphatase profiling studies, we show that the expression of leukocyte common antigen related (LAR) tyrosine phosphatase is significantly decreased during the early adipogenic stages of MSCs. Knockdown of endogenous LAR induced a dramatic increase in adipogenic differentiation, whereas its overexpression led to decreased adipogenic differentiation in both 3T3-L1 preadipocytes and MSCs. LAR reduces tyrosine phosphorylation of the insulin receptor, in turn leading to decreased phosphorylation of the adaptor protein IRS-1 and its downstream molecule Akt (also known as PKB). We propose that LAR functions as a negative regulator of adipogenesis. Furthermore, our data support the possibility that LAR controls the balance between osteoblast and adipocyte differentiation. Overall, our findings contribute to the clarification of the mechanisms underlying LAR activity in the differentiation of MSCs and suggest that LAR is a candidate target protein for the control of stem-cell differentiation.

JOURNAL OF CELL SCIENCE, 122(22): 4160-4167.

Keyword: adipocyte; adipogenesis; mesenchymal stem cells; osteoblast; protein tyrosine phosphatase; leukocyte common antigen related

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Purification and characterization of a novel thermoacid-stable fibrinolytic enzyme from Staphylococcus sp strain AJ isolated from Korean salt-fermented Anchovy-joet

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A novel fibrinolytic enzyme (AJ) was purified from Staphylococcus sp. strain AJ screened from Korean salt-fermented Anchovy-joet. Relative molecular weight of AJ was determined as 26 kDa by using SDS-PAGE and fibrin zymography. Based on a 2D gel, AJ was found to consist of three active isoforms (pI 5.5-6.0) with the same N-terminal amino acid sequence. AJ exhibited optimum pH and temperature at 2.5-3.0 and 85°C, respectively. AJ kept 85% of the initial activity after heating at 100°C for 20 min on the zymogram gel. The Michaelis constant (Km) and Kcat values of AJ towards alpha-casein were 0.38 mM and 19.73 s⁻¹, respectively. AJ cleaved the A alpha-chain of fibrinogen but did not affect the B beta- and gamma-chains, indicating that it is an alpha-fibrinogenase. The fibrinolytic activity was inhibited by diisopropyl fluorophosphate, indicating AJ is a serine protease. Interestingly, AJ was very stable at acidic condition, SDS, and heat (100A degrees C), whereas it was easily degraded at neutral and alkaline conditions. In particular, AJ formed an active homo-dimer in the pH range from 7.0 to 8.0. To our knowledge, a similar combination of acid and heat stability has not yet been reported for other fibrinolytic enzymes. JOURNAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY, 36(3): 417-426.

Keyword: fibrinolytic enzyme; Staphylococcus sp strain AJ; anchovy-joet; thermoacid-stable; fibrin zymography
Article 68

**Generation of expression vectors for high-throughput functional analysis of target genes in *Schizosaccharomyces pombe***

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An immediate challenge in the post-genomic era is to assign a biological function to proteins unraveled by genome analysis. This report is based on studies conducted using *Schizosaccharomyces pombe*, a simple model organism, and presents various vector systems as tools for high-throughput functional analysis of human genes. We constructed *S. pombe* expression vectors for efficient cloning of genes via the Gateway system. We modified the pREP and pSLF series vectors, which are widely used for gene expression in *S. pombe*. The vectors constructed have a uniform backbone of *S. pombe* autonomously replicating sequence (ARS) elements with different selective markers, namely, *ura4* and *Saccharomyces cerevisiae* LEU2 complementing *leu1*. These vectors contain 3 different strengths of the inducible promoter *nmt1*, which affect the expression levels of the cloned open reading frames (ORFs). Further, target proteins can be fused with an N-terminal or C-terminal tag such as triple hemagglutinin (3x HA), enhanced green fluorescent protein (EGFP), or *Discosoma* red fluorescent protein (DsRed). We tested the feasibility of the constructed vectors by using 3 human genes, namely, RAB18, SCC-112, and PTEN. Proper expression of tagged RAB18 was confirmed by western blot analysis. Further, localization of RAB18, SCC112, and PTEN was demonstrated. The constructed vectors can be utilized for high-throughput functional analysis of heterologous genes.


**Keyword**: expression vector; EGFP; DsRed; HA; gateway system; fission yeast

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Article 69

**Temperature of peptide ions generated by matrix-assisted laser desorption ionization and their dissociation kinetic parameters**

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Product ion yields in postsource decay and photodissociation at 193 and 266 nm were measured for some peptide ions without a basic amino acid residue ([Y₆ + H]⁺, [F₅ + H]⁺, and [YPFVEPI + H]⁺) generated by matrix-assisted laser desorption ionization (MALDI). Data indicated statistical nature for the dissociation processes. Assuming that peptide ions formed by MALDI are in thermal equilibrium at temperature T and that their dissociation rate constants are specified by the critical energy (E₀) and entropy (ΔS DOUBLE-DAGGER), a method based on kinetic analysis was devised to determine these parameters simultaneously. The matrix used was found to affect the effective temperature of peptide ions, 2,5-dihydroxybenzoic acid (400-430 K) < sinapinic acid (440 K) < alpha-cyano-4-hydroxycinnamic acid (460-510 K), in agreement with previous perceptions. ED of around 0.6 eV and Delta S-double dagger of -24 eu were smaller than previous quantum chemical results for small model peptide ions.

**JOURNAL OF PHYSICAL CHEMISTRY B**, 113(7): 2071-2076.

**Keyword**: surface-induced dissociation; singly protonated peptides; flight mass-spectrometer; reaction-rate constants
Glyceraldehyde-3-phosphate, a glycolytic intermediate, plays a key role in controlling cell fate via inhibition of caspase activity

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Glyceraldehyde-3-phosphate is a key intermediate in several central metabolic pathways of all organisms. Aldolase and glyceraldehyde-3-phosphate dehydrogenase are involved in the production or elimination of glyceraldehyde-3-phosphate during glycolysis or gluconeogenesis, and are differentially expressed under various physiological conditions, including cancer, hypoxia, and apoptosis. In this study, we examine the effects of glyceraldehyde-3-phosphate on cell survival and apoptosis. Overexpression of aldolase protected cells against apoptosis, and addition of glyceraldehyde-3-phosphate to cells delayed apoptosis. Additionally, delayed apoptotic phenomena were observed when glyceraldehyde-3-phosphate was added to a cell-free system, in which artificial apoptotic process was induced by adding dATP and cytochrome c. Surprisingly, glyceraldehyde-3-phosphate directly suppressed caspase-3 activity in a reversible noncompetitive mode, preventing caspase-dependent proteolysis. Based on these results, we suggest that glyceraldehyde-3-phosphate, a key molecule in several central metabolic pathways, functions as a molecule switch between cell survival and apoptosis.

MOLECULES AND CELLS, 28(6): 559-563.

Keyword: aldolase; apoptosis; caspase-3; GAPDH; glyceraldehyde-3-phosphate; induced apoptosis

2′-benzoyloxyccinnamaldehyde inhibits tumor growth in H-ras12V transgenic mice via downregulation of metallothionein

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Cinnamaldehydes have been reported to induce apoptosis in human carcinomas through the generation of reactive oxygen species (ROS). 2′-benzoyloxyccinnamaldehyde (BCA) has been reported to inhibit tumor formation in H-ras12V transgenic mice. To see the antitumor effects of BCA, BCA was administrated intraperitoneally (50 mg/kg) to H-ras12V transgenic mice for 3 wk, and it was found that the hepatic tumor volume and the total number of tumors were decreased in BCA-treated mice as compared to control H-ras12V transgenic mice. To identify possible target genes responsible for BCA antitumor effects in H-ras12V transgenic mice, cDNA microarray analyses were performed comparing gene expression between BCA treated and control transgenic mice. We found that 42 genes were downregulated, and 40 genes were upregulated in the BCA-treated transgenic mice. The downregulated genes included several genes involved in ROS regulation and immune response (aconitase, metallothionein-1, metallothionein-2, and purine nucleoside phosphorylase). The expression of ROS-related genes, metallothionein 1 and metallothionein 2, was decreased more than twofold with BCA treatment (P < 0.001). It was confirmed by RTPCR and immunohistochemical analyses. The inhibition of tumor formation and growth in H-ras12V transgenic mice by BCA was mediated through inhibition of the expression of the ROS scavengers metallothionein 1 and metallothionein 2.

NUTRITION AND CANCER-AN INTERNATIONAL JOURNAL, 61(5): 723-734.

Keyword: oxidative stress; expression patterns; gene-expression; cancer-cells; 2′-hydroxycinnamaldehyde; cinnamaldehydes; inactivation; hepatocytes
Far upstream element-binding protein-1, a novel caspase substrate, acts as a cross-talker between apoptosis and the c-myc oncogene

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Far upstream element-binding protein-1 (FBP-1) binds to an upstream element of the c-myc promoter and regulates the c-myc mRNA level. Earlier, FBP-1 was identified as a candidate substrate of caspase-7. Here, we report that FBP-1 is cleaved by executor caspases, both in vitro and during apoptosis. Cleavage occurs at the caspase consensus site (DQPD⁷) located within the classical bipartite nuclear localization signal sequence. In cells subjected to apoptotic stimuli, the caspase-mediated cleavage of FBP-1 leads to its decreased presence in the nucleus, concomitant with the marked downregulation of c-Myc and its various target proteins. By contrast, cells transfected with a non-cleavable mutant of FBP-1 (D74A) maintain higher levels of c-Myc and are protected from apoptosis. On the basis of these results, we suggest that the oncogenic potential of c-Myc is 'switched off' after apoptosis induction as a consequence of the caspase-mediated cleavage of FBP-1.


**Keyword**: apoptosis; caspase; c-Myc; FBP-1; FUSE

Enhanced S100A4 protein expression is clinicopathologically significant to metastatic potential and p53 dysfunction in colorectal cancer

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To investigate the expression levels of S100A4 in human colorectal carcinoma (CC) and its relationship with clinicopathological parameters and metastatic potential, 73 pathological specimens from patients with CC were examined for S100A4 expression by RT-PCR and immunohistochemistry. An increase of S100A4 mRNA was observed in 19/23 (82.6%) CC specimens, and S100A4 was up-regulated in 40/73 (54.7%) CC cases compared with non-neoplastic mucosal tissues. Upregulation of S100A4 was significantly related to invasion, nodal status, distant metastasis and p53 expression. Next, we investigated whether S100A4 could affect p53 transactivation and stability. Interestingly, it was revealed that treatment with exogenous S100A4 protein reduced transcriptional activity of p53 and abrogated the modification of calcium binding, affinity of S100A4 protein. These findings suggested that S100A4 might be involved in the progression and metastasis of human CC, presumably via modulation of the wild-type p53 protein.


**Keyword**: S100A4; p53 transactivation; metastasis; colorectal carcinoma
Comparative proteomic analysis of mouse melanoma cell line B16, a metastatic descendant B16F10, and B16 overexpressing the metastasis-associated tyrosine phosphatase PRL-3

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Metastasis is a complex, multistep process by which a cancer cell leaves the primary tumor, travels to a distant site via the circulatory system, and establishes a secondary cancer. A deeper understanding of the molecular events underlying metastasis will provide information that will be useful for the development of new diagnostic and therapeutic strategies. The B16 and B16F10 mouse melanoma cell lines are widely used as model system for studying many aspects of cancer biology including metastasis. Compared with B16, which has a low metastatic potential, the highly metastatic cell line B16F10 displayed a higher metastatic ability along with higher expression levels of the metastasis-associated phosphatase of regenerating liver-3 (PRL-3). B16 cells transfected with PRL-3 cDNA (B16-PRL3) had metastatic abilities comparable to those of B16F10 cells. To study the molecular mechanisms that underlie metastasis, the proteomes of the B16, B16F10, and B16-PRL3 cell lines were compared using two-dimensional differential in-gel electrophoresis. Proteins that varied significantly in levels between these cell lines were selected and identified using mass spectrometry. Interestingly, many proteins, especially those present in membrane fractions, were similarly up- or downregulated in both the B16F10 and B16-PRL3 cells lines compared to B16 cell lines. The list of similarly regulated proteins included heat shock protein 70, fascin-1, septin-6, ATP synthase P subunit, and bone morphogenic protein receptor type IB. These proteins may play a causal role in PRL-3-mediated metastasis. These investigations open an avenue for the further characterization of the molecular mechanisms that underlie metastasis.

ONCOLOGY RESEARCH, 17(11-12): 601-612.

**Keyword**: B16 Differential in-gel electrophoresis (DIGE); melanoma; metastasis; proteomics; difference gel-electrophoresis; cutaneous malignant-melanoma; breast-cancer; colorectal-cancer; gene-expression; prostate-cancer; fascin; heat-shock-protein-70

Crystal structure of the catalytic domain of human MKP-2 reveals a 24-mer assembly

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The overall folding of MKP-2C is typical of DSPs, with a central twisted five-stranded beta sheet surrounded by six alpha helices and MKP-2C in the crystal structure constitutes a hollow spherical complex composed of 24 subunits with pseudooctahedral symmetry. We found a peak of apparently tetrameric MKP-2C under highly basic conditions (pH 12.5), but failed to see a clear indication of 24-mer complexes. The catalytic activities of MKP-2 are also unusual relative to other MKPs. The crystal structure predicts that, in the 24-mer complex, the MKB domain points to the inside of sphere where cognate MAPK substrates would be unreachable to it. There are no clues as to how buried MKBs become available and recognize MAPK, or how this process might influence the preferences for different MAPK substrates. Based on the crystal structure, however, we can develop the hypothesis that catalytic activity is controlled according to the status of oligomers. Accordingly, when MKP-2 exists as a 24-mer complex, it exhibits no substrate preference for any of the three MAPKs. After the complex is disassembled into monomers (in response to an unknown signal), the MKB domain is exposed to solvent and substrate specificity can be realized.

PROTEINS-STRUCTURE FUNCTION AND BIOINFORMATICS, 76(3): 763-767.

**Keyword**: protein tyrosine phosphatase; MKP-2; crystal structure
Cytoskeleton-associated proteins are enriched in human embryonic-stem cell-derived neuroectodermal spheres

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The ability to generate neural lineages from human embryonic stem cells (hESCs) in a controlled manner would further investigation of human neurogenesis and development of potential cell therapeutic applications to treat neurological diseases; however, generating such neural stem cells (NSCs) remains a challenge. In an attempt to characterize the cellular mechanisms involved in hESC differentiation into neuroprogenitor cells, we performed 2-DE using protein extracts from hESC-derived embryoid bodies (EBs) and neuroectodermal spheres (NESs) bearing neuroprogenitors. Of 47 differentially expressed protein spots, 28 nonredundant spots were shown to be upregulated in the NESs; these protein spots included neurogenesis-related proteins (TAF1, SEPT2, NPH3, and CRABP), as expected. Interestingly, 6 of these 28 protein spots were cytoskeleton-associated proteins (CSAP) such as Fascin-1, Cofilin-1, and Stathmin-1. Western-blot analyses confirmed the increased levels of these proteins in the NESs. Furthermore, immunostaining analysis showed that both Fascin-1 and Stathmin-1 were preferentially expressed in the inner rims of neural rosettes, which are characteristic features of neuroprogenitors in culture. We also confirmed prominent expression of Fascin-1 in (sub-)ventricular zone in E15.5 mouse fetal brain. Our results suggest that, in addition to the induction of those genes involved in neural development, hESC differentiation into the NES is associated with a marked reorganization of the cellular cytoskeleton.

PROTEOMICS, 9(5): 1128-1141.

Keyword: cytoskeleton; human embryonic stem cell; neural stem cells; neuroectodermal sphere; rosette

Proteomic analysis of liver tissue from HBx-transgenic mice at early stages of hepatocarcinogenesis

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The hepatitis B virus X-protein (HBx), a multifunctional viral regulator, participates in the viral life cycle and in the development of hepatocellular carcinoma (HCC). We previously reported a high incidence of HCC in transgenic mice expressing HBx. In this study, proteomic analysis was performed to identify proteins that may be involved in hepatocarcinogenesis and/or that could be utilized as early detection biomarkers for HCC. Proteins from the liver tissue of HBx-transgenic mice at early stages of carcinogenesis (dysplasia and hepatocellular adenoma) were separated by 2-DE, and quantitative changes were analyzed. A total of 22 spots displaying significant quantitative changes were identified using LC-MS/MS. In particular, several proteins involved in glucose and fatty acid metabolism, such as mitochondrial 3-ketoacyl-CoA thiolase, intestinal fatty acid-binding protein 2 and cytoplasmic malate dehydrogenase, were differentially expressed, implying that significant metabolic alterations occurred during the early stages of hepatocarcinogenesis. The results of this proteomic analysis provide insights into the mechanism of HBx-mediated hepatocarcinogenesis. Additionally, this study identifies possible therapeutic targets for HCC diagnosis and novel drug development for treatment of the disease.

PROTEOMICS, 9(22): 5056-5066.

Keyword: animal proteomics; dysplasia; hepatitis B virus X-protein; hepatocellular adenoma; hepatocellular carcinoma
Prostacyclin stimulates embryonic development via regulation of the cAMP response element-binding protein-cyclo-oxygenase-2 signalling pathway in cattle

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Prostacyclin (PGI(2)) in oviducal fluid is synthesised from arachidonic acid by cyclo-oxygenase (COX) and prostacyclin synthetase and enhances the implantation and live birth potential of mouse embryos. In the present study, we investigated the developmental competence of bovine embryos by examining the effects of the PGI(2) analogue iloprost on blastocyst development, quality and COX-2 expression during IVF and somatic cell nuclear transfer (SCNT). Bovine IVF and SCNT embryos were cultured in CR1-aa medium supplemented with 0.3% bovine serum albumin in either the presence or absence of 1 μM iloprost at 38.5 degrees C and 5% CO2. After 3 days of culture, cleaved embryos were cultured for 4 days in the same medium supplemented with 10% fetal bovine serum. For both IVF and SCNT embryos, iloprost improved the blastocyst developmental rate and cell numbers. In the presence of iloprost, the proportion of expanded blastocysts was significantly higher among the IVF embryos and fewer apoptotic cell nuclei were observed. Expression of COX-2 mRNA and protein, evaluated using real-time polymerase chain reaction and immunoblotting, respectively, was increased in the presence of iloprost. These results suggest that PGI(2) improves the developmental competence of embryos via regulation of the cAMP response element-binding protein-COX-2 signalling pathway in cattle.

REPRODUCTION FERTILITY AND DEVELOPMENT, 21(3): 400-407.

Keyword: iloprost; in-vitro; cloned embryos; bovine embryos; smooth-muscle

Improvement in antigen-delivery using fabrication of a grooves-embedded microneedle array

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The skin, which has immunocompetent cells, is an attractive target for vaccine delivery. Intradermal immunization is the most effective immunization, though it requires considerable technical skill. A promising approach to intradermal immunization is microneedle array technology. This paper presents a new fabrication method for grooves-embedded microneedle arrays of a bio-compatible polymer and the immunization characteristics to ovalbumin delivered into mice by the microneedle arrays through the skin. The microneedles fabricated using a hot embossing process have a three-dimensional sharp tip, smooth or grooves-embedded shafts, and large bases. The height, base width, and thickness are 880 +/- 20, 710 +/- 15, and 145 +/- 15 μm, respectively. To perform an immune response test, the ovalbumin-coated microneedle arrays were inserted into mouse skin and then, the titer of antibody to ovalbumin was analyzed. The increased number and deeper grooves of the microneedle induced a higher antibody response. These results suggest that the grooves-embedded microneedle array is loaded with more antigens than the smooth one and that the antigens are well delivered into the skin, though they are located on the deep grooves of the microneedles. In Summary, the fabrication of grooves-embedded microneedles can Supply an improved tool for intradermal immunization.


Keyword: drug delivery system (DDS); ovalbumin; intradermal immunization; vaccination
Abnormal gene expression in extraembryonic tissue from cloned porcine embryos

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The birth rate of cloned animals following somatic cell nuclear transfer (SCNT) is very low and the surviving animals have various developmental defects. We compared the morphology and transcriptional profile of extraembryonic tissue from three 26-d old SCNT pig fetuses with that from control fetuses. Transcriptional profiling using long-oligonucleotide microarray technology revealed 34 genes that were differentially expressed between the three groups. The differential expression of several genes involved in translational regulation was confirmed by real-time quantitative PCR and Western blot analysis. Interestingly, the expression of a translational inhibition-related gene encoding a eukaryotic translation initiation factor 4E-binding protein was significantly elevated in the SCNT samples. We concluded that the low birth rate of cloned animals could be related to abnormal expression of translational regulators in extraembryonic tissue during early pregnancy.

THERIOGENOLOGY, 71(2): 323-333.

Keyword: transcriptional profiling; cloned embryo; somatic cell nuclear transfer; extraembryonic tissue
3. Division of Biosystems Research

- Industrial Biotechnology & Bioenergy Research Center
- Plant Systems Engineering Research Center
- Bioinformatics Research Center
- Industrial Bio-materials Research Center
- Environmental Biotechnology Research Center
Reactive oxygen species: regulation of plant growth and development

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In plants, reactive oxygen species (ROS) are continuously produced from aerobic metabolic processes such as the photosynthetic and respiratory reactions. The cellular accumulation of ROS, which are highly reactive, is highly cytotoxic. Therefore, all the aerobic organisms have been evolved to develop efficient ROS-scavenging mechanisms. In recent years, the role of ROS in the regulation of plant growth and development has been identified. Increased ROS production is functionally coupled to the effects of plant growth regulators. The specific ROS that are related to specific plant hormones may control plant growth and development. The recent discovery of a tip-high, Ca²⁺-interdependent, ROS gradient produced by NADPH oxidase and its close association with polarized growth will provide information on the dual role of ROS in plants, as both the toxic byproducts of aerobic metabolism and the key regulators of growth and developmental pathways.

ADVANCES IN BOTANICAL RESEARCH: OXIDATIVE STRESS AND REDOX REGULATION IN PLANTS, 52: 25-46.

Keywords: activated protein-kinase; redox signaling pathways; stomatal guard-cells; pollen-tube growth; abscisic-acid; hydrogen-peroxide; nadph oxidase; oxidative stress; antioxidant defense; polarized growth

Novel cold-adapted alkaline lipase from an intertidal flat metagenome and proposal for a new family of bacterial lipases

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A new lipase, LipEH166, isolated from an intertidal flat metagenome, showed no amino acid similarity to any known lipolytic enzyme except in the consensus region. This suggested that LipEH166 and its homologues belong to a new family of lipolytic enzymes. Partial characterization indicated that LipEH166 is a novel cold-adapted alkaline lipase.


Keywords: diversity; sediment; DNA; LipEH166; intertidal flat metagenome
Novel GH10 xylanase, with a fibronectin Type 3 domain, from *Cellulosimicrobium* sp strain HY-13, a bacterium in the gut of *Eisenia fetida*

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The gene encoding a novel modular xylanase from *Cellulosimicrobium* sp. strain HY-13 was identified and expressed in *Escherichia coli*, and its truncated gene product was characterized. The enzyme consisted of three distinct functional domains, an N-terminal catalytic GH10 domain, a fibronectin type 3 domain, and C-terminal carbohydrate-binding module 2.

*APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, 75(22): 7275-7279.

**Keywords**: biochemical-characterization; symbiotic bacterium

Nucleotide sequence and genomic organization of a newly identified member of the genus *Carmovirus*, soybean yellow mottle mosaic virus, from soybean

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The viral genome of soybean yellow mottle mosaic virus (SYMMV) from infected soybean (*Glycine max*) in Korea was cloned and sequenced. The complete monopartite single-stranded RNA genome of SYMMV consists of 4009 base pairs with six putative open reading frames and includes 5'- and 3'-untranslated regions of 39 and 229 nucleotides, respectively. The nucleotide and coat protein sequences of SYMMV share the highest sequence identity with those of cowpea mottle virus. Based on its genomic organization, its predicted amino acid sequence, and its phylogenetic relatedness to known carnoviruses, we report that SYMMV is a new member of the genus *Carmovirus* in the family *Tombusviridae*.


**Keywords**: turnip crinkle virus; necrotic-spot-virus; length cDNA-clone; necrosis-virus; subgenomic RNA; in-vitro
Inhibitory effects of calycosin isolated from the root of *Astragalus membranaceus* on melanin biosynthesis

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Tyrosinase is a key enzyme for melanin biosynthesis, and hyperpigmentation disorders are associated with abnormal accumulation of melanin pigments, which can be reduced by treatment with depigmenting agents. A methanol extract of *Astragalus membranaceus* showed inhibitory activity against mushroom tyrosinase. The active compound was purified from the methanol extract of *A. membranaceus* and, following several chromatographic methods, was identified as calycosin via spectroscopic analysis. The results showed that calycosin exhibited tyrosinase inhibitory activity with an IC₅₀ value of 38.4 μM. Moreover, calycosin showed a melanin biosynthesis inhibition zone in a culture plate of *Streptomyces bikiniensis*, which is commonly used as an indicator organism. Furthermore, calycosin dramatically reduced melanin synthesis of Melan-a cells without any apparent cytotoxicity and reduced expression of melanogenic enzyme, tyrosinase. These results suggest that calycosin may be an effective skin-lightening agent that regulates the expression of melanogenic enzymes.


**Keywords**: *Astragalus membranaceus*; calycosin; tyrosinase; melanin biosynthesis

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Aquastatin A, a new inhibitor of enoyl-acyl carrier protein reductase from *Sporothrix* sp FN611

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Bacterial enoyl-acyl carrier protein (ACP) reductase has been confirmed as a novel target for antibacterial drug development. In this study, we determined that a fungal metabolite from *Sporothrix* sp. FN611 potently inhibited the enoyl-ACP reductase (FabI) of *Staphylococcus aureus*. Its structure identified the metabolite as aquastatin A by the MS and NARI data. Aquastatin A inhibited *S. aureus* FabI with an IC₅₀ of 3.2 μM. It also prevented the growth of *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) with minimum inhibitory concentration of 16-32 μg/ml. Aquastatin A also exerted an inhibitory effect against the FabK isoform, an enoyl-ACP reductase of *Streptococcus pneumoniae*, with an IC₅₀ of 9.2 μM. The degalactosylation of aquastatin A did not affect the FabI and FabK-inhibitory or antibacterial activities, thereby suggesting that the sugar moiety within its molecular structure was not involved in these activities. The inhibitory effects of aquastatin A and its degalactosylated derivative on enoyl-ACP reductases and bacterial viability are reported for the first time in this study; these effects point to the potential that aquastatin A may be developed into a new broad-spectrum antibacterial and anti-MRSA agent.

*BIOLOGICAL & PHARMACEUTICAL BULLETIN*, 32(12): 2061-2064.

**Keywords**: aquastatin A; enoyl-acyl carrier protein reductase; *Staphylococcus aureus*; FabI; fungal metabolite; inhibitor
**Rubus coreanus** extract attenuates acetaminophen induced hepatotoxicity; involvement of cytochrome P450 3A4

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Foods of plant origin, especially fruits and vegetables, have attracted attention because of their potential benefits to human health. In this report, *Rubi Fructus* (RF), the dried unripe fruit of *Rubus coreanus* Miq (Rosaceae) and ellagic acid (EA) purified from RF were used to test their potential hepatoprotective effect against acetaminophen (AAP)-induced hepatotoxicity in rats. RF extract (RFext) and EA reduced the elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) in serum and the content of lipid peroxide in liver by AAP administration, while the increment of the cellular glutathione (GSH) content and the induction of glutathione S-transferase (GST) and glutathione peroxidase (GSH-PX) which were decreased by AAP administration. RFext and EA from RFext did not affect the two major form of cytochrome P450s, cytochrome P450 2E1 (CYP2E1) and cytochrome P450 1A2 (CYP1A2), but down-regulated the cytochrome P450 3A4 (CYP3A4) related to the conversion of AAP to N-acetyl-P-benzoquinone imine (NAPQI). These results suggest that RFext and EA from RF exhibit a hepatoprotective effect not only by increasing antioxidant activities but also by down-regulating CYP3A4 in the AAP-intoxicated rat.

**Keywords**: hepatoprotective effect; acetaminophen; *Rubus coreanus*; ellagic acid; anti-oxidant effect; cytochrome p450

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**Metabolic profiles of genetically modified potatoes using a combination of metabolite fingerprinting and multivariate analysis**

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Comprehensive metabolite fingerprinting of transgenic potatoes that constitutively express human beta amyloid, curdlan synthase (CRDS), and glycogen synthase (glgA); and of wild-type potatoes was carried out using FT-IR and H-1 NMR spectroscopy in combination with multivariate analyses. Comparison of metabolic patterns between transgenic and wild-type potatoes revealed that there were neither quantitative nor qualitative differences in metabolites between transgenic potatoes expressing human beta amyloid, CRDS or glgA, and non-transformed control potatoes. However, there were metabolic differences between two control potato lines - one that was fresh and the other stored. After 1 week of storage, comprehensive metabolite patterns were significantly modified. Although the differences between CRDS and glgA transgenic and control potato lines were small, PCA analysis of FT-IR and H-1 NMR spectral data identified two distinct control lines. These results suggest that the comprehensive metabolite changes in control potato lines, which occurred after 1 week of storage, were greater than the differences between CRDS and glgA transgenic and wild-type potato lines. Thus, the combination of FT-IR and H-1 NMR spectral data and multivariate analysis was valuable for the detection of comprehensive differences in metabolic profiles between transgenic and non-transformed control plants, even though peak-signal overlap prevented assignment of pure compounds. The combination of FT-IR and H-1 NMR spectral data and multivariate analysis is a simple and rapid method for evaluation of the metabolic equivalence of GM crops.

**Keywords**: FT-IR spectroscopy; genetically modified potatoes; H-1 NMR spectroscopy; multivariate analysis; principal component analysis (PCA)
Characterization of full-length enriched expressed sequence tags of dehydration-treated white fibrous roots of sweetpotato

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Sweetpotato (Ipomoea batatas (Q. Lam.) is relatively tolerant to unfavorable growth conditions such as drought, yet has not been exploited to provide a better understanding of the molecular basis of drought stress tolerance. We obtained 983 high-quality expressed sequence tags of 100 bp or longer (average length of 700 bp) from cDNA libraries of detached white fibrous root tissues by subjecting them to dehydration for 6 h. The 431 cDNAs were each assigned a function by alignment using the BLASTX algorithm. Among them, three genes associated with various abiotic stresses and nine genes not previously associated with drought stress were selected for expression pattern analysis through detailed reverse transcription-polymerase chain reaction. The direct and indirect relationships of the 12 genes with drought tolerance mechanisms were ascertained at different developmental stages and under various stress conditions.


Keywords: abiotic stress; drought stress; expressed sequence tag; root; sweetpotato

Multiple hTAFIII31-binding motifs in the intrinsically unfolded transcriptional activation domain of VP16

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Transcriptional activation domain (TAD) in virion protein 16 (VP16) of herpes simplex virus does not have any globular structure, yet exhibits a potent transcriptional activity. In order to probe the structural basis for the transcriptional activity of VP16 TAD, we have used NMR spectroscopy to investigate its detailed structural features. Results show that an unbound VP16 TAD is not merely "unstructured" but contains four short motifs (residues 424-433, 442-446, 465-467 and 472-479) with transient structural order. Pre-structured motifs in other intrinsically unfolded proteins (IUPs) were shown to be critically involved in target protein binding. The 472-479 motif was previously shown to bind to hTAFIII31, whereas the hTAFIII31-binding ability of other motifs found in this study has not been addressed. The VP16 TAD represents another IUP whose pre-structured motifs mediate promiscuous binding to various target proteins.

BMB REPORTS, 42(7): 411-417.

Keywords: herpes simplex virus; hTAF(II)31; intrinsically unfolded protein; NMR; transcriptional activation domain; VP16
CONVIRT A web-based tool for transcriptional regulatory site identification using a conserved virtual chromosome

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Techniques for analyzing protein-DNA interactions on a genome-wide scale have recently established regulatory roles for distal enhancers. However, the large sizes of higher eukaryotic genomes have made identification of these elements difficult. Information regarding sequence conservation, exon annotation and repetitive regions can be used to reduce the size of the search region. However, previously developed resources are inadequate for consolidating such information. CONVIRT is a web resource for the identification of transcription factor binding sites and also features comparative genomics. Genomic information on ortholog-independent conserved regions, exons, repeats and sequences is integrated into the virtual chromosome, and statistically over-represented single or combinations of transcription factor binding sites are sought. CONVIRT provides regulatory network analysis for several organisms with long promoter regions and permits inter-species genome alignments. CONVIRT is freely available at http://biosoft.kaist.ac.kr/convirt.

BMB REPORTS, 42(12): 823-828.

Keywords: cis-element; comparative genomics; phylogenetic footprinting; TFBS; transcription factor

Simultaneous improvement of catalytic activity and thermal stability of tyrosine phenol-lyase by directed evolution

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The tyrosine phenol-lyase from Symbio bacterium toebii was engineered to improve both its stability and catalytic activity by the application of random mutagenesis and subsequent reassembly of the acquired mutations. Activity screening of the random library produced four mutants with a two-fold improved activity, whereas parallel screening after heat treatment at 65 degrees C identified three mutants with half-inactivation temperatures improved by up to 5.6 degrees C. The selected mutants were then reassembled using the staggered extension PCR method, and subsequent screening of the library produced seven mutants with up to three-fold improved activity and half-inactivation temperatures improved by up to 11.2 degrees C. Sequence analyses revealed that the stability-improved hits included A13V, E83K and T407A mutations, whereas the activity-improving hits included the additional T129I or T451A mutation. In particular, the A13V mutation was propagated in the hits with improved stability during the reassembly screening process, indicating the critical nature of the N-terminal moiety for enzyme stability. Furthermore, homology modeling of the enzyme structure revealed that most of the stability mutations were located around the dimer-dimer interface, including the N-terminus, whereas the activity-improving mutations were located further away, thereby minimizing any interference that would be detrimental to the co-improvement of the stability and catalytic activity of the enzyme.


Keywords: N-terminal arm; protein engineering; structural relevance; Symbio bacterium toebii; tyrosine phenol-lyase
**Lutimaribacter saemankumensis gen. nov., sp nov., isolated from a tidal flat of the Yellow Sea**

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A Gram-negative, non-motile, rod-shaped bacterial strain, designated SMK-117ᵀ, belonging to the Alphaproteobacteria, was isolated from a tidal flat of the Yellow Sea, Korea, and was subjected to a polyphasic taxonomic study. Strain SMK-117ᵀ grew optimally at pH 7.0-8.0 and 30 degrees C in the presence of 2 % (w/v) NaCl. Neighbour-joining and maximum-likelihood phylogenetic trees based on 16S rRNA gene sequences showed that strain SMK-117ᵀ clustered with Maritimibacter alkaliphilus HTCC2654ᵀ, with which it exhibited a sequence similarity of 95.3%. Strain SMK-117ᵀ contained Q-10 as the predominant ubiquinone and C₁₈:₁ω7c and 11-methyl C₁₈:₁ω7c as the major fatty acids. The major polar lipids were phosphatidylcholine, phosphaticlylglycerol, phosphatidylethanolamine, an unidentified aminolipid and two unidentified phospholipids. The DNA G + C content was 63.5 mol%. Strain SMK-117ᵀ was differentiated from members of the genera Maritimibacter and Oceanicola on the basis of differences in the fatty acid and polar lipid profiles. The phenotypic, chemotaxonomic and phylogenetic data indicated that strain SMK-117ᵀ represents a novel genus and species, for which the name Lutimaribacter saemankumensis gen. nov., sp. nov. is proposed. The type strain is SMK-117ᵀ (=KCTC 22244ᵀ =CCUG 55760ᵀ).

**Spirosoma panaciterrae sp nov., isolated from soil**

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A Gram-negative, yellowish bacterial strain, designated Gsoil 1519ᵀ, was isolated from soil of a ginseng field in Pocheon province (South Korea) and characterized using a polyphasic approach to determine its taxonomic position. Comparative 16S rRNA gene sequence analysis showed that strain Gsoil 1519ᵀ belongs to the family 'Flexibacteraceae' and is related to Spirosoma rigui KCTC 12531ᵀ (91.8% similarity) and Spirosoma linguale LMG 10896ᵀ (91.5% similarity). Phylogenetic distances from any other recognized species within the family 'Flexibacteraceae' were greater than 14.7%. The G+C content of the genomic DNA of strain Gsoil 1519ᵀ was 50.1%. The detection of MK-7 as the predominant menaquinone and a fatty acid profile with C₁₆:₁ω5c, summed feature 4 (C₁₆:₁ω7c and/or iso-C₁₅:₀ 2-OH), iso-C₁₅:₀ and C₁₆:₀ as the major acids supported the affiliation of strain Gsoil 1519ᵀ to the genus Spirosoma. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 1519ᵀ should be classified in the genus Spirosoma as a representative of a novel species, for which the name Spirosoma panaciterrae sp. nov. is proposed. The type strain is Gsoil 1519ᵀ (=KCTC 22263ᵀ =DSM 21099ᵀ).

**INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 59(2): 331-335.**

**Keywords**: Spirosoma panaciterrae; performance liquid-chromatography; bacterium; water
Pedobacter composti sp. nov., isolated from compost

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A Gram-negative, aerobic, rod-shaped, non-motile, non-spore-forming bacterial strain, designated TR6-06T, was isolated from a compost sample in South Korea and characterized taxonomically by using a polyphasic approach. The organism grew optimally at 30 degrees C and pH 6.5-7.0. The isolate was positive for catalase and oxidase tests, but negative for gelatinase and urease and for indole and H2S production. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain TR6-06T was most closely affiliated with members of the genus Pedobacter of the family Sphingobacteriaceae. Strain TR6-06T exhibited 16S rRNA gene sequence similarity values of 89.9-93.5% to the type strains of species of the genus Pedobacter. The G+C content of the genomic DNA of strain TR6-06T was 41.9 mol%. The predominant respiratory quinone was MK-7. The major fatty acids were iso-C15:0, iso-C17:0 3-OH, C16:1 omega 7c and anteiso-C15:0. These chemotaxonomic data support the affiliation of strain TR6-06T to the genus Pedobacter. However, on the basis of its phenotypic properties and phylogenetic distinctiveness, strain TR6-06T (=KCTC 12638T =LMG 23490T) should be classified as the type strain of a novel species, for which the name Pedobacter composti sp. nov. is proposed.

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 59(2): 345-349.

Keywords: Pedobacter composti; performance liquid-chromatography; sequence alignment; bacterium; soil

Lysobacter panaciterrae sp. nov., isolated from soil of a ginseng field

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A Gram-negative, aerobic, rod-shaped, non-spore-forming bacterial strain, designated Gsoil 068T, was isolated from soil of a ginseng field in Pocheon Province (South Korea), and was characterized to determine its taxonomic position by using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that strain Gsoil 068T belonged to the family Xanthomonadaceae, class Gammaproteobacteria, and was related most closely to Lysobacter brunescens ATCC 29482T and Lysobacter gummosus ATCC 29489T (96.1% sequence similarity). The G+C content of the genomic DNA of strain Gsoil 068T was 67.0 mol%. The detection of a quinone system with ubiquinone Q-8 as the predominant component and a fatty acid profile with iso-C15:0, iso-C17:0 3-OH, C16:1 omega 7c and iso-C11:0 3-OH as the major components supported the affiliation of strain Gsoil 068T to the genus Lysobacter. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 068T is considered to represent a novel species of the genus Lysobacter, for which the name Lysobacter panaciterrae sp. nov. is proposed. The type strain is Gsoil 068T (=KCTC 12601T =DSM 17927T).


Keywords: Lysobacter panaciterrae; performance liquid-chromatography; greenhouse soils; bacterium; sludge; genus
Paenibacillus pectinilyticus sp nov., isolated from the gut of Diestrammena apicalis

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During a search for exo-enzyme-producing bacteria in the gut of an insect, Diestrammena apicalis, a novel bacterium capable of degrading pectin was isolated. The isolate, designated strain RCB-08T, comprised Gram-positive, endospore-forming, motile rods capable of growth at 15-30 degrees C and pH 6.0-8.7. The DNA G+C content of the isolate was 51.5 mol% and the predominant cellular fatty acid was anteiso-C15:0 (74.1%). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain RCB-08T was affiliated with a cluster within the Paenibacillaceae, and was related most closely to Paenibacillus chondroitinus NBRC 15376T with a sequence similarity of 96.7%. The DNA-DNA relatedness value for strain RCB-08T with P. chondroitinus NBRC 15376T was 15.0%. Strain RCB-08T hydrolysed pectin, but not cellulose, casein, starch or xylan. Strain RCB-08T could be clearly distinguished from other Paenibacillus species on the basis of characteristics observed using a polyphasic approach. Therefore strain RCB-08T is considered to represent a novel species of the genus Paenibacillus, for which the name Paenibacillus pectinilyticus sp. nov. is proposed. The type strain is RCB-08T (=KCTC 13222T =CECT 7358T).


Keywords: Paenibacillus pectinilyticus; xylanolytic bacterium; bacillus-curdlanolyticus; genus paenibacillus; sequence alignment

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Microbacterium insulae sp nov., isolated from soil

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A Gram-positive, non-motile, rod- or coccoid-shaped Microbacterium-like bacterium, designated strain DS-66T, was isolated from soil of Dokdo, Korea, and its exact taxonomic position was investigated by using a polyphasic approach. Strain DS-66T grew optimally at 30 degrees C and pH 6.5-7.0 in the presence of 0.5-1.0% (w/v) NaCl. Phylogenetic analysis based on 16S RNA gene sequences showed that strain DS-66T belonged to the genus Microbacterium. Strain DS-66T had a peptidoglycan type based on B2 beta with partial substitution of glutamic acid by 3-hydroxy glutamic acid (Glu/Hyg-Gly-Lys-Orn), and galactose, rhamnose and ribose as whole-cell sugars. The acyl type was glycolyl. Strain DS-66T contained MK-13, MK-12 and MK-14 as predominant menaquinones and anteiso-C15:0, anteiso-C17:0, iso-C16:0 and iso-C16:0 as major fatty acids. Major polar lipids were diphosphatidylglycerol, phosphatidyl glycerol, an unidentified phospholipid and an unidentified glycolipid. The DNA G+C content was 69.9 mol%. Phylogenetic distinctiveness, DNA-DNA relatedness data and differential phenotypic properties demonstrated that strain DS-66T is distinguishable from recognized Microbacterium species. On the basis of the data presented, strain DS-66T is considered to represent a novel species of the genus Microbacterium, for which the name Microbacterium insulae sp. nov. is proposed. The type strain is DS-66T (=KCTC 19247T=CCUG 54523T).

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Keywords: Microbacterium insulae; bacterial-cell-walls; genus microbacterium; identification; aureobacterium; systematics
**Marinimicrobium locisalis** sp nov., isolated from a marine solar saltern, and emended description of the genus *Marinimicrobium*

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A Gram-negative, motile and rod-shaped bacterial strain, designated ISL-43T, was isolated from a marine solar saltern of the Yellow Sea, Korea, and its taxonomic position was investigated by means of a polyphasic study. Strain ISL-43T grew optimally at pH 7.0-8.0 and 30 degrees C and in the presence of approximately 2% NaCl. It contained Q-8 as the predominant ubiquinone and C16:0, C19:0ω8C, C16:1ω7c and/or iso-C15:02-OH, and C18:1ω7c as the major fatty acids. The DNA G + C content was 58.4 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ISL-43T fell within the genus *Marinimicrobium*, clustering with *Marinimicrobium agarilyticum* M18T with a bootstrap value of 100%. Strain ISL-43T exhibited DNA-DNA relatedness values of 17 and 10% to *M. agarilyticum* KCTC 12357T and *M. koreense* KCTC 12356T, respectively. On the basis of phenotypic, phylogenetic and genetic data, strain ISL-43T represents a novel species within the genus *Marinimicrobium*, for which the name *Marinimicrobium locisalis* sp. nov. is proposed. The type strain is ISL-43T (=KCTC 22484T=CCUG 56757T).

**Psychroflexus salinarum** sp nov., isolated from a marine solar saltern

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A Gram-negative, non-motile and rod-shaped bacterial strain, ISL-14T, was isolated from a marine solar saltern of the Yellow Sea, Korea, and its taxonomic position was investigated by a polyphasic study. Strain ISL-14T grew optimally at pH 7.0-8.0, 30 degrees C and in the presence of approximately 2% (w/v) NaCl. It contained MK-6 as the predominant menaquinone and anteiso-C15:0, iso-C15:0 and iso-C16:0 3-OH as the major fatty acids. The DNA G + C content was 38.5 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain ISL-14T belonged to the genus *Psychroflexus*. The levels of similarity between the 16S rRNA gene sequence of strain ISL-14T and those of the type strains of recognized *Psychroflexus* species were 95.8-96.8%. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain ISL-14T represents a novel species within the genus *Psychroflexus*, for which the name *Psychroflexus salinarum* sp. nov. is proposed. The type strain is ISL-14T (=KCTC 22483T=CCUG 56752T).

**Keywords**: *Psychroflexus salinarum*; comb. nov; bacterium; reclassification; identification; sediment; taxa; lake
**Salinihabitans flavidus** gen. nov., sp. nov., isolated from a marine solar saltern

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A Gram-negative, non-motile and rod-shaped bacterial strain, ISL-46\(^T\), belonging to the *Alphaproteobacteria*, was isolated from a marine solar saltern in Korea, and subjected to a polyphasic taxonomic study. Strain ISL-46\(^T\) grew optimally at pH 7.0-8.0 and 30 degrees C and in the presence of 7% (w/v) NaCl. It contained Q-10 as the predominant ubiquinone and C\(_{19}\) cyclo omega 8c(1) C\(_{18:1}\) omega 7c and 11-methyl C\(_{18:1}\) omega 7c as the major fatty acids. The DNA G+C content was 63.5 mol%. Strain ISL-46\(^T\) exhibited 16S rRNA gene sequence similarity values of 94.1-95.3% to members of the phylogenetically related genera *Roseivivax*, *Salipiger*, *Citreicella*, *Yangia* and *Citreimonas*. Strain ISL-46\(^T\) could be differentiated from the above-mentioned genera by differences in compositions of the major fatty acids and in some phenotypic properties. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain ISL-46\(^T\) is considered to represent a novel genus and species, for which the name *Salinihabitans flavidus* gen. nov., sp. nov. is proposed. The type strain is ISL-46\(^T\) (=KCTC 22485\(^T\)= CCUG 56758\(^T\)).


**Keywords**: Salinihabitans flavidus; roseobacter clade; bacterium; identification; sediment

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**Seohaeicola saemankumensis** gen. nov., sp. nov., isolated from a tidal flat

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A Gram-negative, non-motile and rod-, oval- or coccoid-shaped bacterial strain, SD-15\(^T\), was isolated from a tidal flat of the Yellow Sea, Korea. The novel strain, which was phylogenetically closely related to the genera *Phaeobacter*, *Leisingera* and *Marinovum*, was studied using a polyphasic taxonomic approach. Strain SD-15\(^T\) grew optimally at pH 7.0-8.0 and 30 degrees C in the presence of 2% (w/v) NaCl. It contained Q-10 as the predominant ubiquinone and C\(_{18:1}\) omega 7c and 11-methyl C\(_{18:1}\) omega 7c as the major fatty acids. The major polar lipids were phosphatidylcholine, phosphatidylethanolamine and an unidentified lipid. The DNA G+C content was 63.4 mol%. Strain SD-15\(^T\) exhibited the highest 16S rRNA gene sequence similarity values (95.1-96.4%) to the type strains of species of the genus *Phaeobacter*, *Leisingera methylohalidivorans* MB2\(^T\) and *Marinovum algicola* ATCC 51440\(^T\). Strain SD-15\(^T\) could be differentiated from members of the genera *Phaeobacter*, *Leisingera* and *Marinovum* by differences in the contents of some fatty acids, by the absence of aminolipid and by differences in some phenotypic properties. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain SD-15\(^T\) represents a new genus and novel species, for which the name *Seohaeicola saemankumensis* gen. nov., sp. nov. is proposed. The type strain of the type species is *Seohaeicola saemankumensis* SD-15\(^T\) (=KCTC 22175\(^T\)=CCUG 55328\(^T\)).


**Keywords**: Seohaeicola saemankumensis; bacterium; roseobacter; identification; leisingera
**Article 103**

*Sphingomonas hankookensis* sp nov., isolated from wastewater

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A Gram-negative, non-motile, rod-shaped bacterial strain, ODN7\(^T\), was isolated from a wastewater treatment plant in Korea, and its taxonomic position was investigated by use of a polyphasic taxonomic approach. Strain ODN7\(^T\) grew optimally at around pH 7.5 and 30 degrees C and in the presence of 0-0.5 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain ODN7\(^T\) fell within the cluster comprising species of the genus *Sphingomonas*, clustering with *Sphingomonas panni* C52\(^T\), with which it shared highest 16S rRNA gene sequence similarity (98.9%). The chemotaxonomic properties of strain ODN7\(^T\) were consistent with those of the genus *Sphingomonas*. The predominant ubiquinone was Q-10, and the major fatty acids were C\(_{18:1}\) omega 7c, C\(_{16:1}\) omega 9c and/or iso-C\(_{15:0}\) 2-OH, and C\(_{16:0}\). Major polar lipids were sphingoglycolipid, phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine. The DNA G+C content of strain ODN7\(^T\) was 67.2 mol%. Strain ODN7\(^T\) exhibited levels of DNA-DNA relatedness of 15-32% to the type strains of phylogenetically related *Sphingomonas* species and could be differentiated from these species based on differences in phenotypic characteristics. On the basis of the data presented, strain ODN7\(^T\) is considered to represent a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas hankookensis* sp. nov. is proposed. The type strain is ODN7\(^T\) (=KCTC 22579\(^T\)= CCUG 57509\(^T\)).

**INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 59(11): 2788-2793.**

**Keywords:** *Sphingomonas hankookensis*; ribosomal-RNA sequence; bacterial systematics; soil; identification; strains

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**Article 104**

*Nocardioides caeni* sp nov., isolated from wastewater

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A Gram-positive, non-motile, rod- or coccoid-shaped bacterial strain, designated MN8\(^T\), was isolated from sludge of domestic wastewater in Korea, and its taxonomic position was investigated by use of a polyphasic study. Strain MN8\(^T\) grew optimally at pH 6.5-7.5 and 30 degrees C and in the presence of 0-0.5% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MN8\(^T\) fell within the cluster comprising *Nocardioides* species, clustering with *Nocardioides simplex* KCTC 9106\(^T\), *Nocardioides aromaticivorans* H-1\(^T\), *Nocardioides kongjuensis* A2-4\(^T\) and *Nocardioides nitrophenolicus* NSP41\(^T\), with which it shared 98.4-99.0% 16S rRNA gene sequence similarity. The chemotaxonomic properties of strain MN8\(^T\) were consistent with those of the genus *Nocardioides*: the cell-wall peptidoglycan type was based on LL-2,6-diaminopimelic acid, the predominant menaquinone was MK-8(H4) and the major fatty acids were iso-C\(_{16:0}\) and C\(_{18:1}\) omega 9c. The DNA G+C content was 71.5 mol%. Strain MN8\(^T\) exhibited levels of DNA-DNA relatedness of 13-31 % to the type strains of phylogenetically related *Nocardioides* species and could be differentiated from these species based on differences in phenotypic characteristics. On the basis of the data presented, strain MN8\(^T\) is considered to represent a novel species of the genus *Nocardioides*, the cell-wall peptidoglycan type was based on LL-2,6-diaminopimelic acid, the predominant menaquinone was MK-8(H4) and the major fatty acids were iso-C\(_{16:0}\) and C\(_{18:1}\) omega 9c. The DNA G+C content was 71.5 mol%. Strain MN8\(^T\) exhibited levels of DNA-DNA relatedness of 13-31 % to the type strains of phylogenetically related *Nocardioides* species and could be differentiated from these species based on differences in phenotypic characteristics. On the basis of the data presented, strain MN8\(^T\) is considered to represent a novel species of the genus *Nocardioides*, the cell-wall peptidoglycan type was based on LL-2,6-diaminopimelic acid, the predominant menaquinone was MK-8(H4) and the major fatty acids were iso-C\(_{16:0}\) and C\(_{18:1}\) omega 9c. The DNA G+C content was 71.5 mol%. Strain MN8\(^T\) exhibited levels of DNA-DNA relatedness of 13-31 % to the type strains of phylogenetically related *Nocardioides* species and could be differentiated from these species based on differences in phenotypic characteristics. On the basis of the data presented, strain MN8\(^T\) is considered to represent a novel species of the genus *Nocardioides*, for which the name *Nocardioides caeni* sp. nov. is proposed. The type strain is MN8\(^T\) (=KCTC 19600\(^T\)= CCUG 57506\(^T\)).

**INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 59(11): 2794-2797.**

**Keywords:** *Nocardioides caeni*; degrading bacterium; cell-wall; systematics; sediment; trees; genus
**Terrabacter terrigena** sp. nov., isolated from soil

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A Gram-positive-staining, non-motile and rod-shaped bacterium, strain ON10ᵀ, was isolated from soil around a wastewater treatment plant in Korea and its taxonomic position was investigated by using a polyphasic approach. Strain ON10ᵀ grew optimally at pH 6.5-7.0 and 30 °C in the presence of 0.5% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain ON10ᵀ clustered with the clade comprising *Terrabacter* species, with which it exhibited 16S rRNA gene sequence similarity values of 98.4-98.8%. The cell-wall peptidoglycan type was based on LL-diaminopimelic acid and the cell-wall sugars were glucose, mannose, arabinose and xylose. The predominant menaquinone was MK-8(H₄). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, an unidentified phospholipid and an unidentified lipid. The major fatty acids were iso-C₁₅:0 and iso-C₁₄:0. The DNA G + C content was 71.6 mol%. Strain ON10ᵀ exhibited DNA-DNA relatedness levels of 17-28 % to the type strains of *Terrabacter* species and could also be differentiated from these species by differences in phenotypic characteristics. On the basis of the data obtained, strain ON10ᵀ was considered to represent a novel species of the genus *Terrabacter*, for which the name *Terrabacter terrigena* sp. nov. is proposed. The type strain is ON10ᵀ (=KCTC 19602ᵀ = CCUG 57508ᵀ).

**Salimicrobium flavidum** sp. nov., isolated from a marine solar saltern

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A Gram-variable-staining, motile and coccolid-, ovoid- or rod-shaped bacterium, strain ISL-25ᵀ, was isolated from a marine solar saltern of the Yellow Sea, Korea, and its taxonomic position was investigated by means of a polyphasic study. Strain ISL-25ᵀ grew optimally at pH 7.0-8.0 and 30-37 °C. Strain ISL-25ᵀ contained meso-diaminopimelic acid as the cell-wall peptidoglycan, MK-7 as the predominant menaquinone and anteiso-C₁₅:0, anteiso-C₁₇:0 and iso-C₁₆:0 as the major fatty acids. The DNA G + C content was 49.3 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ISL-25ᵀ belongs to the genus *Salimicrobium*. The similarity values between the 16S rRNA gene sequence of strain ISL-25ᵀ and those of the type strains of the three currently recognized *Salimicrobium* species were 97.6-98.3 %. Mean DNA-DNA relatedness values between strain ISL-25ᵀ and the type strains of the genus *Salimicrobium* were 9-15 %. Differential phenotypic properties of strain ISL-25ᵀ, together with the phylogenetic and genetic distinctiveness, revealed that this strain could be differentiated from other *Salimicrobium* species. Therefore, strain ISL-25ᵀ represents a novel species within the genus *Salimicrobium*, for which the name *Salimicrobium flavidum* sp. nov. is proposed. The type strain is ISL-25ᵀ (=KCTC 13260ᵀ = CCUG 56755ᵀ).

**Keywords**: *Terrabacter terrigena*; bacterial systematics; identification; *nocardioides*; sequences; strains; trees

**Keywords**: *Salimicrobium flavidum*; bacteria; strains; genus
**Planomicrobium flavidum** sp nov., isolated from a marine solar saltern, and transfer of **Planococcus stackebrandtii** Mayilraj et al. 2005 to the genus **Planomicrobium** as **Planomicrobium stackebrandtii** comb. nov.

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A Gram-positive, motile and coccoid- or short rod-shaped bacterial strain, ISL-41\(^\text{T}\), was subjected to a polyphasic study to investigate its exact taxonomic position. Strain ISL-41\(^\text{T}\) grew optimally at pH 7.0-8.0 and 30 degrees C. It contained MK-8 and MK-7 as the predominant menaquinones and anteiso-C\(_{15}\)H\(_{29}\), anteiso-C\(_{17}\)H\(_{31}\) and C\(_{16}\)H\(_{30}\) omega 7c alcohol as the major fatty acids. The DNA G + C content was 45.9 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain ISL-41\(^\text{T}\) belonged to the genus **Planomicrobium**. The levels of similarity between the 16S rRNA gene sequence of strain ISL-41\(^\text{T}\) and those of the type strains of recognized **Planomicrobium** species and **Planococcus stackebrandtii** were 97.4-98.6%. Mean DNA-DNA relatedness values between strain ISL-41\(^\text{T}\) and the type strains of **Planomicrobium** species and **Planococcus stackebrandtii** were 13-25%. Differential phenotypic properties, together with the phylogenetic and genetic distinctiveness, showed that strain ISL-41\(^\text{T}\) could be differentiated from recognized **Planomicrobium** species and **Planococcus stackebrandtii**.

On the basis of the phenotypic, phylogenetic and genetic data, strain ISL-41\(^\text{T}\) is considered to represent a novel species within the genus **Planomicrobium**, for which the name **Planomicrobium flavidum** sp. nov. is proposed. The type strain is ISL-41\(^\text{T}\) (=KCTC 13261\(^\text{T}\) = CCUG 56756\(^\text{T}\)). It is also proposed that **Planococcus stackebrandtii** be transferred to the genus **Planomicrobium** as **Planomicrobium stackebrandtii** comb. nov. (type strain K22-03\(^\text{T}\) = MTCC 6226\(^\text{T}\) = DSM 16419\(^\text{T}\) = JCM 12481\(^\text{T}\)).

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**Keywords**: **Planomicrobium flavidum**; bacterial systematics; fermented seafood; sediment; alkanoclasticus; identification; psychrophilus; mcmeekini; jeotgal; strains

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**Gordonia hankookensis** sp nov., isolated from soil

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A Gram-positive, aerobic and non-motile bacterial strain, designated ON-33\(^\text{T}\), was subjected to a study based on a polyphasic approach to determine its exact taxonomic position. Strain ON-33\(^\text{T}\) grew optimally at pH 7.0-7.5 and 30 degrees C. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain ON-33\(^\text{T}\) fell within the clade comprising **Gordonia** species, clustering with **Gordonia soli** CC-AB07\(^\text{T}\), with which it exhibited 16S rRNA gene sequence similarity of 98.5%. The chemotaxonomic properties of strain ON-33\(^\text{T}\) were consistent with those shared by members of the genus **Gordonia**. The peptidoglycan type was based on meso-diaminopimelic acid and the whole-cell sugars were arabinose and galactose. The predominant menaquinone was MK-9(H\(_2\)). The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol. The major fatty acids were C\(_{16}\)H\(_{31}\), iso-C\(_{15}\)H\(_{29}\) 2-OH and or C\(_{16}\)H\(_{31}\) omega 7c, 10-methyl C\(_{18}\)H\(_{31}\) and C\(_{18}\)H\(_{31}\) omega 9c. The DNA G + C content was 66.9 mol%. Strain ON-33\(^\text{T}\) exhibited a DNA-DNA relatedness value of 13% to **G. soli** DSM 44995\(^\text{T}\) and could be differentiated from **G. soli** by differences in phenotypic characteristics. On the basis of the data obtained, strain ON-33\(^\text{T}\) is considered to represent a novel species of the genus **Gordonia**, for which the name **Gordonia hankookensis** sp. nov., is proposed. The type strain is ON-33\(^\text{T}\) (=KCTC 19599\(^\text{T}\) = CCUG 57507\(^\text{T}\)).

**INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY**, 59(12): 3172-3175.

**Keywords**: **Gordonia hankookensis**; bacterial systematics; sludge; identification; strains; genus
Antiatherosclerotic effects of Artemisia princeps Pampanini cv. Sajabal in LDL receptor deficient mice

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Antiatherosclerotic effects of ethanolic extracts of Artemisia princeps Pampanini cv. Sajabal (ESJ) were investigated in low-density lipoprotein receptor deficient (LDLR−/−) mice. The Western diet-induced high levels of total cholesterol and triglyceride were similar in the ESJ and control groups. However, circulating oxidized LDL was significantly decreased in the ESJ group (p < 0.05). ESJ also markedly decreased aortic expression levels of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-1 beta (IL-1 beta), and reduced the aortic lesion formation and macrophage accumulation by 36.7% (p < 0.05) and 43% (p < 0.01) in the control group, respectively. Additionally, ESJ inhibited atherogenic properties with cytokine-induced surface expression of cell adhesion molecules, chemokines, and monocyte adhesion to the human umbilical vein endothelial cells (HUVECs), and simultaneously suppressed nuclear factor-kappa B (NF-kappa B) activation.

These results suggest that ethanolic extracts of Artemisia princeps Pampanini cv. Sajabal contribute to the antiatherosclerotic and anti-inflammatory activities in LDLR−/− mice.


Keywords: Artemisia princeps Pamp. cv; Sajabal; atherosclerosis; inflammation; oxidized LDL; NF-kappa B

Antifungal activity of CHE-23C, a dimeric sesquiterpene from Chloranthus henryi

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An antifungal compound was isolated from methanol extracts of stems and roots of Chloranthus henryi Hemsl. using ethyl acetate extraction and various chromatographic techniques. On the basis of spectroscopic analyses including mass and various NMR, the structure of the compound was identified as a dimeric sesquiterpene, CHE-23C. The compound showed potent antifungal activities (MICs = 1-32 μg/mL) in vitro against various phytopathogenic fungi such as Alternaria kikuchiana, Botrytis cinerea, Colletotrichum lagenarium, Magnaporthe grisea, Pythium ultimum, and Phytophthora infestans. In particular, it exhibited 91 and 100% disease-control activity in vivo against tomato late blight (P. infestans) and wheat leaf rust (Puccinia recondita) at concentrations of 33 and 100 μg/mL, respectively. The disease-control activity of this compound was stronger than that of the commercially available fungicide chlorothalonil, but weaker than that of dimethomorph. Therefore, the compound might serve as an interesting lead to develop effective antifungal agents.

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Keywords: Chloranthus henryi; CHE-23C; antifungal activity; Phytophthora infestans; Puccinia recondita

Keywords: Chloranthus henryi; CHE-23C; antifungal activity; Phytophthora infestans; Puccinia recondita
Vinaxanthone, a new FabI inhibitor from *Penicillium* sp.

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Bacterial enoyl-ACP reductase (FabI) has been validated as a novel antibacterial target for tackling infections caused by multidrug-resistant pathogens. A few FabI inhibitors, however, are known. This study isolated a new FabI inhibitor from *Penicillium* sp. A screening programme led to the selection of a *Penicillium* sp. producing a strong FabI-inhibitory metabolite. The chemical structure of the isolated FabI inhibitor was elucidated by mass spectrometry and nuclear magnetic resonance spectral data. The antibacterial target of the inhibitor was validated by overexpression assays. The isolated FabI inhibitor was elucidated to be vinaxanthone. It selectively inhibited *Staphylococcus aureus* FabI with an IC₅₀ of 0.9 M; it did not affect FabK, an enoyl-ACP reductase of *Streptococcus pneumoniae*. Consistent with its inhibition of FabI, the inhibitor prevented intracellular fatty acid synthesis while it did not affect protein biosynthesis. It also prevented the growth of *S. aureus* as well as methicillin-resistant *S. aureus* (MRSA) and quinolone-resistant *S. aureus*. Importantly, *fabI*-overexpressing *S. aureus* showed reduced susceptibility to the inhibitor compared with the wild-type strain, demonstrating that its antibacterial action is mediated by inhibition of FabI. Vinaxanthone is a new FabI-directed antibacterial of natural origin that could have potential for further development as a new anti-MRSA agent.


**Keywords**: enoyl-ACP reductase; antibacterial; fatty acid synthesis; target validation; *Staphylococcus aureus*

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Genome sequence of the probiotic bacterium *Bifidobacterium animalis* subsp *lactis* AD011

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*Bifidobacterium animalis* subsp. *lactis* is a probiotic bacterium that naturally inhabits the guts of most mammals, including humans. Here we report the complete genome sequence of *B. animalis* subsp. *lactis* AD011 that was isolated from an infant fecal sample. Biological functions encoded in a single circular chromosome of 1,933,695 bp, smallest among the completely sequenced bifidobacterial genomes, are suggestive of their probiotic functions, such as utilization of bifidogenic factors and a variety of glycosidic enzymes and biosynthesis of polysaccharides.


**Keywords**: bile; *Bifidobacterium animalis* subsp *lactis* AD011
Identification of a polymyxin synthetase gene cluster of *Paenibacillus polymyxa* and heterologous expression of the gene in *Bacillus subtilis*

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Polymyxin, a long-known peptide antibiotic, has recently been reintroduced in clinical practice because it is sometimes the only available antibiotic for the treatment of multidrug-resistant gram-negative pathogenic bacteria. Lack of information on the biosynthetic genes of polymyxin, however, has limited the study of structure-function relationships and the development of improved polymyxins. During whole genome sequencing of *Paenibacillus polymyxa* E681, a plant growth-promoting rhizobacterium, we identified a gene cluster encoding polymyxin synthetase. Here, we report the complete sequence of the gene cluster and its function in polymyxin biosynthesis. The gene cluster spanning the 40.6-kb region consists of five open reading frames, designated *pmxA*, *pmxB*, *pmxC*, *pmxD*, and *pmxE*. The *pmxC* and *pmxD* genes are similar to genes that encode transport proteins, while *pmxA*, *pmxB*, and *pmxE* encode polymyxin synthetases. The inserational disruption of *pmxE* led to a loss of the ability to produce polymyxin. Introduction of the *pmx* gene cluster into the *amyE* locus of the *Bacillus subtilis* chromosome resulted in the production of polymyxin in the presence of extracellularly added L-2,4-diaminobutyric acid. Taken together, our findings demonstrate that the *pmx* gene cluster is responsible for polymyxin biosynthesis. *JOURNAL OF BACTERIOLOGY*, 191(10): 3350-3358.

Keywords: free enzyme-system; resistant acinetobacter-baumannii; negative bacterial-infections; alpha, gamma-diaminobutyric acid; phylogenetic analysis

Reduction of shoot hyperhydricity in micropropagated potato plants via antisense inhibition of a chCu/ZnSOD gene

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The occurrence of hyperhydricity in transgenic potato plants, which carry a chloroplastic Cu/ZnSOD gene of the lily in either a sense or antisense orientation, was investigated during *in vitro* culturing. Hyperhydric symptoms included curled leaves with a succulent appearance that were mainly observed in Cu/ZnSOD sense plants. By contrast, antisense plants showed a low frequency of hyperhydricity and a high rate of *ex vitro* survival. Therefore, a knockdown approach for a chCu/ZnSOD gene could be a practical method to reduce hyperhydricity in plant micropropagation. *JOURNAL OF KOREAN SOCIETY FOR APPLIED BIOLOGICAL CHEMISTRY*, 52(4): 397-400.

Keywords: ethylene; hyperhydricity; H2O2; micropropagation; potato; superoxide dismutase
Rengyolone inhibits apoptosis via etoposide-induced caspase downregulation

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In the course of screening for substances inhibiting apoptosis of U937 human leukemia cells induced by etoposide (10 \(\mu\)g/ml), \textit{Forsythiae fructus}, which showed a high level of inhibition, was selected. The regulating compounds were purified from the ethyl acetate extract by silica gel column chromatography and HPLC. The active substance was purified and identified as rengyolone by spectroscopic methods. This compound showed inhibitory activity on caspase-3 induction, a major protease of the apoptosis cascade, with an IC\(_{50}\) value of 38.96 \(\mu\)M after 8 h of etoposide treatment in U937 cells. The expression level of caspase-3 and poly(ADP-ribose) polymerase (PARP) were dose-dependently inhibited by the compound, suggesting that rengyolone inhibits etoposide-induced apoptosis via downregulation of caspases.


**Keywords**: apoptosis; U937 cells; rengyolone

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Monitoring bacterial population dynamics using real-time PCR during the bioremediation of crude-oil-contaminated soil

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We evaluated the activity and abundance of the crude-oil-degrading bacterium \textit{Nocardiia} sp. H17-1 during bioremediation of oil-contaminated soil, using real-time PCR. The total petroleum hydrocarbon (TPH) degradation rate constants (\(k\)) of the soils treated with and without H17-1 were 0.103 d\(^{-1}\) and 0.028 d\(^{-1}\), respectively. The degradation rate constant was 3.6 times higher in the soil with H17-1 than in the soil without H17-1. In order to detect and quantify the \textit{Nocardiia} sp. H17-1 in soil samples, we quantified the genes encoding 16S ribosomal RNA (16S rRNA), alkane monooxygenase (\textit{alkB4}), and catechol 2,3-dioxygenase (23CAT) with real-time PCR using SYBR green. The amounts of H17-1 16S rRNA and \textit{alkB4} detected increased rapidly up to 1,000-folds for the first 10 days, and then continued to increase only slightly or leveled off. However, the abundance of the 23CAT gene detected in H17-1-treated soil, where H17-1 had neither the 23CAT gene for the degradation of aromatic hydrocarbons nor the catechol 2,3-dioxygenase activity, did not differ significantly from that of the untreated soil (alpha=0.05, \(p>0.22\)). These results indicated that H17-1 is a potential candidate for the bioaugmentation of alkane-contaminated soil. Overall, we evaluated the abundance and metabolic activity of the bioremediation strain H17-1 using real-time PCR, independent of cultivation.


**Keywords**: bioaugmentation; crude oil; \textit{Nocardiia} sp.; real-time PCR; total petroleum hydrocarbon
A new spore display method is presented that enables recombinant proteins to be displayed on the surface of Bacillus spores via fusion with InhA, an exosporium component of Bacillus thuringiensis. The green fluorescent protein and β-galactosidase as model proteins were fused to the C-terminal region of InhA, respectively. The surface expression of the proteins on the spores was confirmed by flow cytometry, confocal laser scanning microscopy, measurement of the enzyme activity, and an immunogold electron microscopy analysis. InhA-mediated anchoring of foreign proteins in the exosporium of Bacillus spores can provide a new method of microbial display, thereby broadening the potential for novel applications of microbial display.

**Keywords**: Bacillus thuringiensis; spore display; exosporium; InhA

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Microbial community structure in hexadecane- and naphthalene-enriched gas station soil

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Shifts in the activity and diversity of microbes involved in aliphatic and aromatic hydrocarbon degradation in contaminated soil were investigated. Subsurface soil was collected from a gas station that had been abandoned since 1995 owing to ground subsidence. The total petroleum hydrocarbon content of the sample was approximately 2,100 mg/kg, and that of the soil below a gas pump was over 23,000 mg/kg. Enrichment cultures were grown in mineral medium that contained hexadecane (H) or naphthalene (N) at a concentration of 200 mg/l. In the H-enrichment culture, a real-time PCR assay revealed that the 16S rRNA gene copy number increased from $1.2 \times 10^5$ to $8.6 \times 10^6$ with no lag phase, representing an approximately 70-fold increase. In the N-enrichment culture, the 16S rRNA copy number increased about 13-fold after 48 h, from $6.3 \times 10^4$ to $8.3 \times 10^5$. Microbial communities in the enrichment cultures were studied by denaturing gradient gel electrophoresis and by analysis of 16S rRNA gene libraries. Before the addition of hydrocarbons, the gas station soil contained primarily Alpha- and Gammaproteobacteria. During growth in the H-enrichment culture, the contribution of Bacteroidetes to the microbial community increased significantly. On the other hand, during N-enrichment, the Betaproteobacteria population increased conspicuously. These results suggest that specific phylotypes of bacteria were associated with the degradation of each hydrocarbon.

**Keywords**: denaturing gradient gel electrophoresis; gas station; hexadecane; microbial community; naphthalene
Acetate consumption activity directly determines the level of acetate accumulation during Escherichia coli W3110 growth

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Escherichia coli excretes acetate during aerobic growth oil glycolytic carbon sources, which has been explained as an overflow metabolism when the carbon flux into the cell exceeds the capacity of central metabolic pathways. Non-acetogenic growth of E. coli on gluconeogenic carbon sources like succinate or in carbon-limited slow growth conditions is believed an evidence for the explanation. However, we found that a strain defective in the ackA-pta operon encoding acetate kinase and phosphotransacetylase for acetate synthesis was inducible in noncatabolite repression condition, whereas its isogenic parental strain did not. The ackA promoter was inducible in noncatabolite repression condition, whereas (lie expression of file ackA-pta operon encoding acetate kinase and phosphotransacetylase for acetate synthesis was constitutive. Results in this study suggest that E. coli excretes and scavenges acetate simultaneously in the carbon-limited low growth condition and in non-acetogenic carbon source, and the activity of the acetate consumption pathway directly affects the accumulation level of acetate in the culture broth. JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, 19(10): 1127-1134.

Keywords: acetate metabolism; acetyl CoA synthetase; phosphotransacetylase; acetate kinase; glyoxylate shunt

Genome sequences of Escherichia coli B strains REL606 and BL21(DE3)

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Escherichia coli K-12 and B have been the subjects of classical experiments from which much of our understanding of molecular genetics has emerged. We present here complete genome sequences of two E. coli B strains, REL606, used in a long-term evolution experiment, and BL21(DE3), widely used to express recombinant proteins. The two genomes differ in length by 72,304 bp and have 426 single base pair differences, a seemingly large difference for laboratory strains having a common ancestor within the last 67 years. Transpositions by IS1 and IS150 have occurred in both lineages. Integration of the DE3 prophage in BL21(DE3) apparently displaced a defective prophage in the lambda attachment site of B. As might have been anticipated from the many genetic and biochemical experiments comparing B and K-12 over the years, the B genomes are similar in size and organization to the genome of E. coli K-12 MG1655 and have similar to 99% sequence identity over similar to 92% of their genomes. E. coli B and K-12 differ considerably in distribution of IS elements and in location and composition of larger mobile elements. An unexpected difference is the absence of a large cluster of flagella genes in B, due to a 41 kbp IS1-mediated deletion. Gene clusters that specify the LPS core, 0 antigen, and restriction enzymes differ substantially, presumably because of horizontal transfer. Comparative analysis of 32 independently isolated E. coli and Shigella genomes, both commensals and pathogenic strains, identifies a minimal set of genes in common plus many strain-specific genes that constitute a large E. coli pan-genome. JOURNAL OF MOLECULAR BIOLOGY, 394(4): 644-652.

Keywords: E. coli B; REL606; BL21(DE3); comparative genomics; Similarity of E. coli B and K-12
Stable plastid transformation in *Nicotiana benthamiana*

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Plastids from *Nicotiana benthamiana* were transformed with the vector for dicistronic expression of two genes—aminoglycoside 3'-adenyltransferase (*aadA*) and green fluorescent protein (*gfp*)—in the plastids of *Nicotiana tabacum*. Transplastomic shoots exhibited green fluorescence under UV light. Transformation efficiencies were similar between species. Although the border sequence (*trnI* and *trnA*) for homologous recombination to transform the plastid genome of *N. benthamiana* was identical to that sequence of *N. tabacum*, the exception was a 9-bp addition in the intron of *trnI*. This indicated that the *N. tabacum* sequence used as a border region for recombination was sufficient to insert the foreign gene into the target site between the *trnI* and *trnA* of *N. benthamiana* with similar efficiency. Southern blot analysis detected the presence of *aadA* and *gfp* between *trnI* and *trnA* in the plastid genome of *N. benthamiana*. Northern and western blot analyses revealed high expression of *gfp* in the plastids from petals and leaves. Our results suggest that the plastid transformation system established here is applicable to investigations of the interactions between plastid and nucleus in *N. benthamiana*.

**Keywords**: *Nicotiana benthamiana*, plastid transformation

Genetic discrimination of *Catharanthus roseus* cultivars by pyrolysis mass spectrometry

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Pyrolysis mass spectrometry (PyMS) is a rapid, simple, high-resolution analytical method based on thermal degradation of complex materials in a vacuum. It is widely applied to the discrimination of closely related microbial strains. Leaf samples from eight cultivars (‘Apricot Delight’, ‘Cooler Grape’, ‘Cooler Peppermint’, ‘Equator Grape’, ‘Equator Rose’, ‘Equator White’, ‘Equator White Eye’, and ‘Little Bright Eye’) of *Catharanthus roseus* were subjected to PyMS for spectral fingerprinting. Discriminant analysis (DA) of PyMS data enabled us to assign these cultivars to discrete clusters. A hierarchical dendrogram based on DA provided a possible relationship among them that was in general agreement with a previously reported classification of the cultivars based on DNA fingerprints. Furthermore, those belonging to the same ‘series’ were grouped into a single cluster, which previously could not be achieved through similar approaches based on Fourier transform infrared spectroscopy or \(^1\)H NMR data. Overall results suggest that chemical differences (i.e., in pyrolysate composition) among cultivars, as detected by mass spectrometry, reflect their genetic variation.

**Keywords**: dendrogram; discriminant analysis (DA); genetic relationships; principal component analysis (PCA); pyrolysis mass spectrometry (PyMS)
A Rapid, simple method for the determination of 6-benzyladenine by pyrolysis mass spectrometry

Suk Weon Kim and Jang R. Liu

Pyrolysis mass spectrometry (PyMS) is a rapid, simple, high-resolution analytical method based on thermal degradation of complex material in a vacuum. PyMS was used for the quantitative determination of 6-benzyladenine (BA) supplemented to agar-solidified culture media (ASM) in this study. When subjected to PyMS, pure BA generated prominent fingerprint peaks. The peaks at m/z 68 and 123 were chosen for the quantitative measurement of BA because of the highest signal among those generated from pure BA and because of one of the highest masses among those with a prominent signal, respectively. To establish a standard curve for BA concentration in ASM, the combined peak intensity at m/z 68 and 123 was plotted against BA concentration ranged from as low as 0.44 μM after logarithmic transformation of both parameters. A linear regression line was yielded, which indicates BA concentration in ASM is directly proportional to the peak intensity, with R² = 0.9052, significant at the 99% level. These results suggest that PyMS enables the quantitative determination of growth regulators and other related compounds in plant materials in a rapid, simple, sensitive, accurate manner.


Keywords: 6-benzyladenine; linear regression; pyrolysis mass spectrometry; quantitative determination; rapid method

Transgenic sweetpotato (Ipomoea batatas L. cv. Yulmi) plants expressing the Arabidopsis nucleoside diphosphate kinase 2 (AtNDPK2) gene under the control of an oxidative stress-inducible peroxidase (SWPA2) promoter (referred to as SN plants) were developed and evaluated for enhanced tolerance of SN plants under various abiotic stress conditions. The level of AtNDPK2 expression and NDPK activity in SN plants following methyl viologen (MV) treatment was positively correlated with the plant's tolerance to MV. Interestingly, we observed that antioxidant enzyme activities such as peroxidase, ascorbate peroxidase, and catalase increased in MV-treated SN plants. In addition, SN plants showed enhanced tolerance to cold, high salinity, and drought stresses by an increase in the activity of H₂O₂ scavenging enzymes. These results indicate that overexpression of AtNDPK2 in sweetpotato might efficiently modulate oxidative stress from various environmental stresses.

MOLECULAR BREEDING, 24(3): 233-244.

Keywords: H₂O₂ scavenging enzyme; nucleoside diphosphate kinase 2; oxidative stress; peroxidase promoter; sweetpotato
Backbone resonance assignment of a proteolysis-resistant fragment in the oxygen-dependent degradation domain of the hypoxia inducible factor 1 alpha

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Hypoxia-inducible factor 1 alpha (HIF1 alpha) is a transcription factor that plays a key role in the adaptation of cells to low oxygen stress and oxygen homeostasis. The oxygen-dependent degradation (ODD) domain of HIF1 alpha responsible for the negative regulation of HIF1 alpha in normoxia is intrinsically unfolded. Here, we carried out the backbone 1H, 15N, and 13C resonance assignment of a proteolysis-resistant fragment (residues 404-477) in the HIF1 alpha ODD domain using NMR spectroscopy. About 98% (344/352) of all the 1HN, 15N, 13C alpha, 13C beta, and 13CO resonances were unambiguously assigned. The results will be useful for further investigation of the structural and dynamic states of the HIF1 alpha ODD domain and its interaction with binding partners.

MOLECULES AND CELLS, 27(4): 493-496.

Keywords: hypoxia-inducible factor 1 alpha; NMR spectroscopy; oxygen-dependent degradation domain; resonance assignment

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Genome evolution and adaptation in a long-term experiment with Escherichia coli

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The relationship between rates of genomic evolution and organismal adaptation remains uncertain, despite considerable interest. The feasibility of obtaining genome sequences from experimentally evolving populations offers the opportunity to investigate this relationship with new precision. Here we sequence genomes sampled through 40,000 generations from a laboratory population of Escherichia coli. Although adaptation decelerated sharply, genomic evolution was nearly constant for 20,000 generations. Such clock-like regularity is usually viewed as the signature of neutral evolution, but several lines of evidence indicate that almost all of these mutations were beneficial. This same population later evolved an elevated mutation rate and accumulated hundreds of additional mutations dominated by a neutral signature. Thus, the coupling between genomic and adaptive evolution is complex and can be counterintuitive even in a constant environment. In particular, beneficial substitutions were surprisingly uniform over time, whereas neutral substitutions were highly variable.

NATURE, 461(7268): 1243-1247.

Keywords: population-genetics; molecular evolution; bacterial evolution; parallel changes
Molecular characterization of the sweet potato peroxidase *SWPA4* promoter which responds to abiotic stresses and pathogen infection

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Previously, the *swpa4* peroxidase gene has been shown to be inducible by a variety of abiotic stresses and pathogenic infections in sweet potato (*Ipomoea batatas*). To elucidate its regulatory mechanism at the transcriptional level under various stress conditions, we isolated and characterized the promoter region (2374 bp) of *swpa4* (referred to as *SWPA4*). We performed a transient expression assay in tobacco protoplasts with deletions from the 5'-end of *SWPA4* promoter fused to the beta-glucuronidase (GUS) reporter gene. The -1408 and -374 bp deletions relative to the transcription start site (+1) showed 8 and 4.5 times higher GUS expression than the cauliflower mosaic virus 35S promoter, respectively. In addition, transgenic tobacco plants expressing GUS under the control of -2374, -1408 or -374 bp region of *SWPA4* promoter were generated and studied in various tissues under abiotic stresses and pathogen infection. Gel mobility shift assays revealed that nuclear proteins from sweet potato cultured cells specifically interacted with 60-bp fragment (-178/-118) in -374 bp promoter region. In silico analysis indicated that four kinds of cis-acting regulatory sequences, reactive oxygen species-related element activator protein 1 (AP1), CCAAT/enhancer-binding protein alpha element, ethylene-responsive element (ERE) and heat-shock element, are present in the -60 bp region (-178/-118), suggesting that the -60 bp region might be associated with stress inducibility of the *SWPA4* promoter.


**Keywords**: differential expression; oxidative stress; enhanced tolerance; superoxide-dismutase; ascorbate peroxidase; suspension-cultures; tobacco; chloroplasts

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Rapid discrimination of commercial strawberry cultivars using fourier transform infrared spectroscopy data combined by multivariate analysis

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To determine whether pattern recognition based on metabolite fingerprinting for whole cell extracts can be used to discriminate cultivars metabolically, leaves and fruits of five commercial strawberry cultivars were subjected to Fourier transform infrared (FT-IR) spectroscopy. FT-IR spectral data from leaves were analyzed by principal component analysis (PCA) and Fisher's linear discriminant function analysis. The dendrogram based on hierarchical clustering analysis of these spectral data separated the five commercial cultivars into two major groups with originality. The first group consisted of Korean cultivars including 'Maehyang', 'Seolhyang', and 'Gumhyang', whereas in the second group, 'Ryukbo' clustered with 'Janghee', both Japanese cultivars. The results from analysis of fruits were the same as of leaves. We therefore conclude that the hierarchical dendrogram based on PCA of FT-IR data from leaves represents the most probable chemotaxonomical relationship between cultivars, enabling discrimination of cultivars in a rapid and simple manner.

*Plant Biotechnology Reports*, 3(1): 87-93.

**Keywords**: *Fragaria ananassa*; fourier transformation infrared spectroscopy; linear discriminant function analysis; principal component analysis
**Article 129**

**Somatic embryogenesis and plant regeneration in zygotic embryo explant cultures of rugosa rose**

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Rugosa rose (Rosa rugosa) is cultivated as a garden flower and an important genetic resource for the breeding of roses (R. hybrida). This study describes culture conditions for high frequency plant regeneration from zygotic embryo explants via somatic embryogenesis in rugosa rose. Mature zygotic embryo, cotyledon, and radicle explants formed embryogenic calluses at frequencies of 38, 6.7, and 8.8% when cultured on half-strength Murashige and Skoog medium (A1/2MS) supplemented with 2.26, 9.05, and 9.05 μM 2,4-dichlorophenoxyacetic acid, respectively. Embryogenic calluses produced numerous somatic embryos, which then developed into plantlets on A1/2MS without growth regulators. Regenerated plantlets were grown to whole plants in a growth chamber.

*PLANT BIOTECHNOLOGY REPORTS*, 3(3): 199-203.

**Keywords**: Rosa rugosa; somatic embryogenesis; tissue culture

**Article 130**

**Transgenic plants with cyanobacterial genes**

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Over the years, cyanobacteria have been regarded as ideal model systems for studying fundamental biochemical processes like oxygenic photosynthesis and carbon and nitrogen assimilation. Additionally, they have been used as human foods, sources for vitamins, proteins, fine chemicals, and bioactive compounds. Aiming to increase plant productivity as well as nutritional values, cyanobacterial genes involved in carbon metabolism, fatty acid biosynthesis, and pigment biosynthesis have been intensively exploited as alternatives to homologous gene sources. In this short review, transgenic plants with cyanobacterial genes generated over the last two decades are examined, and the future prospects for transgenic crops using cyanobacterial genes obtained from functional genomics studies of numerous cyanobacterial genomes information are discussed.


**Keywords**: cyanobacteria genes; heterologous expression; transgenic plants
**Article 131**

**Differential antioxidation activities in two alfalfa cultivars under chilling stress**

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To understand the adaptability of alfalfa (*Medicago sativa* L.) to chilling stress, we analyzed the antioxidative mechanism during seed germination. The germination rates of six alfalfa cultivars were studied comparatively at 10A degrees C. Xinmu No. 1 and Northstar were selected as chilling stress-tolerant and stress-sensitive cultivars for further characterization. After chilling treatment, Xinmu No. 1 showed higher seedling growth than Northstar. Xinmu No. 1 exhibited low levels of hydrogen peroxide and lipid peroxidation compared with Northstar. In addition, shoots in Xinmu No. 1 treated with chilling showed higher activities of the superoxide dismutase, ascorbate peroxidase (APX), and catalase than those of Northstar, whereas Xinmu No. 1 showed higher APX activity in roots that Northstar. These results indicated that high antioxidation activity in Xinmu No. 1 under chilling stress is well associated with tolerance to chilling condition during germination.


**Keywords**: alfalfa; antioxidant enzymes; chilling stress; germination; lipid peroxidation

**Article 132**

**Plant regeneration from the root-derived embryonic tissues of *Rosa hybrida* L. cv. charming via a combined pathway of somatic embryogenesis and organogenesis**

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This study describes culture conditions for a plant regeneration system via a combined pathway of somatic embryogenesis and organogenesis in root explant cultures of the commercial rose cultivar 'Charming'. Root explants formed white calluses at a frequency of 30% after 6 weeks of culture on Schenk and Hildebrandt (SH) medium supplemented with 11 mg l⁻¹ 2,4-dichlorophenoxyacetic acid. After 6 weeks of transfer to SH medium without growth regulators, initial white calluses gave rise to globular somatic embryos at a frequency of 2.8%, which were subsequently dedifferentiated to embryonic tissues. Somatic embryos or embryonic tissues initially derived from root explants did not undergo development beyond cotyledonal stage. To produce adventitious shoots, embryonic tissues were sliced and cultured on SH medium with 0.5 mg l⁻¹ 6-benzyladenine. After 4 weeks of culture, 28% of embryonic tissue explants formed adventitious shoots. Regenerated shoots were rooted on half strength SH medium with 0.1 mg l⁻¹ alpha-naphthaleneacetic acid and subsequently grown to maturity. Root-derived embryonic tissues were proliferated by subculture, while retaining the capacity for shoot production for a few years.

*PLANT BIOTECHNOLOGY REPORTS*, 3(4): 341-345.

**Keywords**: organogenesis; *Rosa hybrida* L.; root explants; somatic embryo conversion; somatic embryogenesis
Changes in activities of antioxidant enzymes and their gene expression during leaf development of sweetpotato

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To understand the functions of antioxidant enzymes during leaf development in sweetpotato, we investigated the activities of several antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX) and catalase (CAT). Significant increases were observed in the activities of SOD, POX and APX during the late stage of leaf development, whereas CAT activity increased during the early developmental stage. By RT-PCR analysis, various POX and APX genes showed differential expression patterns during leaf development. Four POX genes \textit{swpa3, swpa4, swpa6, swpb4} and one APX gene \textit{swAPX1} exhibited high levels of gene expression during the senescence stage of leaf development, but two POX genes, \textit{swpa1} and \textit{swpa7} were preferentially expressed at both the mature green and the late senescence stages of leaf development. These results indicate that hydrogen peroxide \textit{(H\textsubscript{2}O\textsubscript{2})}-related antioxidant enzymes are differentially regulated in the process of leaf development of sweetpotato.


**Keywords**: antioxidant enzyme; leaf development; senescence; sweetpotato

Airborne induction and priming of plant defenses against a bacterial pathogen

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Herbivore-induced plant volatiles affect the systemic response of plants to local damage and hence represent potential plant hormones. These signals can also lead to "plant-plant communication," a defense induction in yet undamaged plants growing close to damaged neighbors. We observed this phenomenon in the context of disease resistance. Lima bean (\textit{Phaseolus lunatus}) plants in a natural population became more resistant against a bacterial pathogen, \textit{Pseudomonas syringae pv syringae}, when located close to conspecific neighbors in which systemic acquired resistance to pathogens had been chemically induced with benzothiadiazole (BTH). Airborne disease resistance induction could also be triggered biologically by infection with avirulent \textit{P. syringae}. Challenge inoculation after exposure to induced and noninduced plants revealed that the air coming from induced plants mainly primed resistance, since expression of \textit{PATHOGENESIS-RELATED PROTEIN2 (PR-2)} was significantly stronger in exposed than in nonexposed individuals when the plants were subsequently challenged by \textit{P. syringae}. Among others, the plant-derived volatile nonanal was present in the headspace of BTH-treated plants and significantly enhanced \textit{PR-2} expression in the exposed plants, resulting in reduced symptom appearance. Negative effects on growth of BTH-treated plants, which usually occur as a consequence of the high costs of direct resistance induction, were not observed in volatile organic compound-exposed plants. Volatile-mediated priming appears to be a highly attractive means for the tailoring of systemic acquired resistance against plant pathogens.


**Keywords**: systemic acquired-resistance; bean \textit{phaseolus-lunatus}; \textit{arabidopsis-thaliana}; methyl salicylate; signal-transduction; volatiles induce; innate immunity; trade-offs; \textit{zea-mays}
Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses

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To understand the adaptability of alfalfa (Medicago sativa L.) to environmental stresses, we analyzed the activity of several antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT), in alfalfa shoots and roots subjected to salt and drought stresses during germination. The germination rate of six alfalfa cultivars was comparatively studied under 200 mM NaCl or 35% PEG treatment. Alfalfa Xinmu No. 1 and Northstar varieties were selected as stress-tolerant and -sensitive cultivars, respectively, and were used for further characterization. After NaCl or PEG treatment, Xinmu No. 1 showed enhanced seedling growth, compared with Northstar. Xinmu No. 1 also exhibited low levels of hydrogen peroxide (H₂O₂) production and lipid peroxidation, compared with Northstar. In addition, Xinmu No. 1 showed higher enzymatic activity of SOD, APX, CAT, and POD in its shoots and roots than Northstar. These results seem to indicate that Xinmu No. 1 cultivar’s tolerance to salt or drought stresses during germination is associated with enhanced activity of antioxidant enzymes. This study highlights the importance of antioxidant enzymes in the establishment of alfalfa seedlings under drought and salinity conditions typical of desertification.

PLANT PHYSIOLOGY AND BIOCHEMISTRY, 47(7): 570-577.

Keywords: alfalfa; antioxidant enzymes; drought; germination; salt

Isolation and characterization of a cellulase-free endo-beta-1,4-xylanase produced by an invertebrate-symbiotic bacterium, Cellulosimicrobium sp HY-13

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A xylanolytic bacterium, Cellulosimicrobium sp. HY-13, was isolated from the digestive tract of an earthworm, Eisenia fetida. The purified cellulase-free endo-beta-1,4-xylanase (XylK) produced by strain HY-13 was found to contain an N-terminal amino acid sequence of APSTLEAAAE and to have a relative molecular mass of 36 kDa. It was most active at pH 6.0 and 55 degrees C and had Vₘₐₓ and Kₘ values toward oat spelt xylan of 4067 IU/mg and 2.78 mg/ml, respectively. XylK primarily degraded xylan to a series of xylooligosaccharides composed of xylobiose to xylotetraose, but it could not further hydrolyze xylobiose to xylose. The results of the present study suggest that the relatively highly active XylK lacking exo-xylanolytic activity is a promising candidate for the efficient production of non-digestible xylooligosaccharides that may have beneficial effects to gastrointestinal health via promotion of the growth of probiotics.

PROCESS BIOCHEMISTRY, 44(9): 1055-1059.

Keywords: Cellulosimicrobium sp HY-13; earthworm; Eisenia fetida; endo-beta-1,4-xylanase; invertebrate; xylooligosaccharides
Efficient, galactose-free production of *Candida antarctica* lipase B by GAL10 promoter in Delta gal80 mutant of *Saccharomyces cerevisiae*

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An efficient yeast gene expression system with GAL10 promoter that does not require galactose as an inducer was developed using Delta gal80 mutant strain of *Saccharomyces cerevisiae*. We constructed several combinations of gal mutations (Delta gal1, Delta gal80, Delta mig1, Delta mig2, and Delta gal6) of *S. cerevisiae* and tested for their effect on efficiency of recombinant protein production by GAL10 promoter using a lipase, *Candida antarctica* lipase B (CalB), as a reporter. While the use of Delta gal1 mutant strain required the addition of a certain amount of galactose to the medium, Delta gal80 mutant strain did not require galactose. Furthermore, it was found that the recombinant CAB could be produced more efficiently (1.6-fold at 5 L-scale fermentation) in Delta gal80 mutant strain than in the Delta gal1 mutant. The Delta gal80 mutant strain showed glucose repressible mode of expression of GAL10 promoter. Using Delta gal80 mutant strain of *S. cerevisiae*, CalB was efficiently produced in a glucose-only fermentation at volumes up to 500 L.


**Keywords**: GAL10 promoter; Saccharomyces cerevisiae; delta gal80; galactose-free; recombinant protein; *Candida antarctica* lipase B (CalB)

Functional expression in *Bacillus subtilis* of mammalian NADPH-cytochrome P450 oxidoreductase and its spore-display

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The technology for over-expressing NADPH-cytochrome P450 reductase (CPR), a diflavin-containing enzyme, offers the opportunity to develop enzymatic systems for environmental detoxication and bioconversions of drugs, pesticides and fine chemicals. In this study, *Bacillus subtilis* was chosen to express rat CPR (rCPR) because of its capacities for high protein production and spore formation. rCPR was expressed in *B. subtilis* DB104 under the transcriptional control of an IPTG-inducible fusion promoter of P_groE and P_tac. The expressed rCPR was released into the culture medium after sporulation by autolysis of the host cell. It was associated with and displayed on the spore surfaces; this was confirmed by measuring rCPR activity in purified spores and analyzing its accessibility to anti-rCPR antibodies using flow cytometry. The spore-displayed rCPR was able to reduce cytochrome c and ferricyanide, and also assisted in the O-deethylation of 7-ethoxyresorufin and 7-ethoxy-4-trifluoromethylcoumarin (EFC) by human cytochrome P450 1A2, indicating that it was functionally active. Spore surface display of rCPR in *B. subtilis* appears to be useful for preparing cytochrome P450-related enzymes, and spore biocatalysts of rCPR are likely to have wide biotechnological applications.


**Keywords**: NADPH-cytochrome P450 reductase; CPR; *Bacillus* expression; spore-display; whole-cell biocatalyst
Rhizosphere bacteria help plants tolerate abiotic stress

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Plant-growth-promoting rhizobacteria (PGPR) are associated with plant roots and augment plant productivity and immunity; however, recent work by several groups shows that PGPR also elicit so-called 'induced systemic tolerance' to salt and drought. As we discuss here, PGPR might also increase nutrient uptake from soils, thus reducing the need for fertilizers and preventing the accumulation of nitrates and phosphates in agricultural soils. A reduction in fertilizer use would lessen the effects of water contamination from fertilizer run-off and lead to savings for farmers.

Keywords: growth-promoting rhizobacteria; nutrient use efficiency; systemic resistance; confer resistance; arabidopsis

Hepatitis B virus-X protein recruits histone deacetylase 1 to repress insulin-like growth factor binding protein 3 transcription

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Hepatitis B virus (HBV), a major causative agent of hepatocellular carcinoma (HCC), encodes an oncogenic X-protein (HBx) which has been known as a transcriptional transactivator on multiple viral and cellular promoters. In the report, we verified that HBx transcriptionally repress insulin-like growth factor binding protein-3 (IGFBP-3) by promoting HBx/histone deacetylase 1 (HDAC1) complex formation. HBx recruited HDAC1 forms complex with Sp1 in a p53-independent manner) and deacetylates Sp1 which resulted in the diminished binding of Sp1 on targeted DNA during transcriptional repression. Deacetylation of Sp1 by HBx recruited HDAC1 likely to be a part of the mechanism that controls HBx induced IGFBP-3 repression and the modification of chromatin structure.

Keywords: insulin-like growth factor binding protein-3; histone deacetylase 1; immunoprecipitation; chromatin immunoprecipitation; trichostatin A; insulin-like growth factor type 1
4. Division of Bio R&D Infrastructure

- Microbial Resource Center (Korea Biological Resource Center)
- Plant Resource Center
- Human Derived Material Center
- Genome Resource Center
- Animal Model Resource Center
PESTAS: a web server for EST analysis and sequence mining

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We have developed a web server for the high-throughput annotation of expressed sequence tags (ESTs) called pipeline for EST analysis service (PESTAS). PESTAS processes entire datasets with an automated pipeline of 13 analytic services, then deposits the data into the MySQL database and transforms it into three kinds of reports: preprocessing, assembling and annotation. All annotated information is provided to the scientist and can be downloaded through a web browser. To get more relevant functional annotation results, a curation function was introduced with which biologists can easily change the best-hit annotation information. We included a gene chip module that detects gene expression differences between libraries by comparing accession number counts from BLAST search results. PESTAS also provides access to the pathway information of KEGG, which is useful for mapping the relationships among networks of annotated enzymes, and is especially valuable for those researchers interested in biological pathways.


Keywords: program; EST analysis

GarlicESTdb: an online database and mining tool for garlic EST sequences

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Background: Allium sativum, commonly known as garlic, is a species in the onion genus (Allium), which is a large and diverse one containing over 1,250 species. Its close relatives include chives, onion, leek and shallot. Garlic has been used throughout recorded history for culinary, medicinal use and health benefits. Currently, the interest in garlic is highly increasing due to nutritional and pharmaceutical value including high blood pressure and cholesterol, atherosclerosis and cancer. For all that, there are no comprehensive databases available for Expressed Sequence Tags(EST) of garlic for gene discovery and future efforts of genome annotation. That is why we developed a new garlic database and applications to enable comprehensive analysis of garlic gene expression.

Description: GarlicESTdb is an integrated database and mining tool for large-scale garlic (Allium sativum) EST sequencing. A total of 21,595 ESTs collected from an in-house cDNA library were used to construct the database. The analysis pipeline is an automated system written in JAVA and consists of the following components: automatic preprocessing of EST reads, assembly of raw sequences, annotation of the assembled sequences, storage of the analyzed information into MySQL databases, and graphic display of all processed data. A web application was implemented with the latest J2EE (Java 2 Platform Enterprise Edition) software technology (JSP/EJB/JavaServlet) for browsing and querying the database, for creation of dynamic web pages on the client side, and for mapping annotated enzymes to KEGG pathways, the AJAX framework was also used partially. The online resources, such as putative annotation, single nucleotide polymorphisms (SNP) and tandem repeat data sets, can be searched by text, explored on the website, searched using BLAST, and downloaded. To archive more significant BLAST results, a curation system was introduced with which biologists can easily edit best-hit annotation information for others to view. The GarlicESTdb web application is freely available at http://garlicdb.kribb.re.kr.

Conclusion: GarlicESTdb is the first incorporated online information database of EST sequences isolated from garlic that can be freely accessed and downloaded. It has many useful features for interactive mining of EST contigs and datasets from each library, including curation of annotated information, expression profiling, information retrieval, and summary of statistics of functional annotation. Consequently, the development of GarlicESTdb will provide a crucial contribution to biologists for data-mining and more efficient experimental studies.

Keywords: program; gene; EST sequences
Aerobic, alkaliphilic to alkalitolerant and mesophilic bacteria were isolated and characterized from soil and sediment samples collected from Bigeum Island, South Korea. The total numbers of microorganisms in the soil and sediment samples were found to be $10^3$-$10^5$ cfu/g and $10^2$-$10^7$ cfu/g, respectively. A total of 163 isolates were isolated and subjected to further characterization on the basis of pH, temperature and salt tolerance. Among the 163 isolates, 54 were selected based on their tolerance attributes to temperature, pH and NaCl. Out of the 54 isolates, 27 were further selected based on their multiple tolerance ability and enzyme profile and were subjected to 16S rRNA gene sequencing and phylogenetic analysis. The latter indicated that most of the Bigeum Island isolates were related to the phylum Actinobacteria. The phylogenetic tree based on 16S rRNA gene sequences placed the 27 isolates into 9 different major bacterial genera, each genus comprising pure cultures that shared a parts per thousand currency sign97% sequence identity and 18 putative novel species. Most of the strains were alkalitolerant and mesophilic, and produced biotechnologically important enzymes at alkaline pH.


**Keywords**: microbial diversity; polyphasic approach; Bigeum Island

We report here that a novel 1,869 bp repetitive sequence identified from the false killer whale (*Pseudorca crassidens*) could be a new molecular phylogenetic marker in cetaceans. Results of PCR amplification and southern blot hybridization using 16 species' genomic DNAs from five different families revealed that the repetitive sequence is highly conserved within all Delphinidae species. Notably, specific primers designed for this repetitive sequence effectively amplified the targeted repetitive units, which were critically dependent upon the genetic phylogenies in the members of the Delphinidae cetaceans. Therefore, the novel sequences can be used as a useful phylogenetic marker for understanding the molecular evolutionary studies in members of the Delphinidae family of cetaceans.


**Keywords**: false killer whale; DNA marker; repetitive sequence; phylogeny; whale genome; southern blot
Metatranscriptome analysis of lactic acid bacteria during Kimchi fermentation with genome-probing microarrays

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We constructed genome probing microarrays (GPM) that are specific to 39 lactic acid bacteria (LAB) in an effort to monitor microbial diversity and biological activity during the fermentation of kimchi, a traditional Korean vegetable product known to contain various health-promoting and immunity-boosting factors. Metagenomes and metatranscriptomes extracted from periodically sampled kimchi soup were labeled, hybridized and comparatively analyzed using GPMs. Each metatranscriptome was prepared by subtracting 16S rRNA and 23S rRNA from the total RNA, and selectively synthesizing mRNA-specific cDNAs from the rRNA-subtracted samples. Metagenomic analysis revealed 23 LAB related to kimchi fermentation [defined as bacteria with more than a 1% average relative composition (ARC)]. Metatranscriptome analysis revealed that, with the exception of two microorganisms, all LAB probed in the microarray contributed to kimchi fermentation. Moreover, the relative compositions of the major LAB remained unchanged (there was less than a 1.5% difference between the maximum and minimum values) in our metagenome analysis, while our metatranscriptome analysis revealed significant differences in the relative compositions of major LAB during fermentation (relative compositions changed by 2.4% to 9.5%). These data indicate that microorganisms that are less abundant in the flora (those with less than a 5% ARC in the metagenomic analysis) also participated in kimchi fermentation with relatively high activities.

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Keywords: metagenome; metatranscriptome; GPM; Kimchi; lactic acid bacteria

Paenibacillus harense sp nov., isolated from desert sand in China

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A Gram-positive, endospore-forming, rod-shaped bacterium, designated strain B519T, was isolated from a desert sand sample of Gansu Province, China. Strain B519T was strictly aerobic and cells were motile by means of peritrichous flagella. The strain grow optimally at 32-35 degrees C and pH 6.5-7.0. Chemotaxonomic data supported the affiliation of the new isolate to the genus Paenibacillus, including menaquinone-7 (MK-7) as the major isoprenoid quinone, DNA G+C content of 49.9 mol%, cell-wall type A1 gamma (meso-diaminopimelic acid as the diagnostic diamino acid) and anteiso-C15:0, iso-C15:0, C16:0 and iso-C16:0 as the major fatty acids. Comparative 16S rRNA gene sequence analysis showed that strain B519T was most closely related to Paenibacillus alkaliterrae KSL-134T (98.0% Similarity). DNA-DNA relatedness between strain B519T and P. alkaliterrae KSL-134T was about 12.3%. On the basis of phenotypic characteristics and molecular properties, strain B519T is considered to represent a novel species of the genus Paenibacillus, for which the name Paenibacillus harense sp. nov. is proposed. The type strain is B519T (=KCTC 3951T =DSM 16969T).


Keywords: Paenibacillus harense; bacterial systematics; genus paenibacillus; soil; identification; rhizosphere
**Leifsonia kribbensis** sp nov., isolated from soil

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A yellow-pigmented actinobacterium, designated strain MSL-13ᵀ, was isolated from a soil sample collected from Bigeum Island, Republic of Korea, and its taxonomic position was determined by using a polyphasic approach. Strain MSL-13ᵀ showed phenotypic and chemotaxonomic properties consistent with its classification in the genus *Leifsonia*. 16S rRNA gene sequence analysis of strain MSL-13ᵀ with sequences from *Leifsonia naganoensis* DB103ᵀ, *Leifsonia aquatica* DSM 20146ᵀ, *Leifsonia xylī* subsp. cyanodontis JCM 9733ᵀ, *Leifsonia poae* VKM Ac-1401ᵀ and *Leifsonia shinshuensis* DB102ᵀ revealed similarities of 96.22, 96.19, 95.77, 95.44 and 95.37%, respectively, with differences of 39-65 nt among 1483 total nucleotides aligned. Based on differences in phenotypic and genotypic characteristics, strain MSL-13ᵀ (=KCTC 19267ᵀ =DSM 19272ᵀ) is designated as the type strain of a novel species of the genus *Leifsonia*, for which the name *Leifsonia kribbensis* sp. nov. is proposed.


**Keywords**: *Leifsonia kribbensis*; phylogenetic trees; bacteria; clavibacter; identification

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**Pseudomonas sabulinigri** sp nov., isolated from black beach sand

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A novel Gram-negative, aerobic, motile, short rod-shaped bacterium, designated J64ᵀ, was isolated from black sand collected from Soesoggak, Jeju Island, Korea. Cells grew at 4-37 degrees C, at pH 5.5-10.0 and with 0-10% NaCl. The strain was found to be oxidase- and catalase-positive. Phylogenetic analyses showed that strain J64ᵀ belongs to the genus *Pseudomonas*, forming a monophyletic group with *Pseudomonas pachastrellae*, *Pseudomonas pertucinogena* and 'Pseudomonas denitrificans'. The 16S rRNA gene sequence similarity between strain J64ᵀ and type strains of all *Pseudomonas* species with validly published names was below 96.6%. Low levels of DNA-DNA relatedness were found with respect to type strains of *P. pachastrellae* and *P. pertucinogena*, supporting the classification of strain J64ᵀ into a novel species of the genus *Pseudomonas*. Strain J64ᵀ contained C₁₈:₁ omega 7c (37.2 %), C₁₆:₀ (20.4%), summed feature 3 (17.4%; comprising iso-C₁₅:₀ 2-OH and/or C₁₆:₁ omega 7c) and C₁₂:₀ (7.6%) as major cellular fatty acids. On the basis of the phenotypic and phylogenetic data, strain J64ᵀ represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas sabulinigri* sp. nov. is proposed. The type strain is J64ᵀ (=KCTC 22137ᵀ =14963ᵀ).


**Keywords**: *Pseudomonas sabulinigri*; sequence alignment; trees
A novel Gram-positive, aerobic, short-rod-shaped bacterium, designated strain J112\textsuperscript{T}, was isolated from black sand collected from Soesoggak, Jeju Island, Korea. The strain was found to be oxidase-negative and catalase-positive. Cells grew at 10-37 degrees C, at pH 5.5-8.0 and with 1-10% NaCl. Growth occurred on marine agar but not on R2A or trypticase soy agar. A phylogenetic analysis based on 16S rRNA gene sequences showed that the strain belongs to the radiation of the genus \textit{Nocardioides}. Strain J112\textsuperscript{T} shared the highest 16S rRNA gene sequence similarities with \textit{Nocardioides marinisabuli}\textsuperscript{T} (99.2%), \textit{Nocardioides terrigena}\textsuperscript{T} (97.3%), \textit{Nocardioides kribbensis}\textsuperscript{T} (97.1%) and type strains of other \textit{Nocardioides} species with validly published names (< 97%). The DNA-DNA hybridization values between strain J112\textsuperscript{T} and the three most closely related strains were low enough to justify the assignment of this strain to a novel species. On the basis of these phenotypic, phylogenetic and chemotaxonomic data, strain J112\textsuperscript{T} represents a novel species of the genus \textit{Nocardioides}, for which the name \textit{Nocardioides basaltis} sp. nov. is proposed.


**Keywords**: \textit{Nocardioides basaltis}; tidal flat sediment; chromatography; soil

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A Gram-negative, coccoid- or rod-shaped bacterium was isolated from black sand collected from Soesoggak beach, Jeju Island, Korea. The isolate, designated J3\textsuperscript{T}, grew at 15-45 degrees C, at pH 5.5-10.0 and in 0-8% NaCl. It was oxidase- and catalase-positive. Strain J3\textsuperscript{T} reduced nitrate to nitrite, but did not reduce nitrite to nitrogen gas. Phylogenetic analysis of the 16S rRNA gene sequence showed that strain J3\textsuperscript{T} was closely related to \textit{Nitratireductor aquibiodomus}\textsuperscript{T} and belonged to the genus \textit{Nitratireductor}. Major cellular fatty acids were C\textsubscript{18:1} \textit{omega} 7c (82.0%), C\textsubscript{19:0} \textit{omega} cyclo (4.3%) and C\textsubscript{18:0} (4.0%), a profile that is typical of members of the genus \textit{Nitratireductor} and distinct from those of other genera in the family \textit{Phyllobacteriaceae}. Differences in physiological characteristics and fatty acid profiles, as well as low DNA-DNA hybridization values, further established that strain J3\textsuperscript{T} was distinct from \textit{N. aquibiodomus} NL21\textsuperscript{T}. Thus, strain J3\textsuperscript{T} (=KCTC 22119\textsuperscript{T} =JCM 14935\textsuperscript{T}) should be classified as the type strain of a novel species in the genus \textit{Nitratireductor}, for which the name \textit{Nitratireductor basaltis} sp. nov. is proposed.


**Keywords**: \textit{Nitratireductor basaltis}; sequence alignment; montreal biodome; Canada
Nocardioides sediminis sp nov., isolated from a sediment sample

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A strictly aerobic, motile, short-rod-shaped, Gram-positive-staining actinomycete strain, designated MSL-01 T, was isolated from a sediment sample from Bigeum Island of Korea. 16S rRNA gene sequence analysis revealed that the isolate MSL-01 T belonged to the genus Nocardioides, with the highest sequence similarity to Nocardioides terrigena DS-17 T (98.54%), but the DNA-DNA relatedness to this type strain was 34%. The physiological properties of strain MSL-01 T differ from those of Nocardioides terrigena DS-17 T and other species of Nocardioides. The diamino acid in the cell-wall peptidoglycan of strain MSL-01 T is LL-diaminopimelic acid, the major menaquinone is MK-8(H4) and iso-C16:0 is the major fatty acid component. The name Nocardioides sediminis sp. nov. is proposed for the novel species, with the type strain MSL-01 T (=DSM 19263 T =KCTC 19271 T).


Keywords: Nocardioides sediminis; bacterial systematics; sequences; trees; genus; soil

Alishewanella aestuarii sp nov., isolated from tidal flat sediment, and emended description of the genus Alishewanella

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A Gram-negative strain, B11 T, was isolated from tidal flat sediment in Yeosu, Republic of Korea. Strain B11 T did not require NaCl for growth and grew between 18 and 44 degrees C. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain B11 T was associated with the genus Alishewanella and was closely related to the type strain of Alishewanella fetalis (98.3% similarity). Within the phylogenetic tree, the novel isolate shared a branching point with A. fetalis. Analysis of 16S rRNA gene sequences and DNA-DNA relatedness, as well as physiological and biochemical tests, indicated genotypic and phenotypic differences between strain B11 T and the type strain of A. fetalis. Thus, strain B11 T is proposed as a representative of a novel species, Alishewanella aestuarii sp. nov.; the type strain is B11 T (=KCTC 22051 T =DSM 19476 T).


Keywords: Alishewanella aestuarii; sequence alignment; bacterium; water; sea; software
Paracoccus aestuarii sp nov., isolated from tidal flat sediment

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A Gram-negative micro-organism, designated strain B7T, was isolated from tidal flat sediment and subjected to a polyphasic taxonomic study involving morphological, physiological, biochemical and 16S rRNA gene sequence analyses. A phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain B7T belonged to the genus Paracoccus and was closely related phylogenetically to Paracoccus marinus KKL-A5T (97.5% sequence similarity), Paracoccus marinus KKL-A5T (97.5%), Paracoccus carotinifaciens E-396 (97.3%), Paracoccus homiensis DD-R11T (97.2%), Paracoccus serinophilus M BT-A4T (96.9%) and other type strains of the genus Paracoccus (95.2-96.7%). The G+C content of the genomic DNA and the major isoprenoid quinone of the type strain were 62.0 mol% and ubiquinone-10, respectively. The major fatty acid components were C\textsubscript{18:1} omega 7c (68.9%) and C\textsubscript{18:0} (18.1%); this profile, with C\textsubscript{18:1} omega 7c as the predominant fatty acid, was characteristic of members of the genus Paracoccus. The 16S rRNA gene sequence analysis, DNA-DNA hybridization studies and physiological and biochemical tests identified genotypic and phenotypic differences between strain B7T and recognized Paracoccus species. On the basis of these data, therefore, strain B7T represents a novel species of the genus Paracoccus, for which the name Paracoccus aestuarii sp nov. is proposed. The type strain is B7T (=KCTC 22049T=DSM 19484T=JCM 15119T).


Keywords: Paracoccus aestuarii; astaxanthin-producing bacterium; sequence alignment; coccus

Paenibacillus pueri sp nov., isolated from Pu'er tea

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Pu'er tea is a fermented drink made from the leaves of the tea plant, Camellia sinensis. Two novel bacteria, designated strains b09i-3T and b13i-1, were isolated during the process of fermentation of this tea. These isolates were Gram-positive, endospore-forming, motile rods that grew at 25-42 degrees C and pH 5.5-10.4. The DNA G+C content was 56.6-58.4 mol%, the predominant isoprenoid quinone was MK-7 and the predominant cellular fatty acid was anteiso-C-15:0 (49.0-50% of the total). Phylogenetic analysis based on 16S rRNA gene sequences showed that strains b09i-3T and b13i-1 shared 99.9% similarity and were affiliated with a cluster within the family Paenibacillaceae. Strains b09i-3T and b13i-1 were related most closely to Paenibacillus ginsengihumi DCY16T (97% 16S rRNA gene sequence similarity). Levels of DNA-DNA relatedness between the two novel isolates and P. ginsengihumi DCY16T were below 56%. The phylogenetic and phenotypic characteristics of these novel isolates allowed them to be distinguished clearly from recognized species of the genus Paenibacillus. Based on these data, strains b09i-3T and b13i-1 are considered to represent a novel species of the genus Paenibacillus, for which the name Paenibacillus pueri sp. nov. is proposed. The type species is b09i-3T (=KCTC 13223T=CECT 7360T).


Keywords: Paenibacillus pueri; bacterial systematics; sequence alignment; identification; soil; ASH
**Article 155**

*Gordonia kroppenstedtii* sp nov., a phenol-degrading actinomycete isolated from a polluted stream

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A phenol-degrading actinomycete, strain NP8-5⁵, was isolated from a polluted stream in Gumi, Korea. The strain was aerobic, Gram-positive-staining, non-motile and non-spore-forming, displayed a rod-coccus growth cycle, exhibited white opaque colonies on complex media and showed chemotaxonomic markers that were consistent with its classification in the genus *Gordonia*. Phylogenetic analysis, based on 16S rRNA gene sequencing, also showed that strain NP8-5⁵ belonged to the genus *Gordonia*, sharing the highest levels of sequence similarity with *Gordonia araii* IFM 10211¹, *G. hydrophobica* DSM 44015¹ and *G. sinesedis* NCIMB 13802¹ (96.4, 96.0 and 95.9 %, respectively) and forming a separate lineage within this genus. Combined phylogenetic and phenotypic data supported the conclusion that strain NP8-5⁵ represents a novel species of the genus *Gordonia*, for which the name *Gordonia kroppenstedtii* sp. nov. is proposed. The type strain is NP8-5⁵ (=KCTC 19360⁵ =DSM 45133⁵).


**Keywords**: *Gordonia kroppenstedtii*; sequence alignment; chromatography; nocardia; bacteria

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**Article 156**

*Bacillus acidiproducens* sp nov., vineyard soil isolates that produce lactic acid

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Two novel spore-forming lactic acid bacteria, strains SL213¹ and SL1213, were isolated from vineyard soils in Korea. Cells of both isolates were rod-shaped bacilli and contained subterminal, ellipsoid spores. Strains were facultatively anaerobic, catalase-positive, oxidase-negative and motile with single flagella. meso-Diaminopimelic acid, glucose and galactose were detected in whole-cell hydrolysates. Major fatty acids found in the strains were anteiso-C₁₅:₀, iso-C₁₅:₀, iso-C₁₆:₀, C₁₆:₀ and anteiso-C₁₇:₀. The G + C contents of the DNA were 46.1 and 46.3 mol%, 16S rRNA gene sequences from the two strains were almost identical (99.9%) and placed them in the genus *Bacillus*, according to phylogenetic analysis. The type strains most closely related to SL213¹ were *Bacillus coagulans* ATCC 7050¹ and *Bacillus badius* ATCC 14574¹, with 16S rRNA gene sequence similarities of 96.9 and 95.9%, respectively. Levels of DNA-DNA relatedness between strain SL213¹ and strain SL1213, *B. coagulans* ATCC 7050¹ and *B. badius* ATCC 14574¹ were 92.5, 49.0 and 27.5%, respectively. On the basis of 16S rRNA gene sequences and chemotaxonomic and phenotypic evidence given in this study, we report that SL213¹ represents a novel species, for which the name *Bacillus acidiproducens* sp. nov. is proposed. The type strain is SL213¹ (=KCTC 13078¹ =JCM 14638¹).

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**Keywords**: *Bacillus acidiproducens*; emended description; coagulans; chromatography; fermentation; strains; Korea; sea
A novel Gram-negative and facultative anaerobic strain, designated MS1\textsuperscript{T}, was isolated from gajami sikhae, a traditional fermented food in Korea made from flatfish. Strain MS1\textsuperscript{T} was motile, rod-shaped and oxidase- and catalase-positive, and required 1-2\% (w/v) NaCl for growth. Growth occurred at temperatures ranging from 4 to 40 degrees C and the pH range for optimal growth was pH 6.5-9.0. Strain MS1\textsuperscript{T} was capable of reducing trimethylamine oxide, nitrate and thiosulfate. Phylogenetic analysis placed strain MS1\textsuperscript{T} within the genus *Alishewanella*. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MS1\textsuperscript{T} was related closely to *Alishewanella aestuarii* B11\textsuperscript{T} (98.67\% similarity) and *Alishewanella fetalis* CCUG 30811\textsuperscript{T} (98.04\% similarity). However, DNA-DNA reassociation experiments between strain MS1\textsuperscript{T} and reference strains showed relatedness values <70\% (42.6 and 14.8\% with *A. aestuarii* B11\textsuperscript{T} and *A. fetalis* CCUG 30811\textsuperscript{T}, respectively). Genotypic, physiological and biochemical analyses allowed the differentiation of strain MS1\textsuperscript{T} from type strains of species belonging to the genus *Alishewanella*. Therefore, we propose that strain MS1\textsuperscript{T} (= KCTC 22429\textsuperscript{T} = JCM 15561\textsuperscript{T}) is assigned to a novel species, *Alishewanella jeotgali* sp. nov.

**Keywords**: *Alishewanella jeotgali*; DNA; hybridization; trees

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A novel red-pigmented halophilic archaeon, strain A29\textsuperscript{T}, was isolated from shrimp jeotgal, a traditional salt-fermented food from Korea. This strain grows in the ranges 10-30\% (w/v) NaCl, 17-50 degrees C and pH 6.5-8.5, with optimal growth occurring at 15-20\% NaCl, 37-45 degrees C and pH 7.0-7.5. The isolate is Gram-negative and non-motile. Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain A29\textsuperscript{T} is associated with the genus *Haloterrigena* and closely related to the species *Haloterrigena thermotolerans* (99.0\% similarity). However, DNA-DNA hybridization experiments revealed that the level of hybridization between strain A29\textsuperscript{T} and related strains of *Haloterrigena* is less than 70\%. The polar lipid fraction consists of phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me) and mannose-2,6-disulfate(1-2)-glucose glycerol diether (S\textsubscript{2}-DGD). The G + C content of genomic DNA of the type strain is 62.3 mol\%. On the basis of this polyphasic taxonomic study, strain A29\textsuperscript{T} should be placed in the genus *Haloterrigena* as a novel species, for which the name *Haloterrigena jeotgali* sp. nov. is proposed. The type strain of the new species is A29\textsuperscript{T} (= KCTC 4020\textsuperscript{T} = DSM 18794\textsuperscript{T} = JCM 14585\textsuperscript{T} = CECT 7218\textsuperscript{T}).

**Keywords**: *Haloterrigena jeotgali*; shrimp jeotgal; seafood; lake; bacterium; china; xinjiang; trees

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A novel Gram-negative and facultative anaerobic strain, designated MS1\textsuperscript{T}, was isolated from gajami sikhae, a traditional fermented food in Korea made from flatfish. Strain MS1\textsuperscript{T} was motile, rod-shaped and oxidase- and catalase-positive, and required 1-2\% (w/v) NaCl for growth. Growth occurred at temperatures ranging from 4 to 40 degrees C and the pH range for optimal growth was pH 6.5-9.0. Strain MS1\textsuperscript{T} was capable of reducing trimethylamine oxide, nitrate and thiosulfate. Phylogenetic analysis placed strain MS1\textsuperscript{T} within the genus *Alishewanella*. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MS1\textsuperscript{T} was related closely to *Alishewanella aestuarii* B11\textsuperscript{T} (98.67\% similarity) and *Alishewanella fetalis* CCUG 30811\textsuperscript{T} (98.04\% similarity). However, DNA-DNA reassociation experiments between strain MS1\textsuperscript{T} and reference strains showed relatedness values <70\% (42.6 and 14.8\% with *A. aestuarii* B11\textsuperscript{T} and *A. fetalis* CCUG 30811\textsuperscript{T}, respectively). Genotypic, physiological and biochemical analyses allowed the differentiation of strain MS1\textsuperscript{T} from type strains of species belonging to the genus *Alishewanella*. Therefore, we propose that strain MS1\textsuperscript{T} (= KCTC 22429\textsuperscript{T} = JCM 15561\textsuperscript{T}) is assigned to a novel species, *Alishewanella jeotgali* sp. nov.

**INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY**, 59(9): 2313-2316.

**Keywords**: *Alishewanella jeotgali*; DNA; hybridization; trees

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A novel red-pigmented halophilic archaeon, strain A29\textsuperscript{T}, was isolated from shrimp jeotgal, a traditional salt-fermented food from Korea. This strain grows in the ranges 10-30\% (w/v) NaCl, 17-50 degrees C and pH 6.5-8.5, with optimal growth occurring at 15-20\% NaCl, 37-45 degrees C and pH 7.0-7.5. The isolate is Gram-negative and non-motile. Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain A29\textsuperscript{T} is associated with the genus *Haloterrigena* and closely related to the species *Haloterrigena thermotolerans* (99.0\% similarity). However, DNA-DNA hybridization experiments revealed that the level of hybridization between strain A29\textsuperscript{T} and related strains of *Haloterrigena* is less than 70\%. The polar lipid fraction consists of phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me) and mannose-2,6-disulfate(1-2)-glucose glycerol diether (S\textsubscript{2}-DGD). The G + C content of genomic DNA of the type strain is 62.3 mol\%. On the basis of this polyphasic taxonomic study, strain A29\textsuperscript{T} should be placed in the genus *Haloterrigena* as a novel species, for which the name *Haloterrigena jeotgali* sp. nov. is proposed. The type strain of the new species is A29\textsuperscript{T} (= KCTC 4020\textsuperscript{T} = DSM 18794\textsuperscript{T} = JCM 14585\textsuperscript{T} = CECT 7218\textsuperscript{T}).

**INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY**, 59(9): 2359-2363.

**Keywords**: *Haloterrigena jeotgali*; shrimp jeotgal; seafood; lake; bacterium; china; xinjiang; trees
Aidingimonas halophila gen. nov., sp. nov., a moderately halophilic bacterium isolated from a salt lake

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Two Gram-negative, facultatively anaerobic, catalase-positive, oxidase-negative, non-motile, rod-shaped and moderately halophilic bacterial strains, designated YIM 90637T and BH 017, were isolated from a salt lake in Xinjiang province, north-west China, and subjected to a polyphasic taxonomic study. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the two novel isolates were affiliated with the family Halomonadaceae; the two strains shared 100% sequence similarity, but showed similarities of 94.7% with the type strain of Modicisalibacter tunisiensis, 93.2-94.7% with members of the genus Chromohalobacter, 93.2-95.0% with members of the genus Halomonas and less than 92.0% with other members of the family Halomonadaceae. However, DNA-DNA relatedness data and phenotypic properties demonstrated that strains YIM 90637T and BH 017 were representatives of the same species. The major fatty acids were C19:0 cyclo omega 8c and C16:0. The relative amount of C19:0 cyclo omega 8c was notably higher than that found in most species of the family Halomonadaceae for which fatty acid composition has been determined. The genomic G + C content was 57.2-57.5 mol% and the only respiratory quinone was ubiquinone 9. Based on evidence from the polyphasic taxonomic study, it was concluded that the two strains should be classified as representatives of a novel species in a new genus, for which name Aidingimonas halophila gen. nov., sp. nov. is proposed; the type strain of Aidingimonas halophila is YIM 90637T (=KCTC 12885T = CCTCC AB 207002T).

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 59(12): 3088-3094.

Keywords: Aidingimonas halophila; family Halomonadaceae; emended description; deoxyribonucleic-acid; deleya; reclassification; systematics; zymobacter; sequences

Carnobacterium jeotgali sp. nov., isolated from a Korean traditional fermented food

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A Gram-positive, facultatively anaerobic bacterium, designated strain MS3T, was isolated from a traditional Korean fermented food made with freshwater shrimp. Strain MS3T was able to grow at 4-37 degrees C, at pH 5.5-9.0 and in the presence of 0-5% (w/v) NaCl. Optimal growth occurred at 30 degrees C, at pH 8.5 and in the presence of 2% NaCl. The strain was catalase- and oxidase-negative. It was able to metabolize various carbohydrates as energy sources. 16S rRNA gene sequence analysis showed that strain MS3T was most closely related to Carnobacterium pleistocenium FTIR1T (98.95% similarity), but the level of DNA-DNA relatedness between the two taxa was less than 16.0%. The genomic G + C content of strain MS3T was 43.9 mol% and the major fatty acid components were C16:0, C16:1 omega 9c and C18:1 omega 9c. On the basis of its genotypic, physiological and biochemical characteristics, strain MS3T is considered to represent a novel species of the genus Carnobacterium, for which the name Carnobacterium jeotgali sp. nov. is proposed. The type strain is MS3T (=KCTC 13251T = JCM 15539T).

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 59(12): 3168-3171.

Keywords: Carnobacterium jeotgali; hybridization; bacteria; DNA
A simple technique to convert sitting-drop vapor diffusion into hanging-drop vapor diffusion by solidifying the reservoir solution with agarose

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A simple protocol to convert sitting-drop vapor-diffusion plating into a hanging-drop vapor-diffusion experiment in protein crystallization is reported. After making a sitting-drop plate, agarose solution was added to solidify the reservoir solution, and the plates were incubated upside down. Crystallization experiments with hen egg white lysozyme, thaumatin and glucose isomerase showed that the ‘upside-down sitting-drop’ method could produce single crystals with all the benefits of the hanging-drop crystallization method.

JOURNAL OF APPLIED CRYSTALLOGRAPHY, 42(5): 975-976.

Keywords: protein crystallization; kinetics; vapor diffusion

Henriciella marina gen. nov., sp nov., a novel member of the family Hyphomonadaceae isolated from the East Sea

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A bacterial strain, designated Iso4T, was isolated from the East Sea of Korea and was subjected to a poly-phasic taxonomy study including phenotypic and chemotaxonomic characteristics as well as 16S rRNA gene sequence analysis. Cells of the strain were Gram-negative, motile, non-budding, non-stalked, and strictly aerobic. Strain Iso4T grew optimally at 20°C in the presence of 1 to 2% (w/v) NaCl and at pH 6.9 similar to 7.6. The major respiratory quinone was Q-10 and the major cellular fatty acids were C18:1 omega 7c (53.5%), C17:1 omega 5c (11.7%), C17:1 omega 6c (8.1%), C16:0 (7.8%), C15:0 (4.8%), C15:0 (2.9%), and C16:1 omega 7c (2.2%). The DNA G+C content of strain Iso4T was 56.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain Iso4T formed a monophyletic clade in the family Hyphomonadaceae, supported by high bootstrap value and was most closely related to the genus Hyphomonas (92 similar to 94%), a member of marine bacteria in the family. The phenotypic, genotypic, and chemotaxonomic evidences also suggest strain Iso4T represents a novel genus and species in the family Hyphomonadaceae, for which the name Henriciella gen. nov., sp. nov. is proposed. The type strain is Iso4T (=KCTC 12513T =DSM 19595T =KCTC 151161).


Keywords: Henriciella marina gen. nov.; sp nov.; Hyphomonadaceae; taxonomy; East Sea
**Paenibacillus filicis** sp nov., isolated from the rhizosphere of the fern

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A Gram-positive and endospore-forming bacterial strain, designated S4ᵀ, was isolated from the rhizosphere of ferns in Daejeon, Republic of Korea. This isolate is strictly aerobic, motile, and rod-like in shape, and it is positive for catalase, oxidase, esterase lipase, and beta-galactosidase activities. In addition, this strain grows when cultured at temperatures between 15 and 37°C and at pH values ranging from 5.5 to 9.0. The DNA G+C content was determined to be 53.2 mol%. Strain S4ᵀ has meso-diaminopimelic acid in the cell-wall peptidoglycan; it also contains menaquinone 7 (MK-7) as the predominant isoprenoid quinone and anteiso-C₁₅:₀ (57.5%), iso-C₁₆:₀ (11.3%), and C₁₆:₀ (9.4%) as the major cellular fatty acids. Phylogenetic analysis based on alignments of the 16S rRNA gene sequence showed that S4ᵀ is affiliated with a cluster of strains within the genus *Paenibacillaceae* and is most closely related to *Paenibacillus chinjuensis* WN9ᵀ, with 96.8% similarity. Based on the phylogenetic and phenotypic characteristics of strain S4ᵀ, we believe that this isolate should be distinguished from all type species of the genus *Paenibacillus* and should thus represent a novel taxon within the genus *Paenibacillus*. We propose naming this type species *Paenibacillus filicis* sp. nov. for the rhizosphere isolate; the type strain will be known as S4ᵀ (=KCTC 13693ᵀ =KACC 14197ᵀ =JCM 16417ᵀ). **JOURNAL OF MICROBIOLOGY**, 47(5): 524-529.

**Keywords**: novel bacterium; fern; rhizosphere; *Paenibacillus filicis*

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**Paenibacillus pinihumi** sp nov., a cellulolytic bacterium isolated from the rhizosphere of *Pinus densiflora*

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A novel cellulolytic bacterium, strain S23ᵀ, was isolated from the rhizosphere of the pine trees in Daejeon, Republic of Korea. This isolate was Gram-positive, strictly aerobic, rod-shaped, catalase-negative, oxidase-positive, motile by means of peritrichous flagella, and tested positive for alkaline phosphatase, esterase lipase, leucine arylamidase, alpha-galactosidase, and beta-galactosidase activities. The DNA G+C content was 49.5 mol%. The main cellular fatty acids were anteiso-C₁₅:₀ (51.9%), iso-C₁₆:₀ (14.7%), and iso-C₁₅:₀ (13.2%). The major isoprenoid quinone was menaquinone 7 (MK-7). Diagnostic diaminoc acid in the cell-wall peptidoglycan was *meso*-diaminopimelic acid. Comparative 16S rRNA gene sequence analysis showed that this strain clustered with *Paenibacillus* species. The 16S rRNA gene sequence similarity values between S23ᵀ and other *Paenibacillus* species were between 89.9% and 95.9%, and S23ᵀ was most closely related to *Paenibacillus tarimensis* SA-7-6ᵀ. On the basis of phylogenetic and phenotypic properties of strain S23ᵀ, the isolate is considered as a novel species belonging to the genus *Paenibacillus*. Therefore, the name, *Paenibacillus pinihumi* sp. nov., is proposed for the rhizosphere isolate; the type strain is S23ᵀ (=KCTC 13695ᵀ =KACC 14199ᵀ =JCM 16419ᵀ). **JOURNAL OF MICROBIOLOGY**, 47(5): 530-535.

**Keywords**: cellulose; novel bacterium; pine tree; rhizosphere; *Paenibacillus pinihumi*
**Paenibacillus pini** sp nov., a cellulolytic bacterium isolated from the rhizosphere of pine tree

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Strain S22T, a novel cellulolytic bacterium was isolated from the rhizosphere of pine trees. This isolate was Gram-reaction positive, motile and rods, and formed terminal or subterminal ellipsoidal spores. S22T represented positive activity for catalase, oxidase, esterase (C4), esterase lipase (C8), beta-galactosidase, leucine arylamidase, and hydrolysis of esculin. It contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall. The predominant isoprenoid quinone was menaquinone 7 (MK-7), and the major cellular fatty acids were anteiso-C₁₅:₀ (52.9%), iso-C₁₆:₀ (11.3%), and iso-C₁₅:₀ (10.0%). The DNA G+C content was 43.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that this isolate belonged to the family Paenibacillaceae. S22T exhibited less than 97.0% 16S rRNA gene similarity with all relative type strains in the genus Paenibacillus, and the most closely related strains were Paenibacillus anaericanus MH21T and Paenibacillus ginsengisoli Gsoil 1638T, with equal similarities of 95.8%. This polyphasic evidence suggested that strain S22T should be considered a novel species in the genus Paenibacillus, For which the name, Paenibacillus pini sp. nov., is proposed. The type strain is S22T (=KCTC 13694T =KACC 14198T =JCM 16418T).

**Keywords**: Paenibacillus pini; cellulose; pine tree; rhizosphere

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**Virgibacillus xinjiangensis** sp nov., isolated from a salt lake of Xin-jiang province in China

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A strictly aerobic Gram-positive, moderately halophilic spore forming bacterium, designated strain SL6-1T, was isolated from a salt lake in Xin-jiang province, China. Growth of strain SL6-1T was observed at NaCl concentrations of 0 similar to 20% (w/v) (the optimum being 5 similar to 7%, w/v). The peptidoglycan type of strain SL6-1T was Al gamma-meso-diaminopimelic acid and its major cellular fatty acids were iso-C₁₄:₀ and iso-C₁₆:₀ and anteiso-C₁₅:₀. The major respiratory isoprenoid quinone was MK-7 and the G+C content of the genomic DNA was 44.5 mol%. The major cellular phospholipids were phosphatidylglycerol and diphosphatidylglycerol. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain SL6-1T formed a phylogenetic lineage within the genus Virgibacillus. Based on 16S rRNA gene sequence similarity, the strain was most closely related to Virgibacillus olivae E(30)8T, Virgibacillus kekensis YIM kkny16T, Virgibacillus marismortui DSM 12325T with 97.1%, 97.1%, and 97.0% gene sequence similarities, respectively and the sequence similarities to other related taxa were less than 96.7%. The DNA relatedness values between strain SL6-1T and V. olivae E(30)8T, V. kekensis YIM kkny16T, V. marismortui DSM 12325T were 16.7%, 51.0%, and 22.8%, respectively. On the basis of physiological, biochemical and phylogenetic properties, strain SL6-1T represents a novel species, for which the name Virgibacillus xinjiangensis sp. nov. is proposed. The type strain is SL6-1T (=KCTC 13128T =DSM 19031T).

**Keywords**: Virgibacillus xinjiangensis sp nov.; halotolerant; Gram-positive
Acinetobacter antiviralis sp nov., from tobacco plant roots

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Acinetobacter strain KNF2022T was isolated from tobacco plant roots during the screening of antiviral substances having inhibitory effects on Tobacco mosaic virus (TMV) and examined by phenotypic, chemotaxonomic, and genetic characterization. It was a nonmotile, Gram-negative bacterium. This strain contained Q-9 as the main respiratory quinone. The major cellular fatty acids of the isolate were 16:0, 18:1ω9c, and 16:1ω7c/15 iso 2OH. The DNA base composition was 44 mol%. Phylogenetic analysis based on the 16S rRNA sequence revealed that the isolate formed an evolutionary lineage distinct from other Acinetobacter species. Based on the evaluation of morphologic, physiologic, and chemotaxonomic characteristics, DNA-DNA hybridization values, and 16S rRNA sequence comparison, we propose the new species Acinetobacter antiviralis sp nov., the type strain of which is KNF2022T (=KCTC 0699BP). JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, 19(3): 250-256.

Keywords: Acinetobacter antiviralis sp nov.; antiviral activity; polyphasic taxonomy

Glycerol affects the acyl moieties of teicoplanin components produced by Actinoplanes teichomyceticus MSI2210

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Teicoplanin, a glycopeptide antibiotic, is composed of five main components, denoted T-A2-1 to T-A2-5. We investigated the use of glycerol as a carbon source affecting the teicoplanin components and its acyl moieties. As a result, we show the change of teicoplanin components, as well as an increase of total teicoplanin yields, caused by the addition of glycerol to the production medium. Analysis of the total cell lipids upon the addition of glycerol also showed a corresponding change in the proportion of teicoplanin, suggesting that glycerol strongly affects a change of teicoplanin branched acyl moieties. MICROBIOLOGICAL RESEARCH, 164(5): 588-592.

Keywords: teicoplanin; antibiotic; carbon source; acyl moiety; fermentation
The rust fungus *Gymnosporangium* in Korea including two new species, *G. monticola* and *G. unicorne*

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A survey was conducted of the rust fungus *Gymnosporangium* in Korea. We recollected previously known species, namely *Gymnosporangium asialicum*, *G. clavariiforme*, *G. globosum*, *G. japonicum* and *G. yamadae*. *Gymnosporangium nidus-avis* and *G. sabinae* are reported for the first time from Korea, and two new species, *G. monticola* sp. nov. and *G. unicorne* sp. nov., are recognized. Previous single reports of *G. miyabei* and *G. shiraianum* could not be confirmed. The LSU rDNA was sequenced from freshly collected specimens. Phylogenetic analyses show that species of *Gymnosporangium* form a monophyletic group with strong bootstrap support within the rust fungi. The two new species are unique based on both A and B Molecular as well as morphological characteristics. Analyses of phenotypic characters mapped onto the phylogenetic tree show that teliospore length followed by telia shape and telia length are conserved; these are morphological characters useful in differentiating species of *Gymnosporangium*. Each of the nine species of *Gymnosporangium* in Korea is described and illustrated, and keys based on aecia and telia stages are provided. Lectotype specimens for several names described in *Gymnosporangium* are designated.


**Keywords**: aecia stage; forest pathogens; LSU rDNA; pucciniales; systematics; telia stage
5. Division of Reading R&D

- Korean Bioinformation Center
- Viral Infectious Disease Research Center
- DAEJEON-KRIBB-FHCRC Research Cooperation Center
- International Biological Material Research Center
BioBarcode: a general DNA barcoding database and server platform for Asian biodiversity resources

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Background: DNA barcoding provides a rapid, accurate, and standardized method for species-level identification using short DNA sequences. Such a standardized identification method is useful for mapping all the species on Earth, particularly when DNA sequencing technology is cheaply available. There are many nations in Asia with many biodiversity resources that need to be mapped and registered in databases.

Results: We have built a general DNA barcode data processing system, BioBarcode, with open source software - which is a general purpose database and server. It uses mySQL RDBMS 5.0, BLAST2, and Apache httpd server. An exemplary database of BioBarcode has around 11,300 specimen entries (including GenBank data) and registers the biological species to map their genetic relationships. The BioBarcode database contains a chromatogram viewer which improves the performance in DNA sequence analyses.

Conclusion: Asia has a very high degree of biodiversity and the BioBarcode database server system aims to provide an efficient bioinformatics protocol that can be freely used by Asian researchers and research organizations interested in DNA barcoding. The BioBarcode promotes the rapid acquisition of biological species DNA sequence data that meet global standards by providing specialized services, and provides useful tools that will make barcoding cheaper and faster in the biodiversity community such as standardization, depository, management, and analysis of DNA barcode data. The system can be downloaded upon request, and an exemplary server has been constructed with which to build an Asian biodiversity system http://www.asianbarcode.org.

BMC GENOMICS, 10: Art. No. S8 Suppl. 3.

Keywords: DNA barcoding database, Asian biodiversity resources

MitoVariome: a variome database of human mitochondrial DNA

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Background: Mitochondrial sequence variation provides critical information for studying human evolution and variation. Mitochondrial DNA provides information on the origin of humans, and plays a substantial role in forensics, degenerative diseases, cancers, and aging process. Typically, human mitochondrial DNA has various features such as HVSI, HVSII, single-nucleotide polymorphism (SNP), restriction enzyme sites, and short tandem repeat (STR).

Results: We present a variome database (MitoVariome) of human mitochondrial DNA sequences. Queries against MitoVariome can be made using accession numbers or haplogroup/continent. Query results are presented not only in text but also in HTML tables to report extensive mitochondrial sequence variation information. The variation information includes repeat pattern, restriction enzyme site polymorphism, short tandem repeat, disease information as well as single nucleotide polymorphism. It also provides a graphical interface as Gbrowse displaying all variations at a glance. The web interface also provides the tool for assigning haplogroup based on the haplogroup-diagnostic system with complete human mitochondrial SNP position list and for retrieving sequences that users query against by using accession numbers.

Conclusion: MitoVariome is a freely accessible web application and database that enables human mitochondrial genome researchers to study genetic variation in mitochondrial genome with textual and graphical views accompanied by assignment function of haplogrouping if users submit their own data. Hence, the MitoVariome containing many kinds of variation features in the human mitochondrial genome will be useful for understanding mitochondrial variations of each individual, haplogroup, or geographical location to elucidate the history of human evolution.

BMC GENOMICS, 10: Art. No. S12 Suppl. 3.

Keywords: genome database; sequence; mitomap
PutidaNET: Interactome database service and network analysis of *Pseudomonas putida* KT2440

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Background: *Pseudomonas putida* KT2440 (*P. putida* KT2440) is a highly versatile saprophytic soil bacterium. It is a certified bio-safety host for transferring foreign genes. Therefore, the bacterium is used as a model organism for genetic and physiological studies and for the development of biotechnological applications. In order to provide a more systematic application of the organism, we have constructed a protein-protein interaction (PPI) network analysis system of *P. putida* KT2440.

Results: PutidaNET is a comprehensive interaction database and server of *P. putida* KT2440 which is generated from three protein-protein interaction (PPI) methods. We used PSIMAP (Protein Structural Interactome MAP), PEIMAP (Protein Experimental Interactome MAP), and Domain-domain interactions using iPfam. PutidaNET contains 3,254 proteins, and 82,019 possible interactions consisting of 61,011 (PSIMAP), 4,293 (PEIMAP), and 30,043 (iPfam) interaction pairs except for self interaction. Also, we performed a case study by integrating a protein interaction network and experimental 1-DE/MS-MS predicted interaction partners and functional analyses such as gene ontology assignment, KEGG pathway assignment, and server of *P. putida* KT2440. PutidaNET is freely available at [http://sequenceome.kobic.kr/](http://sequenceome.kobic.kr/). *BMC GENOMICS*, 10: Art. No. S18 Suppl. 3.

**Keywords**: molecular interaction database; protein interactions; gene ontology

MitoInteractome: mitochondrial protein interactome database, and its application in ‘aging network’ analysis

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Background: Mitochondria play a vital role in the energy production and apoptotic process of eukaryotic cells. Proteins in the mitochondria are encoded by nuclear and mitochondrial genes. Owing to a large increase in the number of identified mitochondrial protein sequences and completed mitochondrial genomes, it has become necessary to provide a web-based database of mitochondrial protein information.

Results: We present 'MitoInteractome', a consolidated web-based portal containing a wealth of information on predicted protein-protein interactions, physico-chemical properties, polymorphism, and diseases related to the mitochondrial proteome. MitoInteractome contains 6,549 protein sequences which were extracted from the following databases: SwissProt, MitOP, MitOproteome, HPRD and Gene Ontology database. The first general mitochondrial interactome has been constructed based on the concept of 'homologous interaction' using PSIMAP (Protein Structural Interactome MAP) and PEIMAP (Protein Experimental Interactome MAP). Using the above mentioned methods, protein-protein interactions were predicted for 74 species. The mitochondrial protein interaction data of humans was used to construct a network for the aging process. Analysis of the 'aging network' gave us vital insights into the interactions among proteins that influence the aging process.

Conclusion: MitoInteractome is a comprehensive database that would (1) aid in increasing our understanding of the molecular functions and interaction networks of mitochondrial proteins, (2) help in identifying new target proteins for experimental research using predicted protein-protein interaction information, and (3) help in identifying biomarkers for diagnosis and new molecular targets for drug development related to mitochondria. MitoInteractome is available at [http://mitointeractome.kobic.kr/](http://mitointeractome.kobic.kr/). *BMC GENOMICS*, 10: Art. No. S20 Suppl. 3.

**Keywords**: molecular interaction database; gene ontology; mitochondria
Gevab: a prototype genome variation analysis browsing server

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Background: The first Korean individual diploid genome sequence data (KOREF) was publicized in December 2008. Results: A Korean genome variation analysis and browsing server (Gevab) was constructed as a database and web server for the exploration and downloading of Korean personal genome(s). Information in the Gevab includes SNPs, short indels, and structural variation (SV) and comparison analysis between the NCBI human reference and the Korean genome(s). The user can find information on assembled consensus sequences, sequenced short reads, genetic variations, and relationships between genotype and phenotypes.

Conclusion: This server is openly and publicly available online at http://koreagenome.org/en/ or directly http://gevab.org. BMC BIOINFORMATICS, 10: Art. No. S3 Suppl. 15.

Keywords: database; NCBI; genome variation analysis

Protein comparison at the domain architecture level

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Background: The general method used to determine the function of newly discovered proteins is to transfer annotations from well-characterized homologous proteins. The process of selecting homologous proteins can largely be classified into sequence-based and domain-based approaches. Domain-based methods have several advantages for identifying distant homology and homology among proteins with multiple domains, as compared to sequence-based methods. However, these methods are challenged by large families defined by 'promiscuous' (or 'mobile') domains.

Results: Here we present a measure, called Weighed Domain Architecture Comparison (WDAC), of domain architecture similarity, which can be used to identify homolog of multidomain proteins. To distinguish these promiscuous domains from conventional protein domains, we assigned a weight score to Pfam domain extracted from RefSeq proteins, based on its abundance and versatility. To measure the similarity of two domain architectures, cosine similarity (a similarity measure used in information retrieval) is used. We combined sequence similarity with domain architecture comparisons to identify proteins belonging to the same domain architecture. Using human and nematode proteomes, we assigned a weight score to Pfam domain extracted from RefSeq proteins, based on its abundance and versatility. To measure the similarity of two domain architectures, cosine similarity (a similarity measure used in information retrieval) is used. We combined sequence similarity with domain architecture comparisons to identify proteins belonging to the same domain architecture. Using human and nematode proteomes, we compared WDAC with an unweighted domain architecture method (DAC) to evaluate the effectiveness of domain weight scores. We found that WDAC is better at identifying homology among multidomain proteins.

Conclusion: Our analysis indicates that considering domain weight scores in domain architecture comparisons improves protein homology identification. We developed a web-based server to allow users to compare their proteins with protein domain architectures. BMC BIOINFORMATICS, 10: Art. No. S5 Suppl. 15.

Keywords: web-based server; database; evolution; homology
Previously, we reported that the oral administration of high molecular mass poly-gamma-glutamate (gamma-PGA) induced antitumor immunity but the mechanism underlying this antitumor activity was not understood. In the present study, we found that application of high molecular mass gamma-PGA induced secretion of tumor necrosis factor (TNF)-alpha from the bone-marrow-derived macrophages of wild type (C57BL/6 and C3H/HeN) and Toll-like receptor 2 knockout (TLR2−/−) mice, but not those of myeloid differentiation factor 88 knockout (MyD88−/−) and TLR4-defective mice (C3H/HeJ). Production of interferon (IFN)-gamma-inducible protein 10 (IP-10) in response to treatment with gamma-PGA was almost abolished in C3H/HeJ cells. In contrast to LPS, gamma-PGA-induced productions of TNF-alpha and IP-10 could not be blocked by polymyxin B. Furthermore, gamma-PGA-induced interleukin-12 production was also impaired in immature dendritic cells (iDCs) from MyD88−/− and C3H/HeJ mice. Downregulation of MyD88 and TLR4 expression using small interfering RNA (siRNA) significantly inhibited gamma-PGA-mediated intracellular signaling was markedly inhibited in C3H/HeJ cells. The antitumor effect of gamma-PGA was completely abrogated in C3H/HeJ mice compared with control mice (C3H/HeN) but significant antitumor effect was generated by the intratumoral administration of C3H/HeN mice-derived iDCs followed by 2,000 kDa gamma-PGA in C3H/HeJ. These findings strongly suggest that the antitumor activity of gamma-PGA is mediated by TLR4.

Cancer Immunology Immunotherapy, 58(11): 1781-1794.

**Keywords**: poly-gamma-glutamate; MyD88; TLR4; anti-tumor effect; dendritic cell
The occurrence of acrylamide is frequently observed in processed foods. Therefore, the harmful effects of acrylamide on metabolic enzymes are important to understand. We studied the inhibitory effects of acrylamide on the brain creatine kinase (CK-BB). We found that CK-BB was kinetically inactivated by acrylamide accompanied by the disruption of the hydrophobic surface. Acrylamide mainly interacted with the thiol (-SH) residue of CK-BB and resulted in alkylation. A computational docking simulation supported that acrylamide directly bound to the active site of CK-BB where cysteine and glycine residues interacted mainly. The inhibition kinetics combined with computational prediction can be useful in order to have insights into the mechanisms regarding environmentally hazardous factors at the molecular level.


Keywords: brain creatine kinase; acrylamide; inhibition kinetics

CleanEST: a database of cleansed EST libraries

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The EST division of GenBank, dbEST, is widely used in many applications such as gene discovery and verification of exon-intron structure. However, the use of EST sequences in the dbEST libraries is often hampered by inconsistent terminology used to describe the library sources and by the presence of contaminated sequences. Here, we describe CleanEST, a novel database server that classified dbEST libraries and removes contaminants. We classified all dbEST libraries according to species and sequencing center. In addition, we further classified human EST libraries by anatomical and pathological systems according to eVOC ontologies. For each dbEST library, we provide two different cleansed sequences: 'pre-cleansed' and 'user-cleansed'. To generate pre-cleansed sequences, we cleansed sequences in dbEST by alignment of EST sequences against well-known contamination sources: UniVec, Escherichia coli, mitochondria and chloroplast (for plant). To provide user-cleansed sequences, we built an automatic user-cleansing pipeline, in which sequences of a user-selected library are cleansed on-the-fly according to user-selected options. The server is available at http://cleanest.kobic.re.kr/ and the database is updated monthly.


Keywords: expressed sequence tags; identification; genomes; system
Implication of aberrant glycosylation in cancer and use of lectin for cancer biomarker discovery

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Aberrant glycosylation is frequently found in cancer, and efforts for biomarker discovery include the preparation of aberrant glycoproteins as promising analytes. Several lectins that bind to aberrant glycans and can be thus used to capture and enrich aberrant glycoproteins in the frontal stage during biomarker discovery are to be introduced.


\textbf{Keywords}: Aberrant glycosylation; biomarker; cancer; lectin; glycosyltransferase

The effect of size and quality of potato microtubers on quality of seed potatoes in the cultivar 'Superior'

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Potato microtubers produced \textit{in vitro} of the cultivar 'Superior' were assessed to decide up to what size it can be used for the seed potato with respect to storability, dormancy period, and sprouting vigor. The larger microtubers lost moisture content more slowly and retained firmness longer when stored at 4 degrees C. In the sprouting test, the larger ones had less period of dormancy and showed more vigorous sprouting ability. The starch increased with increasing size of the microtuber and showed the distribution of about 70-80\% of dry matter content. The amounts of sugars were positively co-related with the size of the potato. The internal factors such as dry matter and carbohydrate content reveal that potato microtuber follows the field-grown potatoes in all aspects. The results suggest that the size of microtubers can be used as an index for grading their quality as seed potatoes, and the size of the microtuber should be at least 0.5 g to be used as seed potato.

\textit{SCIENTIA HORTICULTURAE}, 120(1): 127-129.

\textbf{Keywords}: solanum tuberosum; sprouting vigor; storability; dormancy; firmness; seed potato
6. Bio-Therapeutics Research Institute

- Therapeutic Antibody Research Center
- Stem Cell Research Center
- Immune Modulator Research Center
- Chemical Biology Research Center
Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus

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Porcine epidemic diarrhea virus (PEDV) is the predominant cause of severe enteropathogenic diarrhea in swine. The lack of effective therapeutical treatment underlines the importance of research for new antivirals. In this study, we identified Q7R, which actively inhibited PEDV replication with a 50% inhibitory concentration (IC₅₀) of 0.014 g/mL. The 50% cytotoxicity concentration (CC₅₀) of Q7R was over 100 μg/mL and the derived therapeutic index was 7142. Several structural analogues of Q7R, quercetin, apigenin, luteolin and catechin, also showed moderate anti-PEDV activity. Antiviral drugs and natural compounds revealed ribavirin, interferon-alpha, coumarin and tannic acid have relative weaker efficacy compared to Q7R. Q7R did not directly interact with or inactivate PEDV particles and affect the initial stage of PEDV infection by interfering of PEDV replication. Also, the effectiveness of Q7R against the other two viruses (TGEV, PRCV) was lower compared to PEDV. Q7R could be considered as a lead compound for development of anti-PEDV drugs to be used during the early stage of PEDV replication and the structure-activity data of Q7R may usefully guideline to design other related antiviral agents. ANTIVIRAL RESEARCH, 81(1): 77-81.

Keywords: porcine epidemic diarrhea virus; antiviral activity; quercetin 7-rhamnoside; houttuynia cordat

Diacylglycerol acyltransferase-inhibitory compounds from *Erythrina senegalensis*

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Inhibition of acyl CoA:diacylglycerol acyltransferase (DGAT) is proposed to be a drug target for the treatment of obesity and type 2 diabetes. Bioassay-guided fractionation of the CH₂Cl₂-soluble extract of the stem bark of *Erythrina senegalensis*, using an in vitro DGAT enzyme assay, resulted in the isolation of eight known prenylflavonoids, 8-prenyleutone (1), auriculatin (2), erysenegalensein O (3), erysenegalensein D (4), erysenegalensein N (5), derrone (6), alpinumisoflavone (7), and 6,8-diprenylgenistein (8). Compounds 1, 2-4, 6, and 8 inhibited DGAT activity, with IC₅₀ values ranging from 1.1 +/- 0.3 to 15.1 +/- 1.1 μg/mL. On the basis of the data obtained, we propose isoflavonoids with isoprenyl groups as a novel class of DGAT inhibitors. ARCHIVES OF PHARMACAL RESEARCH, 32(1): 43-47.

Keywords: *Erythrina senegalensis*; prenylflavonoid; diacylglycerol acyltransferase
Free radical scavenging and antielastase activities of flavonoids from the fruits of Thuja orientalis

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Bioassay-guided fractionation of the MeOH extract of Thuja orientalis fruits using a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay led to the isolation of 9 flavonoids: cupressuflavone (1), amentoflavone (2), robustaflavone (3), afzelin (4), (+)-catechin (5), quercitrin (6), hypolaetin 7-O-beta-xylopyranoside (7), isoquercitrin (8) and myricitrin (9). Their chemical structures were determined by spectroscopic analyses. The free radical scavenging and human neutrophil elastase (HNE) inhibitory activities were evaluated for the isolated compounds. By DPPH scavenging assay, compounds 5, 6, 7, 8 and 9 showed anti-oxidant activities with IC₅₀ values of 28.66, 31.19, 18.30, 26.63 and 15.10 μM, respectively. By ABTS [2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] scavenging assay, these compounds also exhibited potent anti-oxidant activities with IC₅₀ values of 6.77, 13.96, 6.97, 22.79 and 9.96 μM, respectively. Of note, compounds 1, 2 and 3 showed significant HNE inhibitory activities with IC₅₀ values of 8.09, 1.27 and 1.33 μM, respectively.


Keywords: Thuja orientalis; flavonoid; DPPH; ABTS; elastase

Pharmacophore-based 3D-QSAR of HIF-1 inhibitors

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(Aryloxyamino)benzoic acids and nicotinic/isonicotinic acids represent an important new class of small molecules that inhibit the activation of Hypoxia-Inducible Factor (HIF)-1. In order to understand the factors affecting inhibitory potency of HIF-1 inhibitors, 3 dimensional-quantitative structure activity relationship (3D-QSAR) studies were performed. Since no receptor structure are available, the pharmacophore-based alignment was used for comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). The CoMFA and CoMSIA models gave reasonable statistics (CoMFA: q² = 0.564, r²=0.945; CoMSIA: q² = 0.575, r²=0.929). Both CoMFA and CoMSIA results indicate that the steric interaction is a major factor, while CoMSIA suggests importance of hydrogen bonding. These findings about steric and H-bonding effects can be useful to design new inhibitors.

ARCHIVES OF PHARMACAL RESEARCH, 32(3): 317-323.

Keywords: CoMFA; CoMSIA; drug design; pharmacophore; HIF-1 inhibitor; 3D-QSAR
Chemical constituents from the Leaves of *Ilex paraguariensis* inhibit human neutrophil elastase

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Human neutrophil elastase (HNE), a serine protease with broad target specificity, is the only enzyme responsible for the degradation of elastin which is an insoluble elastic fibrous protein in animal connective tissue. Biologically, elastase activity significantly increased with age, which results in a reduced skin elasticity and in the appearance of wrinkles or stretchmarks. In the course of our screening program for HNE inhibitors from natural source, the MeOH extract of *Ilex paraguariensis* leaves showed strong HNE inhibitory effect. Bioassay-guided fractionation led to the isolation of a new pyrrole alkaloid (1), along with seventeen known compounds (2-18) from the MeOH extract of *Ilex paraguariensis* leaves, and their chemical structures were elucidated on the basis of spectroscopic analysis. All isolated compounds were evaluated for HNE inhibitory activity, and the result demonstrated that dicaffeoylquinic acid derivatives (12, 13, 14, 15 and 16) and flavonoids (8 and 17) exhibited potent HNE inhibitory activity with IC₅₀ values ranging from 1.4 to 7.3 μM.

ARCHIVES OF PHARMACAL RESEARCH, 32(9): 1215-1220.

**Keywords**: human neutrophil elastase; *Ilex paraguariensis*; dicaffeoylquinic acid derivatives; pyrrolezanthine-6-methyl ether

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A novel benzimidazole analogue inhibits the hypoxia-inducible factor (HIF)-1 pathway

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Hypoxia-inducible factor (HIF)-1 is a therapeutic target in solid tumors. We report the novel benzimidazole analogue AC1-004, obtained from a chemical library using an HRE-dependent cell-based assay in colorectal carcinoma HCT-116 cells. The accumulation of hypoxia-induced HIF-1 alpha was inhibited by compound AC1-004 in various cancer cells, including HCT-116, MDA-MB435, SK-HEP1, and Caki-1. Further, AC1-004 down-regulated VEGF and EPO, target genes of HIF-1, and inhibited *in vitro* tube formation of HUVEC, suggesting its potential inhibitory activity on angiogenesis. Importantly, AC1-004 was found to regulate the stability of HIF-1 alpha through the Hsp90-Akt pathway, leading to the degradation of HIF-1 alpha. An *in vivo* antitumor study demonstrated that AC1-004 reduced tumor size significantly (i.e., by 58.6%), without severe side effects. These results suggest the benzimidazole analogue AC1-004 is a novel HIF inhibitor that targets HIF-1 alpha via the Hsp90-Akt pathway, and that it can be used as a new lead in developing anticancer drugs.


**Keywords**: hypoxia; HIF-1 alpha inhibitor; benzimidazole; angiogenesis; hsp90; akt
**Article 188**

**Tumor necrosis factor-alpha enhances IL-15-induced natural killer cell differentiation**

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The differentiation of natural killer (NK) cells is regulated by various factors including soluble growth factors and transcription factors. Here, we have demonstrated that tumor necrosis factor-alpha (TNF-alpha) is a positive regulator of NK cell differentiation. TNF-alpha augmented the IL-15-induced expression of NK1.1 and CD122 in mature NK cells, and TNF-alpha alone also induced NK cell maturation as well as IL-15. TNF-alpha also increased IFN-gamma production in NK cells in the presence of IL-15. Meanwhile, mRNA expression of several transcription factors, including T-bet and GATA-3, was increased by the addition of TNF-alpha and IL-15. In addition, TNF-alpha increased nuclear factor-kappa B (NF-kappa B) activity in NK cells and inhibition of NF-kappa B impeded TNF-alpha-enhanced NK cell maturation. Overall, these data suggest that TNF-alpha significantly increased IL-15-driven NK cell differentiation by increasing the expression of transcription factors that play crucial roles in NK cell maturation and inducing the NF-kappa B activity.

**Keywords**: TNF-alpha; NK cells; differentiation; t-bet; GATA-3; NF-kappa B


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**Article 189**

**Genistein-derivatives from Tetracera scandens stimulate glucose-uptake in L6 myotubes**

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An EtOAc-soluble partition of the MeOH extract of a branch of *Tetracera scandens* (Dilleniaceae family) was subjected to a glucose-uptake assay, which led to the isolation and identification of five isoflavones of previously known structure namely, genistein (1), its derivatives 3',5'-diprenylgenistein (2), 6,8-diprenylgenistein (3), derrone (4) and alpinumisoflavone (5). Of these, compounds 2-5 exhibited significant glucose-uptake activity in basal and insulin-stimulated L6 myotubes. The findings from adenosine monophosphate-activated kinase (AMPK) activation and glucose transport protein4 (GLUT4) and GLUT1 over-expression revealed certain characteristics of compounds 2-5. These compounds inhibited protein tyrosine phosphatase 1B (PTP1B) activities with IC₅₀ values ranging from 20.63 +/- 0.17 to 37.52 +/- 0.31 μM. No muscle cell toxicity was reported with compounds 3-5, while compounds 1 and 2 reduced muscle cell viability with IC₅₀ values of 34.27 +/- 0.35 and 18.69 +/- 0.19 μM, respectively. It was concluded that *T. scandens* and its constituents exerted highly desirable activities on type 2 diabetes mellitus treatment since they significantly stimulated the uptake of glucose, AMPK phosphorylation, GLUT4 and GLUT1 mRNA expressions and PTP1B inhibition in L6 myotubes.


**Keywords**: *Tetracera scandens*; isoflavone; diabetes; glucose-uptake; L6 myotube
A novel class of highly potent multidrug resistance reversal agents: disubstituted adamantyl derivatives

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Novel disubstituted adamantyl derivatives were synthesized and evaluated in a P-glycoprotein dependent multidrug resistance cancer cell line. The hit to lead optimization provided potent MDR reversal agents. Some potent adamantyl derivatives were more than 10-fold more potent than verapamil without considerable intrinsic cytotoxicity. The 3-trifluorophenyl derivative 14f did not affect the metabolism of CYP450 3A4, whereas most of MDR revertants had a weak inhibitory effect.

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, 19(18): 5376-5379.

Keywords: multidrug resistance; MES-SA/DX5; ABC transporter; p-glycoprotein; adamantane; CYP3A4

Isolation of the protein tyrosine phosphatase 1B inhibitory metabolite from the marine-derived fungus Cosmospora sp SF-5060

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In the course of bioassay-guided study on the EtOAc extract of a culture broth of the marine-derived fungus Cosmospora sp. SF-5060, aquastatin A (1) was isolated as a protein tyrosine phosphatase 1B (PTP1B) inhibitory component produced by the fungus. The compound was isolated by various chromatographic methods, and the structure was determined mainly by analysis of NMR spectroscopic data. Compound 1 exhibited potent inhibitory activity against PTP1B with IC50 value of 0.19 μM, and the kinetic analyses of PTP1B inhibition by compound 1 suggested that the compound is inhibiting PTP1B activity in a competitive manner. Aquastatin A (1) also showed modest but selective inhibitory activity toward PTP1B over other protein tyrosine phosphatases, such as TCPTP, SHP-2, LAR, and CD45. In addition, the result of hydrolyzing aquastatin A (1) suggested that the dihydroxypentadecyl benzoic acid moiety in the molecule is responsible for the inhibitory activity.


Keywords: marine-derived fungus; Cosmospora sp.; protein tyrosine phosphatase 1B (PTP1B); fungal metabolite; competitive inhibitor
Protein tyrosine phosphatase 1B inhibitors isolated from *Morus bombycis*

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Bioassay-guided fractionation of the chloroform-soluble fraction of *Morus bombycis*, using an in vitro PTP1B inhibitory assay led to the identification of three 2-arylbenzofurans, albafuran A (1), mulberrofuran W (2) and mulberrofuran D (6), along with three chalcone-derived Diels-Alder products, kuwanon J (3), kuwanon R (4), and kuwanon V (5). Compounds 1-6 showed remarkable inhibitory activity against PTP1B with \(IC_{50}\) values ranging from 2.7 to 13.8 \(\mu M\). Inhibition kinetics were analyzed by Lineweaver-Burk plots, which suggested that compounds 1-6 inhibited PTP1B in a mixed-type manner. The present results indicate that the respective lipophilic and hydroxyl groups of 2-arylbenzofurans and chalcone-derived Diels-Alder products play an important role in inhibition of PTP1B.

**Keywords**: morus bombycis; 2-Arylbenzofuran derivatives; chalcone-derived diels-alder types; protein tyrosine phosphatase 1B (PTP1B)

Enhancement of recombinant antibody production in HEK 293E cells by WPRE

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In an effort to make a fast and convenient approach for efficient production of recombinant antibody, transient gene expression was performed in human embryonic kidney 293E (HEK293E) cells, which have been widely used as a mammalian host for transient expression of recombinant proteins. Woodchuck hepatitis virus post-transcriptional regulation element (WPRE) was employed to increase the antibody production. Under the influence of WPRE, the antibody production was increased by 5.5-fold through the enhancement of total mRNA levels of HC and LC, and the efficient export of nuclear mRNA into the cytoplasm. Using WPRE, 1.9 mg of cumulative recombinant antibody was obtained in transiently transfected adherent HEK293E cells from one 100 mm dish transfection with 10 mL medium exchange every 3 days for 24 days of cultivation. In addition, the highest recombinant antibody concentration of 81 mg/L was obtained. This simple and efficient approach of antibody production is expected to provide a sufficient amount of antibody for screening experiments.

**Keywords**: transient expression; HEK 293E cells; WPRE; mRNA stability; mRNA export; recombinant antibody
Synthesis and biological evaluation of decursin, prantschimgin and their derivatives

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The synthesis of coumarin-based natural products and their derivatives is described. In vitro MDR reversal activities of the synthesized compounds were evaluated in P-glycoprotein over-expressing human sarcoma cell line MES-SA/DX5. Some of the coumarin derivatives were found to show potent MDR reversal activity. In particular, pyridyl derivative (15e) exhibited more potency than verapamil.


Keywords: coumarins; decursin; prantschimgin; multidrug resistance; p-glycoprotein

Synthesis of a novel series of imidazo[1,2-alpha]pyridines as Acyl-CoA: Cholesterol Acyltransferase (ACAT) inhibitors

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A novel series of imidazo[1,2-alpha]pyridines was designed, synthesized, and tested for their ability to inhibit acyl-CoA: cholesterol acyltransferase. Preliminary lead optimization efforts resulted in the identification of ACAT inhibitors represented by analogues 5b, 5c, 6a, 6c, 7b, and 7c. The ACAT inhibitory activity of these compounds was further established by potent inhibition of cholesteryl ester formation in HepG2 cells by a representative analogue 7b.

BULLETIN OF THE KOREAN CHEMICAL SOCIETY, 30(6): 1297-1304.

Keywords: imidazo[1,2-alpha]pyridines; Acyl CoA: cholesterol acyl transferase (ACAT); HepG2 cells; structure-activity relationship
Pancreatic adenocarcinoma up-regulated factor (PAUF), a novel up-regulated secretory protein in pancreatic ductal adenocarcinoma

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The identification of novel tumor-specific proteins or antigens is of great importance for diagnostic and therapeutic applications in pancreatic cancer. Using oligonucleotide microarrays, we identified a broad spectrum of differentially expressed pancreatic cancer-related genes. Of these, we selected an overexpressed sequence. The full-length cDNA of this novel gene was localized on the Homo sapiens 16p13.3 chromosomal locus, and its nucleotide sequence matched the Homo sapiens similar to common salivary protein 1 (LOC124220). We named the gene pancreatic adenocarcinoma up-regulated factor. The pancreatic adenocarcinoma up-regulated factor was secreted into the culture medium of pancreatic adenocarcinoma up-regulated factor-overexpressing Chinese hamster ovary cells, had an apparent molecular mass of similar to 25 kDa, and was N-glycosylated. The induction of pancreatic adenocarcinoma up-regulated factor in Chinese hamster ovary cells increased cell proliferation, migration, and invasion ability in vitro. Subcutaneous injection of mice with Chinese hamster ovary/pancreatic adenocarcinoma up-regulated factor cells resulted in 3.8-fold greater tumor sizes compared to Chinese hamster ovary/mock cells. Reverse transcription-polymerase chain reaction and western blotting with antirecombinant human pancreatic adenocarcinoma up-regulated factor antibodies confirmed that pancreatic adenocarcinoma up-regulated factor was highly expressed in six of eight pancreatic cancer cell lines. Immunohistochemical staining of human pancreatic cancer tissues also showed pancreatic adenocarcinoma up-regulated factor overexpression in the cytoplasm of cancer cells. Transfection with pancreatic adenocarcinoma up-regulated factor-specific small-interfering RNA reduced cancer cell migration and invasion in vitro. Treatment with antirecombinant human pancreatic adenocarcinoma up-regulated factor in vitro and in vivo reduced proliferation, migration, invasion, and tumorigenic ability. Collectively, our results suggest that pancreatic adenocarcinoma up-regulated factor is a novel secretory protein involved in pancreatic cancer progression and might be a potential target for the treatment of pancreatic cancer. CANCER SCIENCE, 100(5): 828-836.

Keywords: growth-factor receptor; gene-expression; cell-lines; cancer; carcinoma

VDUP1 potentiates Ras-mediated angiogenesis via ROS production in endothelial cells


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Vitamin D3 up-regulated protein 1 (VDUP1) is a tumor suppressor of which expression is reduced in a variety of cancer cells, and enforced expression inhibits the tumor cell proliferation. It inhibits the activity of thioredoxin, thus contributing cellular ROS generation. Since ROS is a critical factor for angiogenesis, we investigated the role of VDUP1 in angiogenesis and endothelial proliferation. The expression of VDUP1 was upregulated by overexpression of an oncogene, Ras. Enforced expression of VDUP1 increases ROS production and proliferation of Ras-overexpressing endothelial cells. Overexpression of VDUP1 increases the resistance to the anchorage-dependent cell death and tube formation of the Ras-overexpressing endothelial cell. In addition, the removal of ROS by ROS scavenger attenuates the effect of VDUP1 on tube formation. These results suggest that VDUP1 is involved in Ras-mediated angiogenesis via ROS generation in endothelial cells.

CELLULAR AND MOLECULAR BIOLOGY, 55(S): OL1096-OL1103.

Keywords: angiogenesis; VDUP1; ROS; anoikis; Ras
Rational biosynthetic engineering for optimization of geldanamycin analogues

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A rational biosynthetic engineering approach was applied to the optimization of the pharmacological properties of the benzoquinone ansamycin, geldanamycin. Geldanamycin and its natural or semisynthetic derivatives have the potential to serve as anticancer chemotherapeutic agents. However, these first-generation Hsp90 inhibitors share an unfavorable structural feature that causes both reduced efficacy and toxicity during clinical evaluation. We report the rationally designed biosynthesis of C15 hydroxylated non-quinone geldanamycin analogues by site-directed mutagenesis of the geldanamycin polyketide synthase (PKS), together with a combination of post-PKS tailoring genes. A 15-hydroxyl-17-demethoxy non-quinone analogue, DHQ3, exhibited stronger inhibition of Hsp90 ATPase activity (4.6-fold) than geldanamycin. Taken together, the results of the present study indicate that rational biosynthetic engineering allows the generation of derivatives of geldanamycin with superior pharmacological properties.

CHEMBIOCHEM, 10(7): 1243-1251.

Keywords: biosynthesis; geldanamycin; natural products; polyketides; site-directed mutagenesis

The first total synthesis of moracin O and moracin P, and establishment of the absolute configuration of moracin O

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The first total synthesis of the naturally occurring benzofurans, moracins O and P was achieved using a Sonogashira cross coupling reaction followed by in situ cyclization, and the absolute configuration of natural moracin O was established. CHEMICAL COMMUNICATIONS, (14): 1879-1881.

Keywords: cultivated mulberry tree; diels-alder adducts; morus root bark; asymmetric dihydroxylations; quinone methides; acid analogs
L1 cell adhesion molecule is a novel independent poor prognostic factor of extrahepatic cholangiocarcinoma

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Purpose: Cholangiocarcinomas (CC) are associated with poor survival, but diagnostic markers and therapeutic targets have not yet been elucidated. We previously found aberrant expression of L1 cell adhesion molecule in intrahepatic CC and a role for L1 in the progression of intrahepatic CC. Here, we analyzed L1 expression in extrahepatic CC (ECC) and evaluated its prognostic significance.

Experimental Design: We examined L1 expression in tumors from 75 ECC patients by immunohistochemistry. We analyzed the correlations between L1 expression and clinicopathologic factors as well as patient survival.

Results: L1 was not expressed in normal extrahepatic bile duct epithelium but was aberrantly expressed in 42.7% of ECC tumors. High expression of L1 was detected at the invasive front of tumors and was significantly associated with perineural invasion (P<0.01). Univariate analysis indicated that various prognostic factors such as histologic grade 3, advanced pathologic T stage and clinical stage, perineural invasion, nodal metastasis, and high expression of L1 were risk factors predicting patient survival. Multivariate analyses done by Cox's proportional hazards model showed that high expression of L1 (hazard ratio, 2.171; 95% confidence interval, 1.162-4.055; P = 0.015) and nodal metastasis (hazard ratio, 2.088; 95% confidence interval, 1.159-3.764; P = 0.014) were independent risk factors for patient death.

Conclusions: L1 was highly expressed in 42.7% of ECC and its expression was significantly associated with perineural invasion. High expression of L1 and nodal metastasis were independent poor prognostic factors predicting overall survival in patients with ECC.

CLINICAL CANCER RESEARCH, 15(23): 7345-7351.

Keywords: L1 cell adhesion molecule; extrahepatic cholangiocarcinoma; prognosis; survival analysis

Antiviral activities of cell-free supernatants of yogurts metabolites against some RNA viruses

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The antiviral activity of the cell-free supernatants (CFS) containing the metabolites of five yogurts fermented under anaerobic conditions with Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus plantarum, Streptococcus thermophilus and Bifidobacterium bifidum, respectively, was initially evaluated against seven RNA viruses using virus-induced cytopathic effects reduction method, while comparing that of De Man, Rogosa, and Sharpe (MRS) broths containing metabolites fermented with the same bacteria. All the CFS of yogurt showed high antiviral activity against seven RNA viruses, while the CFS of MRS broth exhibited similar or lower antiviral activity. Each CFS of yogurt and MRS broth showed no cytotoxicity with normal morphology in Vero or MDCK cells. Furthermore, CFS of each yogurt was more effective than that of MRS broth against the three enteroviruses and CFS of yogurt containing metabolites fermented with L. plantarum exhibited strong anti-influenza virus activity among that of the other yogurt and MRS broth. Therefore, CFS of the yogurt containing metabolites fermented with probiotic bacteria showed high potential to be used for developing fermented milk-based foods or drugs.

EUROPEAN FOOD RESEARCH AND TECHNOLOGY, 228(6): 945-950.

Keywords: yogurt; antiviral activity; RNA viruses; cytotoxicity; MRS broth
Inhibitory effects of quercetin 3-rhamnoside on influenza A virus replication

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Influenza viruses cause significant morbidity and mortality in humans through epidemics or pandemics. The lack of effective therapeutical treatment underlines the importance of research for new antiviral compounds. Flavonoids widely exist in the plant kingdom, and their antiviral activities against various viruses have been recently reported. In this study, the anti-influenza A/WS/33 virus of quercetin 3-rhamnoside (Q3R) from Houttuynia cordata was evaluated using a cytopathic effect (CPE) reduction method, the assay results demonstrated that Q3R possessed strong anti-influenza A/WS/33 virus reducing the formation of a visible CPE. Q3R also did inhibit virus replication in the initial stage of virus infection by indirect interaction with virus particles. However, oseltamivir has relative weaker efficacy compared to Q3R. Therefore, these findings provide important information for the utilization of Q3R for influenza treatment.


Keywords: quercetin 3-rhamnoside (Q3R); Houttuynia cordata; antiviral activity; influenza

ATM blocks tunicamycin-induced endoplasmic reticulum stress

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Endoplasmic reticulum stress (ER-stress) is associated with ataxia telangiectasia mutated (ATM) gene. We present here conclusive data showing that ATM blocks ER-stress induced by tunicamycin or ionizing radiation (IR). X-box protein-1 (XBP-1) splicing, GRP78 expression and caspase-12 activation were increased by tunicamycin or IR in Atm-deficient AT5BIVA fibroblasts. Activation of caspase-12 and caspase-3 by tunicamycin was significantly reduced in cells transfected with wild-type Atm (AT5BIVA/wtATM). Atm knockdown by siRNA, however, noticeably elevated ER-stress and chemosensitivity to tunicamycin. In summary, we present substantial data demonstrating that ATM blocks the ER stress signaling associated with cancer cell proliferation.


Keywords: ataxia telangiectasia mutated; ER stress; tunicamycin; x-box protein-1
Article 204

Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma

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Asthma is an inflammatory disease of the airways, and the current focus in managing asthma is the control of inflammation. Resveratrol (3,4,5-trihydroxystilbene) is a polyphenolic stilbene found in the skins of red fruits, including grapes, that may be responsible for some of the health benefits ascribed to Consumption of red wine. We investigated the suppressive effects of resveratrol oil asthmatic parameters such as cytokine release, eosinophilia, airway hyperresponsiveness, and mucus hypersecretion, in an OVA-induced allergic mouse model of asthma. Resveratrol significantly inhibited increases in T-helper-2-type cytokines such as IL-4 and IL-5 in plasma and bronchoalveolar lavage fluid (BALF), and also effectively suppressed airway hyperresponsiveness, eosinophilia, and mucus hypersecretion, in the asthmatic mouse model. The efficacy of resveratrol was similar to that of dexamethasone, a glucocorticoid used as a positive control. These results suggest that resveratrol may have applications in the treatment of bronchial asthma.


Keywords: asthma; resveratrol; airway hyperresponsiveness; eosinophilia; cytokine

Article 205

Anti-inflammatory activity of (-)-aptosimon isolated from Daphne genkwa in RAW264.7 cells

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In the present study, we investigated that (-)-aptosimon, isolated from flower buds of Daphne genkwa inhibited, cyclooxygenase-2 (COX-2) and inducible nitric oxide (NO) synthase (iNOS) expression in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Similarly, (-)-aptosimon suppressed tumor necrosis factor (TNF)-alpha production. Our results clearly indicated that (-)-aptosimon inhibited LPS-induced nuclear factor-kappa B (NF-kappa B) activation. by preventing degradation of the inhibitor kappa B-alpha (IKB-alpha). (-)-Aptosimon also inhibited interleukin-4 (IL-4) and interleukin-13 (IL-13) production in ConA-induced splenocytes. In conclusion, the anti-inflammatory effects of (-)-aptosimon are attributed to the suppression of pro-inflammatory cytokines and mediators by blocking NF-kappa B activation. These data suggest that (-)-aptosimon as a potential therapeutic agent for inflammation-associated disorders.

INTERNATIONAL IMMUNOPHARMACOLOGY, 9(7-8): 878-885.

Keywords: anti-inflammatory; (-)-aptosimon; iNOS; COX-2; IL-4; IL-13
Isodeoxyhelicobasidin, a novel human neutrophil elastase inhibitor from the culture broth of Volvariella bombycina

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In the course of our screening program for HNE inhibitors, we isolated a novel compound, isodeoxyhelicobasidin, from the culture broth of Volvariella bombycina. We report herein the fermentation, isolation, structure elucidation and biological activities of isodeoxyhelicobasidin. Compound isodeoxyhelicobasidin dose-dependently inhibited HNE activity with an IC₅₀ value of 9.0 µM, also showed antibacterial activity against several gram-positive bacteria. In conclusion, compound isodeoxyhelicobasidin was a new analog of helicobasidin and lagopodin B, which were earlier isolated from H. mompa Tanaka and Coprinus cinereus, respectively, and the potent HNE inhibitory activity of isodeoxyhelicobasidin suggested that it could be useful for the development of anti-aging cosmetics.


Keywords: human neutrophil elastase; isodeoxyhelicobasidin; sesquiterpenoid; Volvariella bombycina

Inhibition of protein tyrosine phosphatase 1B by lupeol and lupenone isolated from Sorbus commixta

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Protein tyrosine phosphatase 1B (PTP1B) appears to be an attractive target for the development of new drugs for type 2 diabetes and obesity. In our preliminary test, a MeOH extract of the stem barks of Sorbus commixta Hedl. (Rosaceae) showed strong PTP1B inhibitory activity. Bioassay-guided fractionation of the MeOH extract resulted in the isolation of two lupane-type triterpenes, lupenone (1) and lupeol (2). Compounds 1 and 2 inhibited PTP1B with IC₅₀ values of 13.7 +/- 2.1 and 5.6 +/- 0.9 µM, respectively. Kinetic studies revealed that both the compounds 1 and 2 are non-competitive inhibitors of PTP1B that decrease Vₘₐₓ values with no effect on Kₘ values.


Keywords: protein tyrosine phosphatase 1B; sorbus commixta Hedl.; Rosaceae; lupenone; lupeol; non-competitive inhibitors
Thioredoxin-interacting protein regulates hematopoietic stem cell quiescence and mobilization under stress conditions

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Hematopoietic stem cells (HSCs) are maintained in a quiescent state in bone marrow (BM) niches by intrinsic and extrinsic signals. The mechanisms regulating the quiescence and mobilization of HSCs, however, remain unclear. In this study, we report that the expression of thioredoxin-interacting protein (TXNIP) is decreased during HSC activation. In Txnip\textsuperscript{-/-} mice, the long-term reconstituting HSC population is decreased and exhausted, and its capacity to repopulate is rapidly lost. These effects are associated with hyperactive Wnt signaling, an active cell cycle, and reduced p21 expression under conditions of stress. TXNIP deficiency reduced the CXCL12- and osteopontin-mediated interaction between HSCs and the bone marrow, and impaired homing and retention in the osteoblastic niche, resulting in mobilized HSCs. Therefore, we propose that TXNIP is essential for maintaining HSC quiescence and the interaction between HSCs and the BM niche.


\textbf{Keywords}: self-renewal; bone-marrow; growth-factor; beta-catenin; niche; VDUP1

RasGRP1 is required for human NK cell function

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Cross-linking of NK activating receptors activates phospholipase-gamma and subsequently induces diacylglycerol and Ca\textsuperscript{2+} as second messengers of signal transduction. Previous studies reported that Ras guanyl nucleotide-releasing protein (RasGRP) 1, which is activated by diacylglycerol and Ca\textsuperscript{2+}, is crucial for TCR-mediated Ras-ERK activation. We now report that RasGRP1, which can also be detected in human NK cells, plays an essential role in NK cell effector functions. To examine the role of RasGRP1 in NK cell functions, the expression of RasGRP1 was suppressed using RNA interference. Knockdown of RasGRP1 significantly blocked ITAM-dependent cytokine production as well as NK cytotoxicity. Biochemically, RasGRP1-knockdown NK cells showed markedly decreased ability to activate Ras, ERK, and JNK. Activation of the Ras-MAPK pathway was independently shown to be indispensable for NK cell effector functions via the use of specific pharmacological inhibitors. Our results reveal that RasGRP1 is required for the activation of the Ras-MAPK pathway leading to NK cell effector functions. Moreover, our data suggest that RasGRP1 might act as an important bridge between phospholipase-gamma activation and NK cell effector functions via the Ras-MAPK pathway.


\textbf{Keywords}: phospholipase-c-gamma; kappa-B activation; t-cells; cytotoxicity; receptors; Ras
Suppressive effects of *Anthriscus sylvestris* constituents on the expression and production of matrix metalloproteinase-9 using luciferase transfected raw 264.7 cell based assay system

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Bioactivity-guided fractionation of *Anthriscus sylvestris* extract, using matrix metalloproteinase-9 (MMP-9) assay, led to the isolation of deoxypodophyllotoxin (1), falcarindiol (2), (-)-hinokinin (3), and (-)-hibalactone (4). All compounds obtained were evaluated for the inhibitory activities against MMP-9 in luciferase and zymographic assays. Of these compounds, compounds 1 (IC₅₀, 0.5 nm) and 2 (IC₅₀ 8.4 M) were found to inhibit MMP-9 expression in the PGL4.14-MMP-9-Luc plasmid transfected Raw 264.7 cells, and MMP-9 production in a gelatin zymographic assay.


**Keywords**: *Anthriscus sylvestris; deoxypodophyllotoxin; falcarindiol; luciferase assay; matrix metalloproteinase-9; zymography*

Cytotoxic terpenoids from the methanolic extract of *Bridelia cambodiana*

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Bioactivity-guided isolation of *Bridelia cambodiana*, with a cytotoxicity assay using a small panel of cancer cell lines, led to the isolation and identification of five active compounds, 24-methylstanosta-9(11),25-dien-3 beta-ol, 3-epi-glutinol, betulinic acid, ursolic acid, and maslinic acid, along with eight triterpenoids and three steroids.


**Keywords**: *Bridelia cambodiana; cytotoxicity; euphorbiaceae; triterpenoids*
Aspochalasin I, a melanogenesis inhibitor from *Aspergillus* sp.

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In the course of screening for the melanogenesis inhibitors, aspochalasin I was isolated from solid-state culture of *Aspergillus* sp. Fb020460. Its structure was determined by spectroscopic analysis including mass spectroscopy and NMR analysis. Aspochalasin I potently inhibited melanogenesis in Mel-Aβ cells with an IC₅₀ value of 22.4 μM without cytotoxicity.


**Keywords**: melanogenesis; aspochalasin I; *Aspergillus* sp.

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Clitocybin D, a novel human neutrophil elastase inhibitor from the culture broth of *Clitocybe aurantiaca*

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Clitocybin D, a novel human neutrophil elastase inhibitor, was isolated from the culture broth of *Clitocybe aurantiaca*. This compound was purified by solvent extraction, silica gel column chromatography, Sephadex LH₂₀ column chromatography, and preparative HPLC. The compound was determined to be 4-(4,6-dihydroxy-3-methoxy-3H-isoindol-1-yl)-benzoic acid on the basis of 1D and 2D NMRs and MS spectroscopic analysis. Analysis of the human neutrophil elastase (HNE) inhibitory activity of the isolated compound revealed that it showed significant HNE inhibitory activity with an IC₅₀ value of 17.8 μM.

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**Keywords**: *Clitocybe aurantiaca*; citocybin D; human neutrophil elastase
Reticulone, a novel free radical scavenger produced by *Aspergillus* sp.

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Bioassay-guided fractionation of the culture broth of *Aspergillus* sp. FN070449 (KCTC 26428) using a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay led to the isolation of two compounds: reticulone (1) and reticulol (2). Their chemical structures were elucidated on the basis of UV, IR, NMR, and MS spectroscopic analyses. Compound 1 exhibited more potent free radical scavenging activity on ABTS(center dot+) (2,2′-azino-bis [3-ethylbenzthiazoline-6-sulphonic acid]) and DPPH radicals than did butylated hydroxyanisole (BHA) and caffeic acid.


**Keywords**: *Aspergillus* sp.; reticulone; DPPH; ABTS; antioxidants

Hypoxia-inducible factor-1 inhibitory benzofurans and chalcone-derived diels-alder adducts from *morus* species

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Hypoxia-inducible factor-1 (HIF-1) is the central mediator of cellular responses to low oxygen concentrations and vital to many aspects of cancer biology. Bioassay-guided fractionation of the chloroform-soluble extracts of *Morus* species using a hypoxia response element (HRE)-dependent reporter assay led to identification of six benzofurans (1-6) and two chalcone-derived Diels-Alder adducts (7, 8) from Mori Cortex Radicis and three prenylated benzofurans (9-11) and four chalcone-derived Diels-Alder adducts (12-15) from *Morus bombycis*. The structure of the new 2-arylben-zofuran-type compound, moracin Q (3), was elucidated by spectroscopic methods, and the absolute configuration of 2 was determined for the first time. The selected compounds (1-3, 5, 7, 9, 10, and 12) from the cell-based reporter assay were found to inhibit hypoxia-induced HIF-1 alpha accumulation in a dose-dependent manner in human hepatocellular carcinoma cell-line Hep3B cells. Furthermore, these compounds were also active against hypoxia-induced vascular endothelial growth factor (VEGF) secretion in Hep3B cells.


**Keywords**: cultivated mulberry tree; root bark; constituents; luciferase; flavonoids; flavanone
Cytotoxic triterpenoids from the rhizomes of *Astilbe chinensis*

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Six new triterpenoids (1-6) with a carboxylic acid functionality at C-27 were isolated from the rhizomes of a Korean native perennial herb, *Astilbe chinensis*, along with nine known triterpenoids. The structures of 1-6 were elucidated on the basis of spectroscopic data interpretation. All compounds isolated were evaluated for cytotoxic effects against a small panel of human cancer lines.


**Keywords**: hela-cells; apoptosis; induction

Isolation of betulinic acid, its methyl ester and guaiane sesquiterpenoids with protein tyrosine phosphatase 1B inhibitory activity from the roots of *Saussurea lappa* C.B.Clarke

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Activity-guided fractionation of a MeOH extract of the roots of *Saussurea lappa* C.B.Clarke (Compositae), using an *in vitro* protein tyrosine phosphatase 1B (PTP1B) inhibition assay, led to the isolation of four active constituents: betulinic acid (1), betulinic acid methyl ester (2), mokko lactone (3) and dehydrocostus lactone (4), along with nine inactive compounds. Our findings indicate that betulinic acid (1) and its methyl ester 2, as well as the two guaiane sesquiterpenoids 3 and 4 are potential lead moieties for the development of new PTP1B inhibitors.


**Keywords**: protein tyrosine phosphatase 1B; *Saussurea lappa* C.B.Clarke; betulinic acid; betulinic acid methyl ester; mokko lactone; dehydrocostus lactone
Antiviral activity of raoulic acid from *Raoulia australis* against Picornaviruses

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RNA viruses are a major source of respiratory diseases worldwide. The lack of effective therapeutical treatment underlines the importance of research for new antiviral compounds. Raoulic acid is a principal ingredient of the plant *Raoulia australis* Hook. F. Antiviral assay using cytopathic effect (CPE) reduction method showed that raoulic acid possessed strong antiviral activity against human rhinovirus 2 (HRV2) with a 50% inhibition concentration (IC₅₀) value of less than 0.1 µg/ml, human rhinovirus 3 (HRV3) with a IC₅₀ value of 0.19 µg/ml, coxsackle B3 (CB3) virus with IC₅₀ values of 0.33 µg/ml, coxsackle B4 (CB4) virus with IC₅₀ values of 0.40 µg/ml, and enterovirus 71 (EV71) virus with IC₅₀ values of less than 0.1 µg/ml. However, the compound did not possess antiviral activity against influenza A (Flu A/PR, Flu A/WS, H1N 1) and B viruses at four concentrations ranging from 0.1 to 100 µg/ml.

**Keywords**: *Raoulia australis*; Raoulic acid; antiviral activity

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A peroxisome proliferator-activated receptor-gamma agonist and other constituents from *Chromolaena odorata*

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Peroxisome proliferator-activated receptors (PPARs) are key regulators of lipid and glucose metabolism and have become important therapeutic targets for various diseases. The phytochemical investigation of the chloroform-soluble extract of *Chromolaena odorata* led to the isolation of a PPAR-gamma agonist, (9S,13R)-12-oxo-phytodienoic acid (1), together with 12 other compounds. The Structures of chromomoric acid G (2), a new dehydrogenated derivative of 1, and chromolanone (3) were elucidated based on spectroscopic methods. Compound 1 showed a significant effect on PPAR-gamma activation in comparison with rosiglitazone. However, Compound 2 was inactive, suggesting that the dehydrogenation of the prostaglandin-like Structure in 1 abrogates its PPAR-gamma agonistic activity.

**Keywords**: *Chromolaena odorata*; asteraceae; (9s, 13r)-12-oxo-phytodienoic acid; chromomoric acid G; PPAR-gamma
**Pseudomonas aeruginosa eliminates natural killer cells via phagocytosis-induced apoptosis**

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**Pseudomonas aeruginosa** (PA) is an opportunistic pathogen that causes the relapse of illness in immunocompromised patients, leading to prolonged hospitalization, increased medical expense, and death. In this report, we show that PA invades natural killer (NK) cells and induces phagocytosis-induced cell death (PICD) of lymphocytes. **In vivo** tumor metastasis was augmented by PA infection, with a significant reduction in NK cell number. Adoptive transfer of NK cells mitigated PA-induced metastasis. Internalization of PA into NK cells was observed by transmission electron microscopy. In addition, PA invaded NK cells via phosphoinositide 3-kinase (PI3K) activation, and the phagocytic event led to caspase 9-dependent apoptosis of NK cells. PA-mediated NK cell apoptosis was dependent on activation of mitogen-activated protein (MAP) kinase and the generation of reactive oxygen species (ROS). These data suggest that the phagocytosis of PA by NK cells is a critical event that affects the relapse of diseases in immunocompromised patients, such as those with cancer, and provides important insights into the interactions between PA and NK cells.


**Keywords**: induced macrophage apoptosis; neutrophil apoptosis; epithelial-cells; protein-kinases; host-defense; in-vivo; NK cell; lymphocyte; bacteremia
7. Division of Bio-Infra Structure

- Bio-Evaluation Center
- Korea National Primate Research Center
- Biomedical Mouse Resource Center
- Biotechnology Process Engineering Center
Phosphate-responsive promoter of a Pichia pastoris sodium phosphate symporter

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To develop a functional phosphate-regulated promoter in Pichia pastoris, a phosphate-responsive gene, P-HO89, which encodes a putative sodium (Na+) coupled phosphate symporter, was isolated. Sequencing analyses revealed a 1,731-bp open reading frame encoding a 576-amino-acid polypeptide with 12 putative transmembrane domains. The properties of the PHO89 promoter (P_PHO89) were investigated using a bacterial lipase gene as a reporter in 5-liter jar fermentation experiments. P_PHO89 was tightly regulated by phosphate and was highly activated when the cells were grown in a phosphate-limited external environment. Compared to translation elongation factor 1 alpha and the glyceraldehyde-3-phosphate dehydrogenase promoter, P_PHO89 exhibited strong transcriptional activity with higher specific productivity (amount of lipase produced/cell/h). Furthermore, a cost-effective and simple P_PHO89-based fermentation process was developed for industrial application. These results demonstrate the potential for efficient use of P_PHO89 for controlled production of recombinant proteins in P. pastoris.


Keywords: heterologous-protein-production; Saccharomyces cerevisiae; Escherichia coli; alkaline-phosphatase; regulated promoter

Antitumor activity of cytokine-induced killer cells in nude mouse xenograft model

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Malignant glioma is the most common primary brain tumor in adults and the median survival for patients is less than a year. Despite aggressive treatments including surgical resection, radiotherapy, and chemotherapy, only modest improvement has been achieved in the survival of patients with glioma. In this study, the antitumor activity of cytokine-induced killer (CIK) cells against human glioma cancer was evaluated in vitro and in vivo. Human peripheral blood mononuclear cells were cultured with IL-2-containing medium in anti-CD3 antibody-coated flasks for 5 days, followed by incubation in IL-2-containing medium for 9 days. The number of cells increased more than 200-fold and the viability was > 90%. The resulting populations were consisted of 96% CD3+, 2% CD3-CD56+, 68% CD3+CD56-, 2% CD4+, < 1% CD4-CD56-, 80% CD8+, and 49% CD8-CD56-. This heterogeneous cell population was called as CIK cells. At an effector-target cell ratio of 30:1, CIK cells destroyed 43% of U-87 MG human glioma cells, as measured by the 51Cr-release assay. In addition, CIK cells at doses of 0.3, 1, and 3 million cells per mouse inhibited 23%, 40%, and 50% of U-87 MG tumor growth in nude mouse xenograft assays, respectively. This study suggests that CIK cells may be used as an adoptive immunotherapy for glioma cancer patients.


Keywords: cytokine-induced killer cells; adoptive immunotherapy; U-87 MG glioma
Structural modifications of outer membrane vesicles to refine them as vaccine delivery vehicles

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In an effort to devise a safer and more effective vaccine delivery system, outer membrane vesicles (OMVs) were engineered to have properties of intrinsically low endotoxicity sufficient for the delivery of foreign antigens. Our strategy involved mutational inactivation of the MsbB (LpxM) lipid A acyltransferase to generate OMVs of reduced endotoxicity from \textit{Escherichia coli} (\textit{E. coli}) O157:H7. The chromosomal tagging of a foreign FLAG epitope within an OmpA-fused protein was exploited to localize the FLAG epitope in the OMVs produced by the \textit{E. coli} mutant having the defined msbB and the ompA::FLAG mutations. It was confirmed that the desired fusion protein (OmpA::FLAG) was expressed and destined to the outer membrane (OM) of the \textit{E. coli} mutant from which the OMVs carrying OmpA::FLAG are released during growth. A luminal localization of the FLAG epitope within the OMVs was inferred from its differential immunoprecipitation and resistance to proteolytic degradation. Thus, by using genetic engineering-based approaches, the native OMVs were modified to have both intrinsically low endotoxicity and a foreign epitope tag to establish a platform technology for development of multifunctional vaccine delivery vehicles.

\textit{BIOCHIMICA ET BIOPHYSICA ACTA-BIOMEMBRANES}, 1788(10): 2150-2159.

\textbf{Keywords}: OMV; \textit{E. coli} O157; MsbB (LpxM); OmpA fusion; vaccine vehicle

A novel delta-lactam-based histone deacetylase inhibitor, KBH-A42, induces cell cycle arrest and apoptosis in colon cancer cells

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In this study, we investigated the anti-tumor activity of KBH-A42[N-hydroxy-3-(2-oxo-1-(3-phenylpropyl)-1,2,5,6-tetrahydropyridin-3-yl)propanamide], a novel synthetic histone deacetylase (HDAC) inhibitor. KBH-A42 inhibited a variety of HDAC isoforms in enzyme assays and suppressed growth of various cancer cell lines. Among the cell lines examined, colon cancer cells, including SW620, SW480 and HCT-15, were the cell types most sensitive to KBH-A42. KBH-A42 inhibition of cancer cell growth was comparable to or stronger than that of suberoylanilide hydroxamic acid (SAHA), a well-known HDAC inhibitor approved by the FDA to treat cutaneous T cell lymphomas. In SW620 cells, KBHA42 increased the acetylation of histones, mediated cell cycle arrest (G1 arrest at low doses and G2 arrest at high doses), and induced apoptosis. The cell cycle arrest and apoptosis induced by KBH-A42 might be mediated through up-regulation of p21\textsuperscript{Waf1} and activation of caspases, respectively. In addition, KBHA42 inhibited SW620 tumor growth in a human tumor xenograft model. Taken together, our results indicate that KBH-A42 exerts an anti-tumor activity \textit{in vitro} and \textit{in vivo} and is a promising therapeutic candidate to treat human cancers.


\textbf{Keywords}: HDAC; KBH-A42; colon cancer; cell cycle arrest; apoptosis
Low dose estrogen supplementation reduces mortality of mice in estrogen-dependent human tumor xenograft model

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Breast cancer is one of the most frequent female cancers in the Western world. Perturbation of estrogen levels by hormone replacement therapy or pregnancy is associated with a variety of diseases, including breast cancer. Estrogen supplementation is required to establish appropriate animal models for estrogen-related diseases. In this report, we demonstrated that supplementation with high doses of 17 beta-estradiol results in deaths in estrogen-dependent MCF-7 tumor xenograft model. Renal damage and bladder stone formation was implicated as a major cause of death. The mortality rate was significantly reduced when mice received a low dose of 17 beta-estradiol. We also confirmed that low dose of 17 beta-estradiol supplementation can support the growth of tumors in MCF-7 tumor xenograft model. These results suggest that low dose estrogen supplementation may be more appropriate in estrogen-dependent tumor xenograft models.

BIOLOGICAL & PHARMACEUTICAL BULLETIN, 32(1): 150-152.

Keywords: tumor xenograft model; estrogen; breast cancer; MCF-7

Efficient proteolytic cleavage by insertion of oligopeptide linkers and its application to production of recombinant human interleukin-6 in Escherichia coli

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Efficient expression and purification of bioactive recombinant human interleukin-6 (hIL6) was successfully achieved in Escherichia coli (E. coli) by fusion of the maltose-binding protein (MBP) with hIL6 and the insertion of oligopeptide linkers. MBP/hIL6 was over-expressed in the soluble form at a concentration of approximately 2.5 g/L. For hIL6 recovery, enterokinase, factor Xa, and thrombin were employed to cleavage MBP from the fusion constructs. However, undesired and non-specific cleavage fragments as well as rhIL6 were obtained following the cleavage. The introduction of oligopeptide linkers at the C-terminal end of the fusion construct could improve the efficiency and the rate of the enzymatic cleavage reaction. and the rhIL6 purification was achieved by using MBP affinity chromatography. factor Xa cleavage, and reverse-phase chromatography, resulting in an overall yield as high as 33% (equivalent to 0.27 ghIL6/L) at purity over 98%. The biological activity of the purified recombinant hIL6 was demonstrated by confirming the presence of the signal transducer and activator of transcription 3 (STAT3) signaling pathway. This study suggests that the optimized peptide linker specifically designed for both fusion partner and target molecule has a great potential for efficient recombinant protein production.

ENZYME AND MICROBIAL TECHNOLOGY, 44(5): 254-262.

Keywords: Interleukin-6; enzymatic cleavage; enterokinase; factor Xa; thrombin; maltose-binding protein
The roles of glycosphingolipids in the proliferation and neural differentiation of mouse embryonic stem cells

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Glycosphingolipids including gangliosides play important regulatory roles in cell proliferation and differentiation. UDP-glucose:ceramide glucosyltransferase (Ugcg) catalyze the initial step in glycosphingolipids biosynthesis pathway. In this study, Ugcg expression was reduced to approximately 80% by short hairpin RNAs (shRNAs) to evaluate the roles of glycosphingolipids in proliferation and neural differentiation of mouse embryonic stem cells (mESCs). HPTLC/immunofluorescence analyses of shRNA-transfected mESCs revealed that treatment with Ugcg-shRNA decreased expression of major gangliosides, GM3 and GD3. Furthermore, MTT and Western blot/immunofluorescence analyses demonstrated that inhibition of the Ugcg expression in mESCs resulted in decrease of cell proliferation (P < 0.05) and decrease of activation of the ERK1/2 (P < 0.05), respectively. To further investigate the role of glycosphingolipids in neural differentiation, the embryoid bodies formed from Ugcg-shRNA transfected mESCs were differentiated into neural cells by treatment with retinoic acid. We found that inhibition of Ugcg expression did not affect embryoid body (EB) differentiation, as judged by morphological comparison and expression of early neural precursor cell marker, nestin, in differentiated EBs. However, RT-PCR/immunofluorescence analyses showed that expression of microtubule-associated protein 2 (MAP-2) for neurons and glial fibrillary acidic protein (GFAP) for glial cells was decreased in neural cells differentiated from the shRNA-transfected mESCs. These results suggest that glycosphingolipids are involved in the proliferation of mESCs through ERK1/2 activation, and that glycosphingolipids play roles in differentiation of neural precursor cells derived from mESCs.

EXPERIMENTAL AND MOLECULAR MEDICINE, 41(12): 935-945.

Keywords: cell differentiation; ceramide glucosyltransferase; extracellular signal-regulated MAP kinases; glycosphingolipids; embryonic stem cells; neurons

Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and along the transportation routes of the Incheon port in South Korea

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In South Korea, imported genetically modified (GM) soybean and maize have been approved for both human consumption and use in animal feed, but not for use in cultivation in fields. This study was conducted to survey the spread of GM soybean and maize in South Korea using multiplex-PCR analysis methods. Cultivated soybean, wild soybean, and maize leaf samples were collected from 26 major areas of soybean cultivation throughout eight provinces. Roadside areas near a major grain port in Incheon were also surveyed to investigate the escape and spread of GM seeds and plants. Amplification results showed that no GM soybean or maize was collected from cultivated fields, However, four GM maize plants were found in samples collected from the roadside near a grain transporting company at the Incheon Port. Based on PCR analysis using GM maize event-specific primers, it was suggested that a maize plant may be Mon810, while the other plants may be stacked events: Mon863 x Mon810 or Mon88017 x Mon810.


Keywords: GMO detection; maize; PCR; soybean
Lineage specific evolutionary events on SFTPB gene: Alu recombination-mediated deletion (ARMD), exonization, and alternative splicing events

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SFTPB gene encoding pulmonary surfactant protein has been investigated to have transcript variants related with transposable elements (TEs). To investigate the alternative splicing event on SFTPB gene, various primate samples (tissue RNA and genomic DNA) were used. Two different transcript variants (T1-2 and T2-2) were newly identified by RT-PCR analysis. T1-1,-2 and T2-1,-2 are TE-related and original transcripts, respectively. T1-1 transcript was investigated to be a lung and human specific alternative transcript. T2-1 transcript shows dominant expression pattern in lung tissues. T1-related TEs (LTR7B and AluSx) were investigated by genomic PCR and sequencing procedure of different primate genomic DNAs. Sequencing analysis indicated that they had been integrated into our common ancestor genome before the divergence of simian and prosimian and Alu recombination-mediated deletion (ARMD) event had been Occurred on our common ancestor genome after the divergence of New World monkeys and Old World monkeys in SFTPB gene 3' UTR region. Taken together, integration event of LTR7B and AluSx on SFTPB gene seem to cause lineage specific events via general exonization event (New World monkeys and hominoids), and alternative splicing (human) during the primate evolution.


Keywords : SFTPB gene; LTR7B; ARMD; exonization; alternative splicing

Dynamic evolution of tRNAThr-derived Hpal SINEs and effect on genomes of Oncorhynchus species

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Short interspersed nuclear elements (SINEs) are the most abundant non-autonomous retroelements in many vertebrate genomes. The events that led to their integration may have had marked effects on the evolution of host genomes. One well-investigated SINE lineage is in the pacific salmon (genus Oncorhynchus). Experimental approaches and bioinformatics have been used to investigate the dynamic features and evolutionary impact of these SINEs. Four gene-related Hpal SINES in the CD4L-2a, NOS, MHC and IL1B genes were identified by bioinformatics tool. To investigate these SINES, PCR amplification and sequencing were performed on eight species of the genus Oncorhynchus and one of Salina. Unexpectedly, the CD4L-2a, MHC and IL-1B gene loci proved to be dimorphic for the Hpal SINE insertion; this may be attributable to lineage sorting. Sequence transduction and horizontal transmission events also occurred in CD4L-2a. To elucidate the impact of Hpal SINES on pacific salmon genomes and the diversity of transcriptomes, 243,668 mRNA sequences from the GenBank database were analyzed. A total of 163 mRNA sequences were identified as fused with Hpal SINES. Among these, 87 ESTs were annotated into 41 functional genes. Our data suggest that SINES could contribute to the genomic diversity of the pacific salmon by exonization and could move more dynamically within this genome by lineage sorting, sequence transduction and horizontal transmission.


Keywords : Hpal SINES; Oncorhynchus; lineage sorting; horizontal transmission; bioinformatics
Inhibition of human cervical carcinoma growth by cytokine-induced killer cells in nude mouse xenograft model

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Cervical cancer is a major cause of cancer mortality in women worldwide and is an important public health problem for adult women in developing countries. Despite aggressive treatment with surgery and chemoradiation, the outcomes for cervical cancer patients remain poor. In this study, the antitumor activity of cytokine-induced killer (CIK) cells against human cervical cancer was evaluated in vitro and in vivo. Human peripheral blood mononuclear cells were cultured with IL-2-containing medium in anti-CD3 antibody-coated flasks for 5 days, followed by incubation in IL-2-containing medium for 9 days. The resulting populations of CIK cells comprised 95%CD3+, 3%CD3-CD56+, 35% CD3+CD56+, 11% CD4(+), <1% CD4+CD56+, 80% CD8+, and 25% CD8+CD56+. At an effector-target cell ratio of 100:1, CIK cells destroyed 56% of KB-3-1 human cervical cancer cells, as measured by the 51Cr-release assay. In addition, CIK cells at doses of 3 and 10 million cells per mouse inhibited 34% and 57% of KB-3-1 tumor growth in nude mouse xenograft assays, respectively. This study suggests that CIK cells may be used as an adoptive immunotherapy for cervical cancer patients.


Keywords: cytokine-induced killer cells; adoptive immunotherapy; KB-3-1 cervical cancer

Molecular characterization of the DYST1C1 gene and its application as a cancer biomarker

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DYST1C1 has three alternatively spliced transcripts. Therefore, we expect that alternative transcripts of DYST1C1 are used as a biomarker to detect specific cancer. RT-PCR analysis is conducted in order to detect expression of the DYST1C1 gene and the PCR products were analyzed using the Image J program to compare the expression levels of each transcript. We found one of the transcripts was directly associated with an HERV-H LTR element that could be translated into protein sequence. Four new alternative transcripts were identified by RT-PCR analysis with various human tissue samples including 10 normal and adjacent tumor tissue sets. Semi-quantitative RT-PCR analysis showed the transcriptional activity of V3 and V2 was higher in tumor than in normal tissue samples, especially in the colorectal tissue samples. Our results indicated that alternatively spliced transcript variants of the DYST1C1 gene could be used as cancer biomarkers to detect colorectal cancer.

JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY, 135(2): 265-270.

Keywords: dyslexia susceptibility 1 candidate 1 (DYST1C1); human endogenous retrovirus (HERV); long terminal repeat (LTR); alternative splicing
Multi-immunogenic outer membrane vesicles derived from a MsbB-deficient *Salmonella enterica* serovar typhimurium mutant

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To develop low endotoxic and multi-immunogenic outer membrane vesicles (OMVs), a deletion mutant of the *msbB* gene in *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) was used as a source of low endotoxic OMV, and an expression vector of the canine parvovirus (CPV) VP2 epitope fused to the bacterial OmpA protein was constructed and transformed into the *Salmonella* Delta msbB mutant. In a lethality test, BALB/c mice injected intraperitoneally with the *Salmonella* Delta msbB mutant survived for 7 days, whereas mice injected intraperitoneally with the wild type survived for 3 days. Moreover, all mice inoculated orally with the Delta msbB mutant survived for 30 days, but 80%, of mice inoculated orally with the wild type survived. The OmpA::CPV VP2 epitope fusion protein was expressed successfully and associated with the outer membrane and OMV fractions from the mutant *S. Typhimurium* transformed with the fusion protein-expressing vector. In immunogenicity tests, sera obtained from the mice immunized with either the *Salmonella* msbB mutant or its OMVs containing the OmpA::CPV VP2 epitope showed bactericidal activities against wild-type & Typhimurium and contained specific antibodies to the CPV VP2 epitope. In the hemagglutination inhibition (HI) assay as a measurement of CPV-neutralizing activity in the immune sera, there was an 8-fold increase of HI titer in the OMV-immunized group compared with the control. These results suggested that the CPV-neutralizing antibody response was raised by immunization with OMV containing the OmpA::CPV VP2 epitope, as well as the protective immune response against *S. Typhimurium* in BALB/c mice.


**Keywords**: outer membrane vesicle; *S. Typhimurium*; low endotoxicity; multi-immunogenicity; canine parvovirus

Assessment of gene flow from genetically modified anthracnose-resistant chili pepper (*Capsicum annuum* L.) to a conventional crop

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We conducted a 2-year field assessment of the gene flow from genetically modified (GM) chili pepper (*Capsicum annuum* L.), containing the PepEST (pepper esterase) gene, to a non-GM control line "WT512" and two commercial hybrid cultivars, "Manidda" and "Cheongpung Myeongwol (CM)." After seeds were collected from the pollen-recipient non-GM plants, hybrids between them and the GM peppers were screened by a hygromycin assay. PCR with the targeting hpt gene was performed to confirm the presence of transgenes in hygromycin-resistant seedlings. Out of 7,071 "WT512" seeds and 6,854 "Manidda" seeds collected in 2006, eight and 12 hybrids, respectively, were detected. In 2007, 33 hybrids from 3,456 "WT512" seeds and 50 hybrids from 3,457 "CM" seeds were found. The highest frequency of gene flow, 6.19%, was observed in that 2007 trial. These results suggest that a limited isolation distance would be sufficient to prevent gene flow from GM to conventionally bred chili peppers.

**JOURNAL OF PLANT BIOLOGY**, 52(3): 251-258.

**Keywords**: *Capsicum annuum*; chili pepper; gene flow; genetically modified (GM) crop
Importance of the porcine ADAM3 disintegrin domain in sperm-egg interaction

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In the mouse, ADAM3, a well-characterized testis-specific protein of the A disintegrin and metalloprotease (ADAM) family, has a crucial role in fertilization by mediating sperm binding to the egg zona pellucida. However, little is known about ADAM3 in other species, such as domestic pigs. We have identified porcine ADAM3 and analyzed the protein. RT-PCR and trypsinization of sperm surface proteins revealed that porcine ADAM3 is expressed at high levels in the testis and on the sperm surface. Furthermore, an IVF inhibition assay with a recombinant porcine ADAM3 disintegrin domain showed that treatment of the disintegrin domain effectively prevented pig sperm-egg interactions. In the present study, we demonstrated the presence of ADAM3a and ADAM3b molecules in the pig and examined their roles in fertilization.


Keywords: a disintegrin and metalloprotease (ADAM); disintegrin domain; porcine; sperm-egg interaction

Identification and molecular characterization of PERV gamma1 long terminal repeats

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Porcine endogenous retroviruses (PERVs) gamma1 in the pig genome have the potential to act as harmful factors in xenotransplantation (pig-to-human). Long terminal repeats (LTRs) are known to be strong promoter elements that could control the transcription activity of PERV elements and the adjacent functional genes. To investigate the transcribed PERV gamma1 LTR elements in pig tissues, bioinformatic and experimental approaches were conducted. Using RT-PCR amplification and sequencing approaches, 69 different transcribed LTR elements were identified. And 69 LTR elements could be divided into six groups (15 subgroups) by internal variation including tandem repeated sequences, insertion and deletion (INDEL). Remarkably, all internal variations were indentified in U3 region of LTR elements. Taken together, the identification and characterization of various PERV LTR transcripts allow us to extend our knowledge of PERV and its transcriptional study.

MOLECULES AND CELLS, 27(1): 119-123.

Keywords: bioinformatics; LTR element; PERV; pig; xenotransplantation
Gain of new exons and promoters by lineage-specific transposable elements-integration and conservation event on \textit{CHRM3} gene

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The \textit{CHRM3} gene is a member of the muscarinic acetylcholine receptor family that plays important roles in the regulation of fundamental physiological functions. The evolutionary mechanism of exon-acquisition and alternative splicing of the \textit{CHRM3} gene in relation to transposable elements (TEs) were analyzed using experimental approaches and in silico analysis. Five different transcript variants (T1, T2, T3, T3-1, and T4) derived from three distinct promoter regions (T1: L1HS, T2, T4: original, T3, T3-1: THE1C) were identified. A placenta (T1) and testis (T3 and T3-1)-dominated expression pattern appeared to be controlled by different TEs (L1HS and THE1C) that were integrated into the common ancestor genome during primate evolution. Remarkably, the T1 transcript was formed by the integration event of the human specific L1HS element. Among the 12 different brain regions, the brain stem, olfactory region, and cerebellum showed decreased expression patterns. Evolutionary analysis of splicing sites and alternative splicing suggested that the exon-acquisition event was determined by a selection and conservation mechanism. Furthermore, continuous integration events of transposable elements could produce lineage specific alternative transcripts by providing novel promoters and splicing sites. Taken together, exon-acquisition and alternative splicing events of \textit{CHRM3} genes were shown to have occurred through the continuous integration of transposable elements following conservation.


\textbf{Keywords} : alternative splicing; \textit{CHRM3} gene; exon-acquisition; L1HS element; transposable elements

Gene flow from genetically modified to conventional chili pepper (\textit{Capsicum annuum L.})

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Gene flow from genetically modified (GM) chili pepper (\textit{Capsicum annuum L.}), containing the CMVP0-CP (cucumber mosaic virus pathotype 0 - coat protein) gene to a non-GM control variety 'P915' and two commercial F\textsubscript{1}, hybrids, 'Manidda' and 'Taesan', was assessed over two growing seasons in the field. Seeds were collected from non-GM chili peppers at a range of distances from the GM chili pepper plot, and hybrids between GM and non-GM plants were screened using the kanamycin assay. Event-specific PCR was performed to confirm the presence of transgenes in the kanamycin-resistant seedlings. From a total of 11,194 'P915' seeds, there were 67 hybrids; there were 40 hybrids of 7499 seeds, and 102 hybrids of 5340 seeds for 'Manidda' and 'Taesan', respectively. The gene flow frequency was as high as 17.89% between GM and 'Taesan' chili pepper at the closest distance from the GM plot.


\textbf{Keywords} : \textit{Capsicum annuum}; chili pepper; gene flow; genetically modified crop
A framework for molecular genetic assessment of a transgenic watermelon rootstock line

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Transgenic plants over-expressing virus coat protein genes have attracted particular interest from researchers recently, due primarily to their tolerance to viral infection. The transgenic watermelon rootstock analyzed in this study contains the introduced *cucumber green mottle mosaic virus* coat protein (*CGMMV-CP*) gene. The primary objective of this study was to determine the copy number, integration site, and expression level of the transgene element, in order to establish a scientific framework for the molecular genetic assessment of transgenic plant rootstocks. The results of our Southern blot analysis indicated that a single copy of the *CGMMV-CP* gene was inserted into the genome of a transgenic watermelon rootstock. We also identified the genomic sequences flanking the integration site of the transgene via inverse PCR analysis. In an effort to find a sequence usable as an internal positive control for the screening of the transgenic watermelons, we determined that the Sat gene appears as one copy within their genomes and is watermelon-specific. The information from the integrated site and the internal positive control sequence was utilized to establish a new event-specific PCR-based detection method. The expression of both *CGMMV-CP* mRNA and protein was detected in the transgenic watermelon rootstocks but not in watermelon scions, thereby suggesting that the tissues of the watermelon scions are free of the introduced gene products.


**Keywords** : CGMMV-CP; rootstock; sat; scion; transgenic; watermelon

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High-level production of lycopene in metabolically engineered *E. coli*

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Recombinant lycopene was generated by utilizing metabolically engineered *Escherichia coli* with yields being dependent upon inocula state. Yields were especially low in the case of cultures harboring high-copy plasmids that were established with inocula at the stationary growth phase. On the other hand, cultures derived using low-copy plasmid, however, yielded high amounts of lycopene irrespective of inocula state. Nevertheless, it showed still an inocula dependence pattern in lycopene productivity (mg/l/h). To further increase lycopene productivity, we applied a temperature-shift culture technique (37 -> 25 degrees C). Using this method, we effectively enhanced lycopene productivity without any problematic phenomena. As a result, we were able to increase lycopene yield by approximately 20% compared to previous culture methods. In the present study, we were able to reach a final lycopene yield up to 260 mg/l for 60 h, which corresponds to the highest titer to date for the production of lycopene in *E. coli*.

*PROCESS BIOCHEMISTRY*, 44(8): 899-905.

**Keywords** : inocula dependence; lycopene; *Pantoea ananatis*; plasmid copy number; synthetic operon; temperature-shift
The pharmacokinetics and metabolism of 2'-benzoyloxycinnamaldehyde (BCA) was characterized in male Sprague-Dawley rats as part of the preclinical evaluations for developing this compound as an antitumour agent. BCA was not detected in the plasma following either intravenous or oral dose, whereas its putative metabolites 2'-hydroxycinnamaldehyde (HCA) and o-coumaric acid were present at considerable levels. In separate pharmacokinetics studies, HCA exhibited a high systemic clearance and a large volume of distribution, whereas both pharmacokinetic parameters were much lower for o-coumaric acid. The terminal half-life of both metabolites was approximately 2 h. BCA was converted rapidly to HCA in rat serum, liver microsomes and cytosol \( \text{in vitro} \); HCA was subsequently converted to o-coumaric acid in a quantitative manner only in the liver cytosol. In addition, the formation of o-coumaric acid was inhibited significantly by menadione, a specific inhibitor for aldehyde oxidase. Taken collectively, the results suggest that the rapid systemic clearance of HCA is likely due mainly to hepatic clearance occurring from aldehyde oxidase-catalysed biotransformation to o-coumaric acid. In conclusion, the present work demonstrates that the anticancer drug candidate BCA is highly likely to work as its active metabolite HCA in the body. 


Keywords: 2'-Benzoyloxycinnamaldehyde; 2'-hydroxycinnamaldehyde; o-coumaric acid; antitumour; anticancer; pharmacokinetics; drug metabolism; active metabolite
8. Jeonbuk Branch Institute

- Microbe-based Fusion Technology Research Center
- Eco-Friendly Biomaterial Research Center
- Bioindustrial Process Center
Multiple-layer substrate zymography for detection of several enzymes in a single sodium dodecyl sulfate gel

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We have developed a system to detect three hydrolytic enzymes (cellulase, lipase, and protease) using a single sodium dodecyl sulfate (SDS) gel and an electrotransfer system. After electrophoresis, proteins in the gel were transferred to three sandwiched substrate gels containing glycerol tributyrate, azo-carboxy-methyl cellulose (Azo-CMC), and fibrin for detection of cellulase, lipase, and protease, respectively. We show that three cellulases (from a Paenibacillus sp. and two Bacillus sp. strains), one lipase (from a Staphylococcus sp.), and two proteases (from two Bacillus sp. strains) can be detected simultaneously with our zymogram system.

ANALYTICAL BIOCHEMISTRY, 386(1): 121-122.

Keywords: single sodium dodecyl sulfate; Azo-carboxy-methyl cellulose; hydrolytic enzymes

Elimination of by-product formation during production of 1,3-propanediol in Klebsiella pneumoniae by inactivation of glycerol oxidative pathway

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The microbial production of 1,3-propanediol (1,3-PD) by Klebsiella pneumoniae involves the formation of various by-products, which are synthesized through the oxidative pathway. To eliminate the by-products synthesis, the oxidative branch of glycerol metabolism was inactivated by constructing two mutant strains. In one of the mutant strains, the structural genes encoding glycerol dehydrogenase and dihydroxyacetone kinase were deleted from the chromosomal DNA, whereas in the second mutant strain dhaR, which is a putative transcription factor that activates gene expression was deleted from the chromosomal DNA. In the resultant mutant strains lacking the dhaT gene encoding 1,3-PD oxidoreductase, which was simultaneously deleted while replacing the native promoter with the lacZ promoter, the by-product formation except for acetate was eliminated, but it still produced 1,3-PD at a lower level, which might be due to a putative oxidoreductase that catalyzes the production of 1,3-PD. The recombinant strains in which the reductive pathway was recovered produced slightly lower amount of 1,3-PD as compared to the parent strain, which might be due to the reduced activity of DhaB caused by the substitution of the promoter. However, the production yield was higher in the recombinant strain (0.57 mol mol⁻¹) than the wild type Cu strain (0.47 mol mol⁻¹).

APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, 84(3): 527-534.

Keywords: Klebsiella pneumoniae; 1,3-Propanediol; glycerol metabolism; by-product
Characteristic of neuraminidase inhibitory xanthones from *Cudrania tricuspidata*

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Natural polyphenolic compounds generally transpire to show relatively low inhibition against glycosidase including neuraminidase. In addition the inhibition modes of such compounds are rarely competitive. In this manuscript, a series of xanthone derivatives from *Cudrania tricuspidata* are shown to display nanomolar inhibitor activity against neuraminidase (EC 3.2.1.18) as well as competitive inhibition modes. Compound 8 bearing vicinal dihydroxy group on the A-ring displays nanomolar activity (IC_{50} = 0.08 +/- 0.01 μM), a 200-fold increase in activity relative to that of the first reported xanthone-derived neuraminidase inhibitor, mangiferin (IC_{50} = 16.2 +/- 4.2 μM). The 6,7-vicinal dihydroxy group plays a crucial role for inhibitory activity because compound 4, which has one of these hydroxyl groups prenylated was inactive (33% at 200 μM), whereas other compounds (1-3 and 6-8) showed nanomolar activity (0.08-0.27 μM) and competitive inhibition modes. Interestingly all inhibitors manifested enzyme isomerization inhibition against neuraminidase. The most potent inhibitor, compound 8 showed similar interaction with a transition-state analogue of neuraminic acid in active site.

**BIOORGANIC & MEDICINAL CHEMISTRY**, 17(7): 2744-2750.

**Keywords**: *Cudrania tricuspidata*; xanthone; neuraminidase; time-dependent; 2vk6

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Neuraminidase inhibitory activities of flavonols isolated from *Rhodiola rosea* roots and their in vitro anti-influenza viral activities

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Five flavonols (3, 5, and 9-11) were isolated from *Rhodiola rosea*, and compared with commercially available flavonoids (1, 2, 4, 6-8, and 12-14) to facilitate analysis of their structure-activity relationship (SAR). All compounds (1-14) showed neuraminidase inhibitory activities with IC_{50} values ranging from 0.8 to 56.9 μM. The in vitro anti-influenza virus activities of flavonoids 1-6, 8-12, and 14 were evaluated using two influenza viral strains, H1N1 (A/PR/8/34) and H9N2 (A/Chicken/Korea/MS96/96), testing their ability to reduce virus-induced cytopathic effect (CPE) in MDCK cells. We found that the activity of these compounds ranged from 30.2 to 99.1 μM against H1N1- and 18.5 to 133.6 μM against H9N2-induced CPE. Of compounds 1-14, gossypetin (6) exhibited the most potent inhibitory activity, with IC_{50} values of 0.8 and 2.6 μM on neuraminidases from *Clostridium perfringens* and recombinant influenza virus A (rvH1N1), respectively. In contrast, kaempferol (3) exhibited the highest activity against two influenza viruses, H1N1 and H9N2 with EC_{50} values of 30.2 and 18.5 μM, respectively. Activity depended on the position and number of hydroxy groups on the flavonoids backbone. In kinetic studies, all isolated compounds behaved as noncompetitive inhibitors.


**Keywords**: flavonol; influenza virus; MDCK cell; neuraminidase inhibitor; *Rhodiola rosea*
Structural characteristics of flavanones and flavones from *Cudrania tricuspidata* for neuraminidase inhibition

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The structural characteristics of flavonoids (1-3 and 6-8) from the root of *Cudrania tricuspidata* required for neuraminidase inhibition were studied and compared with commercially available flavonoids (4, 5, and 9-12). Alkylated flavanones (1-3) display better inhibition than the corresponding parent compound 4. Importantly, flavanone 1 bearing a C-8 hydrated prenyl group showed extremely high inhibition with IC\(_{50}\) of 380 nM. On the other hand, the parent flavone 5 was more effective than alkylated analogues (6-8). Isolated inhibitors (1-3 and 6-8) showed noncompetitive inhibition in kinetic studies. The binding affinity of flavanones (1-4) for neuraminidase in in silico docking experiments correlated well with their IC\(_{50}\) values and noncompetitive inhibition mode.


**Keywords**: neuraminidase; *Cudrania tricuspidata*; flavonoid

Bacterial expression and purification of human papillomavirus type 18 L1

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The human papillomavirus (HPV) 18 L1 gene, which encodes the L1 major capsid protein, was isolated from a female patient in Pusan, Korea Republic and was cloned into pGEX-4T-1 vector. The HPV-18 L1 gene was expressed in *Escherichia coli* as a fusion protein with a glutathione-S-transferase (GST) tag. The soluble recombinant fusion protein, GST-18 L1 fusion, was isolated to high purity. HPV-18 L1 was purified from the GST-18 L1 fusant after biotinylated thrombin cleavage, and then the treated thrombin was removed serially using streptavidin conjugated resin. The purified HPV-18 L1 was confirmed by western blotting using a rabbit anti-denatured papillomavirus polyclonal antibody. The virus-like particles (VLP) from the purified full-length 18 L1 protein without any extra amino acid sequences was observed through the analysis of the electron microscope. This is the first study to report the expression and purification of HPV-18 L1 in *E. coli*. This expression and purification system offers a simple method of expressing and purifying HPV L1 protein, and could potentially be an effective route for the development and manufacturing of highly purified HPV-18 L1-based cervical cancer vaccines.


**Keywords**: human papillomavirus; bacterial expression; purification; L1 major capsid protein; HPV type 18
An efficient plasmid vector for constitutive high-level expression of foreign genes in Escherichia coli

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The levansucrase gene (lsrA) from Rahnella aquatilis was strongly expressed in a constitutive manner in Escherichia coli when cloned into a pBluescript KS-based pRL1CP plasmid vector. The native promoter upstream of lsrA and the lacZ promoter cooperatively enhanced the expression of lsrA to a level that was comparable to that of the T7 promoter, which is used in commercial pET expression vector system. A putative rho-independent transcription termination signal downstream of lsrA was crucial for gene expression. This plasmid vector also proved to be applicable for efficient expression of other foreign genes in E. coli.


Keywords: Escherichia coli; gene expression; levansucrase gene; Rahnella aquatilis; promoter

Identification of a serine protease from a Bacillus sp using multiple loading of O’Farrell-type isoelectric focusing slab two-dimensional gel

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A protease was purified from Bacillus sp. DJ isolated from Doenjang, a traditional Korean fermented food. Its molecular weight (MW) and isoelectric point (pI) were 18-19 kDa and 6.0-6.5 using 1- or 2-D fibrin zymography, respectively. The protease was optimally active at pH 9 and 55A degrees C. Activity was inhibited by 1 mM PMSF, but not by EDTA, EGTA, aprotinin, or leupeptin, indicating that the protease is a serine protease. By using a new electrophoretic technique, multiple loading of O’Farrell-type isoelectric focusing (IEF) slab gel, the first amino acid residues of the N-terminal sequence of the protease were determined as HPLVLVDPIL, which is 80% identical with serine proteases of the subtilase family.

BIOTECHNOLOGY LETTERS, 31(7): 975-978.

Keywords: isoelectric focusing; MALDI-TOF/MS; protease; two-dimensional electrophoresis; zymography
Improved ethanol tolerance in *Escherichia coli* by changing the cellular fatty acids composition through genetic manipulation

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To investigate the effect of cellular fatty acids composition on ethanol tolerance in *Escherichia coli*, we overexpressed either des, encoding fatty acid desaturase from *Bacillus subtilis*, or fabA, encoding beta-hydroxydecanoyl thio-ester dehydrase from *E. coli*, or both genes together, into *E. coli*. Recombinant *E. coli* harboring *fabA* had elevated tolerance against ethanol compared to wild type strain. In contrast, des decreased resistance to ethanol. Co-expression of both genes together complemented ethanol tolerance of *E. coli*. This result indicates how to engineer bacterial strains to be resistant to higher concentrations of ethanol.

*BIOTECHNOLOGY LETTERS*, 31(12): 1867-1871.

**Keywords**: *Escherichia coli*; fatty acids composition; ethanol tolerance; desaturase; fabA

Mixed-substrate (glycerol tributyrate and fibrin) zymography for simultaneous detection of lipolytic and proteolytic enzymes on a single gel

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A new zymography method for simultaneous detection of two different enzymatic activities (lipolytic and proteolytic) using a single SDS-containing or native-conformation gel and a mixed-substrate (glycerol tributyrate and fibrin) (MS) gel was developed. After routine electrophoresis, SDS in the gel was removed by treatment with Triton X-100. Gel proteins were electrotransferred to the MS gel. To visualize lipolytic activity, the MS gel was incubated at 37 degrees C (for 6 or 24 h) until clear bands against an opaque background were observed. To detect proteolytic activity, the same MS gel was stained with Coomassie brilliant blue. Using this method, we show that six lipolytic enzymes from *Staphylococcus pasteuri* NJ-1 and four proteolytic enzymes from two *Bacillus* strains, *B. licheniformis* DJ-2 and *B. licheniformis* NJ-5, isolated from soil, can be simultaneously detected.

*ELECTROPHORESIS*, 30(S1): 2234-2237.

**Keywords**: electrotransfer; lipase; mixed-substrates; protease; zymography
Purification and characterization of a subtilisin D5, a fibrinolytic enzyme of *Bacillus amyloliquefaciens* DJ-5 isolated from Doenjang

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The fibrinolytic enzyme, subtilisin D5, was purified from the culture supernatant of the isolated *Bacillus amyloliquefaciens* DJ-5. The molecular weight of subtilisin D5 was estimated to the 30 kDa. Subtilisin D5 was optimally active at pH 10.0 and 45 degrees C. Subtilisin D5 had high degrading activity for the A alpha-chain of human fibrinogen and hydrolyzed the B beta-chain slowly, but did not affect the gamma-chain, indicating that it is an alpha-fibrinogenase. Subtilisin D5 was completely inhibited by phenylmethylsulfonyl fluoride, indicating that it belongs to the serine protease. The specific activity (F/C, fibrinolytic/caseinolytic activity) of subtilisin D5 was 2.37 and 3.52 times higher than those of subtilisin BPN’ and Carlsberg, respectively. Subtilisin D5 exhibited high specificity for Meo-Suc-Arg-Pro-Tyr-pNA (S-2586), a synthetic chromogenic substrate for chymotrypsin. The first 15 amino acid residues of the N-terminal sequence of subtilisin D5 are AQSVPYGISQIKAPA; this sequence is identical to that of subtilisin NAT and subtilisin E.

**Food Science and Biotechnology**, 18(2): 500-505.

**Keywords**: *Bacillus amyloliquefaciens*; doenjang; fibrinolytic enzyme; subtilisin D5

Brevundimonas naejangsanensis sp nov., a proteolytic bacterium isolated from soil, and reclassification of *Mycoplana bullata* into the genus *Brevundimonas* as *Brevundimonas bullata* comb. nov.

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A Gram-negative, motile and rod-shaped bacterial strain, BIO-TAS2-2¹, of the class Alphaproteobacteria, was isolated from a soil in Korea and studied using a polyphasic taxonomic approach. Strain BIO-TAS2-2¹ grew optimally at pH 7.5-8.5 and 30 degrees C and in the presence of 0-1.0% (w/v) NaCl. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain BIO-TAS2-2¹ fell within the clade comprising species of the genus *Brevundimonas*, forming a coherent cluster with *Brevundimonas terrae KSL-145¹¹ and *Brevundimonas diminuta* LMG 2089¹¹. It exhibited 16S rRNA gene sequence similarity values of 96.0-98.7% to members of the genus *Brevundimonas* and *Mycoplana bullata* IAM 13153¹¹. Strain BIO-TAS2-2¹ contained Q-10 as the predominant ubiquinone and cyclo-C-18:1 omega 7c and C-16:0 as the major fatty acids. The DNA G + C content was 67.0 mol%. Strain BIO-TAS2-2¹ exhibited DNA-DNA relatedness levels of 12-19% with the type strains of phylogenetically related *Brevundimonas* species and *M. bullata*. The novel strain could be differentiated from *Brevundimonas* species and *M. bullata* by differences in phenotypic characteristics. On the basis of phenotypic, phylogenetic and genetic data, strain BIO-TAS2-2¹ is considered to represent a novel species of the genus *Brevundimonas*, for which the name *Brevundimonas naejangsanensis* sp. nov. is proposed. The type strain is BIO-TAS2-2¹ (=KCTC 2263¹¹ = CCUG 57609¹¹). In this study, it is also proposed that *Mycoplana bullata* be transferred to the genus *Brevundimonas* as *Brevundimonas bullata* comb. nov. (type strain TK0051¹¹ = ATCC 4278¹¹ = DSM 7126¹¹ = JCM 20846¹¹ = LMG 17157¹¹).

**International Journal of Systematic and Evolutionary Microbiology**, 59(12): 3155-3160.

**Keywords**: *Brevundimonas naejangsanensis*; emended description; alkaline soil; identification; caulobacter; systematics; strains; Korea


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By sequence and phylogenetic analyses, the 11 genomic segments of two bovine rotaviruses isolated from clinically infected calves were proven to be derived from the swine-like P[7]G5 genotype. This finding reinforced the hypothesis that interspecies transmission of completely heterologous strains can occur in nature.


Keywords: group-a rotavirus; molecular characterization; porcine rotavirus; p-genotype

Rhodiosin, an antioxidant flavonol glycoside from Rhodiola rosea

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The EtOAc fraction of Rhodiola rosea ethanolic extracts showed a strong antioxidant activity. Through activity-guided fractionation and purification, we isolated two flavonol glycosides, which were identified as the well known flavonoids, rhodionin (1) and rhodiosin (2). To compare their antioxidant activities, we used an authentic aglycone compound, herbacetin (3). Among the compounds tested, rhodiosin (2) exhibited strong antioxidant activity, with IC50 values of 0.21 and 0.15 μM against center dot OH and center dot O2-, respectively. Rhodiosin (2) (100 mg/kg) reduced MDA content in the liver induced by irradiation when given prior to exposure of gamma-radiation.

JOURNAL OF KOREAN SOCIETY FOR APPLIED BIOLOGICAL CHEMISTRY, 52(5): 486-492.

Keywords: antioxidant; gamma-irradiation; Rhodiola rosea; rhodiosin; UWLA
Cancer preventive potential of methanol extracts of *Hypsizigus marmoreus*

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*Hypsizigus marmoreus* has recently become a popular edible mushroom in Asia. Despite its extensive use, the underlying mechanisms of the anticarcinogenic effects on the initiation stage are not precisely known. Therefore, methanol extracts from *H. marmoreus* were prepared and then tested for antiproliferative effects in cancer cells and antimutagenic activities as well as mutagenic capacity using the Ames *Salmonella* mutagenicity test. In addition, the effects on the phase I drug metabolizing enzymes, phase II detoxifying enzymes, and antioxidative activities were evaluated in livers from mice pretreated with methanol extracts from *H. marmoreus* and challenged with benzo[a]pyrene (B[a]P). In the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, methanol extracts from *H. marmoreus* displayed a dose-dependent inhibitory effect against human hepatocarcinoma and colon carcinoma cells. However, equivalent doses did not induce mutagenicity when tested with *Salmonella typhimurium* TA98 and TA100 while exhibiting antimitogenicity against different phases of cell growth, and gradually decreased with further growth of the cells. A cosmid DNA including the EPA biosynthesis gene cluster composed of pfaA-E was isolated from a cosmid library of genomic DNA of *Shewanella* sp. BR-2, named pCosEPA-BR2. An E. coli clone harboring pCosEPA-BR2 produced EPA at a maximum level of 7.5% of total fatty acids, confirming the EPA biosynthesis activity of the cloned gene cluster.


**Keywords**: antimutagenesis; cancer chemoprevention; *Hypsizigus marmoreus*; phase I enzyme; phase II enzyme

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Isolation and characterization of the eicosapentaenoic acid biosynthesis gene cluster from *Shewanella* sp BR-2

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Forty-four eicosapentaenoic acid (EPA)-producing microbial strains were isolated from the intestines of marine fishes. Among them, one strain showing a maximum level of EPA (4.78% of total fatty acids) was identified as *Shewanella* sp. BR-2 on the basis of its 16S rRNA sequence. The EPA content reached a maximum level during the mid-exponential phase of cell growth, and gradually decreased with further growth of the cells. A cosmid library of genomic DNA of *Shewanella* sp. BR-2 was isolated. An E. coli clone harboring pCosEPA-BR2 produced EPA at a maximum level of 7.5% of total fatty acids, confirming the EPA biosynthesis activity of the cloned gene cluster.

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, 19(9): 881-887.

**Keywords**: eicosapentaenoic acid; *Shewanella* sp BR-2; PKS-like module; biosynthesis gene cluster
Cloning, expression, and characterization of a new deoxyribose 5-phosphate aldolase from Yersinia sp EA015

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A new deoC gene encoding deoxyribose 5-phosphate aldolase (DERA) was identified in Yersinia sp. EA015 isolated from soil. The DERA gene had an open reading frame (ORF) of 672 base pairs encoding 223 amino acids to yield a protein of molecular mass 24.8 kDa. The amino acid sequence was 94% identical to that of DERA from Yersinia intermedia ATCC 29909. DERA was over-expressed in Escherichia coli and purified using Ni-NTA affinity chromatography. The specific activity was 137 μmol/min/mg. The Michaelis constant (kₘ value) of DERA was 9.1 mM. DERA was optimally active at pH 6.0 and 50 degrees C. DERA was tolerant to a high concentration (300 mM) of acetaldehyde.


Keywords: aldolase; DERA; Yersinia sp; acetaldehyde
9. Cooperating and Supporting the Other Institution
Real-time observations of intracellular Mg\(^{2+}\) signaling and waves in a single living ventricular myocyte cell

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Despite the important regulatory role of Mg\(^{2+}\) in metabolic pathways, its underlying mechanism is not completely understood at the single-cell level. This study examined the propagation and dynamics of Mg\(^{2+}\) signaling across the cell membrane by employing the real-time visualization of intracellular Mg\(^{2+}\) waves in living ventricular myocytes using a combination of total internal reflection fluorescence microscopy and Nomarski differential interference contrast. Real-time Mg\(^{2+}\) waves and sparks in a living cell membrane were observed using a fluorescent Mg\(^{2+}\) indicator (mag-fluo-4-AM) in the concentration range of 5 aM-5 μM. The intracellular locations of the fluorescent Mg\(^{2+}\) indicator were confirmed by adding Na\(^{+}\)ATP. The Mg\(^{2+}\) sparks and waves showed random temporal propagation patterns in nonhomogeneous substructures. These results show that spatiotemporal intracellular Mg\(^{2+}\) signaling information can be obtained for individual living cells.

**ANALYTICAL CHEMISTRY**, 81(2): 538-542.

**Keywords**: reflection fluorescence microscopy; CA\(^{2+}\) sparks; Mg\(^{2+}\); ion channels; heart-cells

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Midazolam inhibits tumor necrosis factor-alpha-induced endothelial activation: involvement of the peripheral benzodiazepine receptor

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Background: Midazolam is widely used as an intravenous sedative. However, the role of midazolam on vascular endothelial activation is still unknown. The present study explores the action of midazolam on endothelial activation and its role to peripheral benzodiazepine receptor (PBR) in cultured human umbilical vein endothelial cells.

Methods: Intracellular localization of PBR in human umbilical vein endothelial cells was visualized with immunofluorescent staining. Monocyte adhesion and vascular cell adhesion molecule-1 expression were measured with monocyte adhesion assay and Western blot analysis. Involvement of PBR was assessed by using specific antagonists and small interfering RNA against PBR.

Results: PBR was localized in the mitochondria of human umbilical vein endothelial cells. Midazolam significantly inhibited tumor necrosis factor-alpha-induced vascular cell adhesion molecule-1 and monocyte adhesion in a dose-dependent manner (1-30 μM). The midazolam-mediated suppression on the tumor necrosis factor-alpha-induced vascular cell adhesion molecule-1 expression and monocyte adhesion were inhibited by the pretreatment of PK11195 and not inhibited by the flumazenil. Transfection of small interfering RNA for PBR decreased the expression of PBR (18 kDa) in human umbilical vein endothelial cells. Midazolam-mediated suppression on die tumor necrosis factor-alpha-induced vascular cell adhesion molecule-1 expression was abrogated by the transfection of small interfering RNA for PBR.

Conclusion: These results suggest that midazolam has an inhibitory action on the endothelial activation and that its action is related to the activation of peripheral benzodiazepine receptor localized in mitochondria of the endothelial cells.


**Keywords**: in-vitro; atherosclerosis; anesthesia; peripheral benzodiazepine receptor; PBR
Protective role of Clusterin/Apolipoprotein J against neointimal hyperplasia via antiproliferative effect on vascular smooth muscle cells and cytoprotective effect on endothelial cells

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Objective: Clusterin is induced in vascular smooth muscle cells (VSMCs) during atherosclerosis and injury-induced neointimal hyperplasia. However, its functional roles in VSMCs and endothelial cells remain controversial and elusive. This study was undertaken to clarify the role of clusterin in neointimal hyperplasia and elucidate its mechanism of action.

Methods and Results: Adenovirus-mediated overexpression of clusterin (Ad-Clu) repressed TNF-alpha-stimulated expression of MCP-1, fractalkine, ICAM-1, VCAM-1, and MMP-9, leading to inhibition of VSMC migration. Both Ad-Clu and secreted clusterin suppressed VSMC proliferation by inhibiting DNA synthesis, but not by inducing apoptosis. Ad-Clu upregulated p53 and p21, but downregulated cyclins D and E, leading to suppression of pRb phosphorylation and subsequent induction of G1 arrest in VSMCs. Clusterin deficiency augmented VSMC proliferation in vitro and accelerated neointimal hyperplasia in vivo, but concomitantly impaired reendothelialization in wire-injured renal murine arteries. Moreover, Ad-Clu significantly reduced neointimal thickening in balloon-injured rabbit carotid arteries. Clusterin also diminished TNF-alpha-induced apoptosis of human umbilical vein endothelial cells and restored endothelial nitric oxide synthase expression suppressed by TNF-alpha.

Conclusion: These results suggest that upregulation of clusterin during vascular injury may be a protective response against, rather than a causative response to, the development of neointimal hyperplasia.

Keywords: clusterin; VSMC; endothelial cells; proliferation; neointimal hyperplasia

Role of genetic factors and environmental conditions in recombinant protein production for molecular farming

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Plants are generally considered to represent a promising heterologous expression system for the production of valuable recombinant proteins. Minimal upstream plant production cost is a salient feature driving the development of plant expression systems used for the synthesis of recombinant proteins. For such a plant expression system to be fully effective, it is first essential to improve plant productivity by plant biomass after inserting genes of interest into a suitable plant. Plant productivity is related closely to its growth and development, both of which are affected directly by environmental factors. These environmental factors that affect the cultivation conditions mainly include temperature, light, Salinity, drought, nutrition, insects and pests. In addition, genetic factors that affect gene expression at the transcriptional, translational, and post-translational levels are considered to be important factors related to gene expression in plants. Thus, these factors influence both the quality and quantity of recombinant protein produced in transgenic plants. Among the genetic factors, the post-translational process is of particular interest as it influences subcellular localization, protein glycosylation, assembly and folding of therapeutic proteins. Consequently, affecting both protein quantity and biological quality. In this review, we discuss the effects of cultivation condition and genetic factors on recombinant protein production in transgenic plants.

Keywords: environmental factors; glycoprotein; glycosylation; molecular farming; recombinant protein; transgenic plant

ARTERIOSCLEROSIS THROMBOSIS AND VASCULAR BIOLOGY, 29(10): 1558-1564.

BIOTECHNOLOGY ADVANCES, 27(6): 914-923.

Keywords: clusterin; VSMC; endothelial cells; proliferation; neointimal hyperplasia

Keywords: environmental factors; glycoprotein; glycosylation; molecular farming; recombinant protein; transgenic plant
Endothelial progenitor cell homing: prominent role of the IGF2-IGF2R-PLC beta 2 axis

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Homing of endothelial progenitor cells (EPCs) to the neovascular zone is now considered to be an essential step in the formation of vascular networks during embryonic development and also for neovascularization in postnatal life. We report here the prominent role of the insulin-like growth factor 2 (IGF2)/IGF2 receptor (IGF2R) system in promoting EPC homing. With high-level expression of IGF2R in EPCs, IGF2-induced hypoxic conditions stimulated multiple steps of EPC homing in vitro and promoted both EPC recruitment and incorporation into the neovascular area, resulting in enhanced angiogenesis in vivo. Remarkably, all IGF2 actions were exerted predominantly through IGF2R-linked Gı protein signaling and required intracellular Ca²⁺ mobilization induced by the beta 2 isoform of phospholipase C. Together, these findings indicate that locally generated IGF2 at either ischemic or tumor sites may contribute to postnatal vasculogenesis by augmenting the recruitment of EPCs. The utilization of the IGF2/IGF2R system may therefore be useful for the development of novel means to treat angiogenesis-dependent diseases. BLOOD, 113(1): 233-243.

Keywords: acute myocardial-infarction; growth-factor II; matrix metalloproteinases; stem-cell; tumor angiogenesis; arterial injury; precursor cells; insulin; neovascularization

Cooperation between integrin alpha 5 and tetraspan TM4SF5 regulates VEGF-mediated angiogenic activity

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Tetraspan TM4SF5 is highly expressed in a diverse number of tumor types. Here we explore the mechanistic roles of TM4SF5 in angiogenesis. We found that TM4SF5 overexpression correlates with vascular endothelial growth factor (VEGF) expression in SNU449 hepatocytes and with vessel formation in clinical hepatocarcinoma samples. Conditioned media from TM4SF5-expressing cells enhanced viability and tube formation of primary human umbilical vein endothelial cells, and outgrowth of endothelial cells from aorta ring segments, which was abolished by treatment with an anti-VEGF antibody. TM4SF5 retained integrin alpha 5 on the cell surface for VEGF induction, and preincubation with anti-integrin alpha 5 antibody abolished TM4SF5-mediated VEGF expression and secretion. TM4SF5-mediated effects required integrin alpha 5, c-Src, and signal transducediatd activator of tranduction 3 (STAT3). In addition, tumors from nude mice injected with TM4SF5-expressing cells and from clinical human hepatocarcinoma tissues showed enhanced integrin alpha 5 expression, vessel formation, and signaling activity, which were inhibited by administration of anti integrin alpha 5 or -VEGF antibody. This study suggests that TM4SF5 facilitates angiogenesis of neighboring endothelial cells through VEGF induction, mediated by cooperation between TM4SF5 and integrin alpha 5 of epithelial cells. BLOOD, 113(8): 1845-1855.

Keywords: focal adhesion; vascular-permeability; carcinoma cells; Src; motility; kinases; FAK; alpha-5-beta-1
Interleukin-33 (IL-33), a member of the IL-1 cytokine family, is emerging as a new regulator of immune responses and inflammatory vascular diseases. Although IL-33 and its cognate receptor ST2 appear to be expressed in vascular cells, the precise role of IL-33 in the vasculature has not been determined. In this study, we report a novel role of IL-33 as a potent endothelial activator, promoting both angiogenesis and vascular permeability. IL-33 increased proliferation, migration, and morphologic differentiation of human endothelial cells, consistently with increased angiogenesis in vitro. IL-33 also increased endothelial permeability with reduced vascular endothelial-cadherin-facilitated cell-cell junctions in vitro and induced vascular leakage in mouse skin. These effects of IL-33 were blocked by knockdown of ST2. Ligation of IL-33 with ST2 rapidly increased endothelial nitric oxide (NO) production through TRAF6-mediated activation of phosphoinoside-3-kinase, Akt, and endothelial NO synthase. Moreover, pharmacologic or genetic blockade of endothelial NO generation resulted in the inhibition of angiogenesis and vascular hyperpermeability induced by IL-33. These data demonstrate that IL-33 promotes angiogenesis and vascular leakage by stimulating endothelial NO production via the ST2/TRA6-Akt-cNOS signaling pathway. These findings open new perspectives for the role of IL-33 in the pathogenesis of angiogenesis-dependent and inflammatory vascular diseases.

**Keywords**: smooth-muscle-cells; enos-deficient mice; growth-factor; il-1 receptor; ST2 comprise; ve-cadherin; mast-cells

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Prognostic significance of c-Met expression in glioblastomas

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BACKGROUND: The authors investigated whether expression of c-Met protein in glioblastomas is associated with overall survival and biologic features representing tumor invasiveness in patients with glioblastomas. METHODS: Paraffin-embedded specimens of glioblastomas from 62 patients treated in a single institution were assessed by immunohistochemical (IHC) analysis of c-Met expression. On the basis of the clinical data for these patients, the association between c-Met expression and clinicobiologic features representing tumor invasiveness was analyzed. RESULTS: c-Met overexpression was detected in 29.0% (18 of 62) of glioblastomas. In patients with c-Met overexpression, the median survival was 11.7 months (95% confidence interval [95% CI], 9.9 months-13.5 months), compared with a median survival of 14.3 months (95% CI, 7.6 months-21.0 months) for patients whose tumors had no or little expression of c-Met (P = .031). On the radiographic analysis, 9 of 18 patients (50%) with tumors overexpressing c-Met demonstrated invasive and multifocal lesions on the initial magnetic resonance images, whereas only 9 of 44 patients (20.5%) with tumors that expressed no or little c-Met demonstrated these features (P = .30). Using immunohistochemistry, we also found a significant association between c-Met expression and matrix metalloproteinase-2,-9 (P = .020 and P = .013). Furthermore, Myc overexpression was found to be closely correlated with c-Met overexpression on IHC analysis (P = .004). CONCLUSIONS: The authors suggest that c-Met overexpression is associated with shorter survival time and poor treatment response in glioblastomas, the mechanism for which is elevated tumor invasiveness on the molecular and clinical phenotypes. This implies that more effective therapeutic strategies targeting c-Met receptors may have important clinical implications.

**Keywords**: c-Met; glioblastoma; overexpression; survival
Suppression of NF-kappa B activity by NDRG2 expression attenuates the invasive potential of highly malignant tumor cells

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Downregulation of the N-myc downstream-regulated gene 2 (NDRG2) gene is involved in the progression of aggressive forms of cancer, along with the poor prognosis of cancer patients. In the current study, we examined the effect of NDRG2 expression on the metastatic potential of HT1080 human fibrosarcoma and B16F10 murine melanoma cells in both in vitro and in vivo systems. In gelatin zymography, NDRG2 expression remarkably suppressed the matrix metalloproteinase (MMP)-9 activity and slightly inhibited MMP-2 activity of both cell lines. Tumor migration and invasion in vitro were significantly reduced by NDRG2 expression, and NDRG2 inhibited tumor cell proliferation in an anchorage-independent semisolid agar assay. Specifically, we found that NDRG2 affects invasion through suppression of nuclear factor kappa B (NF-kappa B) activity. In animal experiments, subcutaneously injected B16F10-NDRG2 cells showed delayed tumor growth compared with B16F10-mock cells. Furthermore, severe metastasis from primary tumor mass into the draining lymph nodes was observed after injection of B16F10-mock cells, but not with B16F10-NDRG2 cells. Pulmonary metastasis after intravenous injection of B16F10 cells was also reduced by NDRG2 expression. Intra- and peritumoral angiogenesis that is critical for the tumor growth and metastasis was clearly attenuated by NDRG2 expression. Intra- and peritumoral angiogenesis was found in tumors after injection with B16F10-mock cells, while NDRG2 gene in metastatic tumors.

CARCINOGENESIS, 30(6): 927-936.

Keywords : downstream-regulated gene-2; poor-prognosis; cancer; carcinoma; differentiation

Nutlin-3, an Hdm2 antagonist, inhibits tumor adaptation to hypoxia by stimulating the FIH-mediated inactivation of HIF-1 alpha

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The interplay among hypoxia-inducible factor 1-alpha (HIF-1 alpha), p53 and human orthologue of murine double minute 2 (Hdm2) has been introduced as a key event in tumor promotion and angiogenesis. Recently, nutlin-3, a small-molecule antagonist of Hdm2, was demonstrated to inhibit the HIF-1-mediated vascular endothelial growth factor production and tumor angiogenesis. Yet, the mechanism by which nutlin-3 inhibits HIF-1 is an open question. We here addressed the mode-of-action of nutlin-3 with respect to the HIF-1 alpha-p53-Hdm2 interplay. The effect of nutlin-3 on HIF-1 alpha function was examined by reporter analyses, immunoprecipitation and immunoblotting. Nutlin-3 downregulated HIF-1 alpha, which occurred p53-dependently but von Hippel-Lindau-independently. On the contrary, nutlin-3 blunted the hypoxic induction of vascular endothelial growth factor by inactivating HIF-1 even in p53-null cells. The C-terminal transactiivation dducin (reD) of HIF-1 alpha was inactivated by nutlin-3, and furthermore, the factor-inhibiting hypoxia-inducible factor (FIH) hydroxylation of Asn803 was required for the nutlin-3 action. In terms of protein interactions, Hdm2 competed with FIH in reD binding and inhibited the Asn803 hydroxylation both in vivo and in vitro, which facilitated p i0 recruitment. Moreover, nutlin-3 reinforced the FIH binding and Asn803 hydroxylation by inhibiting Hdm2. In conclusion, Hdm2 functionally activates HIF-1 by inhibiting the FIH interaction with CAD, and the Hdm2 inhibition by nutlin-3 results in HIF-1 inactivation and vascular endothelial growth factor suppression. The interplays among HIF-1 alpha, Hdm2, FIH and p300 could be potential targets for treating tumors overexpressing HIF-1 alpha.

CARCINOGENESIS, 30(10): 1768-1775.

Keywords : inducible factor-1 alpha; factor-I; cancer-therapy; p53; mdm2; HIF-1 alpha inhibitor; nutlin-3
The extracellular loop 2 of TM4SF5 inhibits integrin alpha 2 on hepatocytes under collagen type I environment

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Four-transmembrane L6 family member 5 (TM4SF5) and its homolog L6, a tumor antigen, form a four-transmembrane L6 family. TM4SF5 expression causes uncontrolled cell proliferation and angiogenesis. Although other genuine transmembrane 4 superfamily (TM4SF) members co-operate with integrins for cell migration, roles of TM4SF5 in the cellular spreading and migration are unknown. Using hepatocarcinoma cell clones that ectopically express TM4SF5, we found that cross talks via an extracellular interaction between TM4SF5 and integrin alpha 2 in collagen type I environment inhibited integrin alpha 2 functions such as spreading on and migration toward collagen I, which were recovered by suppression of TM4SF5 or structural disturbance of its second extracellular loop using a peptide or mutagenesis. Altogether, the observations suggest that TM4SF5 in hepatocytes negatively regulates integrin alpha 2 function via an interaction between the extracellular loop 2 of TM4SF5 and integrin alpha 2 during cell spreading on and migration through collagen I environment.


Keywords: epithelial-mesenchymal transition; cell motility; tetraspanins; TM4SF5

A near-infrared fluorescence-based optical thermosensor

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A polymeric thermosensor composed of the thermo-responsive block copolymer Pluronic F127 (PF127) and the near-infrared (NIR) dye Cy5.5 can simply monitor, image, and analyze temperature changes. The thermoprobe exhibited linear NIR fluorescent emission changes over a broad temperature range (0-80°C).


Keywords: aggregation; imaging agents; micelles; sensors; polymeric thermosensor
Prognostic implications of and relationship between CpG island hypermethylation and repetitive DNA hypomethylation in hepatocellular carcinoma

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This study aims to determine the relationship between CpG island DNA hypermethylation and global genomic DNA hypomethylation and their prognostic implications in hepatocellular carcinoma. The association of DNA methylation changes with clinicopathologic factors and the chronological ordering of DNA methylation changes along multistep hepatocarcinogenesis were also assessed. Hepatocellular carcinoma (n = 20) and nonneoplastic liver samples (n = 72) were analyzed for their methylation status at 41 CpG island loci and 3 repetitive DNA elements (LINE-1, ALU, and SAT2) using MethyLight or combined bisulfite restriction analysis. After selection of 19 CpG island loci showing cancer-specific DNA methylation, another set of 99 hepatocellular carcinoma samples was analyzed for these loci. The number of methylated genes in hepatocellular carcinoma was significantly higher in hepatocellular carcinoma patients with a cirrhotic liver than in hepatocellular carcinoma patients with a noncirrhotic liver (9.9 versus 7.0, P = 0.001). Hepatocellular carcinoma from female patients showed a higher number of methylated genes than carcinoma patients with a cirrhotic liver than in hepatocellular carcinoma patients with a noncirrhotic liver (9.9 versus 7.0, P = 0.001). Hepatocellular carcinoma from female patients showed a higher number of methylated genes than hepatocellular carcinoma from male patients (11.2 versus 8.4, P = 0.006). The genes CRABP1 and SYK showed significant association between CpG island hypermethylation and patients' poor survival. SAT2 hypomethylation occurred earlier than LINE-1 or ALU hypomethylation along the multistep hepatocarcinogenesis. Depending on the type of CpG island locus, a direct, inverse, or no relationship between CpG island hypermethylation and repetitive DNA hypomethylation was observed in hepatocellular carcinomas. The varying relationships between the hypermethylation of individual CpG island loci and the hypomethylation of repetitive elements suggests that they are not mechanically linked. SYK and CRABP1 hypermethylation may serve as useful tumor markers for prognostication of hepatocellular carcinoma patients.


Keywords: CpG islands; DNA methylation; hepatocellular carcinoma; repetitive DNA element

Screening a genome-wide S. pombe deletion library identifies novel genes and pathways involved in genome stability maintenance

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The maintenance of genome stability is essential for an organism to avoid cell death and cancer. Based on screens for mutant sensitivity against DNA damaging agents a large number of DNA repair and DNA damage checkpoint genes have previously been identified in genetically amenable model organisms. These screens have however not been exhaustive and various genes have been, and remain to be, identified by other means. We therefore screened a genome-wide Schizosaccharomyces pombe deletion library for mutants sensitive against various DNA damaging agents. Screening the library on different concentrations of these genotoxins allowed us to assign a semi-quantitative score to each mutant expressing the degree of sensitivity. We isolated a total of 229 mutants which show sensitivity to one or more of the DNA damaging agents used. This set of mutants was significantly enriched for processes involved in DNA replication, DNA repair, DNA damage checkpoint, response to UV, mating type switching, telomere length maintenance and meiosis, and also for processes involved in the establishment and maintenance of chromatin architecture (notably members of the SAGA complex), transcription (members of the CCR4-Not complex) and microtubule related processes (members of the DASH complex). We also identified 23 sensitive mutants which had previously been classified as "sequence orphan" or as "conserved hypothetical". Among these, we identified genes showing extensive homology to CUP, Stra13, Ybp1/Ybp2, Human Fragile X mental retardation interacting protein NUF1P1, and Aprataxin. The identification of these homologues will provide a basis for the further characterisation of the role of these conserved Proteins in the genetically amenable model organism S. pombe.

DNA REPAIR, 8(5): 672-679.

Keywords: Schizosaccharomyces pombe; DNA repair; DNA damage checkpoint; genome-wide deletion library; CtIP; Aprataxin
Genome-wide comparative analysis of the *Brassica rapa* gene space reveals genome shrinkage and differential loss of duplicated genes after whole genome triplication

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*Brassica rapa* is one of the most economically important vegetable crops worldwide. Owing to its agronomic importance and phylogenetic position, *B. rapa* provides a crucial reference to understand polyploidy-related crop genome evolution. The high degree of sequence identity and remarkably conserved genome structure between *Arabidopsis* and *Brassica* genomes enables comparative tiling sequencing using Arabidopsis sequences as references to select the counterpart regions in *B. rapa*, which is a strong choice of structural and comparative crop genomics. We assembled 65.8 megabase-pairs of non-redundant euchromatic sequence of *B. rapa* and compared this sequence to the Arabidopsis genome to investigate chromosomal relationships, macrosynteny blocks, and microsynteny within blocks. The triplicated *B. rapa* genome contains only approximately twice the number of genes as in Arabidopsis because of genome shrinkage. Genome comparisons suggest that *B. rapa* has a distinct organization of ancestral genome blocks as a result of recent whole genome triplication followed by a unique diploidization process. A lack of the most recent whole genome duplication (3R) event in the *B. rapa* genome, atypical of other Brassica genomes, may account for the emergence of *B. rapa* from the Brassica progenitor around 8 million years ago. This work demonstrates the potential of using comparative tiling sequencing for genome aenoysis of crop species. Based on a comparative analysis of the *B. rapa* sequences and the *Arabidopsis* genome, it appears that polyploidy and chromosomal diploidization are ongoing processes that collectively stabilize the *B. rapa* genome and facilitate its evolution.

Article 274

**Formation of vitamin A lipid droplets in pancreatic stellate cells requires albumin**

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Objective: Quiescent pancreatic stellate cells (PSCs) store vitamin A as cytoplasmic lipid droplets, and, when activated by profibrogenic stimuli, they transform into myofibroblast-like cells characterised by the loss of vitamin A droplets. Activation of stellate cells is central to fibrogenesis, but the mechanism for the formation of vitamin A droplets and its relationship to stellate cell activation remain unclear.

Methods: With use of cultured PSCs, an attempt was made to characterise the function of albumin endogenously expressed in stellate cells.

Results: Albumin is endogenously expressed in quiescent PSCs, localised in cytoplasmic lipid droplets, and, when activated by profibrogenic stimuli, they transform into myofibroblast-like cells characterised by the loss of vitamin A droplets.

Keywords: retinol-binding-protein; TGF-beta; liver fibrosis; analbuminemic rats; serum-albumin; storing cells; pancreatic stellate cells; PSCs; vitamin A
Fenofibrate differentially regulates plasminogen activator inhibitor-1 gene expression via adenosine monophosphate-activated protein kinase-dependent induction of orphan nuclear receptor small heterodimer partner

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Plasminogen activator inhibitor type I (PAI-1) is a marker of the fibrinolytic system and serves as a possible predictor for hepatic metabolic syndromes. Fenofibrate, a peroxisome proliferator-activated receptor alpha (PPAR alpha) agonist, is a drug used for treatment of hyperlipidemia. Orphan nuclear receptor small heterodimer partner (SHP) plays a key role in transcriptional repression of crucial genes involved in various metabolic pathways. In this study, we show that fenofibrate increased PAI-1 gene expression in cultured liver cells and in the normal and diabetic mouse liver by activating the adenosine monophosphate-activated protein kinase (AMPK signaling pathway in a PPAR alpha-independent manner. Administration of transforming growth factor beta (TGF-beta) or a methionine-deficient and choline-deficient (MCD) diet to induce the progressive fibrosing steatohepatitis model iPK 57BL/6 mice was significantly reversed by fenofibrate via AMPK-mediated induction of SHP gene expression with a dramatic decrease iPKPAI-1 messenger RNA (mRNA) and protein expression along with other fibrotic marker genes. No reversal was observed in SHP null mice treated with fenofibrate. Treatment with another PPAR alpha agonist, WY14643, showed contrasting effects on these marker gene expressions in wild-type and SHP null mice, demonstrating the specificity of fenofibrate in activating AMPK signaling. Fenofibrate exhibited a differential inhibitory pattern on PAI-1 gene expression depending on the transcription factors inhibited by SHP. Conclusion: By demonstrating that a PPAR alpha-independent fenofibrate-AMPK-SHP regulatory cascade can play a key role in PAI-1 gene down-regulation and reversal of fibrosis, our study suggests that various AMPK activators regulating SHP might provide a novel pharmacologic option in ameliorating hepatic metabolic syndromes.

HEPATOLOGY, 50(3): 880-892.

Keywords: fibrosis; PAI-1; SHP; PPAR alpha; AMPK

Planning the Human Variome Project: the Spain report

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Human Variome Project Planning Meeting

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The remarkable progress in characterizing the human genome sequence, exemplified by the Human Genome Project and the HapMap Consortium, has led to the perception that knowledge and the tools (e.g., microarrays) are sufficient for many if not most biomedical research efforts. A large amount of data from diverse studies proves this perception inaccurate at best, and at worst, an impediment for further efforts to characterize the variation in the human genome. Because variation in genotype and environment are the fundamental basis to understand phenotypic variability and heritability at the population level, identifying the range of human genetic variation is crucial to the development of personalized nutrition and medicine. The Human Variome Project (HVP; http://www.humanvariomeproject.org/) was proposed initially to systematically collect mutations that cause human disease and create a cyber infrastructure to link locus specific databases (LSDB). We report here the discussions and recommendations from the 2008 HVP planning meeting held in San Feliu de Guixols Spain, in May 2008.

HUMAN MUTATION, 30(4): 496-510.

Keywords: variome; genome; mutation; database; genetic disease
SCAMP5 links endoplasmic reticulum stress to the accumulation of expanded polyglutamine protein aggregates via endocytosis inhibition

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Accumulation of expanded polyglutamine proteins is considered to be a major pathogenic biomarker of Huntington disease. We isolated SCAMP5 as a novel regulator of cellular accumulation of expanded polyglutamine track protein using cell-based aggregation assays. Ectopic expression of SCAMP5 augments the formation of ubiquitin-positive and detergent-resistant aggregates of mutant huntingtin (mHTT). Expression of SCAMP5 is markedly increased in the striatum of Huntington disease patients and is induced in cultured striatal neurons by endoplasmic reticulum (ER) stress or by mHTT. The increase of SCAMP5 impairs endocytosis, which in turn enhances mHTT aggregation. On the contrary, down-regulation of SCAMP5 alleviates ER stress-induced mHTT aggregation and SCAMP5 alleviates ER. Moreover, stereotactic injection into the striatum and intraperitoneal injection of tunicamycin significantly increase mHTT aggregation in the striatum of R6/2 mice and in the cortex of N171-82Q mice, respectively. Taken together, these results suggest that exposure to ER stress increases SCAMP5 in the striatum, which positively regulates mHTT aggregation via the endocytosis pathway.

*Keywords: ubiquitin-proteasome system; inclusion-body formation; neuronal cell-death; er stress; mutant huntingtin; prone proteins; neurodegenerative disease; intranuclear inclusions; autophagic clearance; beta neurotoxicity; SCAMP5*

DAX-1 acts as a novel corepressor of orphan nuclear receptor HNF4 alpha and negatively regulates gluconeogenic enzyme expression

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DAX-1 (dosage-sensitive sex reversal adrenal hypoplasia congenital critical region on X chromosome, gene 1) is an atypical member of the nuclear receptor family and acts as a corepressor of a number of nuclear receptors. HNF4 alpha (hepatocyte nuclear factor 4 alpha) is a liver-enriched transcription factor that controls the expression of a variety of genes involved in cholesterol, fatty acid, and glucose metabolism. Here we show that DAX-1 inhibits transcriptional activity of HNF4 alpha and modulates hepatic gluconeogenic gene expression. Hepatic DAX-1 expression is increased by insulin and SIK1 (salt-inducible kinase 1), whereas it is decreased in high fat diet-fed and diabetic mice. Coimmunoprecipitation assay from mouse liver samples depicts that endogenous DAX-1 interacts with HNF4 alpha in vivo. In vivo chromatin immunoprecipitation assay affirms that the recruitment of DAX-1 on the phosphoenolpyruvate carboxykinase (PEPCK) gene promoter is inversely correlated with the recruitment of PGC-1 alpha and HNF4 alpha under fasting and refeeding, showing that DAX-1 could compete with the coactivator PGC-1 alpha for binding to HNF4 alpha. Adenovirus-mediated expression of DAX-1 decreased both HNF4 alpha- and forskolin-mediated gluconeogenic gene expressions. In addition, knockdown of DAX-1 partially reverses the insulin-mediated inhibition of gluconeogenic gene expression in primary hepatocytes. Finally, DAX-1 inhibits PEPCK and glucose-6-phosphatase gene expression and significantly lowers fasting blood glucose level in high fat diet-fed mice, suggesting that DAX-1 can modulate hepatic gluconeogenesis in vivo. Overall, this study demonstrates that DAX-1 acts as a corepressor of HNF4 alpha to negatively regulate hepatic gluconeogenic gene expression in liver.

*Keywords: adrenal hypoplasia congenita; hormone-receptor; DNA-binding; hepatic gluconeogenesis; transcription factor; coactivator torc2; protein-kinase; insulin; transactivation; shp; DAX-1*
Hepatocyte growth factor family negatively regulates hepatic gluconeogenesis via induction of orphan nuclear receptor small heterodimer partner in primary hepatocytes

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Hepatic gluconeogenesis is tightly balanced by opposing stimulatory (glucagon) and inhibitory (insulin) signaling pathways. Hepatocyte growth factor (HGF) is a pleiotropic growth factor that mediates diverse biological processes. In this study, we investigated the effect of HGF and its family member, macrophage-stimulating factor (MSP), on hepatic gluconeogenesis in primary hepatocytes. HGF and MSP significantly repressed expression of the key hepatic gluconeogenic enzyme genes, phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (Glc-6-Pase) and reduced glucose production. HGF and MSP activated small heterodimer partner (SHP) gene promoter and induced SHP mRNA and protein levels, and the effect of HGF and MSP on SHP gene expression was demonstrated to be mediated via activation of the AMP-activated protein kinase (AMPK) signaling pathway. We demonstrated that upstream stimulatory factor-1 (USF-1) specifically mediated HGF effect on SHP gene expression, and inhibition of USF-1 by dominant negative USF-1 significantly abrogated HGF-mediated activation of the SHP promoter. Elucidation of the mechanism showed that USF-1 bound to E-box-1 in the SHP promoter, and HGF increased USF-1 DNA binding on the SHP promoter via AMPK and DNA-dependent protein kinase-mediated pathways. Adenoviral overexpression of USF-1 significantly repressed PEPCK and Glc-6-Pase gene expression and reduced glucose production. Knockdown of endogenous SHP expression significantly reversed this effect. Finally, knockdown of SHP or inhibition of AMPK signaling reversed the ability of HGF to suppress hepatocyte nuclear factor 4 alpha-mediated up-regulation of PEPCK and Glc-6-Pase gene expression along with the HGF- and MSP-mediated suppression of gluconeogenesis. Overall, our results suggest a novel signaling pathway through HGF/AMPK/USF-1/SHP to inhibit hepatic gluconeogenesis.

Keywords: activated protein-kinase; hepg2 liver-cells; signaling pathway; gene-expression; diabetic-nephropathy; HGF; MSP

GP91phox/NADPH oxidase (NOX) 2 is the main catalytic component of NOX, which mediates the phagocytic killing of ingested pathogens via the production of reactive oxygen species (ROS). However, Mycobacterium tuberculosis (MtB) is relatively resistant to the microbicidal effects of ROS. Thus, the exact roles of NOX2 in the innate immune control against MtB infection are not fully resolved. In this study, we show that NOX2 is essential for TLR2-dependent inflammatory responses and 1,25-dihydroxyvitamin D3 (1,25D3)-mediated antimiicrobial activity against MtB via cathelicidin expression. NOX2-null macrophages prominently abrogated MtB-induced ROS production and inflammatory signaling activation in a TLR2-dependent manner. MtB triggered a physical association between NOX2 and TLR2. In addition, the knockdown of NOX2 inhibited 1,25D3-triggered antimiicrobial activity against viable MtB through the modulation of cathelicidin expression in human macrophages. Treatment of NOX2 knocked down cells with cathelicidin restored the 1,25D3-induced antimiicrobial effect, suggesting that the NOX2-dependent induction of cathelicidin in macrophages is part of a defensive strategy against MtB. Furthermore, cathelicidin expression was required for the MtB-induced release of ROS and the production of proinflammatory cytokines/chemokines, indicating a positive circuit of inflammation in response to MtB. Our data collectively demonstrate a novel regulatory mechanism for TLR2-dependent innate responses to MtB involving crosstalk between NOX2 and TLR2 and the expression of cathelicidin.

Keywords: toll-like receptor; antimicrobial peptide il-37; kinase-c-zeta; NF-kappa B; reactive oxygen; phosphatidylinositol 3-kinase; macrophage activation; dendritic cells; cutting edge; tuberculosis; NADPH oxidase

Article 280

Hepatocyte growth factor family negatively regulates hepatic gluconeogenesis via induction of orphan nuclear receptor small heterodimer partner in primary hepatocytes

Article 281

NADPH oxidase 2 interaction with TLR2 is required for efficient innate immune responses to mycobacteria via cathelicidin expression

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Keywords: toll-like receptor; antimicrobial peptide il-37; kinase-c-zeta; NF-kappa B; reactive oxygen; phosphatidylinositol 3-kinase; macrophage activation; dendritic cells; cutting edge; tuberculosis; NADPH oxidase

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Surface molecules of pathogens play an important role in stimulating host immune responses. Elucidation of file signaling pathways activated by critical surface molecules in host cells provides insight into the molecular pathogenesis resulting from bacteria-host interactions. MspTL is the most abundant outer membrane protein of *Treponema lecitinholyticum*, which is associated with periodontitis, and induces expression of a variety of proinflammatory factors. Although bacteria and bacterial components like LPS and flagellin are known to induce IFN-beta, induction by bacterial surface proteins has not been reported. In the present study, we investigated *MspTL*-mediated activation of signaling pathways stimulating up-regulation of IFN-beta and IFN-stimulated genes in a human monocytic cell line, THP-1 cells, and primary cultured human gingival fibroblasts. *MspTL* treatment of the cells induced IFN-beta and the IFN-stimulated genes IFN-gamma-inducible protein-10 (IP-10) and RANTES. A neutralizing late-IFN-beta Ab significantly reduced the expression of IP-10 and RANTES, as well as STAT-1 activation, which was also induced by *MspTL*. Experiments using specific small interfering RNA showed that *MspTL* activated TANK-binding kinase 1 (TBK1), but not inducible IKB kinase (IKKi). *MspTL* also induced dimerization of IFN regulatory factor-3 (IRF-3) and translocation into the nucleus. The lipid rapid-disrupting agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited the agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited the agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited the agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited the agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited the agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited the agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited the agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited the agents methyl-beta-cyclodextrin, nystatin, and filipin inhibited.
Quantitative analysis of an aberrant glycoform of TIMP1 from colon cancer serum by L-PHA-Enrichment and SISCAPA with MRM mass spectrometry

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Variations in glycosylation levels or in the glycoprofile of a certain glycoprotein in tumor-related sera have been widely reported and can be used as a means of differentiation. However, quantitative mass analysis of glycoproteins is difficult because of their high structural complexity and low mass sensitivity of glycopeptides. Therefore, more powerful technologies are required for the discovery of these potential biomarkers. Tissue inhibitor of metalloproteinase 1 (TIMP1), a glycoprotein typically present at a low concentration in serum, is known to be aberrantly glycosylated in colorectal cancer cell lines as a result of the terminal addition of beta-1,6-N-acetylglucosamine by N-acetylglucosaminyltransferase-V (GnT-V), which is reportedly up-regulated in invasive/metastatic cancer cells. In this report, a highly sensitive method is presented for the quantitative analysis of aberrant GlcNAcylated TIMP1 in the serum of colorectal cancer (CRC) patients. Glycoproteins having an N-linked glycan terminating with beta-1,6-GlcNAc were enriched by phytohemagglutinin-L (L-PHA), a lectin that specifically recognizes the beta-1,6-GlcNAc moiety of N-linked glycan. The L-PHA-enriched glycoproteins were digested in solution with trypsin. With the use of a monoclonal anti-peptide TIMP1 antibody linked covalently to magnetic beads, a unique target peptide (antigen) of TIMP1 was immuno-enriched from the L-PHA-enriched tryptic digests and analyzed quantitatively by multiple reaction monitoring (MRM) mass analysis. The systematic coupling of L-PHA lectin enrichment followed by stable isotope standards and capture by anti-peptide antibodies (SISCAPA) with MRM mass analysis afforded quantification of TIMP1 at attomolar (10^-18) concentrations. An abnormally GlcNAcylated substoichiometric TIMP1 isoform was quantified at approximately 0.8 ng/mL serum, using sample equivalent to only 1.7 μL of serum from a CRC patient. This approach provides a useful tool for the quantification of a specific aberrant glycoform from human serum containing a variety of protein isoforms and may be helpful in studies of biological function as it pertains to protein glycan heterogeneity.

JOURNAL OF PROTEOME RESEARCH, 8(9): 4216-4224.

Keywords: peptide quantitation; aberrant glycoforms; TIMP1; L-PHA; anti-peptide antibody; SISCAPA; MRM

Proteolytic activation of the 1918 influenza virus hemagglutinin

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Proteolytic activation of the hemagglutinin (HA) protein is indispensable for influenza virus infectivity, and the tissue expression of the responsible cellular proteases impacts viral tropism and pathogenicity. The HA protein critically contributes to the exceptionally high pathogenicity of the 1918 influenza virus, but the mechanisms underlying cleavage activation of the 1918 HA have not been characterized. The neuraminidase (NA) protein of the 1918 influenza virus allows trypsin-independent growth in canine kidney cells (MDCK). However, it is at present unknown if the 1918 NA, like the NA of the closely related strain A/WSN/33, facilitates HA cleavage activation by recruiting the protease plasminogen. Moreover, it is not known which pulmonary proteases activate the 1918 HA. We provide evidence that NA-dependent, trypsin-independent cleavage activation of the 1918 HA is cell line dependent and most likely plasminogen independent since the 1918 NA failed to recruit plasminogen and neither exogenous plasminogen nor the presence of the A/WSN/33 NA promoted efficient cleavage of the 1918 HA. The transmembrane serine protease TMPRSS4 wnt fen d to be expressed in ln i tissue and wnt cleavage activation by recruiting the protease plasminogen. Moreover, it is not known which pulmonary proteases activate the 1918 HA. We provide evidence that NA-dependent, trypsin-independent cleavage activation of the 1918 HA is cell line dependent and most likely plasminogen independent since the 1918 NA failed to recruit plasminogen and neither exogenous plasminogen nor the presence of the A/WSN/33 NA promoted efficient cleavage of the 1918 HA. The transmembrane serine protease TMPRSS4 wnt fen d to be expressed in ln i tissue and wnt shown to cleave the 1918 HA. Accordingly, coexpression of the 1918 HA with TMPRSS4 or the previously identified HA-processing protease TMPRSS2 allowed trypsin-independent infection by pseudotypes bearing the 1918 HA, indicating that these proteases might support 1918 influenza virus spread in the lung. In summary, we show that the previously reported 1918 NA-dependent spread of the 1918 influenza virus is a cell line-dependent phenomenon and is not due to plasminogen recruitment by the 1918 NA. Moreover, we provide evidence that TMPRSS2 and TMPRSS4 activate the 1918 HA by cleavage and therefore may promote viral spread in lung tissue.

JOURNAL OF VIROLOGY, 83(7): 3200-3211.

Keywords: pandemic virus; receptor-binding; serine-protease; cathepsin-I; DC-SIGN; 1918 HA
Due to dual susceptibility to both human and avian influenza A viruses, pigs are believed to be effective intermediate hosts for the spread and production of new viruses with pandemic potential. In early 2008, two swine H5N2 viruses were isolated from our routine swine surveillance in Korea. The sequencing and phylogenetic analysis of surface proteins revealed that the Sw/Korea/C12/08 and Sw/Korea/C13/08 viruses were derived from avian influenza viruses of the Eurasian lineage. However, although the Sw/Korea/C12/08 isolate is an entirely avian-like virus, the Sw/Korea/C13/08 isolate is an avian-swine-like reassortant with the PB2, PA, NP, and M genes coming from a 2006 Korean swine H3N1-like virus. The molecular characterization of the two viruses indicated an absence of significant mutations that could be associated with virulence or binding affinity. However, animal experiments showed that the reassortant Sw/Korea/C13/08 virus was more adapted and was more readily transmitted than the purely avian-like virus in a swine experimental model but not in ferrets. Furthermore, seroprevalence in swine sera from 2006 to 2008 suggested that avian H5 viruses have been infecting swine since 2006. Although there are no known potential clinical implications of the avian-swine reassortant virus for pathogenicity in pigs or other species, including humans, at present, the efficient transmissibility of the swine-adapted H5N2 virus could facilitate virus spread and could be a potential model for pandemic, highly pathogenic avian influenza (e.g., H5N1 and H7N7) virus outbreaks or a pandemic strain itself.

*Keywords*: multiple sequence alignment; RT-PCR assays; molecular-basis; H3N2 viruses; H5N2 viruses; phylogenetic analysis

Cells infected by viruses utilize interferon (IFN)-mediated and p53-mediated irreversible cell cycle arrest and apoptosis as part of the overall host surveillance mechanism to ultimately block viral replication and dissemination. Viruses, in turn, have evolved elaborate mechanisms to subvert IFN- and p53-mediated host innate immune responses. Kaposi's sarcoma-associated herpesvirus (KSHV) encodes several viral IFN regulatory factors (vIRF1 to vIRF4) within a cluster of loci, their functions being primarily to inhibit host IFN-mediated innate immunity and deregulate p53-mediated cell growth control. Despite its significant homology and similar genomic location to other vIRFs, vIRF4 is distinctive, as it does not target and antagonize host IFN-mediated signal transduction. Here, we show that KSHV vIRF4 interacts with the murine double minute 2 (MDM2) E3 ubiquitin ligase, leading to the reduction of p53, a tumor suppressor, via proteasome-mediated degradation. The central region of vIRF4 is required for its interaction with MDM2, which led to the suppression of MDM2 autoubiquitination and, thereby, a dramatic increase in MDM2 stability. Consequently, vIRF4 expression markedly enhanced p53 ubiquitination and degradation, effectively suppressing p53-mediated apoptosis. These results indicate that KSHV vIRF4 targets and stabilizes the MDM2 E3 ubiquitin ligase to facilitate the proteasome-mediated degradation of p53, perhaps to circumvent host growth surveillance and facilitate viral replication in infected cells. Taken together, the indications are that the downregulation of p53-mediated cell growth control is a common characteristic of the four KSHV vIRFs and that p53 is indeed a key factor in the host's immune surveillance program against viral infections.

*Keywords*: ring-finger domain; signal-transduction; ubiquitin ligase; KSHV

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**Isolation and genetic characterization of H5N2 influenza viruses from pigs in Korea**

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**Kaposi's sarcoma-associated herpesvirus viral interferon regulatory factor 4 targets MDM2 to deregulate the p53 tumor suppressor pathway**

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**JOURNAL OF VIROLOGY**, 83(9): 4205-4215.

Crucial role for Mst1 and Mst2 kinases in early embryonic development of the mouse

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Mammalian sterile 20-like kinases 1 and 2 (Mst1 and Mst2, respectively) are potent serine/threonine kinases that are involved in cell proliferation and cell death. To investigate the physiological functions of Mst1 and Mst2, we generated Mst1 and Mst2 mutant mice. Mst1−/− and Mst2−/− mice were viable and fertile and developed normally, suggesting possible functional overlaps between the two genes. A characterization of heterozygous and homozygous combinations of Mst1 and Mst2 mutant mice showed that mice containing a single copy of either gene underwent normal organ development; however, Mst1−/−; Mst2−/− mice lacking both Mst1 and Mst2 genes started dying in utero at approximately embryonic day 8.5. Mst1−/−; Mst2−/− mice exhibited severe growth retardation, failed placental development, impaired yolk sac/embryo vascular patterning and primitive hematopoiesis, increased apoptosis in placentas and embryos, and disorganized proliferating cells in the embryo proper. These findings indicate that both Mst1 and Mst2 kinases play essential roles in early mouse development, regulating placental development, vascular patterning, primitive hematopoiesis, and cell proliferation and survival.

MOLECULAR AND CELLULAR BIOLOGY, 29(23): 6309-6320.

Keywords: cell-cycle exit; tumor-suppressor; promotes apoptosis; protein-kinase; nuclear translocation; proliferation arrest; sterile-20 kinase; caspase cleavage; down-regulation; histone h2b

A regulatory polymorphism at position-309 in PTPRCAP is associated with susceptibility to diffuse-type gastric cancer and gene expression

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PTPRCAP (CD45-AP) is a positive regulator of protein tyrosine phosphatase PTPRC (CD45), which activates Src family kinases implicated in tumorigenesis. Single-nucleotide polymorphism (SNP) rs869736 located at position -309 of the PTPRCAP promoter was associated with susceptibility to diffuse-type gastric cancer in the current case-control study. The minor-allele homozygote was significantly associated with a 2.5-fold increased susceptibility to diffuse-type gastric cancer (P = .0021, n = 252), but not to intestinal-type (P = .30, n = 178), versus the major-allele homozygote, when comparing unrelated Korean patients with healthy controls (n = 406). Nine other SNPs were in nearly perfect linkage disequilibrium (r^2 >= 0.97) with this SNP, exhibiting the same association, and spread out for 26 kb on chromosome 11q13.1 covering RPS6KB2, PTPRCAP, CORO1B, and GPR152. Among the four genes, however, only PTPRCAP expression was affected by haplotypes of the 10 SNPs. Endogenous transcript levels of PTPRCAP were linearly correlated with copy numbers (0, 1, and 2) of the risk-haplotype (P = .0060) in 12 lymphoblastoid cells derived from blood samples, but those of the other three genes were not. Furthermore, the cancer-risk, minor-allele T of rs869736 increased both promoter activity and specific nuclear protein-binding affinity than the nonrisk, major-allele G in luciferase reporter and electrophoretic mobility shift assays, respectively. Accordingly, the minor allele of rs869736 correlated with copy numbers (0, 1, and 2) of the 10 SNPs.

Keywords: CD45 phosphotyrosine phosphatase; protein-tyrosine kinases; pylori caga protein; src family kinases; CD45-associated protein; helicobacter-pylori; e-cadherin; haplotype reconstruction; colon-cancer
Molecular phylogeography of East Asian Kirengeshoma (Hydrangeaceae) in relation to Quaternary climate change and landbridge configurations

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Kirengeshoma comprises two species inhabiting warm temperate-deciduous forests in East China/South Japan (Kirengeshoma palmata) and South Korea (Kirengeshoma koreana). A survey of chloroplast (cp) DNA and inter-simple sequence repeats (ISSRs) variation in Kirengeshoma was carried out to determine the population history of a plant taxon around the East China Sea (ECS). CpDNA and ISSRs revealed lower genetic divergence between China and Japan relative to the other contrasts, in line with intrageneric classification. Molecular dating suggests that K. koreana diverged at the Plio-Pleistocene boundary from the Japanese populations, whereas the latter migrated into China during the early-to-mid Pleistocene via the ECS basin. Vicariant segregation of Chinese and Japanese populations likely occurred during the mid-Pleistocene. Mismatch distributions and neutrality tests indicated that Chinese populations expanded their range during the Late Pleistocene, probably during a cold period, whereas those from Japan showed no significant population growth. We conclude that the current distribution and differentiation of components of presently isolated warm temperate-deciduous forests in China, Japan and Korea likely resulted from a combination of relatively ancient vicariant and immigration events, and those from Japan were particularly sensitive to range fragmentation and long-term refugial isolation throughout the Late Pleistocene.

NEW PHYTOLOGIST, 183(2): 480-495.

Keywords: cpDNA; ISSRs; Kirengeshoma; landbridge; phylogeography; Quaternary; vicariance

Mouse period 2 mRNA circadian oscillation is modulated by PTB-mediated rhythmic mRNA degradation

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Circadian mRNA oscillations are the main feature of core clock genes. Among them, period 2 is a key component in negative-feedback regulation, showing robust diurnal oscillations. Moreover, period 2 has been found to have a physiological role in the cell cycle or the tumor suppression. The present study reports that 3'-untranslated region (UTR)-dependent mRNA decay is involved in the regulation of circadian oscillation of period 2 mRNA. Within the mper2 3'UTR, both the CU-rich region and polypyrimidine tract-binding protein (PTB) are more responsible for mRNA stability and degradation kinetics than are other factors. Depletion of PTB with RNAi results in mper2 mRNA stabilization. During the circadian oscillations of mper2, cytoplasmic PTB showed a reciprocal expression profile compared with mper2 mRNA and its peak amplitude was increased when PTB was depleted. This report on the regulation of mper2 proposes that post-transcriptional mRNA decay mediated by PTB is a fine-tuned regulatory mechanism that includes dampening-down effects during circadian mRNA oscillations.


Keywords: tract-binding-protein; induced phase-shifts; gene-expression; posttranscriptional regulation; 3'-untranslated region; peripheral-tissues; tumor suppression; mice lacking
**EVOG: a database for evolutionary analysis of overlapping genes**

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Overlapping genes are defined as a pair of genes whose transcripts are overlapped. Recently, many cases of overlapped genes have been investigated in various eukaryotic organisms; however, their origin and transcriptional control mechanism has not yet been clearly determined. In this study, we implemented evolutionary visualizer for overlapping genes (EVOG), a Web-based DB with a novel visualization interface, to investigate the evolutionary relationship between overlapping genes. Using this technique, we collected and analyzed all overlapping genes in human, chimpanzee, orangutan, marmoset, rhesus, cow, dog, mouse, rat, chicken, Xenopus, zebrafish and Drosophila. This integrated database provides a manually curated database that displays the evolutionary features of overlapping genes. The EVOG DB components included a number of overlapping genes (10074 in human, 10 009 in chimpanzee, 51 001 in marmoset, 219 in rhesus, 3627 in cow, 209 in dog, 10 700 in mouse, 7987 in rat, 1439 in chicken, 597 in Xenopus, 2457 in zebrafish and 4115 in Drosophila). The EVOG database is very effective and easy to use for the analysis of the evolutionary process of overlapping genes when comparing different species. Therefore, EVOG could potentially be used as the main tool to investigate the evolution of the human genome in relation to disease by comparing the expression profiles of overlapping genes. EVOG is available at http://neobio.cs.pusan.ac.kr/evog/. 

**NUCLEIC ACIDS RESEARCH, 37(SI): D698-D702.**

**Keywords**: antisense-RNA; transcripts; evolutionary visualizer; overlapping genes

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**GDSL lipase-like 1 regulates systemic resistance associated with ethylene signaling in Arabidopsis**

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Systemic resistance is induced by necrotizing pathogenic microbes and non-pathogenic rhizobacteria and confers protection against a broad range of pathogens. Here we show that Arabidopsis GDSL LIPASE-LIKE 1 (GLIP1) plays an important role in plant immunity, eliciting both local and systemic resistance in plants. GLIP1 functions independently of salicylic acid but requires ethylene signaling. Enhancement of GLIP1 expression in plants increases resistance to pathogens including *Alternaria brassicicola*, *Erwinia carotovora* and *Pseudomonas syringae*, and limits their growth at the infection site. Furthermore, local treatment with GLIP1 proteins is sufficient for the activation of systemic resistance, inducing both resistance gene expression and pathogen resistance in systemic leaves. The *PDF1.2*-inducing activity accumulates in petiole exudates in a GLIP1-dependent manner and is fractionated in the size range of less than 10 kDa as determined by size exclusion chromatography. Our results demonstrate that GLIP1-elicited systemic resistance is dependent on ethylene signaling and provide evidence that GLIP1 may mediate the production of a systemic signaling molecule(s). 

**PLANT JOURNAL, 58(2): 235-245.**

**Keywords**: Arabidopsis; GDSL lipase; systemic resistance; ethylene; salicylic acid; jasmonic acid
Identification of a serodiagnostic antigen, legumain, by immunoproteomic analysis of excretory-secretory products of *Clonorchis sinensis* adult worms

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*Clonorchis sinensis*, the Chinese liver fluke, is the causative agent of clonorchiasis as well as liver and biliary diseases. The excretory-secretory products (ESPs) of the parasites play important roles in host-parasite interactions. In this study, we have investigated the proteome of ESPs obtained from *C. sinensis* adult worms. Although the full genome database of *C. Sinensis* is not yet available, we have successfully identified 62 protein spots using 2-DE-based mass analysis and EST database of *C. sinensis*. The proteins identified include detoxification enzymes, such as glutathione S-transferase and thioredoxin peroxidase, myoglobin and a number of cysteine proteases that are expressed abundantly. In order to identify potential targets for the diagnosis and therapy of clonorchiasis, we conducted immunoblot analysis of the ESPs proteome using the sera obtained from *C. sinensis* adult worms. Although the full genome database of *C. Sinensis* is not yet available, we have successfully identified 62 protein spots using 2-DE-based mass analysis and EST database of *C. sinensis*. The proteins identified include detoxification enzymes, such as glutathione S-transferase and thioredoxin peroxidase, myoglobin and a number of cysteine proteases that are expressed abundantly. In order to identify potential targets for the diagnosis and therapy of clonorchiasis, we conducted immunoblot analysis of the ESPs proteome using the sera obtained from clonorchiasis patients and identified legumains and cysteine proteases as antigens present in the ESPs. Although the cysteine proteases were previously reported to elicit antigenicity, the legumains are found herein for the first time as a serological antigen of *C. sinensis*. To confirm these findings, we expressed recombinant legumain in *Escherichia coli* and verified that recombinant legumain also functions as a potent antigen against the sera of clonorchiasis patients. Our results illustrate the validity of immuno-proteomic approaches in the identification of serodiagnostic antigens in the parasites.


**Keywords**: antigen; *Clonorchis sinensis*; excretory-secretory products; legumain; proteome

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**p35 interacts with alpha-tubulin and organelle proteins: nuclear translocation of p35 in dying cells**

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We identified heterogeneous nuclear ribonucleoprotein (hnRNP) C1/C2, hnRNP A1, the translocase of the transporter outer membrane 40 (TOM40), and alpha-tubulin as new interaction partners of anti-apoptotic protein p35 using MS-based functional proteomics with GST-p35 fusion protein as a bait, and using a pull-down assay with p35-6His followed by Western blot analysis. p35 was localized in the cytoplasm and in distinct organelles such as the nucleus and mitochondria. p35 was more abundant in the cytoplasm than it was in the nucleus. It co-localized with alpha-tubulin in the cytoplasm in the absence of a death stimulus. However, while cells were undergoing death induced by actinomycin D, cytoplasmic p35 was translocated into the nucleus; this process was inhibited by deletions of the N- and C-terminal domains containing leucine-rich motifs. Gene delivery of p35 using recombinant adenoviruses inhibited cytoplasmic compartmentalization of hnRNP C1/C2 and hnRNP A1 in dying cells. This study demonstrated translocation of p35 into the nuclei, as well as protection of the hnRNPs from redistribution in cells undergoing death. We propose an active role for p35 in maintaining the integrity of nuclear proteins during cell death.

PROTEOMICS, 9(16): 4036-4047.

**Keywords**: apoptosis; hnRNPs; p35; protein arrays; TOM40; alpha-tubulin
Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles

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Although archaea, Gram-negative bacteria, and mammalian cells constitutively secrete membrane vesicles (MVs) as a mechanism for cell-free intercellular communication, this cellular process has been overlooked in Gram-positive bacteria. Here, we found for the first time that Gram-positive bacteria naturally produce MVs into the extracellular milieu. Further characterizations showed that the density and size of Staphylococcus aureus-derived MVs are both similar to those of Gram-negative bacteria. With a proteomics approach, we identified with high confidence a total of 90 protein components of S. aureus-derived MVs. In the group of identified proteins, the highly enriched extracellular proteins suggested that a specific sorting mechanism for vesicular proteins exists. We also identified proteins that facilitate the transfer of proteins to other bacteria, as well to eliminate competing organisms, antibiotic resistance, pathological functions in systemic infections, and MV biogenesis. Taken together, these observations suggest that the secretion of MVs is an evolutionally conserved, universal process that occurs from simple organisms to complex multicellular organisms. This information will help us not only to elucidate the biogenesis and functions of MVs, but also to develop therapeutic tools for vaccines, diagnosis, and antibiotics effective against pathogenic strains of Gram-positive bacteria.


Keywords: gram-positive bacteria; IgG-binding protein; membrane vesicles; microbiology; microvesicles; Staphylococcus aureus

Mapping human genetic diversity in Asia

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Asia harbors substantial cultural and linguistic diversity, but the geographic structure of genetic variation across the continent remains enigmatic. Here we report a large-scale survey of autosomal variation from a broad geographic sample of Asian human populations. Our results show that genetic ancestry is strongly correlated with linguistic affiliations as well as geography. Most populations show relatedness within ethnic/linguistic groups, despite prevalent gene flow among populations. More than 90% of East Asian (EA) haplotypes could be found in either Southeast Asian (SEA) or Central-South Asian (CSA) populations and show clinal structure with haplotype diversity decreasing from south to north. Furthermore, 50% of EA haplotypes were found in SEA only and 5% were found in CSA only, indicating that SEA was a major geographic source of EA populations.

SCIENCE, 326(5959): 1541-1545.

Keywords: population-structure; wide analysis; admixture; geography; uyghurs
Identification of a stroma-mediated Wnt/beta-catenin signal promoting self-renewal of hematopoietic stem cells in the stem cell niche

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With contrasting observations on the effects of beta-catenin on hematopoietic stem cells (HSCs), the precise role of Wnt/beta-catenin signals on HSC regulation remains unclear. Here, we identify a specific mode of Wnt/beta-catenin signal that can regulate HSCs in a stroma-dependent manner. Stabilization of beta-catenin in the bone marrow stromal cells promoted maintenance and self-renewal of HSCs in a contact-dependent manner, whereas direct stabilization in hematopoietic cells caused loss of HSCs. Interestingly, canonical Wnt receptors and beta-catenin accumulation were predominantly enriched in the stromal rather than the hematopoietic compartment of bone marrows. Moreover, the active form of beta-catenin accumulated selectively in the trabecular endosteum in "Wnt 3a-stimulate" or "irradiation-stressed," but not in "steady-state" marrows. Notably, notch ligands were induced in Wnt/beta-catenin activated bone marrow stroma and downstream match signal activation was seen in the HSCs in contact with the activated stroma. Taken together, Wnt/beta-catenin activated stroma and their cross-talk with HSCs may function as a physiologically regulated microenvironmental cue for HSC self-renewal in the stem cell niche.

STEM CELLS, 27(6): 1318-1329.

Keywords: hematopoietic stem cell; self-renewal; wnt; stroma; stem cell-microenvironment interactions
Indexes

- Author Index
- Journal Index
- Keyword Index

Korea Research Institute of Bioscience and Biotechnology
<table>
<thead>
<tr>
<th>Author</th>
<th>Article No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahn, Jiwon</td>
<td>68</td>
</tr>
<tr>
<td>Ahn, Jong Seog</td>
<td>167, 186, 189, 191, 192, 203, 207, 214, 217</td>
</tr>
<tr>
<td>Ahn, Jungoh</td>
<td>137, 221, 226</td>
</tr>
<tr>
<td>Ahn, Kyung-Seop</td>
<td>204, 205, 210, 211, 216</td>
</tr>
<tr>
<td>An, Dong-Shan</td>
<td>161</td>
</tr>
<tr>
<td>An, Sojin</td>
<td>109</td>
</tr>
<tr>
<td>Bae, Eun Young</td>
<td>217</td>
</tr>
<tr>
<td>Bae, Kwang-Hee</td>
<td>38, 39, 47, 66, 70, 72, 74, 77, 197</td>
</tr>
<tr>
<td>Bae, Kyung Sook</td>
<td>83, 97, 136, 154, 163, 169</td>
</tr>
<tr>
<td>Baek, Dong Chul</td>
<td>235</td>
</tr>
<tr>
<td>Baek, Jin-Oh</td>
<td>244</td>
</tr>
<tr>
<td>Baek, Jung Eun</td>
<td>23, 226</td>
</tr>
<tr>
<td>Baek, Kyoung Eun</td>
<td>54</td>
</tr>
<tr>
<td>Baek, Kyung-Hwa</td>
<td>116, 118</td>
</tr>
<tr>
<td>Baek, Seung-Hwa</td>
<td>109</td>
</tr>
<tr>
<td>Bahn, Young Jae</td>
<td>42, 46</td>
</tr>
<tr>
<td>Bhak, Jong</td>
<td>170, 171, 172, 173, 174, 177, 178, 277, 297</td>
</tr>
<tr>
<td>Boovanahalli, S. K.</td>
<td>187, 195</td>
</tr>
<tr>
<td>Byun, Ha Na</td>
<td>174</td>
</tr>
<tr>
<td>Cai, Xing-Fu</td>
<td>211, 216</td>
</tr>
<tr>
<td>Chae, Jung-II</td>
<td>45, 76, 80</td>
</tr>
<tr>
<td>Chang, Ho-Won</td>
<td>145, 148, 149, 150, 152, 153, 158, 162</td>
</tr>
<tr>
<td>Chang, Jong Sun</td>
<td>256, 257</td>
</tr>
<tr>
<td>Chang, Keun Young</td>
<td>140</td>
</tr>
<tr>
<td>Chang, Kyu-Tae</td>
<td>44, 223, 227, 229, 230, 233, 235, 236, 237, 156, 259</td>
</tr>
<tr>
<td>Chang, Young-Hyo</td>
<td>38, 47, 58, 70, 72, 74, 90, 125</td>
</tr>
<tr>
<td>Chi, Seung Wook</td>
<td>210, 211</td>
</tr>
<tr>
<td>Chin, Young-Won</td>
<td>116</td>
</tr>
<tr>
<td>Cho, Dae-Hyun</td>
<td>2, 29</td>
</tr>
<tr>
<td>Cho, Hyunmin</td>
<td>3, 6, 30, 33</td>
</tr>
<tr>
<td>Cho, Mi Young</td>
<td>45</td>
</tr>
<tr>
<td>Cho, Sunwha</td>
<td>39, 66</td>
</tr>
<tr>
<td>Cho, Yee Sook</td>
<td>9, 76</td>
</tr>
<tr>
<td>Cho, Young Keun</td>
<td>20</td>
</tr>
<tr>
<td>Choi, Bang Sil</td>
<td>68</td>
</tr>
<tr>
<td>Choi, Chung-Hae</td>
<td>137, 221</td>
</tr>
<tr>
<td>Choi, Eui-Sung</td>
<td></td>
</tr>
<tr>
<td>Choi, Eun Hwa</td>
<td>53</td>
</tr>
<tr>
<td>Choi, Hwa Jung</td>
<td>182, 201, 202, 218</td>
</tr>
<tr>
<td>Choi, Inpyo</td>
<td>50, 54, 60, 188, 197, 208, 209, 220</td>
</tr>
<tr>
<td>Choi, Jinsun</td>
<td>40</td>
</tr>
<tr>
<td>Choi, Jong Hyun</td>
<td>243, 250, 252, 254</td>
</tr>
<tr>
<td>Choi, Jung Ho</td>
<td>195, 226</td>
</tr>
<tr>
<td>Choi, Kyung Hwa</td>
<td>234, 238</td>
</tr>
<tr>
<td>Choi, Min Ho</td>
<td>244, 249</td>
</tr>
<tr>
<td>Choi, Nack-Shick</td>
<td>67, 243, 250, 252, 253, 254, 259</td>
</tr>
<tr>
<td>Choi, Oksik</td>
<td>198</td>
</tr>
<tr>
<td>Choi, Seung Cheol</td>
<td>112, 120, 141, 142, 144</td>
</tr>
<tr>
<td>Choi, Shin-Jung</td>
<td>60</td>
</tr>
<tr>
<td>Choi, Soo-Keun</td>
<td>61</td>
</tr>
<tr>
<td>Choi, So-Young</td>
<td>113</td>
</tr>
<tr>
<td>Choi, Su-Lim</td>
<td>35</td>
</tr>
<tr>
<td>Choo, Soo-Jin</td>
<td>92</td>
</tr>
<tr>
<td>Chu, In-Sun</td>
<td>184, 186, 206, 212, 213, 214</td>
</tr>
<tr>
<td>Chun, Chang-Soo</td>
<td>298</td>
</tr>
<tr>
<td>Chung, Bong Hyun</td>
<td>137</td>
</tr>
<tr>
<td>Chung, Dong-Min</td>
<td>1, 2, 3, 5, 6, 7, 8, 11, 13, 15, 16, 17, 18, 20, 23, 29, 30, 33, 34, 271</td>
</tr>
<tr>
<td>Chung, Im Sik</td>
<td>67, 253</td>
</tr>
<tr>
<td>Chung, Jin Woong</td>
<td>13, 16</td>
</tr>
<tr>
<td>Chung, Kyung-Sook</td>
<td>188, 197, 208, 220</td>
</tr>
<tr>
<td>Chung, Mi Yeon</td>
<td>61, 68, 187</td>
</tr>
<tr>
<td>Chung, Sang Jeon</td>
<td>183</td>
</tr>
<tr>
<td>Cui, Long</td>
<td>1, 6, 8, 14, 18, 26, 70, 72, 197</td>
</tr>
<tr>
<td>Dastager, Syed G.</td>
<td>183</td>
</tr>
<tr>
<td>Dat, Nguyen Tien</td>
<td>143, 147, 151</td>
</tr>
<tr>
<td>Davarpanah, Seyed Javad</td>
<td>199, 215, 219</td>
</tr>
<tr>
<td>Fang, Yi</td>
<td>121</td>
</tr>
<tr>
<td>Ghang, Ho</td>
<td>86</td>
</tr>
<tr>
<td>Ha, Ga-Hee</td>
<td>174, 177, 277, 297</td>
</tr>
<tr>
<td>Ha, Hye-Lin</td>
<td>49</td>
</tr>
<tr>
<td>Ha, Jong Seong</td>
<td>27, 71</td>
</tr>
<tr>
<td>Han, Dong Chol</td>
<td>10</td>
</tr>
<tr>
<td>Han, Eun Jong</td>
<td>48, 52, 71</td>
</tr>
<tr>
<td>Han, Hyo-Won</td>
<td>248</td>
</tr>
<tr>
<td>Han, Jong-Min</td>
<td>45, 76</td>
</tr>
<tr>
<td>Han, Jung Hyun</td>
<td>71, 109</td>
</tr>
<tr>
<td>Han, Kyou-Hoon</td>
<td>20</td>
</tr>
<tr>
<td>Han, Mi Kyoung</td>
<td>90, 125</td>
</tr>
<tr>
<td>Han, Ying-Hao</td>
<td>83, 136</td>
</tr>
<tr>
<td>Han, Yun Jon</td>
<td>27</td>
</tr>
<tr>
<td>He, Long</td>
<td>243, 252, 253</td>
</tr>
<tr>
<td>Heo, Sun-Yeon</td>
<td>203</td>
</tr>
<tr>
<td>Hoang, Duc Manh</td>
<td>244, 248, 251</td>
</tr>
<tr>
<td>Hoe, Kwang-Lae</td>
<td>189, 192</td>
</tr>
<tr>
<td>Hong, Hyo Jeong</td>
<td>68, 273</td>
</tr>
<tr>
<td>Hoang, Duc Manh</td>
<td>58, 193, 200</td>
</tr>
<tr>
<td>Name</td>
<td>Pages</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Hong, Jiyeon</td>
<td>221</td>
</tr>
<tr>
<td>Hong, Seung-Hyun</td>
<td>19</td>
</tr>
<tr>
<td>Hong, Soon Gyu</td>
<td>169</td>
</tr>
<tr>
<td>Hong, Won-Kyung</td>
<td>249</td>
</tr>
<tr>
<td>Hong, Young-Soo</td>
<td>198, 199, 215, 219</td>
</tr>
<tr>
<td>Huh, Jae-Won</td>
<td>229, 230, 232, 236, 237, 292</td>
</tr>
<tr>
<td>Hur, Cheol-Goo</td>
<td>91, 120, 112</td>
</tr>
<tr>
<td>Hwang, Chae Young</td>
<td>12, 28, 288</td>
</tr>
<tr>
<td>Im, Namhui</td>
<td>187</td>
</tr>
<tr>
<td>Jang, Mi</td>
<td>70, 72</td>
</tr>
<tr>
<td>Jang, Ye Jin</td>
<td>63</td>
</tr>
<tr>
<td>Jeon, Che Ok</td>
<td>148, 149</td>
</tr>
<tr>
<td>Jeon, Jae Heung</td>
<td>81, 88, 114, 181</td>
</tr>
<tr>
<td>Jeon, Jun Ho</td>
<td>197, 208</td>
</tr>
<tr>
<td>Jeon, Yeo-Jin</td>
<td>57</td>
</tr>
<tr>
<td>Jeong, Dae Gwin</td>
<td>17, 46, 64, 75</td>
</tr>
<tr>
<td>Jeong, Haeyoung</td>
<td>112, 120, 126</td>
</tr>
<tr>
<td>Jeong, Hyung Jae</td>
<td>246, 256</td>
</tr>
<tr>
<td>Jeong, Jae Kap</td>
<td>3, 11, 34</td>
</tr>
<tr>
<td>Jeong, Jin Young</td>
<td>188, 208, 209, 220</td>
</tr>
<tr>
<td>Jeong, Misong</td>
<td>193</td>
</tr>
<tr>
<td>Jeong, Moon Sik</td>
<td>203</td>
</tr>
<tr>
<td>Jeong, Sook Jung</td>
<td>228, 234, 238, 239</td>
</tr>
<tr>
<td>Jeong, Soon-Chun</td>
<td>49, 57</td>
</tr>
<tr>
<td>Jeong, So-Young</td>
<td>71, 83, 109</td>
</tr>
<tr>
<td>Jeong, Tae-Sook</td>
<td>121</td>
</tr>
<tr>
<td>Jeong, Won Jong</td>
<td>97, 154</td>
</tr>
<tr>
<td>Jeong, Young Sun</td>
<td>55</td>
</tr>
<tr>
<td>Jeong, Yujin</td>
<td>8</td>
</tr>
<tr>
<td>Jho, Sung Woong</td>
<td>172, 177</td>
</tr>
<tr>
<td>Ji, Mairyun</td>
<td>171</td>
</tr>
<tr>
<td>Ji, Na Young</td>
<td>63</td>
</tr>
<tr>
<td>Jin, Xuejun</td>
<td>187, 215</td>
</tr>
<tr>
<td>Jin, Yinglan</td>
<td>187, 190, 195, 199</td>
</tr>
<tr>
<td>Jin, Yue-Yan</td>
<td>109</td>
</tr>
<tr>
<td>Joung, Hyouk</td>
<td>81, 88, 114, 181</td>
</tr>
<tr>
<td>Ju, Sung Kyu</td>
<td>19</td>
</tr>
<tr>
<td>Ju, Yoon-Jung</td>
<td>147, 151</td>
</tr>
<tr>
<td>Jung, Heung-Chae</td>
<td>138</td>
</tr>
<tr>
<td>Jung, Hyeun</td>
<td>39, 66</td>
</tr>
<tr>
<td>Jung, Jin-Hee</td>
<td>4</td>
</tr>
<tr>
<td>Jung, Joong-Ki</td>
<td>23, 221, 226, 240</td>
</tr>
<tr>
<td>Jung, Min Young</td>
<td>156</td>
</tr>
<tr>
<td>Jung, Seo Hee</td>
<td>121</td>
</tr>
<tr>
<td>Jung, Suk-Kyeong</td>
<td>46, 52, 64, 75</td>
</tr>
<tr>
<td>Jung, Tae-Sung</td>
<td>141, 142</td>
</tr>
<tr>
<td>Jung, Won Seok</td>
<td>25</td>
</tr>
<tr>
<td>Jung, Yong-Taek</td>
<td>99, 100, 105, 107</td>
</tr>
<tr>
<td>Jung, Yongwoon</td>
<td>2</td>
</tr>
<tr>
<td>Jung, Yu-Jin</td>
<td>61</td>
</tr>
<tr>
<td>Kang, Aram</td>
<td>144</td>
</tr>
<tr>
<td>Kang, Chul-Hyung</td>
<td>82</td>
</tr>
<tr>
<td>Kang, Hyo Jin</td>
<td>8, 14, 70</td>
</tr>
<tr>
<td>Kang, Jong Soon</td>
<td>222, 224, 225, 231, 241, 242</td>
</tr>
<tr>
<td>Kang, Min Ah</td>
<td>65</td>
</tr>
<tr>
<td>Kang, Moo Rim</td>
<td>187, 224, 225</td>
</tr>
<tr>
<td>Kang, Sung-Hyun</td>
<td>38, 47, 70, 72, 74, 77</td>
</tr>
<tr>
<td>Kang, Yong-Kook</td>
<td>45, 55, 76</td>
</tr>
<tr>
<td>Kang, Yun Hee</td>
<td>63</td>
</tr>
<tr>
<td>Kaur, Navneet</td>
<td>190, 199</td>
</tr>
<tr>
<td>Khiev, Piseth</td>
<td>211</td>
</tr>
<tr>
<td>Kim, Ae-Kyeong</td>
<td>19</td>
</tr>
<tr>
<td>Kim, Aeri</td>
<td>142</td>
</tr>
<tr>
<td>Kim, Bo Yeon</td>
<td>79, 189, 191, 203, 207, 217</td>
</tr>
<tr>
<td>Kim, Bo-Hye</td>
<td>243, 252</td>
</tr>
<tr>
<td>Kim, Byoung-Chul</td>
<td>172, 173, 174, 177</td>
</tr>
<tr>
<td>Kim, Byoung-Chun</td>
<td>97, 154, 163, 164, 165</td>
</tr>
<tr>
<td>Kim, Byoung-Hyun</td>
<td>116</td>
</tr>
<tr>
<td>Kim, Cha Young</td>
<td>124, 127, 133</td>
</tr>
<tr>
<td>Kim, Chang-Gi</td>
<td>228, 234, 238, 239</td>
</tr>
<tr>
<td>Kim, Chang-Jin</td>
<td>143, 146, 147, 151, 159, 166, 168</td>
</tr>
<tr>
<td>Kim, Chan-Shick</td>
<td>235</td>
</tr>
<tr>
<td>Kim, Chul Ho</td>
<td>244, 248, 249, 251, 258</td>
</tr>
<tr>
<td>Kim, Chulhong</td>
<td>174, 177</td>
</tr>
<tr>
<td>Kim, Chang Hee</td>
<td>189</td>
</tr>
<tr>
<td>Kim, Chunsuk</td>
<td>221</td>
</tr>
<tr>
<td>Kim, Dae In</td>
<td>234, 238</td>
</tr>
<tr>
<td>Kim, Dae-Soo</td>
<td>174, 177, 229, 230, 232, 236, 237, 274, 292</td>
</tr>
<tr>
<td>Kim, Dae-Won</td>
<td>112, 141, 142, 144</td>
</tr>
<tr>
<td>Kim, Deokhooon</td>
<td>174</td>
</tr>
<tr>
<td>Kim, Do Hyung</td>
<td>39, 66, 77</td>
</tr>
<tr>
<td>Kim, Do Young</td>
<td>83, 136, 154</td>
</tr>
<tr>
<td>Kim, Do-Hyung</td>
<td>90, 125</td>
</tr>
<tr>
<td>Kim, Dong Joon</td>
<td>80</td>
</tr>
<tr>
<td>Kim, Dong-II</td>
<td>49</td>
</tr>
<tr>
<td>Kim, Dong-Myung</td>
<td>61, 224</td>
</tr>
<tr>
<td>Kim, Dong-Uk</td>
<td>273, 275</td>
</tr>
<tr>
<td>Kim, Ekyune</td>
<td>223, 233, 235</td>
</tr>
<tr>
<td>Kim, Eun-Mi</td>
<td>164, 165</td>
</tr>
<tr>
<td>Kim, Eun-Young</td>
<td>66, 82</td>
</tr>
<tr>
<td>Kim, Hansoo</td>
<td>40</td>
</tr>
<tr>
<td>Kim, Hee-Sik</td>
<td>116, 118</td>
</tr>
<tr>
<td>Kim, Hui-Seong</td>
<td>205</td>
</tr>
<tr>
<td>Kim, Hwan Mook</td>
<td>61, 187, 222, 224, 225, 228, 231, 234, 238, 239, 241, 242</td>
</tr>
<tr>
<td>Kim, Hyangmi</td>
<td>154</td>
</tr>
<tr>
<td>Kim, Hye-Nan</td>
<td>48</td>
</tr>
<tr>
<td>Kim, Hye-Neung</td>
<td>228, 234, 238</td>
</tr>
<tr>
<td>Kim, Hyoungwoo</td>
<td>237</td>
</tr>
<tr>
<td>Kim, Hyun Soon</td>
<td>81, 88, 114, 165, 181, 263</td>
</tr>
<tr>
<td>Kim, Hyung-Cheol</td>
<td>144</td>
</tr>
<tr>
<td>Kim, Jae Wha</td>
<td>50, 53, 60, 63, 73, 220, 268</td>
</tr>
<tr>
<td>Kim, Jang Hoon</td>
<td>246, 256</td>
</tr>
<tr>
<td>Kim, Jang Hyun</td>
<td>224, 225</td>
</tr>
<tr>
<td>Kim, Janghwan</td>
<td>45, 76</td>
</tr>
<tr>
<td>Kim, Jeong-Min</td>
<td>49, 56</td>
</tr>
<tr>
<td>Kim, Ji Han</td>
<td>171</td>
</tr>
<tr>
<td>Kim, Ji-Hyun F.</td>
<td>112, 113, 120, 126</td>
</tr>
<tr>
<td>Kim, Jin Hee</td>
<td>85, 182</td>
</tr>
<tr>
<td>Kim, Jin-Kyeong</td>
<td>33</td>
</tr>
<tr>
<td>Kim, Jin-A</td>
<td>52</td>
</tr>
<tr>
<td>Kim, Ji-Su</td>
<td>44, 55, 78, 235</td>
</tr>
<tr>
<td>Kim, Jong Hyun</td>
<td>122, 128</td>
</tr>
<tr>
<td>Kim, Jong-Tae</td>
<td>50, 53, 54, 60, 65</td>
</tr>
<tr>
<td>Kim, Jong-Su</td>
<td>1, 24</td>
</tr>
<tr>
<td>Kim, Jung Min</td>
<td>156, 252, 259</td>
</tr>
<tr>
<td>Kim, Jung-Won</td>
<td>52</td>
</tr>
<tr>
<td>Kim, Kang-eun</td>
<td>241, 242</td>
</tr>
<tr>
<td>Kim, Keun-Soo</td>
<td>193</td>
</tr>
<tr>
<td>Kim, Keun-Su</td>
<td>223, 233</td>
</tr>
<tr>
<td>Kim, Kwang Kyu</td>
<td>155, 167</td>
</tr>
<tr>
<td>Kim, Kyung-Ho</td>
<td>145, 148, 149, 150, 152, 153</td>
</tr>
<tr>
<td>Kim, Mi-Jeong</td>
<td>188, 209</td>
</tr>
<tr>
<td>Kim, Mi Na</td>
<td>163, 164, 165</td>
</tr>
<tr>
<td>Kim, Mi Sun</td>
<td>114, 188, 197, 208, 220</td>
</tr>
<tr>
<td>Kim, Min-Soo</td>
<td>152, 153, 157, 158</td>
</tr>
<tr>
<td>Kim, Min-Gon</td>
<td>160, 193, 203, 256</td>
</tr>
<tr>
<td>Kim, Min-Jung</td>
<td>31</td>
</tr>
<tr>
<td>Kim, Moonil</td>
<td>109</td>
</tr>
<tr>
<td>Kim, Myeong-Su</td>
<td>5, 7, 18, 26, 32, 74, 197</td>
</tr>
<tr>
<td>Kim, Myung Hee</td>
<td>223, 235</td>
</tr>
<tr>
<td>Kim, Nam Shin</td>
<td>287</td>
</tr>
<tr>
<td>Kim, Nam-Soon</td>
<td>274</td>
</tr>
<tr>
<td>Kim, Ryong Nam</td>
<td>49, 51, 56, 57, 278</td>
</tr>
<tr>
<td>Kim, Sang Hee</td>
<td>142</td>
</tr>
<tr>
<td>Kim, Sang-Kyu</td>
<td>74</td>
</tr>
<tr>
<td>Kim, Sang-Hyun</td>
<td>18, 29, 32</td>
</tr>
<tr>
<td>Kim, Sang-Yoon</td>
<td>223, 233, 235, 296</td>
</tr>
<tr>
<td>Kim, Semi</td>
<td>170, 174, 177</td>
</tr>
<tr>
<td>Kim, Seon Young</td>
<td>265, 270, 285</td>
</tr>
<tr>
<td>Kim, Seong-Bin</td>
<td>43, 53, 77, 283</td>
</tr>
<tr>
<td>Kim, Seong Mun</td>
<td>113</td>
</tr>
<tr>
<td>Kim, Seong-Hun</td>
<td>21</td>
</tr>
<tr>
<td>Kim, Seung Jun</td>
<td>17, 39, 46, 48, 52, 64, 75</td>
</tr>
<tr>
<td>Kim, Seung-Ho</td>
<td>67, 253</td>
</tr>
<tr>
<td>Kim, Song-Gun</td>
<td>95, 161</td>
</tr>
<tr>
<td>Kim, Soyoung</td>
<td>204</td>
</tr>
<tr>
<td>Kim, Sujin</td>
<td>92</td>
</tr>
<tr>
<td>Kim, Suk-Weon</td>
<td>88, 122, 123, 128, 129, 132</td>
</tr>
<tr>
<td>Kim, Sun Ok</td>
<td>203</td>
</tr>
<tr>
<td>Kim, Sung Uk</td>
<td>44, 51, 83, 110</td>
</tr>
<tr>
<td>Kim, Sung-Jin</td>
<td>233</td>
</tr>
<tr>
<td>Kim, Sun-Hyung</td>
<td>89</td>
</tr>
<tr>
<td>Kim, Tae-Don</td>
<td>188, 197, 208, 209, 291</td>
</tr>
<tr>
<td>Kim, Tae-Hyung</td>
<td>174, 177</td>
</tr>
<tr>
<td>Kim, Un-Young</td>
<td>26</td>
</tr>
<tr>
<td>Kim, Won Kon</td>
<td>39, 66</td>
</tr>
<tr>
<td>Kim, Won-Geol</td>
<td>198</td>
</tr>
<tr>
<td>Kim, Won-Gon</td>
<td>86, 111, 206</td>
</tr>
<tr>
<td>Kim, Woo-Young</td>
<td>171, 174, 177</td>
</tr>
<tr>
<td>Kim, Yang-Hyun</td>
<td>176</td>
</tr>
<tr>
<td>Kim, Yaw Joo</td>
<td>121</td>
</tr>
<tr>
<td>Kim, Yong Sung</td>
<td>272, 289</td>
</tr>
<tr>
<td>Kim, Yong-Hoon</td>
<td>276, 279, 280</td>
</tr>
<tr>
<td>Kim, Yong-Mo</td>
<td>74, 259</td>
</tr>
<tr>
<td>Kim, Yong-Ook</td>
<td>259</td>
</tr>
<tr>
<td>Kim, Yong-Sam</td>
<td>180, 284</td>
</tr>
<tr>
<td>Kim, Yoon-Sik</td>
<td>81, 114</td>
</tr>
<tr>
<td>Kim, Young Jun</td>
<td>50, 60</td>
</tr>
<tr>
<td>Kim, Young-Kook</td>
<td>195, 295</td>
</tr>
<tr>
<td>Kim, Young-Hee</td>
<td>184, 186, 206, 212</td>
</tr>
<tr>
<td>Kim, Young-Hyun</td>
<td>213, 214</td>
</tr>
<tr>
<td>Kim, Yu-Jin</td>
<td>233, 237</td>
</tr>
<tr>
<td>Kim, Yun-Hee</td>
<td>239</td>
</tr>
<tr>
<td>Ko, Jeong Heon</td>
<td>89, 124, 127, 131, 133, 135</td>
</tr>
<tr>
<td>Koh, Jong Sung</td>
<td>180, 284</td>
</tr>
<tr>
<td>Koh, Sang Seok</td>
<td>267</td>
</tr>
<tr>
<td>Koh, Serry</td>
<td>196</td>
</tr>
<tr>
<td>Koo, Deog-Bon</td>
<td>44, 78, 80</td>
</tr>
<tr>
<td>Kwak, Sang-Soo</td>
<td>89, 124, 127, 131, 133, 135</td>
</tr>
<tr>
<td>Kwon, Byoung Mog</td>
<td>37, 48, 52, 71, 187</td>
</tr>
<tr>
<td>Kwon, Dohyoung</td>
<td>241, 242</td>
</tr>
<tr>
<td>Kwon, Dur Han</td>
<td>29</td>
</tr>
<tr>
<td>Kwon, Hyuk-Ryul</td>
<td>182, 201, 202, 218</td>
</tr>
<tr>
<td>Kwon, Hyung-Jun</td>
<td>142</td>
</tr>
<tr>
<td>Kwon, Ji-Na</td>
<td>246, 256</td>
</tr>
<tr>
<td>Kwon, Ohsuk</td>
<td>15, 20</td>
</tr>
<tr>
<td>Kwon, Ok-Kyoungh</td>
<td>10, 12, 28, 288</td>
</tr>
<tr>
<td>Kwon, Osong</td>
<td>4, 21, 25, 258</td>
</tr>
<tr>
<td>Kwon, Suk Youn</td>
<td>204, 205, 216</td>
</tr>
<tr>
<td>Kwon, Sun Beom</td>
<td>203</td>
</tr>
<tr>
<td>Kwon, Yun-Ju</td>
<td>88, 89, 124, 127</td>
</tr>
<tr>
<td>Lee, Bumkyu</td>
<td>163</td>
</tr>
<tr>
<td>Lee, Byungwook</td>
<td>86</td>
</tr>
<tr>
<td>Lee, Chang Soo</td>
<td>228, 238</td>
</tr>
<tr>
<td>Lee, Chang Woo</td>
<td>175, 179</td>
</tr>
<tr>
<td>Lee, Chang-Soon</td>
<td>11, 18, 26, 32, 98</td>
</tr>
<tr>
<td>Lee, Chang-Woo</td>
<td>224, 225, 241, 242</td>
</tr>
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<td>Lee, Choong Hoon</td>
<td>120</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>Lee, Chul Ho</td>
<td>183, 262, 276, 279, 280, 281</td>
</tr>
<tr>
<td>Lee, Chung Il</td>
<td>63</td>
</tr>
<tr>
<td>Lee, Daesil</td>
<td>282</td>
</tr>
<tr>
<td>Lee, Do Hee</td>
<td>74</td>
</tr>
<tr>
<td>Lee, Dong-Seok</td>
<td>27</td>
</tr>
<tr>
<td>Lee, Eun Gyo</td>
<td>23, 221, 226</td>
</tr>
<tr>
<td>Lee, Eung Seuk</td>
<td>200</td>
</tr>
<tr>
<td>Lee, Haeng-Soon</td>
<td>89, 124, 127, 131, 133, 135</td>
</tr>
<tr>
<td>Lee, Hana</td>
<td>38</td>
</tr>
<tr>
<td>Lee, Hee Gu</td>
<td>50, 53, 54, 60, 63, 65, 79, 197, 203, 260</td>
</tr>
<tr>
<td>Lee, Heun-Sik</td>
<td>71</td>
</tr>
<tr>
<td>Lee, Hong-Weon</td>
<td>137, 221, 226</td>
</tr>
<tr>
<td>Lee, Hye Won</td>
<td>243</td>
</tr>
<tr>
<td>Lee, Hyeokweon</td>
<td>221</td>
</tr>
<tr>
<td>Lee, Hyeong-Kyu</td>
<td>204, 205, 210, 211, 216</td>
</tr>
<tr>
<td>Lee, Hyun Sun</td>
<td>183</td>
</tr>
<tr>
<td>Lee, In-Chul</td>
<td>89</td>
</tr>
<tr>
<td>Lee, J. W.</td>
<td>52</td>
</tr>
<tr>
<td>Lee, Jae-Chan</td>
<td>143, 147, 151, 159, 168</td>
</tr>
<tr>
<td>Lee, Jae-Ran</td>
<td>17</td>
</tr>
<tr>
<td>Lee, Jae-Woong</td>
<td>235</td>
</tr>
<tr>
<td>Lee, Jeong Min</td>
<td>2, 29</td>
</tr>
<tr>
<td>Lee, Jiwon</td>
<td>188, 208, 209</td>
</tr>
<tr>
<td>Lee, Jong Suk</td>
<td>83, 136</td>
</tr>
<tr>
<td>Lee, Joohwan</td>
<td>221</td>
</tr>
<tr>
<td>Lee, Joongku</td>
<td>290</td>
</tr>
<tr>
<td>Lee, Jung Joon</td>
<td>185, 187, 198, 199, 215, 219</td>
</tr>
<tr>
<td>Lee, Jung Mi</td>
<td>4, 21</td>
</tr>
<tr>
<td>Lee, Jung Min</td>
<td>6</td>
</tr>
<tr>
<td>Lee, Jung-Sook</td>
<td>93, 155, 167, 254</td>
</tr>
<tr>
<td>Lee, Kang Hyun</td>
<td>97, 163, 164, 165</td>
</tr>
<tr>
<td>Lee, Keun Chul</td>
<td>155, 167</td>
</tr>
<tr>
<td>Lee, Ki Hoon</td>
<td>224, 225</td>
</tr>
<tr>
<td>Lee, Kihon</td>
<td>187, 190, 224, 225, 231, 241, 242</td>
</tr>
<tr>
<td>Lee, Kye Sook</td>
<td>241, 242</td>
</tr>
<tr>
<td>Lee, Kyeong</td>
<td>63, 185, 187, 190, 194, 195, 198, 199, 215, 219</td>
</tr>
<tr>
<td>Lee, Kyung Jin</td>
<td>4</td>
</tr>
<tr>
<td>Lee, Kyung-A</td>
<td>172</td>
</tr>
<tr>
<td>Lee, Kyung-Kwang</td>
<td>44, 78, 80</td>
</tr>
<tr>
<td>Lee, Kyu-Sun</td>
<td>19, 22, 80</td>
</tr>
<tr>
<td>Lee, Lan Hee</td>
<td>5</td>
</tr>
<tr>
<td>Lee, Mee-Young</td>
<td>204, 205</td>
</tr>
<tr>
<td>Lee, Mi-Hwa</td>
<td>82</td>
</tr>
<tr>
<td>Lee, Myung Kyu</td>
<td>17, 269</td>
</tr>
<tr>
<td>Lee, Myung Sun</td>
<td>189</td>
</tr>
<tr>
<td>Lee, Phil Young</td>
<td>38, 77</td>
</tr>
<tr>
<td>Lee, S. Y.</td>
<td>52</td>
</tr>
<tr>
<td>Lee, Sang Chul</td>
<td>17, 39, 66, 70, 72, 74, 77</td>
</tr>
<tr>
<td>Lee, Sangku</td>
<td>213</td>
</tr>
<tr>
<td>Lee, Sang-Rae</td>
<td>223, 233, 235, 237</td>
</tr>
<tr>
<td>Lee, Seok Kee</td>
<td>31</td>
</tr>
<tr>
<td>Lee, Seung Hui</td>
<td>23, 226</td>
</tr>
<tr>
<td>Lee, Seung-Goo</td>
<td>92, 243, 250, 252</td>
</tr>
<tr>
<td>Lee, Seung-Won</td>
<td>120</td>
</tr>
<tr>
<td>Lee, Si-Hyung</td>
<td>90, 125</td>
</tr>
<tr>
<td>Lee, So Young</td>
<td>71, 101, 102</td>
</tr>
<tr>
<td>Lee, Soohyun</td>
<td>293</td>
</tr>
<tr>
<td>Lee, Soo-Young</td>
<td>98</td>
</tr>
<tr>
<td>Lee, Su Ub</td>
<td>140</td>
</tr>
<tr>
<td>Lee, Su-Jin</td>
<td>258</td>
</tr>
<tr>
<td>Lee, Suk Hyung</td>
<td>188, 197, 208, 209, 220</td>
</tr>
<tr>
<td>Lee, Sun Young</td>
<td>70</td>
</tr>
<tr>
<td>Lee, Sunghoon</td>
<td>174, 177, 297</td>
</tr>
<tr>
<td>Lee, Taek Chang</td>
<td>225</td>
</tr>
<tr>
<td>Lee, Tae-Young</td>
<td>176</td>
</tr>
<tr>
<td>Lee, Woo Song</td>
<td>245, 246, 247, 256</td>
</tr>
<tr>
<td>Lee, Yangsoon</td>
<td>196</td>
</tr>
<tr>
<td>Lee, Yong Seok</td>
<td>171, 174, 177</td>
</tr>
<tr>
<td>Lee, Yong-Hwa</td>
<td>114</td>
</tr>
<tr>
<td>Lee, Young Ik</td>
<td>87, 140</td>
</tr>
<tr>
<td>Lee, Young-mi</td>
<td>8</td>
</tr>
<tr>
<td>Lee, Yun Mi</td>
<td>21, 110</td>
</tr>
<tr>
<td>Lim, Hye-Sun</td>
<td>210</td>
</tr>
<tr>
<td>Lim, Jee-Min</td>
<td>146</td>
</tr>
<tr>
<td>Lim, Jeongheui</td>
<td>170</td>
</tr>
<tr>
<td>Lim, Mi Yeon</td>
<td>36</td>
</tr>
<tr>
<td>Lim, So-Hee</td>
<td>17</td>
</tr>
<tr>
<td>Lim, Soon</td>
<td>124</td>
</tr>
<tr>
<td>Lim, Yong Taik</td>
<td>3, 6, 15, 20, 30, 33, 34, 271</td>
</tr>
<tr>
<td>Liu, Jang Ryol</td>
<td>88, 121, 122, 123, 128, 129, 130, 132</td>
</tr>
<tr>
<td>Luo, Lian Hua</td>
<td>251</td>
</tr>
<tr>
<td>Maeng, Jin-Soo</td>
<td>10</td>
</tr>
<tr>
<td>Min, Jeong-Ki</td>
<td>197, 200, 220, 264, 266</td>
</tr>
<tr>
<td>Min, Sung Ran</td>
<td>121, 128, 165</td>
</tr>
<tr>
<td>Moon, Jae Sun</td>
<td>84, 110</td>
</tr>
<tr>
<td>Moon, Jeong Hee</td>
<td>17, 62, 69</td>
</tr>
<tr>
<td>Moon, Ji Hyun</td>
<td>193</td>
</tr>
<tr>
<td>Nam, Ki Hoon</td>
<td>90, 125</td>
</tr>
<tr>
<td>Nam, Ki-Hoan</td>
<td>76</td>
</tr>
<tr>
<td>Nam, Seong-Hyeuk</td>
<td>141, 142</td>
</tr>
<tr>
<td>Nam, Young-Do</td>
<td>145, 148, 149, 150, 152, 153, 157, 158, 160</td>
</tr>
<tr>
<td>Noh, Jung-Ran</td>
<td>276</td>
</tr>
<tr>
<td>Noh, Young-Woock</td>
<td>15, 20, 30, 33</td>
</tr>
<tr>
<td>Oh, Baek-Rock</td>
<td>244</td>
</tr>
<tr>
<td>Oh, Doo-Byoung</td>
<td>4, 21</td>
</tr>
</tbody>
</table>
Song, Wan-Keun 89
Sun, Hu-nan 208
Sung, Youlboong 158
Venkatakrishnan, A. J. 173
Wang, Ai-Guo 27, 56
Wang, Wen-Bin 135
Whang, Jake 137
Whang, Kyung Eun 87
Whon, Tae Woong 161
Won, Eun-Young 47
Won, Misun 61, 68, 185, 187
Woo, Eui Jeon 36, 75
Woo, Joo Rang 52
Woo, Sun-Mi 45, 76
Wu, Cheng-Zhu 198
Xia, Yan 190, 194, 199
Xu, Guang-Hua 86, 184, 186, 206, 213, 214
Yang, Jin Ok 57, 297
Yang, Jun Hyuk 36
Yang, Jungwook 139
Yang, Kyoung-Sil 124
Yeom, Young Il 53, 63, 65, 71, 268, 277
Yeom, Sun Hee 23
Yi, Hoonbok 228
Yi, Hwe-Su 134
Yi, So Yeon 5, 7, 26
Yim, Sung-Kun 138
Yoo, Hyang Sook 49, 57, 180, 277, 297
Yoo, Ick-Dong 184, 186, 206, 212, 213, 214
Yoo, Jin-San 267
Yoon, Byung-Dae 116
Yoon, Hye-Ran 79
Yoon, Jeongwon 2
Yoon, K. S. 52
Yoon, Sei Mee 36
Yoon, Suk Ran 54, 188, 197, 208, 209, 220
Yoon, Sun Young 56
Yoon, Sung Ho 112, 120, 126
Yoon, Sun-Woo 176
Yoon, Tae-Sung 75
Yoon, Won Kee 225, 228, 239
Yoon, Young Ju 48
Youk, Eun Soo 239
Yu, Dae-Yeul 17, 27, 51, 71, 77
Yu, Dong Su 112, 120, 126
Yu, Hyung Eun 242
Yu, Kweon 9, 19, 22
# Journal Index

<table>
<thead>
<tr>
<th>Journal</th>
<th>Article No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acta Crystallogr F</td>
<td>36</td>
</tr>
<tr>
<td>Adv Bot Res</td>
<td>81</td>
</tr>
<tr>
<td>Anal Biochem</td>
<td>243</td>
</tr>
<tr>
<td>Anal Chem</td>
<td>2, 260</td>
</tr>
<tr>
<td>Analyst</td>
<td>1</td>
</tr>
<tr>
<td>Anesthesiology</td>
<td>261</td>
</tr>
<tr>
<td>Angewandte Chemie</td>
<td>3</td>
</tr>
<tr>
<td>Antivir Res</td>
<td>182</td>
</tr>
<tr>
<td>Appl Environ Microb</td>
<td>82, 83, 221</td>
</tr>
<tr>
<td>Appl Microbiol Biot</td>
<td>244</td>
</tr>
<tr>
<td>Arch Pharm Res</td>
<td>37, 183, 184, 185, 186, 222</td>
</tr>
<tr>
<td>Arch Virol</td>
<td>84</td>
</tr>
<tr>
<td>Arterioscl Throm Vas</td>
<td>262</td>
</tr>
<tr>
<td>B Korean Chem Soc</td>
<td>46, 47, 194, 195</td>
</tr>
<tr>
<td>BBA-Biomembranes</td>
<td>223</td>
</tr>
<tr>
<td>Biochem Bioph Res Co</td>
<td>4, 5, 38, 39, 40, 41, 187, 188</td>
</tr>
<tr>
<td>Biochem Pharmacol</td>
<td>224</td>
</tr>
<tr>
<td>Bioinformatics</td>
<td>141</td>
</tr>
<tr>
<td>Biol Pharm Bull</td>
<td>85, 86, 189, 225</td>
</tr>
<tr>
<td>Biomaterials</td>
<td>6</td>
</tr>
<tr>
<td>Biomol Ther</td>
<td>87</td>
</tr>
<tr>
<td>Bioorg Med Chem Lett</td>
<td>42, 190, 191, 192, 247</td>
</tr>
<tr>
<td>Bioorgan Med Chem</td>
<td>245, 246</td>
</tr>
<tr>
<td>Biosens Bioelectron</td>
<td>7, 8</td>
</tr>
<tr>
<td>Biotecnol Adv</td>
<td>263</td>
</tr>
<tr>
<td>Biotecnol Bioproc E</td>
<td>88, 193, 248</td>
</tr>
<tr>
<td>Biotecnol Lett</td>
<td>249, 250, 251</td>
</tr>
<tr>
<td>Blood</td>
<td>264, 265, 266</td>
</tr>
<tr>
<td>BMB Reports</td>
<td>9, 89, 90, 91</td>
</tr>
<tr>
<td>BMC Bioinformatics</td>
<td>43, 174, 175</td>
</tr>
<tr>
<td>BMC Dev Biol</td>
<td>44</td>
</tr>
<tr>
<td>BMC Genomics</td>
<td>170, 171, 172, 173</td>
</tr>
<tr>
<td>BMC Neurosci</td>
<td>45</td>
</tr>
<tr>
<td>BMC Plant Biol</td>
<td>142</td>
</tr>
<tr>
<td>Brain Res</td>
<td>10</td>
</tr>
<tr>
<td>Cancer</td>
<td>267</td>
</tr>
<tr>
<td>Cancer Immunol Immun</td>
<td>176</td>
</tr>
<tr>
<td>Cancer Res</td>
<td>48, 49</td>
</tr>
<tr>
<td>Cancer Sci</td>
<td>196</td>
</tr>
<tr>
<td>Carbon</td>
<td>11</td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>50, 51, 268, 269, 270</td>
</tr>
<tr>
<td>Cell Cycle</td>
<td>12</td>
</tr>
<tr>
<td>Cell Mol Biol</td>
<td>197</td>
</tr>
<tr>
<td>Cell Mol Life Sci</td>
<td>52</td>
</tr>
<tr>
<td>Chembiochem</td>
<td>198</td>
</tr>
<tr>
<td>Chem-Eur J</td>
<td>271</td>
</tr>
<tr>
<td>Chem Commun</td>
<td>13, 14, 15, 199</td>
</tr>
<tr>
<td>Chem Mater</td>
<td>16</td>
</tr>
<tr>
<td>Clin Chim Acta</td>
<td>53, 54</td>
</tr>
<tr>
<td>Clin Cancer Res</td>
<td>200, 272</td>
</tr>
<tr>
<td>Curr Microbiol</td>
<td>143</td>
</tr>
<tr>
<td>Dev Dynam</td>
<td>55</td>
</tr>
<tr>
<td>DNA Repair</td>
<td>273</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>252</td>
</tr>
<tr>
<td>EMBO J</td>
<td>17</td>
</tr>
<tr>
<td>Enzyme Microb Tech</td>
<td>226</td>
</tr>
<tr>
<td>Eur Food Res Technol</td>
<td>201</td>
</tr>
<tr>
<td>Eur J Pharm Sci</td>
<td>202</td>
</tr>
<tr>
<td>Exp Mol Med</td>
<td>56, 227</td>
</tr>
<tr>
<td>Exp Biol Med</td>
<td>57</td>
</tr>
<tr>
<td>FEBS J</td>
<td>92</td>
</tr>
<tr>
<td>FEBS Lett</td>
<td>18, 19, 58, 203</td>
</tr>
<tr>
<td>Food Control</td>
<td>228</td>
</tr>
<tr>
<td>Food Sci Biotechnol</td>
<td>253</td>
</tr>
<tr>
<td>Gene</td>
<td>229</td>
</tr>
<tr>
<td>Genes Genet Syst</td>
<td>144</td>
</tr>
<tr>
<td>Genes &amp; Genomics</td>
<td>230</td>
</tr>
<tr>
<td>Genet Test Mol Biomark</td>
<td>59</td>
</tr>
<tr>
<td>Genome Biol</td>
<td>274</td>
</tr>
<tr>
<td>Genome Res</td>
<td>177</td>
</tr>
<tr>
<td>Gut</td>
<td>275</td>
</tr>
<tr>
<td>Hepatology</td>
<td>276</td>
</tr>
<tr>
<td>Hum Mutat</td>
<td>277</td>
</tr>
<tr>
<td>Int Immunopharmacol</td>
<td>204, 205, 231</td>
</tr>
<tr>
<td>Int J Biol Macromol</td>
<td>178</td>
</tr>
<tr>
<td>Int J Cancer</td>
<td>60, 61</td>
</tr>
<tr>
<td>Int J Food Microbiol</td>
<td>145</td>
</tr>
<tr>
<td>Int J Mass Spectrom</td>
<td>62</td>
</tr>
<tr>
<td>Int J Mol Med</td>
<td>63</td>
</tr>
<tr>
<td>J Agr Food Chem</td>
<td>109, 110</td>
</tr>
<tr>
<td>J Am Chem Soc</td>
<td>20</td>
</tr>
<tr>
<td>J Antibiot</td>
<td>206</td>
</tr>
<tr>
<td>J Antimicrob Chemoth</td>
<td>111</td>
</tr>
<tr>
<td>J Appl Crystallogr</td>
<td>161</td>
</tr>
<tr>
<td>J Bacteriol</td>
<td>21, 112, 113</td>
</tr>
<tr>
<td>J Biol Chem</td>
<td>22, 64, 278, 279, 280</td>
</tr>
<tr>
<td>J Cancer Res Clin</td>
<td>65, 232</td>
</tr>
<tr>
<td>J Cell Sci</td>
<td>66</td>
</tr>
<tr>
<td>J Chem Technol Biot</td>
<td>23</td>
</tr>
<tr>
<td>J Clin Microbiol</td>
<td>255</td>
</tr>
<tr>
<td>J Enzym Inhib Med Ch</td>
<td>207</td>
</tr>
<tr>
<td>J Exp Nanosci</td>
<td>24</td>
</tr>
<tr>
<td>J Immunol</td>
<td>208, 209, 281, 282</td>
</tr>
<tr>
<td>J Ind Microbiol Biot</td>
<td>67</td>
</tr>
<tr>
<td>J Kor Soc Appl Biol Chem</td>
<td>114, 210, 211, 256</td>
</tr>
<tr>
<td>J Med Food</td>
<td>257</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------------------------------------</td>
</tr>
<tr>
<td>J Microbiol</td>
<td>25, 68, 162, 163, 164, 165, 166</td>
</tr>
<tr>
<td>J Mol Biol</td>
<td>120</td>
</tr>
<tr>
<td>J Mol Cell Cardiol</td>
<td>283</td>
</tr>
<tr>
<td>J Nat Prod</td>
<td>215, 216</td>
</tr>
<tr>
<td>J Pathol</td>
<td>27</td>
</tr>
<tr>
<td>J Phys Chem B</td>
<td>69</td>
</tr>
<tr>
<td>J Plant Biol</td>
<td>121, 122, 123, 234</td>
</tr>
<tr>
<td>J Proteome Res</td>
<td>284</td>
</tr>
<tr>
<td>J Reprod Develop</td>
<td>235</td>
</tr>
<tr>
<td>J Virol</td>
<td>285, 286, 287</td>
</tr>
<tr>
<td>Microbiol Res</td>
<td>168</td>
</tr>
<tr>
<td>Mol Cell Biol</td>
<td>28, 288</td>
</tr>
<tr>
<td>Mol Breeding</td>
<td>124</td>
</tr>
<tr>
<td>Mol Cells</td>
<td>70, 125, 236, 237</td>
</tr>
<tr>
<td>Molecules</td>
<td>217</td>
</tr>
<tr>
<td>Mycologia</td>
<td>169</td>
</tr>
<tr>
<td>Nanotechnology</td>
<td>29, 30</td>
</tr>
<tr>
<td>Nature</td>
<td>126</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>289</td>
</tr>
<tr>
<td>New Phytol</td>
<td>290</td>
</tr>
<tr>
<td>Nucleic Acids Res</td>
<td>179, 291, 292</td>
</tr>
<tr>
<td>Nutr Cancer</td>
<td>71</td>
</tr>
<tr>
<td>Oncogene</td>
<td>72</td>
</tr>
<tr>
<td>Oncol Rep</td>
<td>73</td>
</tr>
<tr>
<td>Oncol Res</td>
<td>74</td>
</tr>
<tr>
<td>Physiol Plantarum</td>
<td>127</td>
</tr>
<tr>
<td>Phytomedicine</td>
<td>218</td>
</tr>
<tr>
<td>Plant Biotechnol Rep</td>
<td>128, 129, 130, 131, 132</td>
</tr>
<tr>
<td>Plant Growth Regul</td>
<td>133</td>
</tr>
<tr>
<td>Plant J</td>
<td>293</td>
</tr>
<tr>
<td>Plant Physiol</td>
<td>134</td>
</tr>
<tr>
<td>Plant Physiol Bioch</td>
<td>135</td>
</tr>
<tr>
<td>Plant Sci</td>
<td>238, 239</td>
</tr>
<tr>
<td>Planta Med</td>
<td>219</td>
</tr>
<tr>
<td>PLOS Pathog</td>
<td>220</td>
</tr>
<tr>
<td>Process Biochem</td>
<td>136, 137, 240</td>
</tr>
<tr>
<td>Protein Expres Purif</td>
<td>138, 259</td>
</tr>
<tr>
<td>Protein Peptide Lett</td>
<td>180</td>
</tr>
<tr>
<td>Proteins</td>
<td>75</td>
</tr>
<tr>
<td>Proteomics</td>
<td>76, 77, 294, 295, 296</td>
</tr>
<tr>
<td>Reprod Fert Develop</td>
<td>78</td>
</tr>
<tr>
<td>Science</td>
<td>297</td>
</tr>
<tr>
<td>Sci Hortic-Amsterdam</td>
<td>181</td>
</tr>
<tr>
<td>Sensor Actuat B-Chem</td>
<td>79</td>
</tr>
<tr>
<td>Sensors-Basel</td>
<td>31, 32</td>
</tr>
<tr>
<td>Small</td>
<td>33</td>
</tr>
<tr>
<td>Stem Cells</td>
<td>298</td>
</tr>
<tr>
<td>Theriogenology</td>
<td>80</td>
</tr>
<tr>
<td>Toxicol Appl Pharm</td>
<td>34</td>
</tr>
<tr>
<td>Trends Mol Med</td>
<td>35</td>
</tr>
<tr>
<td>Trends Plant Sci</td>
<td>139</td>
</tr>
<tr>
<td>Virus Res</td>
<td>140</td>
</tr>
<tr>
<td>Xenobiotica</td>
<td>241, 242</td>
</tr>
</tbody>
</table>
### Keyword Index

<table>
<thead>
<tr>
<th>Keyword</th>
<th>Article No</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-aptosimon</td>
<td>205</td>
</tr>
<tr>
<td>(9s, 13r)-12-oxo-phytodieneoic acid</td>
<td>219</td>
</tr>
<tr>
<td>1,3-Propanediol</td>
<td>244</td>
</tr>
<tr>
<td>1918 HA</td>
<td>285</td>
</tr>
<tr>
<td>2-Arylbenzofuran derivatives</td>
<td>192</td>
</tr>
<tr>
<td>2'-Benzyloxycinnamaldehyde</td>
<td>241, 242</td>
</tr>
<tr>
<td>2'-hydroxycinnamaldehyde</td>
<td>71, 241, 242</td>
</tr>
<tr>
<td>2vk6</td>
<td>245</td>
</tr>
<tr>
<td>3D-QSAR</td>
<td>185</td>
</tr>
<tr>
<td>3'-untranslated region</td>
<td>291</td>
</tr>
<tr>
<td>6-benzyladenine</td>
<td>123</td>
</tr>
<tr>
<td>a disintegrin and metalloprotease (ADAM)</td>
<td>235</td>
</tr>
<tr>
<td>ABC transporter</td>
<td>190</td>
</tr>
<tr>
<td>aberrant glycoform</td>
<td>264</td>
</tr>
<tr>
<td>Aberrant glycosylation</td>
<td>180</td>
</tr>
<tr>
<td>abiotic stress</td>
<td>89</td>
</tr>
<tr>
<td>abscissic-acid</td>
<td>81</td>
</tr>
<tr>
<td>ABTS</td>
<td>184, 214</td>
</tr>
<tr>
<td>accumulation</td>
<td>34</td>
</tr>
<tr>
<td>acetaldehyde</td>
<td>255</td>
</tr>
<tr>
<td>acetalaminophen</td>
<td>87</td>
</tr>
<tr>
<td>acetate kinase</td>
<td>119</td>
</tr>
<tr>
<td>acetate metabolism</td>
<td>119</td>
</tr>
<tr>
<td>acetyl CoA synthetase</td>
<td>119</td>
</tr>
<tr>
<td>acetyl phosphate</td>
<td>25</td>
</tr>
<tr>
<td>acid analogs</td>
<td>199</td>
</tr>
<tr>
<td>Acinetobacter antiviralis sp nov. acrylamide</td>
<td>167</td>
</tr>
<tr>
<td>activated protein-kinase</td>
<td>28, 81, 280</td>
</tr>
<tr>
<td>active metabolite</td>
<td>241</td>
</tr>
<tr>
<td>active-site</td>
<td>36</td>
</tr>
<tr>
<td>acute exposure</td>
<td>10</td>
</tr>
<tr>
<td>acute myocardial-infarct</td>
<td>264</td>
</tr>
<tr>
<td>Acyl CoA: cholestrol acyl transferase (ACAT)</td>
<td>195</td>
</tr>
<tr>
<td>acyl moiety</td>
<td>168</td>
</tr>
<tr>
<td>adamantane</td>
<td>190</td>
</tr>
<tr>
<td>ADHD</td>
<td>59</td>
</tr>
<tr>
<td>adipocyte</td>
<td>39, 52, 66</td>
</tr>
<tr>
<td>adipogenesis</td>
<td>39, 66</td>
</tr>
<tr>
<td>admixture</td>
<td>297</td>
</tr>
<tr>
<td>adoptive immunotherapy</td>
<td>222, 231</td>
</tr>
<tr>
<td>adrenal hypoplasia congenita</td>
<td>279</td>
</tr>
<tr>
<td>advanced glycated end products (AGES)</td>
<td>7, 38</td>
</tr>
<tr>
<td>aecia stage</td>
<td>169</td>
</tr>
<tr>
<td>aggregation</td>
<td>271</td>
</tr>
<tr>
<td>Aidingimonas halophila</td>
<td>159</td>
</tr>
<tr>
<td>airway hyperresponsiveness</td>
<td>204</td>
</tr>
<tr>
<td>Akt</td>
<td>187, 283</td>
</tr>
<tr>
<td>aldolase</td>
<td>70, 255</td>
</tr>
<tr>
<td>alfalfa</td>
<td>131, 135</td>
</tr>
<tr>
<td>Alishewanella aestuarii</td>
<td>152</td>
</tr>
<tr>
<td>Alishewanella jeotgali</td>
<td>157</td>
</tr>
<tr>
<td>alkaline soil</td>
<td>254</td>
</tr>
<tr>
<td>alkaline-phosphatase</td>
<td>221</td>
</tr>
<tr>
<td>alkanoclasticus</td>
<td>107</td>
</tr>
<tr>
<td>alpha(1,3)-Fucose</td>
<td>4</td>
</tr>
<tr>
<td>alpha.gamma-diaminobutyric acid</td>
<td>113</td>
</tr>
<tr>
<td>alpha-5-beta-1</td>
<td>265</td>
</tr>
<tr>
<td>alpha-Tubulin</td>
<td>295</td>
</tr>
<tr>
<td>alternative splicing</td>
<td>229, 232, 237</td>
</tr>
<tr>
<td>AMPK</td>
<td>276</td>
</tr>
<tr>
<td>analbuminemic rats</td>
<td>275</td>
</tr>
<tr>
<td>anchovy-jeot</td>
<td>67</td>
</tr>
<tr>
<td>anesthesia</td>
<td>261</td>
</tr>
<tr>
<td>angiogenesis</td>
<td>187, 197</td>
</tr>
<tr>
<td>angiosperm</td>
<td>274</td>
</tr>
<tr>
<td>animal proteomics</td>
<td>77</td>
</tr>
<tr>
<td>annexin II</td>
<td>63</td>
</tr>
<tr>
<td>anoikis</td>
<td>197</td>
</tr>
<tr>
<td>anthraquinone glycosides</td>
<td>37</td>
</tr>
<tr>
<td>Anthricus sylvestris</td>
<td>210</td>
</tr>
<tr>
<td>antibacterial</td>
<td>111</td>
</tr>
<tr>
<td>antibiotic</td>
<td>168</td>
</tr>
<tr>
<td>anticancer</td>
<td>241, 242</td>
</tr>
<tr>
<td>anticancer agents</td>
<td>42, 46</td>
</tr>
<tr>
<td>antifungal activity</td>
<td>110</td>
</tr>
<tr>
<td>antigen</td>
<td>294</td>
</tr>
<tr>
<td>anti-inflammatory</td>
<td>205</td>
</tr>
<tr>
<td>antimicrobial peptide II-37</td>
<td>281</td>
</tr>
<tr>
<td>antimutagenesis</td>
<td>255</td>
</tr>
<tr>
<td>antioxidant</td>
<td>255</td>
</tr>
<tr>
<td>antioxidant defense</td>
<td>81</td>
</tr>
<tr>
<td>anti-oxidant effect</td>
<td>87</td>
</tr>
<tr>
<td>antioxidant enzymes</td>
<td>131, 133, 135</td>
</tr>
<tr>
<td>antioxidants</td>
<td>214</td>
</tr>
<tr>
<td>anti-peptide antibody</td>
<td>284</td>
</tr>
<tr>
<td>antisense-RNA</td>
<td>292</td>
</tr>
<tr>
<td>anti-tumor effect</td>
<td>176</td>
</tr>
<tr>
<td>antitumour</td>
<td>241, 242</td>
</tr>
<tr>
<td>antiviral activity</td>
<td>167, 182, 201, 202, 218</td>
</tr>
<tr>
<td>apoptosis</td>
<td>61, 70, 72, 115, 216, 224, 295</td>
</tr>
<tr>
<td>Aprataxin</td>
<td>273</td>
</tr>
<tr>
<td>aquastatin A</td>
<td>86</td>
</tr>
<tr>
<td>arabidopsis</td>
<td>139, 293</td>
</tr>
<tr>
<td>arabidopsis-thaliana</td>
<td>134, 274</td>
</tr>
<tr>
<td>Arc two-component signal transduction</td>
<td>25</td>
</tr>
<tr>
<td>ArcA response regulator</td>
<td>25</td>
</tr>
<tr>
<td>ARMD</td>
<td>229</td>
</tr>
<tr>
<td>Artemisia princeps Pamp. cv</td>
<td>109</td>
</tr>
<tr>
<td>arterial injury</td>
<td>264</td>
</tr>
<tr>
<td>Term</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>ascorbate peroxidase</td>
<td>127</td>
</tr>
<tr>
<td>ASH</td>
<td>154</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>212, 214</td>
</tr>
<tr>
<td>asposchalin 1</td>
<td>212</td>
</tr>
<tr>
<td>astaxanthin-producing bacterium</td>
<td>153</td>
</tr>
<tr>
<td>asteraceae</td>
<td>219</td>
</tr>
<tr>
<td>asthmal</td>
<td>204</td>
</tr>
<tr>
<td>Astragalus membranaceus</td>
<td>85</td>
</tr>
<tr>
<td>asymmetric dihydroxylations</td>
<td>199</td>
</tr>
<tr>
<td>ataxia telangiectasia mutated</td>
<td>203</td>
</tr>
<tr>
<td>atherosclerosis</td>
<td>109, 262</td>
</tr>
<tr>
<td>attenuated total reflection (ATR)</td>
<td>31</td>
</tr>
<tr>
<td>aureobacterium</td>
<td>98</td>
</tr>
<tr>
<td>autophagic clearance</td>
<td>278</td>
</tr>
<tr>
<td>autophagy</td>
<td>40</td>
</tr>
<tr>
<td>Azo-carboxy-methyl cellulose</td>
<td>243</td>
</tr>
<tr>
<td>B16 Differential in-gel electrophoresis (DIGE)</td>
<td>74</td>
</tr>
<tr>
<td>Bacillus acidiproducens</td>
<td>156</td>
</tr>
<tr>
<td>Bacillus amyloiquefaciens</td>
<td>253</td>
</tr>
<tr>
<td>Bacillus expression</td>
<td>138</td>
</tr>
<tr>
<td>Bacillus thuringiensis</td>
<td>117</td>
</tr>
<tr>
<td>bacillus-circulars</td>
<td>21</td>
</tr>
<tr>
<td>bacillus-curdlanolyticus</td>
<td>97</td>
</tr>
<tr>
<td>bacteremia</td>
<td>220</td>
</tr>
<tr>
<td>bacteria</td>
<td>106, 147, 155, 160</td>
</tr>
<tr>
<td>bacterial evolution</td>
<td>126</td>
</tr>
<tr>
<td>bacterial expression</td>
<td>248</td>
</tr>
<tr>
<td>bacterial systematics</td>
<td>99, 103, 105, 107, 108, 146, 151, 154</td>
</tr>
<tr>
<td>bacterial-cell-walls</td>
<td>98</td>
</tr>
<tr>
<td>bacterium</td>
<td>94, 95, 96, 100, 101, 102, 152, 158</td>
</tr>
<tr>
<td>bean phaseolus-lunatus</td>
<td>134</td>
</tr>
<tr>
<td>benzimidazole</td>
<td>187</td>
</tr>
<tr>
<td>beta neurotoxicity</td>
<td>278</td>
</tr>
<tr>
<td>beta(1,2)-Xylose</td>
<td>4</td>
</tr>
<tr>
<td>beta-catenin</td>
<td>50, 208</td>
</tr>
<tr>
<td>betulinic acid</td>
<td>217</td>
</tr>
<tr>
<td>betulinic acid methyl ester</td>
<td>217</td>
</tr>
<tr>
<td>Bifidobacterium animalis subsp lactis AD011</td>
<td>112</td>
</tr>
<tr>
<td>Bigeum Island</td>
<td>143</td>
</tr>
<tr>
<td>bile</td>
<td>112</td>
</tr>
<tr>
<td>bioaugmentation</td>
<td>116</td>
</tr>
<tr>
<td>biochemical-characterization</td>
<td>83</td>
</tr>
<tr>
<td>bioinformatics</td>
<td>230, 236</td>
</tr>
<tr>
<td>biology</td>
<td>170</td>
</tr>
<tr>
<td>biomarker</td>
<td>180</td>
</tr>
<tr>
<td>biomolecules</td>
<td>32</td>
</tr>
<tr>
<td>biosensor</td>
<td>8, 18, 32</td>
</tr>
<tr>
<td>biosynthesis</td>
<td>198</td>
</tr>
<tr>
<td>biosynthesis gene cluster</td>
<td>255</td>
</tr>
<tr>
<td>BL21(DE3)</td>
<td>120</td>
</tr>
<tr>
<td>bladder-cancer</td>
<td>1</td>
</tr>
<tr>
<td>bone-marrow</td>
<td>208</td>
</tr>
<tr>
<td>bovine embryos</td>
<td>78</td>
</tr>
<tr>
<td>brain creatine kinase</td>
<td>178</td>
</tr>
<tr>
<td>Brassica rapa</td>
<td>274</td>
</tr>
<tr>
<td>breast cancer</td>
<td>48, 74, 225</td>
</tr>
<tr>
<td>Brevundimonas naegangsanensis</td>
<td>254</td>
</tr>
<tr>
<td>Bridelia cambodiana</td>
<td>211</td>
</tr>
<tr>
<td>by-product</td>
<td>244</td>
</tr>
<tr>
<td>CA2+ sparks</td>
<td>255</td>
</tr>
<tr>
<td>Caco-2</td>
<td>242</td>
</tr>
<tr>
<td>calycosin</td>
<td>85</td>
</tr>
<tr>
<td>Canada</td>
<td>150</td>
</tr>
<tr>
<td>cancer</td>
<td>24, 180, 196, 268</td>
</tr>
<tr>
<td>cancer chemoprevention</td>
<td>255</td>
</tr>
<tr>
<td>cancer-cells</td>
<td>71</td>
</tr>
<tr>
<td>cancer-therapy</td>
<td>269</td>
</tr>
<tr>
<td>candida antarctica lipase B (CalB)</td>
<td>137</td>
</tr>
<tr>
<td>canine parvovirus</td>
<td>233</td>
</tr>
<tr>
<td>Capsicum annuum</td>
<td>234, 238</td>
</tr>
<tr>
<td>carbon source</td>
<td>168</td>
</tr>
<tr>
<td>carcinoma</td>
<td>196, 268</td>
</tr>
<tr>
<td>carcinoma cells</td>
<td>265</td>
</tr>
<tr>
<td>cardiac hypertrophy</td>
<td>283</td>
</tr>
<tr>
<td>cardio-facial syndrome</td>
<td>59</td>
</tr>
<tr>
<td>Carnobacterium jeotgali</td>
<td>160</td>
</tr>
<tr>
<td>caspase</td>
<td>72</td>
</tr>
<tr>
<td>caspase cleavage</td>
<td>288</td>
</tr>
<tr>
<td>caspase substrate</td>
<td>26</td>
</tr>
<tr>
<td>caspase-3</td>
<td>70</td>
</tr>
<tr>
<td>cathepsin</td>
<td>53</td>
</tr>
<tr>
<td>cathepsin-1</td>
<td>285</td>
</tr>
<tr>
<td>caulobacter</td>
<td>254</td>
</tr>
<tr>
<td>CD45 phosphotyrosine phosphatase</td>
<td>289</td>
</tr>
<tr>
<td>CD45-associated protein</td>
<td>289</td>
</tr>
<tr>
<td>Cdc25 phosphatase</td>
<td>42, 46</td>
</tr>
<tr>
<td>CDK inhibitors</td>
<td>28</td>
</tr>
<tr>
<td>CDK1</td>
<td>65</td>
</tr>
<tr>
<td>CELISA</td>
<td>8</td>
</tr>
<tr>
<td>cell adhesion molecule</td>
<td>17</td>
</tr>
<tr>
<td>cell cycle arrest</td>
<td>224</td>
</tr>
<tr>
<td>cell differentiation</td>
<td>50, 227</td>
</tr>
<tr>
<td>cell imaging</td>
<td>6</td>
</tr>
<tr>
<td>cell motility</td>
<td>270</td>
</tr>
<tr>
<td>cell-cycle exit</td>
<td>288</td>
</tr>
<tr>
<td>cell-lines</td>
<td>196</td>
</tr>
<tr>
<td>cellular labelling</td>
<td>24</td>
</tr>
<tr>
<td>cellular morphology</td>
<td>24</td>
</tr>
<tr>
<td>cellulose</td>
<td>164, 165</td>
</tr>
<tr>
<td>Cellulosimicrobium sp HY-13</td>
<td>136</td>
</tr>
<tr>
<td>cell-wall</td>
<td>104</td>
</tr>
<tr>
<td>ceramide glucosyltransferase</td>
<td>227</td>
</tr>
<tr>
<td>CGMMV-CP</td>
<td>239</td>
</tr>
<tr>
<td>chalcone-derived diels-alder types</td>
<td>192</td>
</tr>
<tr>
<td>CHE-23C</td>
<td>110</td>
</tr>
<tr>
<td>chili pepper</td>
<td>234, 238</td>
</tr>
<tr>
<td>chilling stress</td>
<td>131</td>
</tr>
<tr>
<td>china</td>
<td>158</td>
</tr>
</tbody>
</table>
chinchilla tracheal epithelium 21
Chloranthus henryi 110
chloroplasts 127
CHRM3 gene 237
chromatin 64
chromatin immunoprecipitation 140
chromatography 149, 155, 156
Chromolaena odorata 219
chromomeric acid G 219
chronic exposure 10
cinnamaldehydes 71
cis-element 91
c-Jun phosphorylation 60
CKS2 65
clavibacter 147
Clitocybe aurantiaca 213
clitosclacin D 213
cloned embryo 78, 80
clonorchis sinensis 294
cluster molecules 16
clustering 262
c-Met 267
c-Myc 72
coactivator torc2 279
coagulans 156
coccus 153
colon cancer 224, 289
colorectal carcinoma 73
comb. nov 100
CoMFA 185
comparative genomics 91, 120
competitive inhibitor 191
CoMSIA 185
confer resistance 139
conformational change 5, 18
conservation 170
constituents 215
core/shell quantum dots 20
Cosmospora sp. 191
coumarins 194
COX-2 205
cpDNA 290
CpG islands 272
CPR 138
crude oil 116
crystal structure 75
CST1 53
CtIP 273
Cudrania tricuspidata 245, 247
cultivated mulberry tree 199, 215
cutaneous malignant-melanoma 74
cutting edge 281
cyanobacteria genes 130
cyclin D1/p21 60
cyclin-dependent kinases 28
CYP3A4 190
cystatin sn 53
cytochrome p450 87
cytokine 204
cytokine-induced killer cells 222, 231
cyttoplasm supports development 44
cytoskeletal localization 12
cytoskeleton 76
cytotoxicity 30, 201, 209, 211
database 174, 175, 177, 277
DAX-1 279
DC-SIGNR 285
de novo design 42, 46
decursin 194
degradation bacterium 104
dehydrocostuslactone 217
deleya 159
delta gal80 137
denaturing gradient gel electrophoresis 118
dendritic cell 33, 176, 281
dendrogram 122
deoxyxypodophyllotoxin 210
deoxyribonucleic-acid 159
DERA 255
desaturase 251
development 9
diabetes 189
diabetic complications 7
diabetic-nephropathy 280
diacylglycerol acyltransferase 183
dicafeoylquinic acid derivatives 186
diels-alder adducts 199
diet 170
difference gel-electrophoresis 74
differential expression 127
differentiation 39, 188, 268
dihydroxyacetone 38
dihydroxyacetone kinase 1 38
discriminant analysis (DA) 122
disintegrin domain 235
diversity 82
DNA 82, 157, 160, 177
DNA damage checkpoint 273
DNA detection 1
DNA marker 144
DNA methylation 272
DNA methyltransferase-1 (Dnmt1) 55
DNA repair 273
DNA sequencer 4
DNA-binding 36, 279
DNA-sequences 274
Dnmt1o 55
Dnmt1s 55
docking 42
doenjang 253
<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>dormancy</td>
<td>181</td>
</tr>
<tr>
<td>down-regulation</td>
<td>288</td>
</tr>
<tr>
<td>downstream-regulated gene-2</td>
<td>268</td>
</tr>
<tr>
<td>DPPH</td>
<td>184, 214</td>
</tr>
<tr>
<td>drosophila</td>
<td>19, 22</td>
</tr>
<tr>
<td>drought</td>
<td>135</td>
</tr>
<tr>
<td>drought stress</td>
<td>89</td>
</tr>
<tr>
<td>drug delivery system (DDS)</td>
<td>79</td>
</tr>
<tr>
<td>drug design</td>
<td>185</td>
</tr>
<tr>
<td>drug metabolism</td>
<td>241</td>
</tr>
<tr>
<td>drug-delivery</td>
<td>20</td>
</tr>
<tr>
<td>DsRed</td>
<td>68</td>
</tr>
<tr>
<td>dual origins</td>
<td>177</td>
</tr>
<tr>
<td>DUSP14</td>
<td>41</td>
</tr>
<tr>
<td>dyslexia susceptibility 1 candidate 1 (DYX1C1)</td>
<td>232</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>77</td>
</tr>
<tr>
<td>E. coli</td>
<td>25</td>
</tr>
<tr>
<td>E. coli B</td>
<td>120</td>
</tr>
<tr>
<td>E. coli O157</td>
<td>223</td>
</tr>
<tr>
<td>earthworm</td>
<td>136</td>
</tr>
<tr>
<td>East Sea</td>
<td>162</td>
</tr>
<tr>
<td>e-cadherin</td>
<td>289</td>
</tr>
<tr>
<td>EGFP</td>
<td>68</td>
</tr>
<tr>
<td>eicosapentaenoic acid</td>
<td>255</td>
</tr>
<tr>
<td>Eisenia fetida</td>
<td>136</td>
</tr>
<tr>
<td>elastase</td>
<td>184</td>
</tr>
<tr>
<td>electrodes</td>
<td>29</td>
</tr>
<tr>
<td>electrotransfer</td>
<td>252</td>
</tr>
<tr>
<td>ELISA</td>
<td>8, 63</td>
</tr>
<tr>
<td>ellagic acid</td>
<td>87</td>
</tr>
<tr>
<td>embryonic stem cells</td>
<td>227</td>
</tr>
<tr>
<td>emended description</td>
<td>156, 159, 254</td>
</tr>
<tr>
<td>endo-beta-1,4-xylanase</td>
<td>136</td>
</tr>
<tr>
<td>endothelial cells</td>
<td>262</td>
</tr>
<tr>
<td>engineered protein</td>
<td>6</td>
</tr>
<tr>
<td>enhanced tolerance</td>
<td>127</td>
</tr>
<tr>
<td>enos-deficient mice</td>
<td>266</td>
</tr>
<tr>
<td>enoyl-ACP reductase</td>
<td>111</td>
</tr>
<tr>
<td>enoyl-acyl carrier protein reductase</td>
<td>86</td>
</tr>
<tr>
<td>enterokinase</td>
<td>226</td>
</tr>
<tr>
<td>environmental factors</td>
<td>263</td>
</tr>
<tr>
<td>enzymatic cleavage</td>
<td>226</td>
</tr>
<tr>
<td>enzyme cascade</td>
<td>8</td>
</tr>
<tr>
<td>eosinophilia</td>
<td>204</td>
</tr>
<tr>
<td>epithelial-cells</td>
<td>220, 282</td>
</tr>
<tr>
<td>epithelial-mesenchymal transition</td>
<td>270</td>
</tr>
<tr>
<td>epitope</td>
<td>58</td>
</tr>
<tr>
<td>ER stress</td>
<td>203, 278</td>
</tr>
<tr>
<td>erk</td>
<td>52</td>
</tr>
<tr>
<td>ERK cascade</td>
<td>12</td>
</tr>
<tr>
<td>erk1/2</td>
<td>40</td>
</tr>
<tr>
<td>ERK-insulin pathway</td>
<td>19</td>
</tr>
<tr>
<td>Erythrina senegalensis</td>
<td>183</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>21, 221, 249, 251</td>
</tr>
<tr>
<td>ESIPT</td>
<td>14</td>
</tr>
<tr>
<td>EST analysis</td>
<td>141</td>
</tr>
<tr>
<td>EST sequences</td>
<td>142</td>
</tr>
<tr>
<td>estrogen</td>
<td>225</td>
</tr>
<tr>
<td>ethanol tolerance</td>
<td>251</td>
</tr>
<tr>
<td>ethylene</td>
<td>114, 293</td>
</tr>
<tr>
<td>euphorbiaceae</td>
<td>211</td>
</tr>
<tr>
<td>evolution</td>
<td>175</td>
</tr>
<tr>
<td>evolutionary visualizer</td>
<td>292</td>
</tr>
<tr>
<td>excretery-secretory products</td>
<td>294</td>
</tr>
<tr>
<td>exon-acquisition</td>
<td>237</td>
</tr>
<tr>
<td>exonization</td>
<td>229</td>
</tr>
<tr>
<td>exosporium</td>
<td>117</td>
</tr>
<tr>
<td>expressed sequence tags</td>
<td>49, 89, 179</td>
</tr>
<tr>
<td>expression data</td>
<td>43</td>
</tr>
<tr>
<td>expression patterns</td>
<td>71</td>
</tr>
<tr>
<td>expression vector</td>
<td>68</td>
</tr>
<tr>
<td>extracellular signal-regulated MAP kinases</td>
<td>227</td>
</tr>
<tr>
<td>extraembryonic tissue</td>
<td>80</td>
</tr>
<tr>
<td>extrahepatic cholangiocarcinoma</td>
<td>200</td>
</tr>
<tr>
<td>eye absent(EYA)</td>
<td>64</td>
</tr>
<tr>
<td>eyes absent 2</td>
<td>283</td>
</tr>
<tr>
<td>fabA</td>
<td>251</td>
</tr>
<tr>
<td>FabI</td>
<td>86</td>
</tr>
<tr>
<td>factor Xa</td>
<td>226</td>
</tr>
<tr>
<td>factor-1</td>
<td>269</td>
</tr>
<tr>
<td>FAK</td>
<td>265</td>
</tr>
<tr>
<td>falcarindiol</td>
<td>210</td>
</tr>
<tr>
<td>false killer whale</td>
<td>144</td>
</tr>
<tr>
<td>family halomonadaceae</td>
<td>159</td>
</tr>
<tr>
<td>fas cin</td>
<td>74</td>
</tr>
<tr>
<td>fatty acid synthesis</td>
<td>111</td>
</tr>
<tr>
<td>fatty acids composition</td>
<td>251</td>
</tr>
<tr>
<td>FBP-1</td>
<td>72</td>
</tr>
<tr>
<td>fermentation</td>
<td>156, 168</td>
</tr>
<tr>
<td>fermented seafood</td>
<td>107</td>
</tr>
<tr>
<td>fern</td>
<td>163</td>
</tr>
<tr>
<td>fez-like</td>
<td>49</td>
</tr>
<tr>
<td>fibrin zymography</td>
<td>67</td>
</tr>
<tr>
<td>fibrinolytic enzyme</td>
<td>67, 253</td>
</tr>
<tr>
<td>fibrosis</td>
<td>276</td>
</tr>
<tr>
<td>firmness</td>
<td>181</td>
</tr>
<tr>
<td>fission yeast</td>
<td>68</td>
</tr>
<tr>
<td>flavanone</td>
<td>215</td>
</tr>
<tr>
<td>flavonoid</td>
<td>184, 215, 247</td>
</tr>
<tr>
<td>flavonol</td>
<td>246</td>
</tr>
<tr>
<td>flight mass-spectrometer</td>
<td>69</td>
</tr>
<tr>
<td>flowering plants</td>
<td>274</td>
</tr>
<tr>
<td>fluorescence resonance energy transfer</td>
<td>5</td>
</tr>
<tr>
<td>focal adhesion</td>
<td>265</td>
</tr>
<tr>
<td>forest pathogens</td>
<td>169</td>
</tr>
<tr>
<td>fourier transformation infrared spectroscopy</td>
<td>128</td>
</tr>
<tr>
<td>FRPase</td>
<td>37</td>
</tr>
<tr>
<td>Fragaria ananassa</td>
<td>128</td>
</tr>
</tbody>
</table>
histone deacetylase 1 140
histone h2b 288
HIF-1 alpha inhibitor 187
hnRNPs 295
hollow polymer microspheres 33
homology 175
horizontal transmission 230
hormone-receptor 279
host-defense 220
houttuynia cordat 182
Houttuynia cordata 202
Hpal SINEs 230
HPV type 18 248
hs60 40
hs90 187
HSQC 47
HT22 40
hTAF(II)31 90
human embryonic stem cell 76
human endogenous retrovirus (HERV) 232
human mesenchymal stem cells 39
human mitochondrial genome 177
human neutrophil elastase 186, 206, 213
human papillomavirus 248
hybridization 157, 160
hydrogen-peroxide 22, 51, 81
hydrolytic enzymes 243
hyperhydricity 114
Hyphomonadaceae 162
hypoaxia 187
hypoaxia-inducible factor 1 56
hypoaxia-inducible factor 1 alpha 125
Hypsizigus marmoreus 255
identification 93, 98, 99, 100, 101, 102, 103, 105, 107, 108, 146, 147, 154, 170, 179, 254

IGG 2
igG-binding protein 296
il-1 receptor 266
IL-13 205
IL-4 205
Ilex paraguariensis 186
iloprost 78
imaging agents 3, 271
imidazo[1,2-alpha]pyridines 195
immobilization methods 2
immunoassays 8
immunoprecipitation 140
inactivation 71
inclusion-body formation 278
indenone 52
induced apoptosis 70
induced cell activation 282
induced macrophage apoptosis 220
induced phase-shifts 291
inducible factor 1-alpha 269
induction 216
inflammation 34, 109
influenza 202
influenza virus 246
influenza-virus entry 35
InhA 117
inhibition kinetics 178
inhibitor 46, 86
innate immunity 134
inocula dependence 240
iNOS 205
insulin 264, 279
insulin-like growth factor binding protein-3 140
insulin-like growth factor type 1 140
interferon response 282
Interleukin-6 226
intestinal flat metagenome 82
intracellular delivery 6
intradermal immunization 79
intraneural inclusions 278
intravenous injection 34
intrinsically unfolded protein 90
invertebrate 136
in-vitro 78, 84, 261
in-vitro differentiation 45
in-vivo 20, 220
ion channels 255
ionotropic glutamate receptor 10
ion-sensitive field-effect transistor 32
ISFET 18, 32
isodeoxyhelicobasidin 206
isoelectric focusing 250
isoflavone 189
ISSRs 290
jasmonic acid 293
jeotgal 107
JNK 41
kappa-B activation 209
KB-3-1 cervical cancer 231
KBH-A42 224
Kimchi 145
kinase-c-zeta 281
kinases 265
kinetics 161
Kirengeshoma 290
Klebsiella pneumoniae 244
Korea 93, 156, 254
KSHV 287
KSHV Bcl-2 47
L1 cell adhesion molecule 200
L1 major capsid protein 248
L1HS element 237
L6 myotube 189
<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>lactic acid bacteria</td>
<td>145</td>
</tr>
<tr>
<td>lake</td>
<td>93, 100, 158</td>
</tr>
<tr>
<td>landbridge</td>
<td>290</td>
</tr>
<tr>
<td>leaf development</td>
<td>133</td>
</tr>
<tr>
<td>lectin</td>
<td>180</td>
</tr>
<tr>
<td>legumain</td>
<td>294</td>
</tr>
<tr>
<td>Leifsonia kribbensis</td>
<td>147</td>
</tr>
<tr>
<td>leisingera</td>
<td>102</td>
</tr>
<tr>
<td>length cDNA-clone</td>
<td>84</td>
</tr>
<tr>
<td>lepidoptera</td>
<td>170</td>
</tr>
<tr>
<td>leukocyte common antigen related</td>
<td>66</td>
</tr>
<tr>
<td>levansucrase gene</td>
<td>249</td>
</tr>
<tr>
<td>lineage markers</td>
<td>9</td>
</tr>
<tr>
<td>lineage sorting</td>
<td>230</td>
</tr>
<tr>
<td>linear discriminant function analysis</td>
<td>128</td>
</tr>
<tr>
<td>linear regression</td>
<td>123</td>
</tr>
<tr>
<td>lipase</td>
<td>252</td>
</tr>
<tr>
<td>LipEH166</td>
<td>82</td>
</tr>
<tr>
<td>lipid peroxidation</td>
<td>131</td>
</tr>
<tr>
<td>lipolysis</td>
<td>52</td>
</tr>
<tr>
<td>liver cancer</td>
<td>57</td>
</tr>
<tr>
<td>liver fibrosis</td>
<td>275</td>
</tr>
<tr>
<td>liver pathogenesis</td>
<td>27</td>
</tr>
<tr>
<td>localized surface plasmon resonance (LSPR)</td>
<td>31</td>
</tr>
<tr>
<td>long terminal repeat (LTR)</td>
<td>232</td>
</tr>
<tr>
<td>low endotoxicity</td>
<td>233</td>
</tr>
<tr>
<td>L-PHA</td>
<td>284</td>
</tr>
<tr>
<td>LSU rDNA</td>
<td>169</td>
</tr>
<tr>
<td>LTR element</td>
<td>236</td>
</tr>
<tr>
<td>LTR7B</td>
<td>229</td>
</tr>
<tr>
<td>luciferase</td>
<td>215</td>
</tr>
<tr>
<td>luciferase assay</td>
<td>210</td>
</tr>
<tr>
<td>lung-cancer cells</td>
<td>51</td>
</tr>
<tr>
<td>lupenone</td>
<td>207</td>
</tr>
<tr>
<td>lupeol</td>
<td>207</td>
</tr>
<tr>
<td>Lutimaribacter saemankumensis</td>
<td>93</td>
</tr>
<tr>
<td>lycopene</td>
<td>240</td>
</tr>
<tr>
<td>lymph-nodes</td>
<td>15</td>
</tr>
<tr>
<td>lymphocyte</td>
<td>220</td>
</tr>
<tr>
<td>Lyso bacter panaciterra</td>
<td>96</td>
</tr>
<tr>
<td>macrophage activation</td>
<td>281</td>
</tr>
<tr>
<td>macrophage-inhibitory cytokine-1</td>
<td>54</td>
</tr>
<tr>
<td>magnetic materials</td>
<td>33</td>
</tr>
<tr>
<td>magnetic nanoparticles</td>
<td>33</td>
</tr>
<tr>
<td>magnetic-resonance</td>
<td>20</td>
</tr>
<tr>
<td>maize</td>
<td>228</td>
</tr>
<tr>
<td>MALDI-TOF/MS</td>
<td>250</td>
</tr>
<tr>
<td>maltose binding protein, MBP</td>
<td>5, 226</td>
</tr>
<tr>
<td>map kinase</td>
<td>28</td>
</tr>
<tr>
<td>marine-bacteria</td>
<td>93</td>
</tr>
<tr>
<td>marine-derived fungus</td>
<td>191</td>
</tr>
<tr>
<td>Marinimicrobium locisalis</td>
<td>99</td>
</tr>
<tr>
<td>mast-cells</td>
<td>266</td>
</tr>
<tr>
<td>matrix metalloproteinase-9</td>
<td>210</td>
</tr>
<tr>
<td>matrix metalloproteinases</td>
<td>264</td>
</tr>
<tr>
<td>MBP</td>
<td>18</td>
</tr>
<tr>
<td>MCF-7</td>
<td>225</td>
</tr>
<tr>
<td>mcmeekinii</td>
<td>107</td>
</tr>
<tr>
<td>MDCK cell</td>
<td>246</td>
</tr>
<tr>
<td>mdm2</td>
<td>269</td>
</tr>
<tr>
<td>mediated up-regulation</td>
<td>51</td>
</tr>
<tr>
<td>melanin biosynthesis</td>
<td>85</td>
</tr>
<tr>
<td>melanogenesis</td>
<td>212</td>
</tr>
<tr>
<td>melanoma</td>
<td>74</td>
</tr>
<tr>
<td>melanoma patients</td>
<td>15</td>
</tr>
<tr>
<td>membrane vesicles</td>
<td>296</td>
</tr>
<tr>
<td>mesenchymal stem cells</td>
<td>66</td>
</tr>
<tr>
<td>MES-SA/DX5</td>
<td>190</td>
</tr>
<tr>
<td>metabolism</td>
<td>242</td>
</tr>
<tr>
<td>metabotropic glutamate receptor</td>
<td>10</td>
</tr>
<tr>
<td>metagenome</td>
<td>145</td>
</tr>
<tr>
<td>metastasis</td>
<td>73, 74</td>
</tr>
<tr>
<td>metatranscriptome</td>
<td>145</td>
</tr>
<tr>
<td>methyl salicylate</td>
<td>134</td>
</tr>
<tr>
<td>Mg2+</td>
<td>255</td>
</tr>
<tr>
<td>MIC-1</td>
<td>54</td>
</tr>
<tr>
<td>mice lacking</td>
<td>291</td>
</tr>
<tr>
<td>micelles</td>
<td>271</td>
</tr>
<tr>
<td>microarray data</td>
<td>43</td>
</tr>
<tr>
<td>Microbacterium insulac</td>
<td>98</td>
</tr>
<tr>
<td>microbial community</td>
<td>118</td>
</tr>
<tr>
<td>microbial diversity</td>
<td>143</td>
</tr>
<tr>
<td>microbiology</td>
<td>296</td>
</tr>
<tr>
<td>micropropagation</td>
<td>114</td>
</tr>
<tr>
<td>microvesicles</td>
<td>296</td>
</tr>
<tr>
<td>mindact trial</td>
<td>43</td>
</tr>
<tr>
<td>mitochondria</td>
<td>173</td>
</tr>
<tr>
<td>mitochondrial dysfunction</td>
<td>22</td>
</tr>
<tr>
<td>mitochondrial protein</td>
<td>36</td>
</tr>
<tr>
<td>mitomap</td>
<td>171</td>
</tr>
<tr>
<td>mixed-substrates</td>
<td>252</td>
</tr>
<tr>
<td>MKP-2</td>
<td>75</td>
</tr>
<tr>
<td>mokko lactone</td>
<td>217</td>
</tr>
<tr>
<td>molecular chaperones</td>
<td>40</td>
</tr>
<tr>
<td>molecular characterization</td>
<td>255</td>
</tr>
<tr>
<td>molecular evolution</td>
<td>126</td>
</tr>
<tr>
<td>molecular farming</td>
<td>263</td>
</tr>
<tr>
<td>molecular interaction</td>
<td>47</td>
</tr>
<tr>
<td>molecular interaction database</td>
<td>172, 173</td>
</tr>
<tr>
<td>molecular-basis</td>
<td>286</td>
</tr>
<tr>
<td>montreal biodome</td>
<td>150</td>
</tr>
<tr>
<td>morgan-elson</td>
<td>1</td>
</tr>
<tr>
<td>morus bombycis</td>
<td>192</td>
</tr>
<tr>
<td>morus root bark</td>
<td>199</td>
</tr>
<tr>
<td>motility</td>
<td>265</td>
</tr>
<tr>
<td>MRI contrast agents</td>
<td>15, 20</td>
</tr>
<tr>
<td>MRM</td>
<td>284</td>
</tr>
<tr>
<td>mRNA export</td>
<td>193</td>
</tr>
<tr>
<td>mRNA stability</td>
<td>193</td>
</tr>
<tr>
<td>MRS broth</td>
<td>201</td>
</tr>
<tr>
<td>Term</td>
<td>Page</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>MsbB (LpxM)</td>
<td>223</td>
</tr>
<tr>
<td>MSn</td>
<td>62</td>
</tr>
<tr>
<td>MSP</td>
<td>280</td>
</tr>
<tr>
<td>MspTL</td>
<td>282</td>
</tr>
<tr>
<td>multidrug resistance</td>
<td>190, 194</td>
</tr>
<tr>
<td>multi-immunogenicity</td>
<td>233</td>
</tr>
<tr>
<td>multiple sequence alignment</td>
<td>286</td>
</tr>
<tr>
<td>multivariate analysis</td>
<td>88</td>
</tr>
<tr>
<td>mutation</td>
<td>278</td>
</tr>
<tr>
<td>mycobacterium-tuberculosis</td>
<td>282</td>
</tr>
<tr>
<td>MyD88</td>
<td>176</td>
</tr>
<tr>
<td>NADPH oxidase</td>
<td>81, 281</td>
</tr>
<tr>
<td>NADPH-cytochrome P450</td>
<td></td>
</tr>
<tr>
<td>reducec10g</td>
<td>138</td>
</tr>
<tr>
<td>nanocrystals</td>
<td>16</td>
</tr>
<tr>
<td>nanoparticles</td>
<td>3, 6, 13, 15, 20, 29, 33</td>
</tr>
<tr>
<td>nanorods</td>
<td>13, 16</td>
</tr>
<tr>
<td>nanostructures</td>
<td>13</td>
</tr>
<tr>
<td>nanotubes</td>
<td>11</td>
</tr>
<tr>
<td>naphtalene</td>
<td>118</td>
</tr>
<tr>
<td>natural products</td>
<td>198</td>
</tr>
<tr>
<td>NCBI</td>
<td>174</td>
</tr>
<tr>
<td>NDRG2</td>
<td>60</td>
</tr>
<tr>
<td>necrosis-factor-alpha</td>
<td>51</td>
</tr>
<tr>
<td>necrosis-virus</td>
<td>84</td>
</tr>
<tr>
<td>necrotic-spot-virus</td>
<td>84</td>
</tr>
<tr>
<td>negative bacterial-infections</td>
<td>113</td>
</tr>
<tr>
<td>negative breast-cancer</td>
<td>43</td>
</tr>
<tr>
<td>neointimal hyperplasia</td>
<td>262</td>
</tr>
<tr>
<td>neovascularization</td>
<td>56, 264</td>
</tr>
<tr>
<td>N-epsilon-(carboxymethyl)lysine (CML)</td>
<td>7</td>
</tr>
<tr>
<td>neural precursors</td>
<td>45</td>
</tr>
<tr>
<td>neural stem cells</td>
<td>76</td>
</tr>
<tr>
<td>neuraminidase</td>
<td>245, 247</td>
</tr>
<tr>
<td>neuraminidase inhibitor</td>
<td>246</td>
</tr>
<tr>
<td>neurodegenerative disease</td>
<td>278</td>
</tr>
<tr>
<td>neuroectodermal sphere</td>
<td>76</td>
</tr>
<tr>
<td>neuronal cell-death</td>
<td>278</td>
</tr>
<tr>
<td>neuronal differentiation</td>
<td>45</td>
</tr>
<tr>
<td>neurons</td>
<td>227</td>
</tr>
<tr>
<td>neutralizing antibody</td>
<td>58</td>
</tr>
<tr>
<td>neutrophil apoptosis</td>
<td>220</td>
</tr>
<tr>
<td>NF-kappa B</td>
<td>109, 188, 281</td>
</tr>
<tr>
<td>niche</td>
<td>208</td>
</tr>
<tr>
<td>Nicotiana benthamiana</td>
<td>121</td>
</tr>
<tr>
<td>Nitratiireductor basaltis</td>
<td>150</td>
</tr>
<tr>
<td>NK cells</td>
<td>188, 220</td>
</tr>
<tr>
<td>NMR</td>
<td>47, 90</td>
</tr>
<tr>
<td>NMR spectroscopy</td>
<td>125</td>
</tr>
<tr>
<td>no association</td>
<td>59</td>
</tr>
<tr>
<td>nocardia</td>
<td>155</td>
</tr>
<tr>
<td>Nocardia sp.</td>
<td>116</td>
</tr>
<tr>
<td>nocardioides</td>
<td>105</td>
</tr>
<tr>
<td>Nocardoides basaltis</td>
<td>149</td>
</tr>
<tr>
<td>Nocardoides caeni</td>
<td>104</td>
</tr>
<tr>
<td>Nocardoides sediminis</td>
<td>151</td>
</tr>
<tr>
<td>non-competitive inhibitors</td>
<td>207</td>
</tr>
<tr>
<td>novel bacterium</td>
<td>163, 164</td>
</tr>
<tr>
<td>NS5A protein</td>
<td>27</td>
</tr>
<tr>
<td>N-terminal arm</td>
<td>92</td>
</tr>
<tr>
<td>n-terminal kinase</td>
<td>51</td>
</tr>
<tr>
<td>nuclear magnetic resonance</td>
<td>58</td>
</tr>
<tr>
<td>nuclear transfer embryos</td>
<td>44</td>
</tr>
<tr>
<td>nuclear translocation</td>
<td>288</td>
</tr>
<tr>
<td>nucleoside diphosphate kinase</td>
<td>124</td>
</tr>
<tr>
<td>nutlin-3</td>
<td>269</td>
</tr>
<tr>
<td>nutrient use efficiency</td>
<td>139</td>
</tr>
<tr>
<td>o-coumaric acid</td>
<td>241, 242</td>
</tr>
<tr>
<td>oleracea</td>
<td>274</td>
</tr>
<tr>
<td>OmpA fusion</td>
<td>223</td>
</tr>
<tr>
<td>OMV</td>
<td>223</td>
</tr>
<tr>
<td>Oncorhynchus</td>
<td>230</td>
</tr>
<tr>
<td>oocyte-specific</td>
<td>55</td>
</tr>
<tr>
<td>order rhodobacterales</td>
<td>93</td>
</tr>
<tr>
<td>organogenesis</td>
<td>132</td>
</tr>
<tr>
<td>osteoblast</td>
<td>66</td>
</tr>
<tr>
<td>osteoclast differentiation</td>
<td>282</td>
</tr>
<tr>
<td>outer membrane vesicle</td>
<td>233</td>
</tr>
<tr>
<td>ovalbumin</td>
<td>79</td>
</tr>
<tr>
<td>overexpression</td>
<td>267</td>
</tr>
<tr>
<td>overlapping genes</td>
<td>292</td>
</tr>
<tr>
<td>oxidative stress</td>
<td>38, 71, 81, 124, 127</td>
</tr>
<tr>
<td>oxidized LDL</td>
<td>109</td>
</tr>
<tr>
<td>oxygen-dependent degradation domain</td>
<td>125</td>
</tr>
<tr>
<td>p35</td>
<td>295</td>
</tr>
<tr>
<td>p53</td>
<td>47, 65, 269</td>
</tr>
<tr>
<td>p53 transactivation</td>
<td>73</td>
</tr>
<tr>
<td>Paenibacillus filicis</td>
<td>163</td>
</tr>
<tr>
<td>Paenibacillus harenae</td>
<td>146</td>
</tr>
<tr>
<td>Paenibacillus pectinilyticus</td>
<td>97</td>
</tr>
<tr>
<td>Paenibacillus pini</td>
<td>165</td>
</tr>
<tr>
<td>Paenibacillus pinhumi</td>
<td>164</td>
</tr>
<tr>
<td>Paenibacillus pueri</td>
<td>154</td>
</tr>
<tr>
<td>PAI-1</td>
<td>276</td>
</tr>
<tr>
<td>pancreatic stellate cells</td>
<td>275</td>
</tr>
<tr>
<td>pandemic virus</td>
<td>285</td>
</tr>
<tr>
<td>Pantoea ananatis</td>
<td>240</td>
</tr>
<tr>
<td>Paracoccus aestuarii</td>
<td>153</td>
</tr>
<tr>
<td>parallel changes</td>
<td>126</td>
</tr>
<tr>
<td>pathologic</td>
<td>56</td>
</tr>
<tr>
<td>PBR</td>
<td>261</td>
</tr>
<tr>
<td>PCNA binding</td>
<td>28</td>
</tr>
<tr>
<td>PCR</td>
<td>228</td>
</tr>
<tr>
<td>Pedobacter composti</td>
<td>95</td>
</tr>
<tr>
<td>peptide quantitation</td>
<td>284</td>
</tr>
<tr>
<td>performance liquid-chromatography</td>
<td>94, 95, 96</td>
</tr>
<tr>
<td>peripheral benzodiazepine receptor</td>
<td>261</td>
</tr>
<tr>
<td>peripheral-tissues</td>
<td>291</td>
</tr>
<tr>
<td>Term</td>
<td>Page</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>periplasmic nuclease</td>
<td>36</td>
</tr>
<tr>
<td>peroxidase promoter</td>
<td>124</td>
</tr>
<tr>
<td>peroxisome proliferators-activated receptor gamma</td>
<td>52</td>
</tr>
<tr>
<td>PERV</td>
<td>236</td>
</tr>
<tr>
<td>p-genotype</td>
<td>255</td>
</tr>
<tr>
<td>p-glycoprotein</td>
<td>190, 194</td>
</tr>
<tr>
<td>pharmacokinetics</td>
<td>34, 241</td>
</tr>
<tr>
<td>pharmacophore</td>
<td>185</td>
</tr>
<tr>
<td>phase I enzyme</td>
<td>255</td>
</tr>
<tr>
<td>phase II enzyme</td>
<td>255</td>
</tr>
<tr>
<td>phosphatase assay</td>
<td>41</td>
</tr>
<tr>
<td>phosphatidylinositol 3-kinase</td>
<td>281</td>
</tr>
<tr>
<td>phospholipase-c-gamma</td>
<td>209</td>
</tr>
<tr>
<td>phosphopeptide</td>
<td>62</td>
</tr>
<tr>
<td>photodissociation</td>
<td>62</td>
</tr>
<tr>
<td>photoluminescence</td>
<td>3</td>
</tr>
<tr>
<td>phylogenetic analysis</td>
<td>113, 274, 286</td>
</tr>
<tr>
<td>phylogenetic footprinting</td>
<td>91</td>
</tr>
<tr>
<td>phylogenetic trees</td>
<td>147</td>
</tr>
<tr>
<td>phylogeny</td>
<td>144</td>
</tr>
<tr>
<td>phylogeography</td>
<td>290</td>
</tr>
<tr>
<td>Phytophthora infestans</td>
<td>110</td>
</tr>
<tr>
<td>PI3K/Akt/mTOR</td>
<td>283</td>
</tr>
<tr>
<td>pig</td>
<td>236</td>
</tr>
<tr>
<td>pig cloned embryos</td>
<td>55</td>
</tr>
<tr>
<td>pine tree</td>
<td>164, 165</td>
</tr>
<tr>
<td>PKS-like module</td>
<td>255</td>
</tr>
<tr>
<td>Planomicrobium flavidum</td>
<td>107</td>
</tr>
<tr>
<td>plant N-glycan</td>
<td>4</td>
</tr>
<tr>
<td>plasmid copy number</td>
<td>240</td>
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<tr>
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<td>121</td>
</tr>
<tr>
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<td>16</td>
</tr>
<tr>
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<td>81</td>
</tr>
<tr>
<td>pollen-tube growth</td>
<td>81</td>
</tr>
<tr>
<td>poly-gamma-glutamate</td>
<td>176</td>
</tr>
<tr>
<td>polygonaceae</td>
<td>37</td>
</tr>
<tr>
<td>Polygonum multiflorum</td>
<td>37</td>
</tr>
<tr>
<td>polyketides</td>
<td>198</td>
</tr>
<tr>
<td>polymerase chain-reaction</td>
<td>49</td>
</tr>
<tr>
<td>polymeric thermosensor</td>
<td>271</td>
</tr>
<tr>
<td>polyl process</td>
<td>16</td>
</tr>
<tr>
<td>polyphasic approach</td>
<td>143</td>
</tr>
<tr>
<td>polyphasic taxonomy</td>
<td>167</td>
</tr>
<tr>
<td>polyploidy</td>
<td>274</td>
</tr>
<tr>
<td>poor-prognosis</td>
<td>268</td>
</tr>
<tr>
<td>population-genetics</td>
<td>126</td>
</tr>
<tr>
<td>population-structure</td>
<td>297</td>
</tr>
<tr>
<td>porcine</td>
<td>235</td>
</tr>
<tr>
<td>porcine epidemic diarrhea virus</td>
<td>182</td>
</tr>
<tr>
<td>porcine pancreas</td>
<td>9</td>
</tr>
<tr>
<td>porcine rotavirus</td>
<td>255</td>
</tr>
<tr>
<td>porphyromonas-gingivalis</td>
<td>282</td>
</tr>
<tr>
<td>positron-emission-tomography</td>
<td>30</td>
</tr>
<tr>
<td>posttranscriptional regulation</td>
<td>291</td>
</tr>
<tr>
<td>potato</td>
<td>114</td>
</tr>
<tr>
<td>PPAR alpha</td>
<td>276</td>
</tr>
<tr>
<td>PPAR-gamma</td>
<td>219</td>
</tr>
<tr>
<td>prantschimgin</td>
<td>194</td>
</tr>
<tr>
<td>precursor cells</td>
<td>264</td>
</tr>
<tr>
<td>preimplantation development</td>
<td>55</td>
</tr>
<tr>
<td>preimplantation mouse embryos</td>
<td>44</td>
</tr>
<tr>
<td>prenylflavonoid</td>
<td>183</td>
</tr>
<tr>
<td>pre-ribosomal-RNA</td>
<td>44</td>
</tr>
<tr>
<td>preS1</td>
<td>58</td>
</tr>
<tr>
<td>principal component analysis (PCA)</td>
<td>88, 122, 128</td>
</tr>
<tr>
<td>processed peptide</td>
<td>19</td>
</tr>
<tr>
<td>progenitor cells</td>
<td>45</td>
</tr>
<tr>
<td>prognosis</td>
<td>200</td>
</tr>
<tr>
<td>program</td>
<td>141, 142</td>
</tr>
<tr>
<td>proliferation</td>
<td>53, 262</td>
</tr>
<tr>
<td>proliferation arrest</td>
<td>288</td>
</tr>
<tr>
<td>promoter</td>
<td>249</td>
</tr>
<tr>
<td>promotes apoptosis</td>
<td>288</td>
</tr>
<tr>
<td>prone proteins</td>
<td>278</td>
</tr>
<tr>
<td>prostate-cancer</td>
<td>74</td>
</tr>
<tr>
<td>prostate-specific antigen</td>
<td>29</td>
</tr>
<tr>
<td>protease</td>
<td>250, 252</td>
</tr>
<tr>
<td>protease activity</td>
<td>26</td>
</tr>
<tr>
<td>protein arrays</td>
<td>295</td>
</tr>
<tr>
<td>protein crystallization</td>
<td>161</td>
</tr>
<tr>
<td>protein engineering</td>
<td>92</td>
</tr>
<tr>
<td>protein interactions</td>
<td>172</td>
</tr>
<tr>
<td>protein stability</td>
<td>12</td>
</tr>
<tr>
<td>protein tyrosine phosphatase</td>
<td>66, 75</td>
</tr>
<tr>
<td>protein tyrosine phosphatase 1B (PTP1B)</td>
<td>191, 192, 207, 217</td>
</tr>
<tr>
<td>protein tyrosine phosphatase RQ</td>
<td>39</td>
</tr>
<tr>
<td>protein-kinase</td>
<td>220, 279, 288</td>
</tr>
<tr>
<td>protein-tyrosine kinases</td>
<td>289</td>
</tr>
<tr>
<td>protein-tyrosine phosphatases</td>
<td>14</td>
</tr>
<tr>
<td>proteolytic indicator</td>
<td>26</td>
</tr>
<tr>
<td>proteome</td>
<td>294</td>
</tr>
<tr>
<td>protocomics</td>
<td>9, 74</td>
</tr>
<tr>
<td>PSCs</td>
<td>275</td>
</tr>
<tr>
<td>Pseudomonas sabulinigri</td>
<td>148</td>
</tr>
<tr>
<td>Psychroflexus salinarum</td>
<td>100</td>
</tr>
<tr>
<td>psychrophilus</td>
<td>107</td>
</tr>
<tr>
<td>PTP inhibitor IV</td>
<td>41</td>
</tr>
<tr>
<td>PTP1b</td>
<td>14</td>
</tr>
<tr>
<td>PTPRT</td>
<td>17</td>
</tr>
<tr>
<td>Puccinia recondita</td>
<td>110</td>
</tr>
<tr>
<td>pucciniales</td>
<td>169</td>
</tr>
<tr>
<td>purification</td>
<td>23, 248</td>
</tr>
<tr>
<td>pylori caga protein</td>
<td>289</td>
</tr>
<tr>
<td>pyrolysis mass spectrometry (PyMS)</td>
<td>122, 123</td>
</tr>
<tr>
<td>pyrrolezanidine-6-methyl ether</td>
<td>186</td>
</tr>
</tbody>
</table>
quantitative determination 123
quantum dots 15, 24, 30, 33
Quaternary 290
quercetin 3-rhamnoside (Q3R) 202
quercetin 7-rhamnoside 182
quinone methides 199
Rahnella aquatilis 249
Raoulia australis 218
Raoulic acid 218
rapid method 123
ras 197, 209
rat mesangial cells 51
reaction intermediate monitoring 62
reaction-rate constants 69
reactive oxygen 51, 281
real-time PCR 116
receptor-binding 285
receptors 209
reclassification 100, 159
recombinant antibody 193
recombinant human erythropoietin (rhEPO) 23
recombinant protein 137, 263
redox signaling pathways 81
reflection fluorescence microscopy 255
regulated promoter 221
REL606 120
ren glycolone 115
repetitive DNA element 272
repetitive sequence 144
resistant acinetobacter-baumannii 113
resonance assignment 125
resveratrol 204
reticulon 214
retinol-binding-protein 275
rhizosphere 146, 163, 164, 165
RhoB 61
Rhodiola rosea 246, 255
rhodisios 255
ribosomal-RNA sequence 103
ring-finger domain 287
RNA viruses 201
root 89
root bark 215
root explants 132
rootstock 239
ROS 197
Rosa hybrida L. 132
Rosa rugosa 129
Rosaceae 207
roseobacter 102
roseobacter clade 101
rosette 76
rosiglitazone 52
RT-PCR assays 286
rubus coreanus 87
S. Typhimurium 233
S100A4 73
Saccharomyces cerevisiae 137, 221
Sajabal 109
salicylic acid 293
Salimicrobium flavidum 106
Salinhabitans flavidus 101
salt 135
salvia-miltiorrhiza bunge 48
SAPK/JNK 61
sat 239
Saussurea lappa C.b.Clarke 217
SCAMP5 278
Schizosaccharomyces pombe 273
scion 239
sea 152, 156
seafood 158
seawater 93
sediment 82, 93, 99, 100, 101, 104, 107
seed potato 181
self-renewal 208, 298
senescence 133
sensors 271
Seohaenicola saemankumensis 102
sequence 171
sequence alignment 95, 97, 148, 150, 152, 153, 154, 155
sequences 105, 151, 159
serine-protease 285
serum 63
serum-albumin 275
sesquiterpenoid 206
SFTPB gene 229
shape-controlled synthesis 16
Shewanella sp BR-2 255
short neuropeptide F 19
SHP 276, 279
shrimp jeotgal 158
signaling pathway 280
signal-transduction 134, 287
silica 3, 33
Similarity of E. coli B and K-12 120
single sodium dodecyl sulfate 243
singly protonated peptides 69
SISCAPA 284
site-directed mutagenesis 198
sludge 96, 108
small nucleolar RNA 44
smooth-muscle 78
smooth-muscle-cells 266
software 152
soil 95, 103, 146, 149, 151, 154
solanum tuberosum 181
sol-gel 3
solid supports 2
somatic cell nuclear transfer 80
somatic embryo conversion 132
somatic embryogenesis 129, 132
sorbus commixta Hedl. 207
southern blot 144
soybean 228
sp nov. 162
sperms-egg interaction 235
Sphingomonas hankookensis 103
Spheroidea panaciterrae 94
spore display 117, 138
sprouting vigor 181
squamous-cell carcinoma 35
Src 265
src family kinases 289
SRC oncoprotein 48
ST2 comprise 266
Staphylococcus aureus 86, 111, 296
Staphylococcus sp strain AJ 67
stat3 serine phosphorylation 48
steatosis 27
stem cell-microenvironment interactions 298
stem-cell 264
sterile-20 kinase 288
stomach neoplasms 56
stomatal guard-cells 81
storability 181
storing cells 275
strain r6 21
strains 99, 103, 105, 106, 107, 108, 156, 254
stroma 298
structural relevance 92
structure-activity relationship 195
subgenomic RNA 84
subtilisin D5 253
superoxide dismutase 114, 127
suppressor gene 50
surface charge 18
surface modification 6
surface plasmon resonance imaging (SPRI) 7
surface proteolytic-enzymes 35
surface-induced dissociation 69
surface-plasmon resonance 13
surfactant 24
survival 267
survival analysis 200
suspension-cultures 127
sweetpotato 89, 124, 133
Symbiobacterium toebii 92
symbiotic bacteria 83
synapse formation 17
synthetic operon 240
system 179
systematics 98, 104, 159, 169, 254
systemic acquired-resistance 134
systemic resistance 139, 293
tanshinone-iia 48
target validation 111
taxa 100
taxonomy 162
t-bet 188
t-cells 209, 282
teicoplanin 168
telia stage 169
temperature-shift 240
Terrabacter terrigena 105
Tetracera scandens 189
tetrapeptide 26
tetraspanins 270
TFBS 91
TGF-beta 275
thermoacid-stable 67
thrombin 226
Thuja orientalis 184
tidal flat sediment 149
time-dependent 245
TIMP1 284
tissue 63
tissue culture 129
tissue microarray analysis 35
TLR4 176
TM4SF5 270
TNFAIP1 61
TNF-alpha 188
tobacco 127
toll-like receptor 281
TOM40 295
total petroleum hydrocarbon 116
tract-binding-protein 291
trade-offs 134
transactivation 279
transcription factor 91, 279
transcriptional activation domain 90
transcriptional profiling 80
transcripts 292
transgenic 239
transgenic mice 27
transgenic pig 23
transgenic plants 130, 263
transient expression 193
transport 242
transposable elements 237
trees 104, 105, 148, 151, 157, 158
treponema-lecithinolyticum 282
trichostatin A 140
triterpenoids 211
trypsin-like protease 35
<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>tuberculosis</td>
<td>281</td>
</tr>
<tr>
<td>tumor angiogenesis</td>
<td>264</td>
</tr>
<tr>
<td>tumor suppression</td>
<td>291</td>
</tr>
<tr>
<td>tumor xenograft model</td>
<td>225</td>
</tr>
<tr>
<td>tumor-suppressor</td>
<td>288</td>
</tr>
<tr>
<td>tunicamycin</td>
<td>203</td>
</tr>
<tr>
<td>turkish children</td>
<td>59</td>
</tr>
<tr>
<td>turnip crinkle virus</td>
<td>84</td>
</tr>
<tr>
<td>two-dimensional electrophoresis</td>
<td>250</td>
</tr>
<tr>
<td>tyrosinase</td>
<td>85</td>
</tr>
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<td>tyrosine phenol-lyase</td>
<td>92</td>
</tr>
<tr>
<td>tyrosine phosphorylation</td>
<td>17</td>
</tr>
<tr>
<td>U-87 MG glioma</td>
<td>222</td>
</tr>
<tr>
<td>U937 cells</td>
<td>115</td>
</tr>
<tr>
<td>ubiquitin ligase</td>
<td>287</td>
</tr>
<tr>
<td>ubiquitin-proteasome system</td>
<td>278</td>
</tr>
<tr>
<td>ultrasensitive detection</td>
<td>1</td>
</tr>
<tr>
<td>uv-irradiation</td>
<td>28</td>
</tr>
<tr>
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<td>255</td>
</tr>
<tr>
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<td>297</td>
</tr>
<tr>
<td>vaccination</td>
<td>79</td>
</tr>
<tr>
<td>vaccine vehicle</td>
<td>223</td>
</tr>
<tr>
<td>vapor diffusion</td>
<td>161</td>
</tr>
<tr>
<td>variome</td>
<td>277</td>
</tr>
<tr>
<td>vascular endothelial growth factor A</td>
<td>56</td>
</tr>
<tr>
<td>vascular-permeability</td>
<td>265</td>
</tr>
<tr>
<td>VDU1</td>
<td>197, 208</td>
</tr>
<tr>
<td>ve-cadherin</td>
<td>266</td>
</tr>
<tr>
<td>vicariance</td>
<td>290</td>
</tr>
<tr>
<td>Virgibacillus xinjiangensis sp nov.</td>
<td>166</td>
</tr>
<tr>
<td>virulence factors</td>
<td>21</td>
</tr>
<tr>
<td>vitamin A</td>
<td>275</td>
</tr>
<tr>
<td>volatiles induce</td>
<td>134</td>
</tr>
<tr>
<td>Volvariella bombycina</td>
<td>206</td>
</tr>
<tr>
<td>VP16</td>
<td>90</td>
</tr>
<tr>
<td>VSMC</td>
<td>262</td>
</tr>
<tr>
<td>water</td>
<td>94, 152</td>
</tr>
<tr>
<td>watermelon</td>
<td>239</td>
</tr>
<tr>
<td>web server</td>
<td>274</td>
</tr>
<tr>
<td>web-based server</td>
<td>175</td>
</tr>
<tr>
<td>well chip</td>
<td>31</td>
</tr>
<tr>
<td>whale genome</td>
<td>144</td>
</tr>
<tr>
<td>whole-cell biocatalyst</td>
<td>138</td>
</tr>
<tr>
<td>wide analysis</td>
<td>297</td>
</tr>
<tr>
<td>wnt</td>
<td>298</td>
</tr>
<tr>
<td>WPRE</td>
<td>193</td>
</tr>
<tr>
<td>xanthone</td>
<td>245</td>
</tr>
<tr>
<td>x-box protein-1</td>
<td>203</td>
</tr>
<tr>
<td>xenotransplantation</td>
<td>236</td>
</tr>
<tr>
<td>xinjiang</td>
<td>158</td>
</tr>
<tr>
<td>xylanolytic bacterium</td>
<td>97</td>
</tr>
<tr>
<td>xylooligosaccharides</td>
<td>136</td>
</tr>
<tr>
<td>Yersinia sp.</td>
<td>255</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Term</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>yogurt</td>
<td>201</td>
</tr>
<tr>
<td>zea-mays</td>
<td>134</td>
</tr>
<tr>
<td>zinc-finger gene</td>
<td>49</td>
</tr>
<tr>
<td>Zucker diabetic fatty rats (ZDF)</td>
<td>7</td>
</tr>
<tr>
<td>zymobacter</td>
<td>159</td>
</tr>
<tr>
<td>zymography</td>
<td>210, 250, 252</td>
</tr>
</tbody>
</table>