Characterization of Adenine-Thiamine Auxotrophic Mutant of *Streptomyces azureus* ATCC 14921

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Mutants with adenine-thiamine(Ath) auxotrophy have been reported in *Salmonella typhimurium*, *Escherichia coli* and *Saccharomyces cerevisiae*. They have single-site mutations, which are divided into two types: one is deficient in some enzymes in the early steps before AIR (5'-phosphoribosyl-5-aminoimidazole) formation in the purine biosynthetic pathway and the other is deficient in AICAR (5'-phosphoribosyl-5-amino-4-imidazole carboxamide) formyltransferase that catalyzes the conversion of AICAR to FAICAR (5'-phosphoribosyl-5-formamide-4-imidazole carboxamide)^{1,2}.

In streptomycetes, Ath mutants were also isolated and Ath gene locus was mapped in *Streptomyces lividans*³, but their properties have not been cleared. In the course of our genetic studies on the thiostrepton-producing *Streptomyces azureus*, we isolated an Ath auxotrophic mutant lacking thiostrepton productivity. In this paper, we demonstrate that this Ath mutant of *S. azureus* lacks AICAR formyltransferase. Some other properties are also described.

Streptomyces azureus ATCC 14921 (wild-type strain PK0) and its derivatives were used in this study and cultivated in a rye flake agar medium, Bennett agar and broth media and minimal medium under the conditions reported previously^{4,5}.

The thiostrepton productivity⁵ and the amount of AICAR⁶ were determined as previously described. AICAR formyltransferase activity was assayed according to the previous paper⁶ and Flaks *et al*⁷.

Adenine, other purines, AICA (5-amino-4-imidazole carboxamide) and thiamine were guaranteed commercial reagents. The thiazole moiety [4-methyl-5-(2-hydroxyethyl)-thiazole] and pyrimidine moiety (2-methyl-4-amino-5-hydroxy-methylpyrimidine) of thiamine were supplied by Takeda Chemical Industries Ltd.

Mutation was induced by UV-irradiation of spores at the lethal rate of more than 99%. Auxotrophic mutants requiring both adenine and thiamine appeared at a frequency of 10⁻⁷ to 10⁻⁶ per spores irradiated. One of the mutants thus obtained (designated ATH) was used throughout this work and found to be deficient in thiostrepton productivity.

To determine the blocked position in the purine biosynthetic pathway of the strain ATH, the growth was monitored in the minimal medium containing purines, thiamine and intermediates single or in combination. As shown in Table 1, the growth was observed in the medium containing thiamine in combination with adenine, guanine and hypoxanthine or in the medium containing adenine in combination with thiamine, pyrimidine moiety of thiamine and histidine. No growth occurred with single addition of adenine, guanine, hypoxanthine or with combined addition of adenine with thiazole moiety of thiamine. This result resembles very closely to those obtained with *pur* mutants of *E. coli*⁷ and *S. typhimurium*⁸ and ad-3 mutant of *S. cerevisiae*, in which purine biosynthesis is blocked at the step just after AICAR formation (Fig. 1). The result also indicates that the blocked step in the purine biosynthesis of the strain ATH seems not to be before AIR forma-

Fig 1. Step blocked on purine synthetic pathway in adenine-thiamine auxotrophic mutant ATH of *Streptomyces azureus*.

▲, blocked step. CAIR, 5'-phosphoribosyl 5-aminoimidazole-4-carboxylic acid; SAICA, [5'-phosphoribosyl 4-(N-succinocarboxamide)-5-aminoimidazole]; SAMP, adenylosuccinic acid.

Table 1. Nutritional requirement of adenine-thiamine auxotrophic mutant ATH of *Streptomyces azureus*.

Addition	Growth
None	-
Adenine	-
Guanine	-
Hypoxanthine	-
Adenine + thiamine	+
Guanine + thiamine	+
Hypoxanthine + thiamine	+
Adenine + pyrimidine moiety of thiamine	+
Adenine + thiazole moiety of thiamine	-
Adenine + histidine	+

+: growth, -: no growth. Mycelial growth was detected on minimum agar medium with or without purines (10 $\mu\text{g/ml}$), thiamine (1 $\mu\text{g/ml}$), thiamine derivatives (1 $\mu\text{g/ml}$) and histidine (50 $\mu\text{g/ml}$).

tion^{1,2,7,8,9}) since both adenine and thiamine (pyrimidine moiety) are synthesized via AIR as shown in Fig. 1.

The *purH* and *ad-3* mutants can grow in histidine in place of thiamine, because histidine gives rise to the synthesis of thiamine by preventing the accumulation of AICAR^{2,7,8,9}). The strain ATH was also able to grow with histidine instead of thiamine (Table 1), although the growth was weaker with histidine than with thiamine. Furthermore, it is known that the *purH* and *ad-3* mutants lack the activity of AICAR formyltransferase. The strain ATH

also exhibited no AICAR formyltransferase activity so that AICAR was accumulated clearly (data not shown). Therefore, we concluded that the strain ATH was AICAR formyltransferase-lacked mutant like the *purH* and *ad-3* mutants.

The spore formation of *Bacillus subtilis* and some streptomycetes are affected by adenine and its analogues^{10,11}). So, the effect of excess purines on the spore formation of mutant ATH was examined. The spores were spread on the surface of Bennett agar plates on which paper disks (8 mm in diameter; each containing 200 μg purine) were then placed and incubated at 28°C. Adenine, guanine and hypoxanthine did not effect the growth of strain ATH for the first 2 days. However, the prolonged incubation resulted in the formation of a clear zone with no or less spore formation around adenine-containing disc (Fig. 2) and to a lesser extent, hypoxanthine-containing disc. No such inhibition occurred with guanine. In our preliminary experiment, excess adenine seems to stimulate abnormally the accumulation of AICAR, but not excess guanine. This difference may be one of the reasons for the inhibition of spore formation by excess adenine (and hypoxanthine). The detailed reasons will be discussed in the next paper.

The same inhibition was observed with another purine auxotrophic mutant Ade²⁶) of *S. azureus*. In contrast, spore formation of the wild-type strain PK0 and its amino acid auxotrophic mutants was not inhibited by excess purine (Table 2). These results indicate that the inhibition of spore formation by excess ade-

Fig 2. Inhibition of spore formation of adenine-thiamine auxotrophic mutant ATH by excess adenine.

A, wild-type strain PK0; B, mutant ATH.

Bennett agar medium was used in this experiment, and a paper disc (8 mm in diameter) contained 200 μg adenine.

Table 2. Effect of excess purines on spore formation of wild-type strain PK0, its amino acid auxotrophic, adenine-thiamine auxotrophic and purine auxotrophic mutants.

Addition	PK0	ARG	AM1	HIS	ATH	Ade2
None(Control)	-	-	-	-	-	-
Adenine	-	-	-	-	+	+
Guanine	-	-	-	-	-	-
Hypoxanthine	-	-	-	-	+	+
Adenine + AICA	-	-	-	-	-	-
Hypoxanthine + AICA	-	-	-	-	-	-

+: inhibition of spore formation, -: no effect. PK0: wild-type strain, ARG: Arg⁻ mutant, AM1: Arg-Met⁻ mutant, HIS: His⁻ mutant, ATH: Ath⁻ mutant, Ade2: Pur⁻ mutant. Spore formation was detected as described in Fig. 2. A paper disc (8 mm in diameter) contained 200 μ g purines. AICA (10 μ g/ml) was added to agar medium.

mine and hypoxanthine is specific to purine auxotrophic mutants of *S. azureus*.

When 10 μ g/ml of AICA, which stimulates both mycelial growth and thio-strepton production of *S. azureus*¹²⁾, was added to the medium, the inhibition of spore formation by excess adenine or hypoxanthine was prevented. The same result was also obtained with the AIR carboxylase deficient mutant Ade2⁶⁾. We suppose that the accumulation of AICAR or other intermediates in the purine synthetic pathway affects the formation of spores in the purine auxotrophic mutants of *S. azureus*. The excess adenine and hypoxanthine would enhance the accumulation of AICAR and other intermediates in the mutants. AICA may enhance the mycelial metabolism so that the accumulated AICAR and other intermediates may be further metabolized. These will be clarified in our future reports.

The effect of cultural conditions and addition of chemicals such as AICA and cysteine on the thio-strepton production of strain ATH were also tested. However, thio-strepton production of strain ATH was never observed. We are now attempting to clarify the relationship between the Ath auxotrophy and blocked thio-strepton production.

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