Note

A 3-Deazauracil-resistant Mutant of Bacillus subtilis with Increased Production of Cytidine

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Bacillus subtilis No. 344 is a cytidine-producing mutant strain derived from wild type strain No. 122. When 3-deazauracil-resistant mutants were derived from strain No. 344, some of the mutants had higher productivities of cytidine. Among them, strain No. 428 accumulated 14.2 mg/ml cytidine in the culture. Cytidine 5'-triphosphate (CTP) synthetase from strain No. 428 changed to be free from feedback inhibition by CTP, compared with the enzyme from strain No. 344.

We have reported the construction of a cytidine-producing mutant, Bacillus subtilis strain No. 344 (cytidine deaminase-deficient, 6-deazauracil-resistant, and 5-fluorocytidine-resistant), that produced 10.4 mg/ml cytidine in the culture broth. This paper deals with the isolation of improved cytidine-producing mutants from strain No. 344, and the clarification of some of their properties.

Minimum medium (M-3) was that of Spizizen with 0.1% (w/v) Casamino acids (Difco Laboratories, Detroit) and 100 μg/ml l-arginine. The stock culture medium (A-1) contained (w/v) 1% Polypepton (Nihon Pharmaceutical, Osaka), 1% meat extract (extract Ehrlich) (Wako Pure Chemical Industries, Osaka), 0.3% yeast extract (Difco), 0.5% NaCl, and 2% maltose (pH 7.2). The fermentation medium (F-1) contained 4% corn steep liquor (Oji Corn Starch, Tokyo), 0.5% corn gluten meal (Japan Corn Starch, Nagoya), 2% urea, 0.5% CaCO₃, and 16% glucose (pH 7.2). Glucose and urea were sterilized separately. Pyrimidine compounds were measured by high-pressure liquid chromatography. The activity of cytidine 5'-triphosphate (CTP) synthetase was assayed by the method described previously.

Growth inhibition of strain No. 344 by various pyrimidine analogs and its reversal by uracil or cytidine was examined. Approximately 10⁶ cells were inoculated into 3 ml of M-3 medium with a pyrimidine analog in the presence or absence of an added pyrimidine compound at the concentration of 1 μmol/ml. The growth was measured by the absorbance at 590 nm after culture for 24 h at 37°C with reciprocal shaking. Among the compounds tested, 3-deazauracil (Sigma Chemical Co., St. Louis) inhibited the growth of strain No. 344 and the inhibition was reversed by cytidine and to a lesser extent by uracil (Fig. 1).

3-Deaauracine is a derivative of 3-deazauracil and has been evaluated as an antitumor agent. 3-Deaauracine is converted to 3-deazauracil 5'-triphosphate (3-deazaUTP) in mammalian cells. 3-Deaauracil inhibits CTP synthetase and the inhibition is competitive with respect to uridine 5'-triphosphate (UTP). 3-Deaauracine inhibits the growth of mammalian cells in vitro and the inhibition is reversed by cytidine and to a lesser extent by uridine. It seems reasonable, therefore, to assume that 3-deazauracil added to the culture of strain No. 344 is converted to 3-deazaUTP, and then inhibits CTP synthetase to diminish the intracellular concentration of cytidine derivatives. The ability of uracil to partially prevent the inhibition may be ascribed not only to its ability to compete with 3-deazauracil for transphosphoribosylation and phosphorylation to 3-deazaUTP, but also to the ability of uracil, which is converted from uracil, to competitively relieve the inhibition of CTP synthetase exerted by 3-deazaUTP.

Figure 1 shows that growth of strain No. 344 was inhibited in the presence of 5 mg/ml 3-deazauracil. Cells of strain No. 344 that had been treated with N-methyl-N'-nitro-N-nitroso-guanidine were spread on M-3 agar plates containing 5 mg/ml 3-deazauracil and then incubated at 37°C for 5 days. The colonies appearing on the plates were picked and tested for their productivity of pyrimidine.

Fig. 1. Growth Inhibition of B. subtilis No. 344 by 3-Deazauracil (○) and Reversal of the Inhibition by Uracl(●) or Cytidine(■).

Cells were inoculated into M-3 medium with or without 1 μmol/ml uracil or cytidine. The growth was measured by the absorbance at 590 nm after culture aerobically for 24 h at 37°C.

Table Properties of Strain Nos. 344 and 428

<table>
<thead>
<tr>
<th>Strain</th>
<th>Resistance to 3-deazauracil (μM)</th>
<th>Productivity of pyrimidine compounds (mg/ml)</th>
<th>Specific activity of CTP synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC₁₀ (mg/ml)</td>
<td>Cytidine</td>
<td>Uracl</td>
</tr>
<tr>
<td>No. 344</td>
<td>1.5</td>
<td>10.4</td>
<td>1.5</td>
</tr>
<tr>
<td>No. 428</td>
<td>12.0</td>
<td>14.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>
compounds. A loopful of cells grown on the agar plate of A-1 medium was inoculated into a 200-ml Erlenmeyer flask containing 20 ml of A-1 medium and then cultured at 37°C for 16 h on a rotary shaker (230 rpm). One milliliter of the culture was transferred to a 200-ml creased flask containing 20 ml of F-1 medium and was then cultured at 37°C for 80 h on the rotary shaker. About 8% of the colonies had improved productivity of cytidine. The total amount of pyrimidine compounds (i.e., uracil, uridine, and cytidine) accumulated in the culture of this series of mutants was equal to that of their parental strain, No. 344, on a molar basis, 60 μmol/ml. Among the mutants, strain No. 428 showed the highest productivity and the strain had higher resistance to 3-deazauracil than its parental strain (Table). The course of cytidine accumulation by strain No. 428 in F-1 medium is shown in Fig. 2. As the cultivation proceeded, cytidine accumulation was increased in parallel with glucose consumption. The titer reached 14.2 mg/ml at 80 h when glucose was completely consumed. Accumulation of uracil and uridine was less than 1.0 mg/ml during the fermentation.

In a B. subtilis wild type strain, activity and formation of CTP synthetase are controlled by cytidine nucleotides.31 We reported that synthesis of CTP synthetase is free from repression by cytidine nucleotides in strain No. 344, but activity of the enzyme is sensitive to inhibition by CTP.11 In this study, the properties of CTP synthetase from strain Nos. 344 and 428 were compared (Table). No apparent change was observed between the two strains in the specific activity of the enzyme. However, the activity of the enzyme from strain No. 344 was reduced by 77% in the presence of 5 mM CTP, while the activity of that from strain No. 428 was reduced only by 15% under the same conditions.

From these results, we conclude that release from feedback inhibition on CTP synthetase caused an easy conversion of UTP to CTP, and, as a result, a large amount of cytidine was accumulated in the 3-deazauracil-resistant mutant strain No. 428.

References