S5-2

CHARACTERIZATION OF GENES ENCODING GLUCOSE PERMEASE OF PHOSPHOTRANSFERASE SYSTEM IN CORYNEBACTERIA AND BREVIBACTERIA

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Gram-positive corvnebacteria, including Brevibacterium lactofermentum, B. flavum, Corvnebacterium glutamicum, and B. ammoniagenes have been used for industrial production of various amino acids and nucleotides. They utilize sugars in large quantity as carbon sources. Four ptsG genes encoding glucose permeases of phosphostransferase system were cloned and sequenced. The deduced amino acid sequences of glucose permeases show higher homologies between B. lactofermentum, B. flavum, and C. glutamicum than B. ammoniagenes. Thee domains are commonly found in four glucose permeases; a hydrophobic region (EIIC) and two hydrophilic domains (EIIA, EIIB) with the same arrangement of structural domains EIIBCA. A B. lactofermentum mutant strain with disrupted ptsG gene was obtained by in vivo homologous recombination between a recombinant plasmid carrying a part of the ptsG gene and the chromosomal ptsG gene. When the mutant strain was grown in the minimal medium supplemented with glucose and sucrose as carbon sources, over eighty percent of glucose was remained, although the mutant could barely grow on glucose as a sole carbon source. The results suggest that glucose permease of PTS plays a major role in the glucose utilization of B. lactofermentum. In addition, it was found that dosage of ptsG gene can influence the viability of Escherichia coli.

S5-3

METABOLIC DESIGN IN AMINO ACID PRODUCING CORYNEBACTERIUM GLUTAMICUM

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Corynebacterium is widely used for the industrial production of amino acids. Application of recombinant DNA technology for strain improvement requires detailed information on the genetics of the target biosynthetic pathway. this context, we analyzed the pathway for methionine biosynthesis at The metA and metB genes encoding the first and the molecular level. second enzymes of methionine biosynthetic pathway were isolated from a C. glutamicum gene library. DNA-sequence analysis of the cloned DNA identified open-reading frames of 1,137 and 1,161 base pairs. The putative protein product showed good amino acid-sequence homology to their counterparts in other organisms. The metA mutant strain generated by the site-specific integration of the cloned DNA into its chromosome lost the ability to grow on glucose minimal medium whereas the metB mutant strain did not. Supplementation of the metA strain with cystathionine restored the growth ability. These data indicate that, in addition to the transsulfuration pathway, other methionine biosynthetic pathways may be present in C. glutamicum.