E001

Characterization of Calcium-Activated Bifunctional Peptidase of the Psychrotrophic *Bacillus cereus*

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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℃. The endopeptidase activity was stimulated by Ca^{++}, Co^{++}, Mn^{++}, Mg^{++} and Ni^{++} 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^{++}, and partially restored by Co^{++} and Mn^{++}, indicating that the enzyme was stabilized and activated by divalent cations and has a serine residue at the active site. Addition of Ca^{++} 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 Km value of endopeptidase is 0 315 mM and Vmax 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

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Methyglyoxal(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 ^{1}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 ^{1}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

[Supported by the BK21 and glycomics program]

E003

Csp Genes of *Corynebacterium glutamium* are Involved in Cold-shock Response

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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_{aceB}-*lacZYA* 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 chloramphenicol 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*

[Supported by a grant from KOSEF.]

E004

Antibacterial and Antiviral Activity of the Culture Soup Produced from *Leuconostoc kimchii*

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The culture soup produced from *Leuconostoc kimchii* has antibacterial activity against gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherchia coli*, *Salmonella typhimurium*, *Shigella sonnei*, *Vibrio vulnificus* The antibacterial activity of the culture soup was stable over 100℃ 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)