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The *ermK* leader peptide alterations leading to differential efficiency of induction by erythromycin

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The *ermK* gene from *Bacillus licheniformis* encodes an inducible rRNA methylase that confers resistance to the macrolide-lincosamide-streptograminB antibiotics. The *ermK* mRNA leader sequence has a total length of 357 nucleotides and encodes a 14-amino acid leader peptide together with its ribosome binding site. The secondary structure of *erm* leader RNA and a leader peptide have been reported as the elements that control expression. In this study, the contribution of specific leader peptide amino acid residues to induction of *ermK* was studied using the PCR-based megaprimer mutation method. *ermK* methylases with altered leader peptide codons were translationally fused to *E. coli* β -galactosidase as reporter gene. The deletion of the codons for Thr-2 through Ser-4 reduced inducibility by erythromycin, whereas that for Thr-2 and His-3 was not. The replacement of the individual codons for Ser-4, Met-5 and Arg-6 with termination codon led to loss of inducibility, but stop mutation of codon Phe-9 restored inducibility by erythromycin. Collectively, these findings suggest that the codons for residue 4, 5 and 6 comprise the critical region for induction. The stop mutation at Leu-7 expressed constitutively *ermK* gene. Thus, ribosome stalling at codon 7 appear to be important for *ermK* induction.

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Pathogenic and immunological properties of alcohol dehydrogenase in *Streptococcus pneumoniae*

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Despite the use of antibiotics and vaccines, *Streptococcus pneumoniae* remains a serious cause of morbidity and mortality in human. In this study, pathogenic and immunological natures of alcohol dehydrogenase (ADH) in *S. pneumoniae* were elucidated. In vitro cytotoxicity test determined by lactate dehydrogenase release from A549 cells revealed that adh mutation significantly reduced cytotoxicity although in vivo intraperitoneal challenge of the adh mutant to BALB/C mouse exhibited marginal increase of survival time than the wild type. To determine the underlying mechanism of reduced virulence in the adh mutant, expression of virulence associated proteins (pneumolysin, choline binding protein, pneumococcal surface protein A, etc) was determined by Western blot analysis. Expression of virulence associated proteins in the adh mutant was not changed, however, autolysis upon exposure to penicillin and expression of autolysin (LytA) in the adh mutant were decreased than those of the wild type. Since all of the 29 clinical isolates of *S. pneumoniae* cross-reacted with antibody raised against ADH, these results suggest that ADH is highly conserved and would be one of the virulence factors in *S. pneumoniae*.

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Isolation of α -Glucosidase Inhibitor Producing Soil Microorganism and Inhibitory Effects of Microbial Metabolites on α -Glucosidase

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To find α -Glucosidase Inhibitors produced by Actinomycetes, 20 soil samples were tested and 53 Actinomycetes were isolated. One of 53 Actinomycetes (strain PM718) showed very potent inhibitory activity in vitro. The