Molecular Metabolic Design for the Biological Production of β-Thymidine

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Abstract

Pyrimidine deoxyribonucleosides (PdNs), such as thymidine and deoxyuridine, are commercially useful starting compounds for the production of antiviral compounds comprising synthetic analogs of pyrimidines, such as azidothymidine (AZT) or azidodeoxyuridine. They are currently produced by organic synthesis utilizing a multi-step process which is very costly, contributing to the high cost of antiviral therapeutics such as AZT. The development of cost-effective biological processes for thymidine prodction is therefore of great interest

In order to develop novel strains for the biological production of thymidine, *Corynebacterium ammoniagenes* ATCC 6782, which does not accumulate pyrimidine nucleoside or nucleotide, was metabolically engineered to secrete a large amount of thymidine. Characteristics including 5-fluorouracil resistance (FUr), hydroxyurea resistance (HUr), trimethoprim resistance (TMr), thymidylate phosphorylase deficiency (*deo*A-), inosine auxotrophy (ino-), 5-fluorocytosine resistance (FCr), thymidine kinase-deficiency, and thymidine resistance (thymr) were successively introduced into mutant strains KR3 and DY5T9-5. They accumulated 408.1 mg/L and 428.2 mg/L of thymidine, respectively, as a major product in shake-flask cultures. The mutant strains did not accumulate thymine at all and accumulated less than 10 mg/L of other pyrimidine nucleosides, such as cytosine, cytidine and deoxycytidine, as byproducts. Thymidylate synthase A gene (*thy*A) which catalyzes the synthesis of TMP from dUMP was cloned from mutant strain DY5T9-5. The recombinant strain DYTTS constructed by introduction of *thy*A gene accumulated 493.1 mg/L of thymidine in flask cultivation.