[SVIII-2]

Proteomic Analysis of Osmotic Stress Regulon Controlled by *sigB* in *Streptomyces coelicolor*

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A gene (*sigB*) encoding an alternative sigma factor σ^B in *Streptomyces coelicolor* A3(2) was isolated and characterized in previous studies and is highly homologous to *Bacillus subtilis* σ^B. RNA analyses revealed that *rsbB*, *rsbA* and *sigB* constitute an operon (*sigB* operon) (Figure 1). Disruption of the *sigB* gene abolished not only the differentiation-associated expression but also the osmotic induction of the *catB* gene encoding a developmentally controlled catalase B, suggesting that σ^B ensures the proper differentiation and osmoprotection of *S. coelicolor* cells, primarily via regulation of the expression of catalase B.

In silico analysis revealed a consensus sequence for σ^B target promoters to identify new σ^B target genes and operons involved in global response to osmotic stress in this organism. About one hundred genes were found to be possibly regulated by σ^B sigma factor, suggesting that osmotic stress response in *S. coelicolor* is controlled by large regulatory network. Comparing the transcription level of these genes in wild-type cell with that in the *sigB* mutant, genes that belong to "real" σ^B regulon will be determined.

Two-dimensional electrophoresis (2-DE) of proteins is currently one of the highest-resolution analytical techniques available for the study of protein expression patterns. This technique allows us to analyze the cellular responses of bacteria to different stresses. Protein expression profiles of wild-type and a *sigB* mutant strain of *S. coelicolor* A3(2), which is impaired in defense against osmotic stress, were compared in the absence and presence of osmotic stress, using 2-dimentional gel electrophoresis. Differential protein spots from each comparison were picked, digested by trypsin, and subjected to MALDI-TOF mass spectrometry. Each protein spot was then identified by peptide mass fingerprinting using Mascot search. These results combined with data obtained by *in silico* analysis provide an important clue to understanding of the relationship between stress response and role of *sigB* regulon in streptomycetes. Biological significance of these results will be speculated by further molecular genetic analysis of the genes encoding the proteins identified by this method.

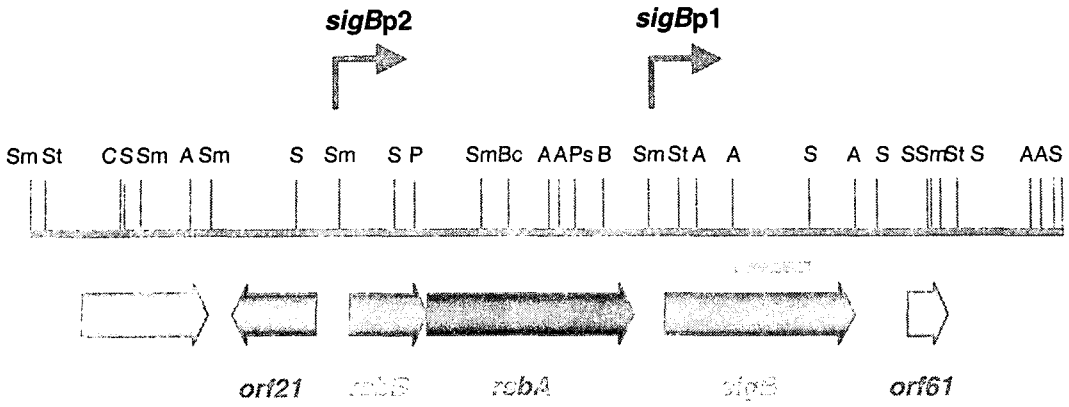

Figure 1. Restriction map and organization of the *sigB* operon

Reference

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