F013

Regulatory Characteristics and Promoter Analysis of the *Vibrio vulnificus malPQ* Operon

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Vibrio vulnificus is the causative agent of foodborne diseases such as gastroenteritis and life-threatening septicemia. It is likely that when the pathogenic bacteria invade human gut, many environmental changes, such as differences in type and concentrations of nutrients, would be encountered. Maltose could be an interesting sugar in this respect, as it is very common in the intestine and could provide a good substrate for the colonizing bacteria. To better characterize maltose metabolism, the *malPQ* genes encoding a maltodextrin phosphorylase and an amylomaltase, respectively, were identified and cloned from *V. vulnificus*. Northern blot and primer extension analyses revealed that *malPQ* genes are transcribed as a single transcriptional operon. A *crp* null mutation decreased amylomaltase production and the level of *malPQ* transcript by reducing the activity of P_{malPQ}. Lrp, a leucine responsive-regulatory protein, is also involved in the regulation of *malPQ* transcription by activating P_{malPQ}. This study was supported by a grant to S.H.C. from the 21C Frontier Microbial Genomics and Applications Center Program, Ministry of Science & Technology(MG02-0201-004-2-1-1), ROK

F014

Identification of the *Vibrio vulnificus fexA* Gene and Evaluation of its Influence on Virulence

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Vibrio vulnificus is the causative agent of food-borne diseases. A mutant exhibiting decreased cytotoxic activity toward INT-407 intestinal epithelial cells was screened from a library of *V. vulnificus* mutants constructed by a random transposon mutagenesis. By a transposon-tagging method, an open reading frame, *fexA* was identified and cloned. The nucleotide and deduced amino acid sequences of the *fexA* were analyzed and the amino acid sequence of FexA from *V. vulnificus* was 84 to 97% similar to those of ArcA. Functions of the FexA were assessed by the construction of an isogenic mutant. The disruption of *fexA* resulted in a significant alteration in growth rate under aerobic as well as anaerobic conditions. When compared to the wild type, the *fexA* mutant exhibited a substantial decrease in motility and cytotoxic activity toward intestinal epithelial cell lines *in vitro*. Furthermore, the intraperitoneal LD_{50} of the *fexA* mutant was approximately 10^1-10^2 times higher than parental wild type. Therefore, it appears that FexA is a novel global regulator controlling numerous genes contributing to pathogenesis as well as growth of *V. vulnificus*.

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F015

Generation of PCR-directed Gene Replacements using λ-Red Recombination Functions in *Escherichia coli.*

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The availability of an efficient chromosomal engineering technology is important in the field of metabolic engineering because one is interested in introducing genetic modifications to develop new and useful cellular traits. We developed a simple and efficient gene replacement method using PCR products containing homologous sequences of 40- to 50-nt. The method is unique in that it requires the λ-Red recombination functions provided under the control of a temperature-dependent CI857 repressor expressed from the P_{lac} promoter in the presence of IPTG to limit the recombinogenic window on a curable plasmid. The recombination functions provided from the plasmid can be easily turned on at 42℃ for 15 min and off at 32℃ in the presence of IPTG. Since this method employs λ-Red recombination functions under the tight control of a temperature-dependent CI857 repressor expressed from the P_{lac} promoter in the presence of IPTG on a curable plasmid, multiple round of gene replacement in any part of *E. coli* chromosome would be possible. The procedures of the method will be widely useful for metabolic engineering of *E. coli* and other bacteria.

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F016

General Control of the *THR4* Gene of the Yeast *Saccharomyces cerevisiae*

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The *THR4* gene of *Saccharomyces cerevisiae* encodes the threonine synthase, which catalyzes the last step in threonine biosynthetic pathway. General control is the regulatory system that controls the biosynthetic pathways of various amino acids in *S. cerevisiae*. To determine whether the *THR4* gene is regulated by general control system, two conditions were examined. i) the effect of known general control mutations on *THR4* expression and ii) amino acid starvation induced by the presence of the histidine analog 3-amino triazole(3AT). We have found that expression of a *THR4-lacZ* fusion increased twofold in the *gcd1* strain, and the expression of the fusion in the *gcn4* strain was reduced to 27% of the wild type. In the presence of 3AT, however, *THR4-lacZ* levels dropped in the wild type strain. In the upstream region of *THR4* are found three putative consensus sequences which might be responsible for the control mediated by Gcn4p. Deletion analysis of the *THR4* promoter revealed a consensus sequence located 180 bp upstream of the *THR4* ORF might be responsible for the control by Gcn4p. These results indicate that expression of the *THR4* gene of *S. cerevisiae* is regulated by general control.