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Molecular Analysis of *pyrH* Gene Encoding UMP Kinase in *Corynebacterium glutamicum*

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To study the expression and regulation mechanism of gene involved in pyrimidine metabolic pathway of *Corynebacterium glutamicum,* the molecular biological approach on the *pyrH* gene was attempted. The *pyrH* gene from *C. glutamicum* was isolated by PCR and was cloned for characterization. To find the protein, the DNA binding affinity chromatography was conducted by using the magnetic bead coated with streptavidin. Among them, the protein PyrR which is very well known in *Bacillus subtilis* as anattenuator-regulator in the pyrimidine metabolism was obtained. The *pyrR* gene was isolated, cloned and characterized.

To reveal the role of PyrR on the expression and regulation of *pyrH* gene in *C. glutamicum,* several recombinant plasmids containing *pyrH* promoter and *pyrR* were constructed. Using the plasmids, it was shown that the expression of GFP under the control of SDF5 was 2.9 fold higher than that of full promoter. However, when the *pyrR* was coexpressed in *C. glutamicum,* the expression of GFP was decreased in both SDF5 and full promoter. These facts suggested that the PyrR from *pyrR* down-regulated the expression of *pyrH.* To reveal the regulation mechanism, the purified PyrR was used for the electrophoretic mobility shift assay (EMSA) of *pyrH* promoter region. When PyrR was added to the reaction mixture, the up-shifted bands were detected in *pyrH* full promoter, SDF5 and even in 92 bp upstream fragment. These results indicated that the PyrR binds to the promoter region, suggesting the possibility that the direct binding of PyrR to the *pyrH* promoter regulates the expression. The putative binding sites of PyrR to the pyrH promoter were identified by comparison of the sequence to *B. subtilis.* To confirm putative sites in binding of PyrR, the putative binding sites were deleted and used in the EMSA. The results showed that the DNA fragment has low affinity to the PyrR. These results suggested that the binding of PyrR to the *pyrH* promoter is happened through the putative binding sites located at upstream and downstream of SDF5 promoter.

To reveal another possibility of attenuation-regulation of PyrR in the *pyrH* gene expression in *C. glutamicum,* the *pyrR* promoter that has putative attenuator region was isolated and used for the

expression of GFP. The GFP expression was lower than that of pyrR promoter that does not contain putative attenuator. This result indicated that the expression of *pyrR* was self-regulated by binding of excess UMP that was derived from uracil to the attenuator region in the cell. These results suggested that the expression of PyrR was autoregulated.

On the other hand, the expression system useful in *corynebacterium* is not well developed so far. Therefore the temperature-inducible expression vector using lambda operator 1 and strong promoter CJ1 and CJ4 those were isolated from *C. glutamicum* and *C. ammoniagenes,* respectively.