F320

Molecular cloning of the histidine biosynthetic genes from *Corynebacterium glutamicum*.

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Histidine biosynthetic genes of *Corynebacterium glutamicum* are cloned and identified by complementation analysis that complements histidine auxotrophs of *E. coli*. According to the many studies on the histidine biosynthetic pathway, eight genes (*hisA,B,C,D,F,G,H,I*) are required for histidine biosynthesis in a total of 10 steps. These 8 genes (*hisA,B,C,D,F,G,H,I*) were cloned, which are located in three regions. That is to say, one group includes *his,A,D,F,H,I* and the other group is *hisB,C*. Another is *hisG*. Among *C. glutamicum* and different species, the similarity is generally observed in the overall lengths and gene order except for *hisD*. Minimal fragment containing *hisF* was subcloned from recombinant DNA including *his,A,D,F,H,I* region to pBluescript II KS(+), which was used in deletion and sequencing of the genes. *hisF* that encodes cyclase is gene of 774 base pair long that has a high similarity with the other bacterial *hisF* and *hisA* genes. *hisF* gene produced by PCR was used for expression of cyclase by pET system. In conclusion, eight genes are very tightly linked to each which encodes most enzymes required for the biosynthesis of histidine in the *C. glutamicum*.

F321

Biological Function of Old protein from Bacteriophage P2.

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P2 is a temperate bacterial virus which infects *E. coli*.. It has a nonessential gene called *old* (overcoming lysogenization defectiveness). The *old* gene product kills *E. coli recB* and *recC* mutants and interferes with the growth of bacteriophage λ. 3 mutant P2s(1, 17, 49) were isolated which were unable to interfere with the growth of λ. We have cloned wild type and mutant *olds* into pMAL vector. MBP-Old proteins were produced by IPTG induction and then purified by one step purification using amylose resin. The Old protein is purified and characterized to have exonuclease activity. But MBP could interfere with precise nuclease assay because it is a large volumn protein of 42KDa. To obtein a protein closer to its natural conformation, *old* and *old* mutant genes were amplified by PCR and cloned into pQE vector. The Old and mutant Olds were expressed as 6×His-tagged proteins and purified using Ni-NTA resin. Various types of nucleic acid were tested as substrates and the biological functions of Old nuclease *in vivo* are discussed.