

## E005

### The Function of Cytochrome *c*<sub>550</sub> is Related to the Sporulation Initiation of *Bacillus subtilis*.

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The effect of cytochrome *c*<sub>550</sub> encoded by *cccA* in *Bacillus subtilis* on the event of sporulation was investigated. The sporulation of *cccA*-overexpressing mutant was significantly accelerated while disruptant strain showed delayed sporulation in spite of the same growth rate. This was proved by determining sporulation rate and detecting activities of some sporulation specific-enzymes. Also, it was observed from northern blot analysis that *cccA*-overexpressing mutant showed high level of *spo0A* transcripts, while disruptant had rarely expression of *spo0A*. These results support that cytochrome *c*<sub>550</sub> play an important role in sporulation initiation through regulation of *spo0A* expression. Changes of redox state by cytochrome *c*<sub>550</sub> may promote signaling in the sporulation phosphorelay.

[Supported by grants from Korea Research Foundation (KRF-2000-041-DS0039)]

## E006

### The *Corynebacterium glutamicum sigH* Gene, Encoding an ECF Sigma Factor, Plays a Key Role in Response to Oxidative Stress Involving Thiol-oxidation

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The *sigH* gene of *Corynebacterium glutamicum* encodes ECF sigma factor  $\sigma^H$ . Besides its role in heat stress response, the gene apparently plays an important role in other stress responses. In this study, we found that deleting the *sigH* gene made *C. glutamicum* cells sensitive to the thiol-specific oxidant diamide. In the mutant strain, the activity of thioredoxin reductase markedly decreased, suggesting that the gene encoding thioredoxin reductase is under the control of  $\sigma^H$ . The expression of *sigH* was stimulated in the stationary growth phase, and modulated by diamide. In addition, the SigH protein was required for the expression of its own gene. These data indicate that the *sigH* gene of *C. glutamicum* stimulates its own expression in the stationary growth phase and also plays a key role in response to oxidative stress involving thiol-oxidation.

## E007

### Upregulation of Aldose Reductase in Glutathione-Deficient *Dictyostelium discoideum*

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We previously reported that disruption of  $\gamma$ -glutamylcysteine synthetase (GCS) gene in *Dictyostelium discoideum* resulted in growth arrest. To find out the mechanism of the growth arrest by glutathione (GSH) depletion, we performed proteomic analysis by 2-dimensional (2D) gel electrophoresis. Comparative analysis on 2D profiles revealed that aldose reductase was highly induced in GCS-null cells. This was a product of *alrA* among 6 putative aldose reductase genes in *Dictyostelium*. The activities of aldose reductase in GCS-null cells cultured with 0.2 mM GSH and without GSH increased 3.4-fold and 6.7-fold, respectively, as compared with that in wild-type cells. It is well known that aldose reductase together with glyoxalase system contributes to detoxification of  $\alpha$ -ketoaldehydes, endogenous metabolites with cytotoxic activity. Since GSH is required for the glyoxalase system as a cofactor, the induction of aldose reductase in the GSH-deficient cells may be due to inability of the glyoxalase system, suggesting that the growth arrest of the *Dictyostelium* GCS-null cells may result from toxicity of  $\alpha$ -ketoaldehydes or their metabolites.

[Supported by grant from KRF (KRF-2003-041-C00318)]

## E008

### Deregulation of *Corynebacterium glutamicum metA* for Strain Construction

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In *C. glutamicum*, the first step of methionine biosynthesis is catalyzed by homoserine acetyltransferase which is regulated by the end product methionine. To deregulate the gene, the native promoter of the *metA* gene encoding homoserine acetyltransferase was replaced with the P<sub>180</sub> promoter which had been isolated in our previous study. The enzymatic activity of homoserine acetyltransferase was measured under various growth conditions. Cells carrying the *metA* gene fused to the P<sub>180</sub> promoter (P<sub>180</sub>*metA*) showed a 24-fold increase in the MetA activity when the cells were grown in a complex medium. A 13-fold increase was observed with the cells carrying the *metA* fused to the *tac* promoter (P<sub>tac</sub>*metA*). In addition, the MetA activity expressed from the P<sub>180</sub>*metA* construct was no longer regulated by methionine. These properties makes the P<sub>180</sub> clone be useful for the deregulated expression of biosynthetic genes in *C. glutamicum* during amino acid fermentation.

[Supported by grants from Korea University, BASF Korea and the ministry of Science and Technology]