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Biotechnological Production of Purine Nucleotides in Corynebacterium ammoniagenes : Current Status and Prospects

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Introduction

Corynebacterium ammoniagenes is gram-positive and food-grade microorganism which is widely used as an industrial producer of purine nucleotides. Among them, especially inosine-5'-monophosphate (5'-IMP) and guanosine-5'-monophosphate (5'-GMP) are commercially important nucleotides that are used as flavor enhancer in various foods and as pharmaceutical intermediates. As well as amino acid production, nucleotide production is another key area of interest in biotechnology. Current total annual worldwide consumption of purine nucleotides is estimated to be over 15,000 tons with an approximate annual market growth rate of five percent. Major producers are Daesang, CJ (Korea), Ajinomoto, Takeda-Kirin Food (Japan) and StarLake (China). At present, the following three processes are known to be major methods for industrial nucleotide production: (a) chemical or enzymatic phosphorylation of corresponding nucleosides, (b) direct fermentation of 5'-IMP and 5'-XMP, (c) enzymatic conversion of 5'-XMP to 5'-GMP by recombinant *E.coli* or *C.ammoniagenes*.

The chemical phosphorylation process, comprising nucleoside fermentation and chemical phosphorylation, is used in Takeda-Kirin Food. Nucleosides such as inosine and guanosine are produced efficiently by fermentation. Direct phosphorylation of unprotected nucleosides is performed when an excess amount of phosphoryl chloride is used as an active phosphate donor. However, this process is relatively complex since it needs two discrete tanks for fermentation and chemical reaction. Also, Ajinomoto has developed a nucleoside phosphorylation reaction using the food additive pyrophosphate as a phosphate donor in order to establish a novel 5'-nucleotide production process, which consists of fermentation of nucleosides and the enzymatic phosphorylation of these nucleosides. They screened for a C5'-selective nucleoside phosphorylating enzyme from various microorganisms and found that the enzyme was an acid phosphatase family with nucleoside phosphotransferase activity.

Compared to the first process, direct fermentation is more economic and simpler method. The third process is the enzymatic production of 5'-GMP from 5'-XMP produced by a *C.ammoniageness* mutant

strain. GMP synthetase (EC 6.3.4.1) is generally used for the conversion of 5'-XMP to 5'-GMP. Since the enzyme reaction requires ATP hydrolysis, ATP has to be recycled to promote 5'-GMP production. The required ATP-regenerating activity is provided by reusing the cells of *C.ammoniagenes* that have finished 5'-XMP fermentation. Addition of overexpressed GMP synthetase promoted the production of 5'-GMP in the fermentor tank used for 5'-XMP fermentation. Therefore, large-scale fermentation processes for 5'-nucleotides have been developed using *C.ammoniagenes* by the combination of classical genetic mutant selection and fermentation improvement with genetic engineering of the biosynthetic genes, deregulating and increasing their level of expression.

Strain development and process optimization

There are 11 enzymatic steps for *de novo* synthesis of 5'-IMP, the first nucleotide intermediate in the purine biosynthetic pathway. Starting from PRPP, which contributes the ribose phosphate moiety of IMP, the purine ring is assembled from two amide nitrogens from glutamine, the amino group of aspartate, the two carbon atoms and amino group of glycine, and three one-carbon units : the carbon atoms from two molecules of 10-formyltetrahydrofolate. The major regulatory enzyme of purine biosynthetic pathway, PRPP amidotransferase (EC 2.4.2.14) is subject to strict end product inhibition by the adenine and guanine nucleotide.

The essential factors for 5'-nucleotide overproduction are as follows: (a) release of the feedback regulation in *de novo* purine nucleotide biosynthesis, (b) lack or decrease of 5'-nucleotide degrading activity, (c) elimination of the permeability barrier for 5'-nucleotide excretion, (d) blockage of branched pathway through auxotrophic mutant. Toward more efficient nucleotide overproduction, the strain development has been successfully achieved by the classical mutagenesis. For example, conferring resistance to purine base analogs to release the feedback regulation of the biosynthetic pathway, conferring drug resistance related to cell wall synthesis to relieve the permeability barrier, conferring resistance to osmotic pressure, and elevating the cellular pool of precursor molecule served as a moiety of the purine base are very critical targets for the construction of industrial hyper-producing strain. Furthermore, in order to elucidate the detailed information of the overall purine biosynthetic pathways and other cellular regulation, whole-genome analysis between wild-type and mutant strain is under progress. In this review, different process for purine nucleotide production and various attempts for rational strain design will be also discussed.

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