

## E001

**Characterization of Calcium-Activated Bifunctional Peptidase of the Psychrotrophic *Bacillus cereus***Jong-Il Kim<sup>\*</sup>, Hyo-Jin Lee, and Sun-Min Lee

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The protease purified from *Bacillus cereus* JH108 has the function of leucine specific endopeptidase. When the enzyme activity was measured by hydrolysis of synthetic substrate (*N*-succinyl-Ala-Ala-Pro-Leu-*p*-nitroanilide) it exhibited optimal activity at pH 9.0, 60°C. The endopeptidase activity was stimulated by Ca<sup>++</sup>, Co<sup>++</sup>, Mn<sup>++</sup>, Mg<sup>++</sup> and Ni<sup>++</sup> and inhibited by metal chelating agents such as EDTA, 1,10-phenanthroline, and EGTA. Addition of serine protease inhibitor, PMSF, resulted in the elimination of the activity. The endopeptidase activity was fully recovered from the inhibition of EDTA by adding 1 mM Ca<sup>++</sup>, and partially restored by Co<sup>++</sup> and Mn<sup>++</sup>, indicating that the enzyme was stabilized and activated by divalent cations and has a serine residue at the active site. Addition of Ca<sup>++</sup> increased the pH and heat stability of endopeptidase activity. These results show that endopeptidase requires calcium ions for activity and/or stability. A Lineweaver-Burk plot analysis indicated that K<sub>m</sub> value of endopeptidase is 0.315 mM and V<sub>max</sub> is 0.222 μmol of *N*-succinyl-Ala-Ala-Pro-Leu-*p*-nitroanilide per min. Bestatin was shown to act as a competitive inhibitor to the endopeptidase.

## E002

**Conversion of Methylglyoxal to Acetol by *Escherichia coli* Aldo-keto Reductases**Dongkyu Park<sup>\*</sup>, Jeeyeon Song, Minsuk Kwon, Jinyoung Yoon, Dongwook Choi, and Chankyu Park

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Methylglyoxal(MG) is a toxic metabolite known to accumulate in various cell types. We detected *in vivo* conversion of MG to acetol in MG-accumulating *E. coli* cells by <sup>1</sup>H-NMR spectroscopy. Search for homologs of the mammalian aldo-keto reductases, reported to exhibit activity to MG, revealed nine ORFs from the *E. coli* genome. Based on both sequence similarities and preliminary screening with <sup>1</sup>H-NMR for crude proteins of the corresponding mutant strains, we selected five genes, *yafB*, *yqhE*, *yeaE*, *yghZ*, and *yajO*. Quantitative assessment of acetol production including its metabolite 1,2-propanediol from the crude extracts of these mutants indicated that the *yafB*, *yqhE*, *yeaE* and *yghZ* genes are involved in the conversion of MG in the presence of NADPH. Although enzymatic properties of these proteins are generally consistent with their activities on MG conversion, the *yqhE* mutation did not exhibit further sensitivity to MG in glyoxalase-deficient strain. The results imply that the glutathione-independent detoxification of MG *in vivo* can occur through multiple pathways, consisting of *yafB*, *yeaE*, and *yghZ* genes, leading to a generation of acetol.

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## E003

***Csp* Genes of *Corynebacterium glutamicum* are Involved in Cold-shock Response**Wan-Soo Kim<sup>1\*</sup>, Younhee Kim<sup>2</sup>, and Heung-Shick Lee<sup>1</sup><sup>1</sup>Department of Biotechnology and Bioinformatics, Korea University, <sup>2</sup>Department of Oriental Medicine, Semyung University

*Corynebacterium glutamicum* genes which appear to play a role in cold shock response were isolated using the *aceB* promoter of *C. glutamicum*. Expression of the gene in *E. coli* resulted in 70% reduction in the expression of the *aceB* gene as monitored by the β-galactosidase activity expressed from the P<sub>aceB</sub>-*lacZ* reporter plasmid. The isolated gene encoded a putative protein composed of 63 amino acids which showed high similarity with cold shock proteins of other bacteria and designated *cspA*. Scrutiny of the *C. glutamicum* ATCC13032 genome sequence identified 2 additional *csp* genes and the expression of the genes were monitored by fusing chromophenicol acetyltransferase (CAT) genes to the promoter region of the genes. For all 3 genes, the highest activity was observed in the late log phase. In addition, expression of the genes was stimulated when the cells carrying the clones were given a cold shock. These data indicate that the *cspA* gene may encode a transcriptional regulator and the *csp* genes are probably involved in the cold shock response in *C. glutamicum*.

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## E004

**Antibacterial and Antiviral Activity of the Culture Soup Produced from *Leuconostoc kitchii***Sung-Hyun Song<sup>\*</sup>, Tae-Hwan Kim, Jin-Hee Park, Dong-Il Jang, Chang-Jin Lee, and Sa-Ouk Kang

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The culture soup produced from *Leuconostoc kitchii* has antibacterial activity against gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherichia coli*, *Salmonella typhimurium*, *Shigella sonnei*, *Vibrio vulnificus*. The antibacterial activity of the culture soup was stable over 100°C but not showed over pH 5. Antibacterial activity of the culture soup was better at 3 days after inoculation. When treatment of Proteinase-K to the culture soup, antibacterial activity remained. Partially purified substance(s) showed antibacterial activity against *B. subtilis* and other strains. The culture soup reveals antiviral activity against influenza virus(H3N2).