Analyses of Bacteriophage Lambda Excisionase Mutants with Non-specific 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.

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 Sau3AI. 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.