Feedback Resistant Pyruvate Carboxylase Gene from Corynebacterium
Technology #12625

Applications

A feedback resistant pyruvate carboxylase enzyme encoded by corynebacterium is applicable to the production of amino acids L-lysine and L-glutamate in an industrial setting.

Problem Addressed

Pyruvate carboxylase is an enzyme which catalyzes a metabolic reaction that converts pyruvate to oxaloacetate (OAA). OAA is the primary molecule from which bacterial cells used in industry, such as corynebacterium (C. glutamicum), can synthesize a variety of amino acids. These amino acids include, but are not limited to, L-lysine, L-glutamate, L-threonine, L-methionine, L-isoleucine, L-arginine, and L-proline. Wildtype pyruvate carboxylase is deactivated by a buildup of aspartic acid, a product of amino acid synthesis directly downstream of OAA, via feedback inhibition. This limits the rate of amino acid synthesis in an industrial setting.

Technology

This invention involves the creation of a mutated pyruvate carboxylase derived from corynebacterium that is resistant to inhibition by aspartic acid. Six separate mutations were introduced in the amino acid sequence of pyruvate carboxylase from the ATCC 21253 strain. This invention also includes a method for the replacement of the original pyruvate carboxylase gene in corynebacterium with the mutated gene. Briefly, the gene encoding pyruvate carboxylase was cloned into a vector including a corynebacterium replication origin and integrated into the corynebacterium host cell genome by homologous recombination to create the new strain NRRL B-11474. When the host cells were permeabilized and assayed for pyruvate carboxylase activity, they showed as much as a nearly 10-fold increase in enzyme activity in the presence of aspartic acid compared to wildtype pyruvate carboxylase activity. The method of integration of the mutated pyruvate carboxylase into host cells may be applied to a variety of microorganisms used in an industrial setting for the production of amino acids.

Advantages

- Increased rate of production of amino acids, including lysine, glutamate, threonine, methionine, isoleucine, arginine, and proline, by corynebacterium in an industrial setting
- Potential integration of the mutated gene to a variety of microorganisms aside from corynebacterium to produce said amino acids

Categories For This Invention:

Life Sciences
Biotechnology
Food
Research Tools
DNA
Protein & Protein Chemistry
Synthetic Biology
Bacterial

Intellectual Property:
Feedback-resistant pyruvate carboxylase gene from corynebacterium
Issued US Patent
7,300,777
Feedback-resistant pyruvate carboxylase gene from corynebacterium
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6,965,021

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External Links:
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