Purine Auxotrophic Mutants with Altered Spore Color in *Streptomyces azureus* ATCC 14921

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Received 27 June 1989/Accepted 1 November 1989

A purine auxotroph with pale purple spores and a derivative with yellowish orange spores were obtained from the wild-type strain of *Streptomyces azureus* ATCC 14921, which has bluish green spores. The changed color or pigmentation in the mutants was limited to the spores. They accumulated AIR (5'-phosphoribosyl-5-aminoimidazole) due to the lack of AIR carboxylase activity.

Streptomyces species are procaryotes that differentiate and produce spores with species- (or strain-) specific color. The spore color has been applied to the conventional grouping of the genus *Streptomyces* (6). Thiostrepton-producing *Streptomyces azureus* ATCC 14921 forms bluish green (or sea green) spores (Fig. 1) and belongs to the blue spore-forming group in the genus *Streptomyces* (6). This paper describes the isolation and some properties of two artificially induced purine auxotrophs of *S. azureus* with altered spore color. There is no report yet concerning the artificial mutation of spore color in the streptomycetes. Studies on the purine auxotrophs have rarely been conducted for the streptomycetes.

S. azureus ATCC 14921 (wild-type strain PK0) and its derivatives were used throughout this work (12). Strain PK0 and its derivatives were propagated by using rye flake agar medium (12). Bennett agar, broth media, and minimal medium were also used to cultivate the strains (11). The conditions for cultivation were the same as those reported previously (11, 12).

Mutation was induced by *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine (NTG) treatment. NTG treatment was carried out under the conditions described by Delic et al. (3). The spore color was judged according to reference 14.

The accumulation of intermediates such as AIR (5'-phosphoribosyl-5-aminoimidazole), SAICAR [5'-phosphoribosyl-4-(*N*-succinocarboxamide)-5-aminoimidazole], and AICAR (5'-phosphoribosyl-5-amino-4-imidazole carboxamide) in the purine biosynthetic pathway was determined as follows. The mycelia were gathered from the plate culture on cellophane-coated minimal agar medium with 10 µg of adenine per ml. A 5-mg (wet weight) portion of the mycelia was extracted with 10 ml of 1 N folic acid for 30 min in an ice bath. The amount of AIR, SAICAR, or AICAR in each extract was determined by the Bratton-Marshall test (1).

The activities of AIR carboxylase, SAICAR synthetase, and SAMP (adenylosuccinic acid) lyase in the purine biosynthetic pathway were assayed as described elsewhere (2, 5, 16). The enzyme extracts were prepared as follows: the harvested mycelia of each strain from the liquid culture were suspended in 1 mM Tris hydrochloride buffer (pH 7.4) and disrupted by ultrasonication, and the extract was obtained by centrifugation.

When spores of the wild-type strain PK0 were treated with

NTG (1,000 µg/ml) and cultivated on rye flake agar, there were some morphologically changed colonies such as bald-type, white, and pale blue colonies. One of them formed pale purple (crocus or heliotrope) spores, unlike the wild-type strain PK0, which formed bluish green spores. The isolated mutant, designated Ade2, required adenine for growth. The purple colonies with the adenine requirement appeared at a frequency of less than 1 per 10⁸ spores treated. The change in color was observed to occur only on spores. Pigments or pigmentation was not found in the aerial and substrate (or submerged) mycelia and culture media of Ade2 or PK0. No mutants with other spore colors and no purine auxotrophs with bluish green spores (except for an adenine-thiamine auxotroph with bluish green spores [11]) were found in this experiment. The spontaneously occurring revertants were obtained at a frequency of about 10⁻⁸. We have determined by phage transduction and protoplast fusion that the initial color change and auxotrophy are due to a single mutation.

Strain Ade2 was further treated with NTG. Another spore color mutant was obtained at a frequency of about 10⁻⁸. This mutant, designated Ade21, formed yellowish orange (peach, salmon pink, or sometimes pale apricot) spores and, like Ade2, also required adenine for growth. The yellowish orange as well as the purple color was also limited to the spores. The spontaneously occurring revertants with purple or bluish green spores and with prototrophy were rarely obtained from Ade21.

Changes were not found in the morphology of spores and the feature of spore chains among strains PK0, Ade2, and Ade21. There were also not found any other differences among the properties of strains PK0, Ade2, and Ade21 such as the sensitivity to actinophage SAT1 (10), the productivity of defective phage SAT2 (12), and the existence of a sex plasmid, pSA1 (9). These results indicate that Ade2 and Ade21 are derivatives of PK0.

To determine the blocked portion in the purine biosynthesis of these two mutants, purines and thiamine (10 µg/ml) were added to the minimal medium, and the growth in each culture was monitored. Guanine, hypoxanthine, and other purines tested as well as adenine were effective, but thiamine had no effect. This result indicates that these mutants are blocked after AIR formation. The clear accumulation of AIR was found in the mutants Ade2 and Ade21 but not in the wild-type strain PK0 (Fig. 2). The accumulation of SAICAR and AICAR was hardly detected in both the mutants and PK0.

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FIG. 1. Lawn of spore color mutants Ade2 and Ade21 of *S. azureus*. (A) Wild-type strain PK0; (B) strain Ade2; (C) strain Ade21.

The SAICAR synthetase and SAMP lyase activities of the mutants were the same as those of PK0 (Table 1), but the AIR carboxylase activity of the mutants was less than that of PK0. These results were very similar to those obtained with

the *purE* mutant of *Escherichia coli* (7) and the *ade-2* mutant of *Saccharomyces cerevisiae* (15), which are deficient in AIR carboxylase (Fig. 3). Therefore, the mutants Ade2 and Ade21 should be blocked just after AIR formation because of the lack of AIR carboxylase activity.

We could not determine any difference between the properties of Ade2 and Ade21 except their spore color, but there is no report yet on such drastic changes in the spore color of streptomycetes. Similar mutations with the accumulation of red or purple pigments and AIR have been observed in *Pur*⁻ mutants of *E. coli*, yeasts, and fungi (4, 7, 8, 13, 15). Therefore, we suppose that the accumulation of AIR may affect the spore color of *S. azureus*. However, there is a clear difference between the mutant Ade2 or Ade21 and *Pur*⁻ mutants of other organisms: in the former, the changed color or pigmentation was limited to the spores. The pigments of spores of the wild type and mutants are being analyzed to clarify these differences.

FIG. 2. Accumulation of AIR in the mycelia of spore color mutants Ade2 and Ade21 grown on minimal agar medium with adenine. Symbols: ▲, wild-type strain PK0; ●, strain Ade2; ■, strain Ade21.

TABLE 1. AIR carboxylase, SAICAR synthetase, and SAMP lyase activities of wild-type strain PK0 and mutants Ade2 and Ade21

Enzyme	Activity (nmol/mg of protein per min) in strain:		
	PK0	Ade2	Ade21
AIR carboxylase	1.1	ND ^a	ND
SAICAR synthetase	3.2	2.2	2.8
SAMP lyase	23.2	25.0	21.8

^a ND, Not determined.

FIG. 3. Step blocked on purine synthetic pathway in the pigment-accumulating and Pur^- mutants. (a) AIR carboxylase; (b) AICAR synthetase; (c) SAMP lyase; $\blacktriangle$, blocked step. CAIR, 5'-Phosphoribosyl 5-aminoimidazole-4-carboxylic acid; FAICAR, 5'-phosphoribosyl 5-formamide-4-imidazole carboxamide; PRPP, 5-phospho- α -D-ribosepyrophosphate.

We are deeply grateful to H. Eguchi (Biotron Institute, Kyushu University) for his helpful advice on the photographs.

LITERATURE CITED

1. Bratton, A. G., and E. K. Marshall, Jr. 1939. A new coupling component for sulfanilamide determination. *J. Biol. Chem.* **128**:537-550.
2. Buchanan, J. M., L. N. Lukens, and R. W. Miller. 1978. N-(5-amino-1-ribosyl-4-imidazolylcarbonyl)-L-aspartic acid 5'-phosphate synthetase. *Methods Enzymol.* **51**:186-193.
3. Delic, V., D. A. Hopwood, and E. J. Friends. 1970. Mutagenesis by N-methyl-N'-nitrosoguanidine (NTG) in *Streptomyces coelicolor*. *Mutat. Res.* **9**:162-182.
4. De Serres, F. J. 1960. Studies with purple adenine mutants in *Neurospora crassa*. *Genetics* **45**:555-565.
5. Flaks, J. G., and L. N. Lukens. 1963. The enzymes of purine nucleotide synthesis *de novo*. *Methods Enzymol.* **6**:52-99.
6. Locci, R. 1989. *Streptomyces* and related genera, p. 2451-2492. In S. T. Williams, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 4. The Williams & Wilkins Co., Baltimore.
7. Love, S. H., and J. S. Gots. 1955. Purine metabolism in bacteria. III. Accumulation of a new pentose containing arylamine by a purine-requiring mutant of *Escherichia coli*. *J. Biol. Chem.* **212**:647-661.
8. Magasanik, B. 1962. Biosynthesis of purine and pyrimidine nucleotides, p. 295. In I. C. Gunsalus and R. Y. Stanier (ed.), *The bacteria*, vol. III. Biosynthesis. Academic Press, Inc., New York.
9. Miyoshi, Y., S. Ogata, and S. Hayashida. 1986. Multicopy derivative of pock-forming plasmid pSA1 in *Streptomyces azureus*. *J. Bacteriol.* **168**:452-454.
10. Ogata, S., H. Suenaga, and S. Hayashida. 1985. A temperate phage of *Streptomyces azureus*. *Appl. Environ. Microbiol.* **49**:201-204.
11. Ogata, S., S. Yamada, and S. Hayashida. 1985. Genetic recombination in *Streptomyces azureus* by protoplast fusion and high production of thiostrepton by the recombinants. *J. Gen. Appl. Microbiol.* **31**:187-191.
12. Ogata, S., S. Yoshino, H. Suenaga, K. Aoyama, N. Kitajima, and S. Hayashida. 1981. Specific lysogenicity in *Streptomyces azureus*. *Appl. Environ. Microbiol.* **42**:135-141.
13. Pouter, R. T. M., and E. H. A. Rikkerink. 1983. Genetic analysis of red, adenine-requiring mutants of *Candida albicans*. *J. Bacteriol.* **156**:1066-1077.
14. Shogakutosyo. 1988. Color guide international: Kokusaiban Iro no Techou. Syougakukan, Tokyo. (In Japanese.)
15. Silver, J. M., and N. R. Eaton. 1969. Functional blocks of the Ad1 and Ad2 mutants of *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* **34**:301-305.
16. Woodward, D. D. 1978. Adenylosuccinate AMP-lyase (*Neurospora crassa*). *Methods Enzymol.* **51**:202-207.