F309 Analyses of Bacteriophage Lambda Excisionase Mutants with Non-specfic DNA Binding Activity

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The Excisionase(Xis) protein of bacteriophage lambda is required for site-specific excision of lambda from the bacterial chromosome. It binds specifically to a 40-bp region of DNA that contains 13-base-pair direct repeats separated by 7 base pairs. When a glutamic acid at amino acid position 40 was substituted with an alanine or a lysine, the mutants lost sequence specificity but bound to DNA in a non-specific manner as determined by *in vitro* gel mobility shift assays. This indicates that the glutamic acid at position 40 serves a critical role, either directly or indirectly, in conferring sequence-specificity to the Xis protein. However, those mutants were still able to carry out excision reaction *in vivo* in the presence of Factor for Inversion Stimulation(FIS). This residual excision activity was mediated through protein-protein interactions between Xis and FIS.

F310 Isolation of the *Vibrio vulnificus* DNA sequences which complement the phenotype of *Escherichia coli* defective in starvation sigma factor

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As an effort on the identification of the genes required for starvation-survival of *V. vulnificus*, the library was prepared of *V. vulnificus* genomic DNA partially digested by *Sau*3AI. Several clones expressing high activity of β-galactosidase were isolated from the transformants of *rpoS*-defective *E. coli* cell containing *rpoS*-dependent promoter, *bolA::lacZ* fusion. One of the *rpoS*-complementing plasmids, pSK22 includes a small sized, 403bp, DNA insert. The deduced polypeptide sequence of the putative ORF is composed of 56aa residues of which size (about 6kDa) was confirmed by SDS-PAGE of IPTG-induced cell extract, and does not show any significant similarity with the known bacterial proteins. In addition to induction of *bolA* promoter by pSK22, another *rpoS*-dependent promoter, *katE* is also induced more than 25-folds by the presence of this plasmid in *E. coli* cell. We have constructed a series of plasmids including the different lengths of the coding region. The effect of these deleted plasmids on the expression of *rpoS*-dependent promoters is under the investigation. We discuss the possible role of this short DAN sequence in ecological and physiological cycle of *V. vulnificus* in nature.