Longan (Dimocarpus longan Lour.) Fruit Extract 1 Stimulates Osteoblast2 Differentiation via Erk1/2-Dependent RUNX2 Activation

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Longan (Dimocarpus longan Lour.) has been used as a traditional oriental medicine and possesses a number of physiological activities. In this study, we used cell-based herbal extract screening to identify longan fruit extract (LFE) as an activator of osteoblast differentiation. LFE up-regulated alkaline phosphatase (ALP) activity, induced mineralization, and activated Runx2 gene expression in MC3T3-E1 cells. Furthermore, treatment of MC3T3-E1 cells with LFE promoted the phosphorylation of extracellular signal-regulated kinase1/2 (Erk1/2); however, abrogation of Erk1/2 activation with PD98059 resulted in down-regulation of phospho-SMAD1/5/8 and Runx2 levels, which in turn reduced ALP activity. Our findings suggest that LFE exerts its osteogenic activity through activation of the ERK signaling pathway and may have potential activity. The research was supported by the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026557).

Keywords: Longan fruit extract, Osteoblast differentiation, Erk1/2 pathway, Herbal medicine

Antifungal Substances from Antagonistic Bacteria Effective to Ginseng Damping-off Disease

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Ginseng has been proven to possess various biological and pharmacological properties, such as antibiotic, anti-inflammatory, anticancer, and antiproliferative properties, as well as other health benefits. Ginseng yield is limited by many factors, including damping-off and root rot in fields as well as during storage prior to consumption. Control of damping-off is normally carried out by the application of fungicides. However, biological control is an efficient and environmentally friendly way to prevent ginseng damping-off disease. In this study, we screened soil-borne bacteria with the potential as a biological control agent and selected several bacteria antagonistic to the plant pathogenic fungi Rhizoctonia solani, causing ginseng damping-off disease. Among them, Streptomyces sp. A1444, A3265, and A3283 and a Bacillus sp. #13 strains exhibited potent antifungal activity against R. solani. Antifungal substances were purified from the culture broth of Streptomyces sp. A3265 by chromatographic methods and identified as guanidylfungin and methylguanidylfungin by spectrophotometric methods. Antifungal substances of strains A1444 and A3283 were identified as guanidylfungin by HPLC analysis. A Bacillus sp. #13 produced volatile antifungal substances.

Keywords: Antifungal substances, Ginseng damping-off, Streptomyces

C-Glycosylation of Selected Flavones Using GUF6CGT1 Glycosyl Transferase from Gentiana triflora

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Plants produce various natural products including diverse flavonoid derivatives. Generally these metabolites are commonly accumulated in the vacuole as their glycosides. C-glycosylflavones, comprising the various pharmacological activities, are biosynthesized from flavone via C-glycosylation of 2-hydroxyflavone or flavone. This is mediated by uridine diphosphate (UDP)-sugar dependent glycosyltransferase. The C-glycosyltransferase catalyzes the transfer of the glucose moiety to the aromatic carbon of the acceptor substrate. The C-glycosylated natural compounds have the specific contribution to the drugs properties like pharmacokinetics, pharmacodynamics, solubility, mechanism and potency. C-glycosylflavones are involved in UV protection, defense against pathogens and inhibition of caterpillar growth. In this study, we tried to biosynthesize C-glycosylflavone in vivo and the product was confirmed by liquid chromatography mass spectrophotometry (LC-MS).

Keywords: C-glycosyltransferase, Glycosyltransferase, 2-hydroxyflavones
Protein Nucleation Studies Using Novel Water Soluble Chelate Agents

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Rapid production of high quality crystals of protein was important to development of drug discovery. It was known that nucleation of protein is an early step in crystallization process. In this study, novel water soluble nitrilotriacetic acid ligands were developed to accelerate the rate of protein nucleations via binding histidines of proteins. Using mCherry fluorescent protein as a model, it turned out that intermolecular interactions nucleations via binding histidines of proteins. Sedimentation velocity ultracentrifugation and size exclusion chromatography indicated that novel water soluble nucleating ligands possess adequate size to interaction with mCherry Fluorescent Protein. Our results suggested that the protein nucleation can be influenced by novel nitrilotriacetic acid chelating agents.

Keywords: Protein nucleations, mCherry fluorescent protein, Water soluble chelate

Structure Determination and Biosynthesis of the Antifungal Butyrolactols from Streptomyces sp.

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In our research for new bioactive secondary metabolites from marine microorganisms, we selectively isolated actinomycete strains from marine sediment samples from the western area of Jeju Island in Republic of Korea and screened their secondary metabolites profiles by LC/MS. During the chemical profile analysis, we observed that one of the actinomycete strains named TH11 (Streptomyces sp.) produces previously reported antifungal butyrolactols A and B, which bear a seven consecutive 1,2-diol moiety, and their new derivatives, butyrolactols C and D. Because the absolute configuration of butyrolactol A has not previously determined, we established the stereochemistry of butyrolactol A by J-based configuration analysis using ROESY and HETLOC data and Mosher’s method. Biosynthetically interestingly, four oxygen-bearing carbons are missing between the 5-membered lactone ring and the chain with a tertiary butyl group in butyrolactol C compared to butyrolactol A. The full genome analysis of the producer enabled us to identify the biosynthetic gene cluster of the butyrolactols. Further analysis indicated that the t-butyl starting unit could be originated from valine catalysed by an AdoCbl-dependent isomerase and polyol groups could be synthesized with glycolate extender units.

Keywords: Secondary metabolite, Stereochemistry, Butyrolactol
Crystal Structure and Biochemical Identification of LOG from Corynebacterium glutamicum
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“Lonely guy” (LOG) has been identified as a cytokinin-producing enzyme in plants and plant-interacting fungi. However, functions of a large number of LOG-like proteins from bacteria remain unclear with misannotation as lysine decarboxylases (LDCs). The gene product of Cg2612 from the soil-dwelling Corynebacterium glutamicum was previously annotated as a LDC. To investigate the function of Cg2612, we determined its crystal structure at a 2.3 Å resolution. Cg2612 functions as a dimer and shows an overall structure similar to other known LOGs, such as LOGs from Arabidopsis thaliana (AtLOG), Claviceps purpurea (CpLOG), and Mycobacterium marinum (MmLOG). Cg2612 also contains a “PGGxGT/C/E” motif that contributes to the formation of an active site similar to other LOGs. Moreover, biochemical studies on Cg2612 revealed that the protein has phosphoribohydrolase activity but not LDC activity. Based on these structural and biochemical studies, we propose that Cg2612 is not an LDC family enzyme, but instead belongs to the LOG family. In addition, the putative prenyl-binding site of Cg2612 (CgLOG) comprised residues identical to those seen in AtLOG and CpLOG, albeit dissimilar to those in MmLOG. Our work provides structural and functional implications for LOG-like proteins from microbes.

Keywords: Phosphoribohydrolase, Cytokinin, C. glutamicum

Genipin Enhances Kaposi’s Sarcoma-associated Herpesvirus Genome Maintenance
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Kaposi’s sarcoma-associated herpesvirus (KSHV) is a gammaherpesvirus that causes acute infection and establishes life-long latency. KSHV causes several human cancers, including Kaposi’s sarcoma, an acquired immune deficiency syndrome (AIDS)-related form of non-Hodgkin lymphoma. Genipin, an aglycone derived from geniposide found in Gardenia jasminoides, is known to be an excellent natural cross-linker, strong apoptosis inducer, and antiviral agent. Although evidence suggests antiviral activity of genipin in several in vitro viral infection systems, no inhibitory effect of genipin on KSHV infection has been reported. Thus, our aim was to determine, using the iSLK-BAC16 KSHV infection system, whether genipin has inhibitory effects on KSHV infection. For this purpose, we evaluated biological effects of genipin on KSHV infection and finally determined the underlying mechanisms responsible for the bioactive effects of genipin.

Keywords: Genipin, Kaposi’s sarcoma-associated herpesvirus, Dysregulation, Lytic reactivation

Deinococcusmycins A-D, new glycolipids from the ant-associated bacterium, Deinococcus sp.
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Bacteria in insect symbiotic systems are considered as an uprising source of novel bioactive small molecules because of the huge biological and chemical diversity of insects and their symbiotic microbes. Our recent studies have focused on the ecosystem of the carpenter ant Camponotus japonicus. We isolated bacterial strains from queen specimen of C. japonicus and chemically analyzed their secondary metabolites by LC/MS. As a result, four new glycolipids, Deinococcusmycins A-D (1-4), were discovered by a Gram-negative bacterial strain belonging to Deinococcus. The structures of Deinococcusmycins A-D (1-4) were determined mainly through NMR and mass spectroscopic data. Analysis of the configuration of amino sugar was based on 1H-1H coupling constants, ROESY NMR correlations and chemical derivatizations. The absolute configuration and double bond geometry were established by PGME derivatization and cross-metathesis. Deinococcusmycins A (1) and B (2) showed significant quinone reductase induction activity.

Keywords: Polyketide, Secondary metabolite, Symbiotic bacteria

Discovery of New Secondary Metabolites from Oil Beetle-Associated Bacteria
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Insect is the most diverse phylogenetic clade of the Animalia kingdom on the Earth. Accordingly symbiotic bacteria in the guts of insect can be considered as infinite sources of new bioactive compounds. Our recent studies have focused on the oil beetle, Meloe proscarabaeus because M. proscarabaeus can be an interesting creature because this insect produces a yellowish toxic substance, cantharidin. The production of cantharidin and its ecology can be related with their symbiotic bacteria. In this study, we isolated bacterial strains from female specimen of M. proscarabaeus and chemically analyzed their secondary metabolites by LC/MS. As a result, a polyketide macrolide, arenicolide A and its new derivatives were discovered from an actinomycete strain, GR10. The structure of arenicolide A was confirmed mainly through NMR and mass spectroscopic analysis. The structures of new congeners and the configurational analysis of arenicolide A will be discussed.

Keywords: Polyketide, Secondary metabolite, Symbiotic bacteria
Bioconversion of Methylated Flavonoids as a Biocatalyst for the Synthesis of 1,3-Diaminopropane

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Sustainable production of commodity chemicals is important for global climate changes. In this study, Escherichia coli is metabolically engineered to produce 1,3-diaminopropane (1,3-DAP), a monomer for polyamide production. In silico flux analysis revealed that heterologous C5 pathway involving dat and ddC from Acinetobacter baumannii encoding 2-ketogluutarate-4-aminotransferase and L-2,4-diaminobutanolate decarboxylase, respectively, was more efficient for 1,3-DAP production than C5 pathway. The ppc and aspC genes were overexpressed to increase flux towards 1,3-DAP synthesis in a strain having feedback resistant aspartokinases. In addition, knocking out pfkA was found to increase 1,3-DAP production by applying 128 synthetic small RNAs. Overexpression of the ppc and aspC in pfkA-deleted strain resulted in even higher production of 1,3-DAP. Fed-batch fermentation of the final engineered E. coli strain allowed production of 13 g/L of 1,3-DAP. [Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation of Korea (NRF) (NRF-2012M2A2A0206656 and NRF-2012M1A2A2026557).]
Acceptor substrates Study Name of Flexibility of Flavonol 7-
Expanded O-Rhamnosyltransferase, AtUGT89C1 from
Arabidopsis thaliana
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Acceptor substrates flexibility of previously characterized flavonol
7-O-rhamnosyltransferase (AtUGT89C1) from Arabidopsis thaliana was explored
with an endogenous nucleotide diphosphate sugar and five
different classes of flavonoids (flavonols, flavones, flavanones, chalcone
and stilbenes) through a biotransformation approach. In contrast to the
previous reports, this study highlights the expanded acceptor substrate
promiscuity of AtUGT89C1 for the regiospecific glycosylation of diverse
class of flavonoids at 7-hydroxyl position using microbial endogenous
sugar thymidine diphosphate (TDP)-L-rhamnos as sugar donor instead
of uridine diphosphate (UDP) -L-rhamnos which is not commonly
biosynthesized in microbes. We successfully examined the biocatalytic
potential of AtUGT89C1 using endogenous sugar (TDP-L-rhamnos) from
E. coli to generate a library of flavonoid 7-O-rhamnosides.

Keywords: AtUGT89C1, Flavonoid 7-O-rhamnosyltransferase, NDP-sugar

Preparation and Evaluation of Fibronectin Attachment Protein
Attached Biomaterials for the Treatment of Bladder Cancer
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Bladder cancer is the common cancers in the world. Fibronectin
attachment protein is a bacterial adhesin highly conserved in all
mycobacterial species, and in vitro and in vivo studies indicated that it
was associated with intravesical instillation of live Mycobacterium bovis
bacillus Calmette-Guérin which is known for the adjuvant therapy of
choice for the treatment of superficial bladder tumors. In the present study, fibronectin
attachment protein was used as a targeting peptide of novel
biomaterial. Novel functionalized polymer biomaterials were prepared via
conjugation with fibronectin attachment protein to increase targeting
efficiency to bladder cancer. The target specificity of biomaterials in the
tumor cells was evaluated by measurement of FRET efficiency of cyanine
7 attached to polymers as a function of internalization times. Besides, the
effected of surface concentration and cytotoxicity of novel polymers
was examined to find optimal structure for development of treatment
of bladder tumor. The results validated that fibronectin attachment protein,
was related with the cellular uptake by T24 bladder tumor cells and
internalization mechanism.

Keywords: Fibronectin Attachment Protein, Bladder cancer, Biomaterials

Structural Identification of Novel ARM-repeat Protein CTNNBL1
as a Human non-snRNP Complex with its Interaction Partner
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The hPrp19-CDC5L complex plays a crucial role during human
pre-mRNA splicing by catalytic activation of the spliceosome. In order to elucidate the molecular architecture of the hPrp19-CDC5L complex,
the crystal structure of CTNNBL1 was determined. Unlike canonical
ARM-repeat proteins such as β-catenin and importin-α, CTNNBL1 was
found to contain a twisted and extended ARM-repeat structure at the
C-terminal domain and, more importantly, the protein formed a stable
dimer. We performed small angle X-ray scattering experiments in various
concentrations of NaCl. We observed that CTNNBL1 existed as a dimer
in physiological NaCl concentrations. Site-directed mutagenesis experiment
of CTNNBL1 confirmed that N-terminal capping region and the first four
ARM repeats are important for dimerization of the protein. We also found
that the positively-charge NLS3-containing region (residues 197-235) of
CDC5L bound to the highly negatively charged patch formed in the
N-terminal ARM-repeat domain of CTNNBL1 and the hPrp19-CDC5L complex
is a heterotetramer consisting of one CTNNBL1 dimer and one
CDC5L dimer. These findings not only present the crystal structure of
a novel ARM-repeat protein, CTNNBL1, but also provide insights into the
detailed molecular architecture of the hPrp19-CDC5L complex.

Keywords: Spliceosome, non-RNP, CTNNBL1/CDC5L complex

Commensal Bacteria Promote IEC Turnover
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The life span of intestinal epithelial cells (IECs) is short (3-5 days),
and its regulation is thought to be homeostasis of the intestinal
epithelium. We have now investigated the role of commensal
bacteria in regulation of IEC turnover in the small intestine. The
proliferative activity of IECs in intestinal crypts as well as the migration
of these cells along the crypt-villus axis was markedly attenuated both
in germ-free mice and in specific pathogen-free (SPF) mice treated with
a mixture of antibiotics, with antibiotics selective for Gram-positive
bacteria more effective in this regard. Oral administration of
chloroform-treated feces of SPF mice to germ-free mice resulted in a
marked increase in IEC turnover, suggesting that sporo-forming
Gram-positive bacteria being most effective in this regard. Oral administration of
chloroform-treated feces of SPF mice to germ-free mice resulted in a
marked increase in IEC turnover, suggesting that sporo-forming
Gram-positive bacteria contribute to this effect. Oral administration of
short-chain fatty acids (SCFAs) as bacterial fermentation products also
restored the turnover of IECs in antibiotic-treated SPF mice as well as
promoted the development of intestinal organoids in vitro. Our results
thus suggest that Gram-positive commensal bacteria are a major
determinant of IEC turnover.

Keywords: Intestinal epithelial cells, Microbiota, Turnover
Borrelidins C-E, New Macrolide Compounds from a Halophilic Nocardiopsis sp. Actinobacterium
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Actinobacteria have been a prominent source of bioactive secondary metabolites. However, over the past three decades, there has been a general decline in the discovery of novel natural products with useful pharmaceutical properties, and at the same time drug resistant diseases became far more widespread. Therefore, inquiry of peculiar place possessing chemically new actinomycetes, rather than typical terrestrial environments, is required to investigate the extensive chemical diversity of this chemically prolific bacterial group. Saltern is probably the most saline environments. It has not been much studied as a source of new bioactive compounds provided by actinomycetes adapted to such extreme habitats. In this context, we selectively isolated halophilic actinomycete strains from saltern surface soil samples collected from Jeung-Do Island in the republic of Korea and chemically screened their secondary metabolites by LC/MS. By means of the screening process, we found out three borrelidin derivatives. The planar structures of the new compounds were determined by spectroscopic analyses of NMR, MS, and UV data. The absolute configuration of the borrelidins C and D were elucidated by the modified Mosher’s method, and borrelidin E was determined by HOMO-decoupling experiments.

Keywords: Borrelidin, Saltern, Secondary metabolite

Overexpression of a Pathway Specific Self-Repressing Regulator Enhances Production of Daunorubicin in bldA Deficient Streptomyces peucetius ATCC 27952
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The dvrO gene is the first regulator to be activated in the daunorubicin (DNR) biosynthesis pathway of Streptomyces peucetius ATCC 27952. DvrO is known for its self-repression capability while it activates rest of the DNR biosynthesis pathway through cascades of regulatory events. S. peucetius was found to contain no functional copy of bldA 3rRNA while a detailed examination of dvrO codons reveals the presence of TTA codon, which is rarely encoded by bldA-3rRNA. Therefore, for evaluating the role of dvrO in DNR production, multiple engineered strains of S. peucetius were generated by heterologously expressing bldA, dvrO and combination of bldA and dvrO. Using these strains, the effects of heterologously expressed bldA and overexpressed dvrO were evaluated on pathway specific regulators, mycelial densities and production of DNR. The transcriptional level of dvrO and master regulator dvr1, was found to be elevated in dvrO containing strain in comparison to dvrO overexpressed strain. Moreover, the dvrO containing strain produces ~1.7 mg higher DNR than bldA deficient wild type strain. Heterologous expression of bldA-3rRNA is accounted for increased transcriptional levels of the DNR pathway specific regulators and enhanced DNR production.

Keywords: bldA-3rRNA, dvrO, dvr1

Bioynthesis of Flavonol Glycosides Using Spinosyn Rhamnosyltransferase from Saccharopolyspora spinosa
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SpnG is a rhamnosyltransferase that catalyzes the key O-glycosylation controlling the biosynthetic maturation of spinosyn. SpnG has the capability to transfer L-Rhamnose from the natural substrate (TDP-Rhamnose) onto spinosyn aglycone. Previous work by chen et. al. proved that this rhamnosyltransferase can also accept glycose other than rhamnose and have accepted TDP-Glucose as the sugar donor. Flavanols are a non-nutrient, bioactive phytochemical compounds within the flavonoid family and are found in high concentrations in a variety of plant-based foods and beverages. In the present study we have carried out the in vitro reaction of commercially available flavonols, namely quercetin, myricetin and kaempferol as a sugar acceptor with both rhamnose and glucose as the sugar donor. Reaction products were analyzed by high performance liquid chromatography (HPLC) and high resolution liquid chromatography quadrupole time of flight electrospray ionization mass spectrometry (HR-LC-QTOF-ESI/MS) which revealed the detection of rhamnosylated and glycosylated products with their corresponding mass but the exact position and configuration of sugar attachment is yet to be identified.

Keywords: Rhamnosyltransferase, Flavonols, Glycosylation

Bioynthesis of Anthraquinone Derivatives in Escherichia coli
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Anthraquinones (also known as anthraquinonoids) is an aromatic organic compound which is a class of naturally occurring phenolic compounds based on the 9,10-anthraquinone skeleton. Anthraquinone glycosides upon hydrolysis yield aglycone, which are usually di-, tri- or tetra hydroxy anthraquinone or derivatives of these compounds. The free anthraquinone aglycone exhibits therapeutic activity however, glycosides certainly enhance the solubility. In certain studies, anthraquinone has been shown to help aid in digestion as a laxative, to reduce inflammation in arthritis patients, to inhibit the growth of cancer cells and also used in the treatment of fungal skin diseases. There are more than one kind of anthraquinone, and have been used for medical treatment are often found naturally in plants. The current research focus on application of post modification enzymes to biosynthesize anthraquinone derivatives in Escherichia coli.

Keywords: Anthraquinones, E. Coli, Biosynthesis
Mohangic Acids A-E, p-Aminoacetophenonic Acids from a Marine Mudflat-Derived \textit{Streptomyces} sp.

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Mohangic acids A-E (1-5) were isolated from a marine \textit{Streptomyces} sp. collected from a mudflat in Buan, Republic of Korea. Comprehensive spectroscopic analysis revealed that the mohangic acids are new members of the p-aminoacetophenonic acid class. The relative and absolute configurations of the mohangic acids were determined by $^J$-based configuration analysis and by the application of bidentate chiral NMR solvents followed by $^{13}$C NNR analysis, chemical derivatization, and circular dichroism spectroscopy. Mohangic acid E (5), which is the first glycosylated compound in the p-aminoacetophenonic acid family, displayed significant quinone reductase induction activity.

Keywords: p-aminoacetophenonic acid class, $^J$-based configuration analysis, Bidentate chiral NMR solvents

Engineering and Evaluation of Novel Lipid Modified Poly(lactide-co-glycolide) Nanoparticles for gene delivery

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Gene therapy is a potent therapeutic approach for a number of diseases such as cancers. Thus, demand of the development of nonviral delivery vectors for gene delivery was increased. In this study, poly(lactic-co-glycolic acid), one of potent polymer for nonviral carriers, was engineered by using BCAT acid-sensitive lipid and was used for the preparation of pH-responsive nanoparticles NPs. Then, plasmid DNA was encapsulated by poly(lactic-co-glycolic acid) NPs. Plasmid DNA release study of novel NPs suggested that the rate of DNA release was mainly influenced by the BCAT lipid hydrolysis. Transfection result turned out that transfection efficiencies of novel NPs were affected by 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine as well as BCAT lipid on novel NPs. Besides, in stability study, the novel NPs showed no visible signs of instability, such as sedimentation or aggregation, for 2 weeks.

Keywords: Gene delivery, Poly(lactic-co-glycolic acid), Nanoparticles

Combining the Biosynthetic Genes from Butirosin and Neomycin Pathway for Production of 2-DOS in \textit{Escherichia coli}

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Aminoglycosides can be generally divided in 2-DOS containing and not containing groups. In case of 2-DOS containing aminoglycosides, 2-DOS is very important intermediate. With an objective of production of aminoglycoside antibiotics in \textit{E. coli}, we started making the production of 2-DOS. \textit{E. coli} offers an advantage as a heterologous host in terms of rapid growth and well-developed genetic tools. The higher pool of 2-DOS is an important condition if 2-DOS containing aminoglycosides are to be made in heterologous hosts. Thus, taking the advantage of \textit{E. coli} as heterologous host for production of 2-DOS, we optimized the first and third steps of the pathway in this study by addition of small subunit BtrC2 along with BtrC and NeoA from butirosin and neomycin biosynthetic pathways respectively. The results were verified using Evaporative Light Scattering Detector (ELSD) analysis and high resolution quantitative time of flight (HR-QTOF) mass analysis.

Keywords: 2-DOS, \textit{E. Coli}, Heterologous production

A Novel Tricyclic Dilactone Isolated from an Actinomycete Derived from Ginseng-Cultivated Soil

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An actinomycete strain BYK1336, identified as \textit{Streptomyces cinerocromogens}, was isolated from 4-year-old ginseng farm. While culturing the strain and analyzing its chemical profile by liquid chromatography coupled with electrospray ionization mass spectrometer (LC-ESI-MS), a previously-unreported secondary metabolite BYK 1336.360 was detected along with the known compounds belonging to the classes of ansatrienin and naphthomycins. The structure of BYK.1336.360 was elucidated by comprehensive analysis of 1D and 2D NMR, mass spectrometric and infrared spectroscopic data. BYK.1336.360 is structurally novel by bearing a cyclooctatriene flanked by six-membered and seven-membered lactone ring.

Keywords: Streptomyces, NMR, Lactone
A Fluorescence-Based Coupled Enzyme Assay to Measure the Concentration of Cofactors NAD(H)

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The nicotinamide adenine dinucleotide typically known as redox factors in-vivo plays a crucial role in energy homeostasis and cell signaling pathways. Therefore, the ratio between the oxidized and reduced state can be used as an important bioactive marker. Although various methods used for detection and quantification of these cofactors were reported to have high sensitivity, current methods are composed of multi-steps and time consuming processes, thereby requiring complex reagents and expensive instrument. A more serious problem of these methods does not simply and simultaneously quantitative another forms of cofactors NADPH in the same sample under identical conditions. Here, we present a coupled enzyme-based fluorometric assay for simple and sensitive measurement of these important redox cofactors. The system suggested here circumvents many difficult issues of previous methods, because this system simply measured an enhanced fluorescence of NADPH for quantification by interaction only with a protein mBFP. As for the purpose, coupled enzymes (a dehydrogenase, NAD+ and NADH kinase) can be used to convert NADP+ and NADH to NADPH. More details are discussed in this presentation.

Keywords: Cofactor, Coupled enzyme assay, Fluorescence

A Prototype Assay Format for Reductase Activity Using mBFP

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Reducase is frequently involved as a component of biocatalyzer enzymes which play an important role in the oxidative metabolism of xenobiotic chemicals and thus has been potentially applied in medicine as well as clinical diagnosis. Most of conventional methods, however, require complex process and much time to assay the activity of reductase at each experiment. Therefore, it is desirable to develop a rapid and simple method for high sensitive detecting the activity of reductase. Here, we suggest a fluorescence-based assay method for measuring the activity of reductase. This assay method can measure the related activity more quickly and sensitively than previous methods by only using a protein mBFP. mBFP reveals an enhanced intensity of NADPH fluorescence by a simple interaction. Consequently, this assay method can be used widely for the quantitative detection of NADPH-dependent reductase and/or dehydrogenase. Using CYP as a model enzyme, we presented a prototype assay format for reductase activity using mBFP and discussed the problems still remained to be solved.

Keywords: Reductase, Cofactor, Simple assay