SHORT COMMUNICATION

A Conditional Aerial Mycelium-negative Mutant of *Streptomyces fradiae* with Deficient Ornithine Carbamoyltransferase Activity

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A mutant defective in ornithine carbamoyltransferase activity and having a concomitant aerial mycelium-negative phenotype was isolated from *Streptomyces fradiae*. The aerial mycelium formation of the mutant could be restored by replacing L-arginine with L-citrulline in the minimal medium. The possibility that the ornithine cycle is connected with the regulation of aerial mycelium formation is discussed.

INTRODUCTION

A systematic genetic study of aerial mycelium formation in *Streptomyces* was carried out by Hopwood *et al.* (1973) using aerial mycelium-negative (Bld⁻) mutants of *S. coelicolor*. Frequent simultaneous loss of the argininosuccinate synthetase activity and the Bld⁺ (or in the authors' nomenclature, Amy⁺) trait have been observed recently in *S. alboniger*, *S. scabies*, *S. violaceus-ruber* (Redshaw *et al.*, 1979) and *S. lavendulae* (Nakano *et al.*, 1980*a*). The insertion and transposition of a plasmid has been suggested by Nakano *et al.* (1980*b*) to affect frequently certain non-specified *bld* and *arg* genes in '*S. kasugaensis*' [a species described by Umezawa *et al.* (1965)]. Yet, it has not been clear what is the relationship between the defect in the arginine pathway and the impaired differentiation. Previous reports suggested the loss or inactivation of an *arg* gene and a *bld* gene. The results reported here raise the possibility of an alternative explanation. To our knowledge, *argF* mutants (defective in ornithine carbamoyltransferase) have not previously been reported in *Streptomyces*. These *argF* mutants may be of interest also with respect to differentiation, since ornithine carbamoyltransferase activity reaches a maximum during sporulation in *Bacillus subtilis* (Deutscher & Kornberg, 1968).

METHODS

Strains. The *Streptomyces* strains used in this study are listed in Table 1.

Media. The minimal media were 'sucrose nitrate agar' (Waksman, 1967), and 'sucrose nitrate liquid medium' (the same as the former except agar was omitted). The complete medium was the 'sporulation medium' of Hopwood & Sermonti (1962).

Mutagenesis. A spore suspension (3 ml) containing 10⁸–10⁹ spores ml⁻¹ was irradiated in a Petri dish with a germicidal lamp (30 W, 254 nm) from 50 cm for 10 min, giving a survival rate of 0.5%.

Preparation of cell homogenates. Cultures were shaken for 68 h at 34 °C in liquid minimal medium supplemented with 1 mM-L-citrulline. The mycelia were then pelleted, washed twice and resuspended in 66.7 mM-KH₂PO₄/Na₂HPO₄ buffer, pH 7.5. The cells were homogenized by sonication. The debris was removed by centrifugation at 1800 g for 15 min, and the supernatant was used for enzyme assays after dialysis in Visking dialysis tubing (Serva) against the same phosphate buffer. (All of the above treatments were performed at 4 °C.)

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Table 1. *Strains of Streptomyces fradiae* examined

Strain	Parent	Mutagen	Phenotype*	Source
LN 47 1-G1			Prototroph, Bld ⁺	Biogal Pharmaceutical Works, Debrecen, Hungary
M2-21	LN 47 1-G1	UV	Prototroph, Bld ⁺ , Str ^R	Our stock
St 3110	M2-21	UV	Arg ⁻ , Bld ⁻ (conditional), Str ^R	Our stock

* Str^R, Resistance to 100 µg streptomycin ml⁻¹.

Enzyme assays. The activity of carbamoyl-phosphate synthetase (EC 6.3.5.5, formerly EC 2.7.2.9) was determined by the chromatographic–videodensitometric technique of Karsai & Elődi (1981) measuring the formation of glutamate from glutamine. Ornithine carbamoyltransferase (EC 2.1.3.3), argininosuccinate synthetase (EC 6.3.4.5), argininosuccinate lyase (EC 4.3.2.1) and arginase (EC 3.5.3.1) were assayed by the same method as described elsewhere (Karsai *et al.*, 1979), except that the reaction mixtures were supplemented with coupled enzymes (Karsai & Elődi, 1982).

Protein was determined by the Lowry method using bovine serum albumin as standard.

Preparation of concentration gradients from nutrients. A cell suspension (0.1 ml) was spread on an agar plate (25 ml minimal medium; 9 cm diam.), then an agar disc (1 cm diam.) was removed from the middle of the plate and 0.25 ml 0.2 M-amino acid solution (pH 7.0) was pipetted into the hole. The plate was incubated at 30 °C. Colour intensity gradients of bromophenol blue and phenol red were fairly constant in parallel model plates.

Chemicals. Arginine, argininosuccinate and citrulline were from Serva, carbamoyl phosphate and glutamine from Calbiochem. Fixion 50 × 8 thin-layer ion-exchange chromatoplates (Reanal, Hungary) were used for the chromatographic studies. All the other chemicals were of reagent grade.

The enzymes applied in coupled assay-systems were isolated in our laboratory; argininosuccinate synthetase from ox liver by the method of Ratner (1970) and arginase from rat liver, according to Schimke (1970).

RESULTS

Isolation of the ornithine carbamoyltransferase-defective mutant

Auxotrophs were generally obtained by UV-mutagenesis from *S. fradiae* at a frequency of 2.4×10^{-3} . Arg⁻ mutants represented about 3% of the total auxotrophs. From strain M2-21 an Arg⁻ mutant clone, St 3110, was isolated, which grew on minimal medium in the presence of 1 mM-L-citrulline, but did not grow on minimal medium supplemented with L-ornithine, suggesting that ornithine carbamoyltransferase was blocked. This was confirmed by enzyme assays (Table 2). The frequency of spontaneous reversion of this mutant to prototrophy was less than 1.5×10^{-8} .

Aerial mycelium formation in the mutant and its parents

Strain St 3110 showed a typical stable Bld⁻ phenotype when cultivated either on complete medium or on minimal medium containing concentration gradients up to about 0.2 M of L-arginine (Fig. 1a) or L-argininosuccinate (data not shown) even after several weeks of incubation. No aerial mycelium was seen with a stereomicroscopic observation of such colonies. However, when the mutant was grown on a minimal medium plate with L-citrulline gradient, aerial mycelium formation was observed over almost the whole plate after 3 d at 30 °C (Fig. 1b). Similar results were obtained with 1 mM concentrations of the same amino acids (data not shown). Bld⁺ revertants have not been found on minimal medium plates supplemented with 1 mM-L-arginine. The parent strains, M2-21 and LN 47 1-G1, were Bld⁺ on minimal medium both with L-arginine (Fig. 1c, d), L-citrulline, or no supplement (data not shown).

DISCUSSION

Mutant St 3110 has well defined permissive and non-permissive conditions for the production of aerial mycelium. It may be useful in studying regulation of differentiation in *Streptomyces*. The restoration of aerial mycelium formation in the mutant by substituting citrulline for arginine in a simple synthetic medium indicates a possible connection between the transport or

Table 2. *Specific activity of the ornithine cycle enzymes in Streptomyces fradiae strains*

Strain	Enzyme activity in cell homogenates [nmol h ⁻¹ (mg protein) ⁻¹]				
	Carbamoylphosphate synthetase	Ornithine carbamoyl-transferase	Argininosuccinate synthetase	Argininosuccinate lyase	Arginase
LN 47 1-G1	43	219.0	65	103	43
St 3110	80	7.3	44	67	48

Fig. 1. Aerial mycelium formation in *S. fradiae* strains. The 9 cm MM plates were inoculated with the strains indicated, and 0.25 ml of 0.2 M-L-arginine or L-citrulline solution (pH 7.0) was applied into the 1 cm holes. Cultures were photographed after 10 d incubation at 30 °C. (a) Strain St 3110 + L-arginine; (b) strain St 3110 + L-citrulline; (c) strain M2-21 + L-arginine; (d) strain LN 47 1-G1 + L-arginine.

intracellular concentrations of metabolites of the ornithine cycle and regulation of differentiation in this strain. The stimulatory effect of certain concentrations (about 25 mM) of arginine on aerial mycelium formation in strain LN 47 1-G1 (Fig. 1d) also supports the possibility of the above connection, which (if present) may be direct or indirect, and may have a physiological regulatory role or not. Since the Bld⁻ phenotype can be reversed by citrulline, at least in this *argF* mutant, it seems unnecessary to assume that inactivation or loss of a *bld* gene also occurs (in addition to that of the *arg* gene) when the simultaneous disappearance of the Arg⁺ and Bld⁺ traits takes place.

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