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A cofactor for thienamycin biosynthesis produced by a blocked mutant of *Streptomyces cattleya*

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Thienamycin (THM; Fig. 1), the first naturally occurring β-lactam antibiotic discovered to possess the novel carbapenem ring system, was isolated from the culture filtrate of *Streptomyces cattleya* [1]. The biosynthesis of THM was investigated by Williamson et al. [3]. They established that the molecule is made up of cysteine, glutamate, acetate, and two methyl groups of methionine. The methyl groups were found to give rise to the hydroxyethyl side-chain of THM. Our intent was to further explore the biosynthetic route of THM using blocked mutants. During our studies we have isolated non-THM-producing strains by NTG, NMU, EMS and/or UV treatment of a THM-producing strain of *S. cattleya*. Applying an agar strip method [2], these nonproducers were tested for cosynthetic ability and classified into five groups. The complementation patterns between each group are shown in Table 1. In this communication, we wish to report the isolation of a bioconvertible substance produced by a Class I mutant.

The blocked mutants used in these studies were MA 5952 (Class I) and MA 5953 (Class XVI), both of which lacked the ability to elaborate THM. However, MA 5953 growing in the presence of previously prepared MA 5952 filtrate was able to produce THM. Utilizing this response as an analytical tool, we were able to determine the presence of the material produced by MA 5952 that allowed MA 5953 to produce THM. The cosynthetic bioassay involves adding the bioconvertible material of MA 5952 to the resting cells of MA 5953. The resting cells were prepared in the same procedure as described by Williamson et al. [3]. After incubation for 2–3 h at 27°C and 220 rpm, the broth was disc assayed on *Staphylococcus aureus* MB 108 plates for THM. Zones of inhibition were obtained after overnight incubation of plates at 37°C.

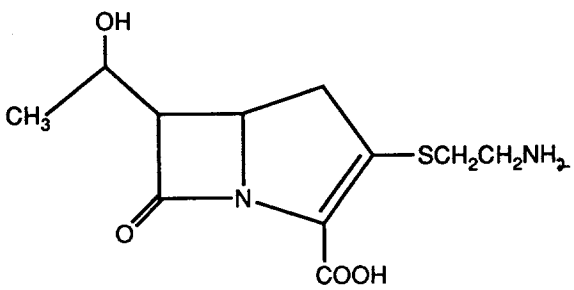

Fig. 1. The structure of thienamycin.

TABLE 1

Cosynthesis between groups of blocked mutants of *S. cattleya*

Mutant class	Co synthesis	
	As convertor	As secretor
I	II	III, XVI
II	—	I, III, XVI
III	I, IV	XVI
XV	—	III, XVI
XVI	I, II, III, XV	—

Streptomyces cattleya MA 5952 was cultured for 24 h at 27°C in a seed medium* (40 ml in a 250-ml baffled flask). These cultures were used as inoculum for the 2-L Erlenmeyer flasks containing 160 ml of production medium†. The fermentation flasks were inoculated with 16 ml of seed culture and incubated at 27°C on a rotary shaker for 4 days. Eight liters of the whole broth was processed as described below.

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* The seed medium contained (per liter): monosodium glutamate monohydrate (5.0 g), NH_4Cl (1.5 g), K_2PO_4 (2.0 g), inositol (0.4 g), NaCl (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $FeSO_4 \cdot 7H_2O$ (0.025 g), $ZnSO_4 \cdot 7H_2O$ (0.01 g), *p*-aminobenzoic acid (0.1 mg), $CoCl_2 \cdot 6H_2O$ (0.01 g), ardamine YEP (10.0 g), glycerol (10.0 g), $CaCO_3$ (0.25 g), pH = 7.0.

† The production medium contained (per liter): monosodium glutamate monohydrate (3.75 g), L-isoleucine (2.4 g), NH_4Cl (0.75 g), K_2HPO_4 (1.0 g), $CoCl_2 \cdot 6H_2O$ (0.01 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $FeSO_4 \cdot 7H_2O$ (0.025 g), glycerol (10.0 g), MES buffer (14.6 g), pH = 6.8.

The mycelia of the harvested fermentation broth were separated by filtration and the bioconvertible activity was adsorbed from the filtrate onto activated carbon. The carbon cake was washed with water, and the activity was then eluted with 50% aqueous acetone. The active eluate was concentrated and loaded onto a Diaion HP-20 column. The column was washed with water to remove salts before eluting with 50% aqueous acetone. The eluate was concentrated *in vacuo* to remove acetone and the remaining water layer was then extracted three times with n-butanol to remove a yellow pigment. The bioconvertible activity stayed in the aqueous phase. The aqueous layer was concentrated and chromatographed in a Diaion HP-20 column using a stepwise gradient of 10%, 20%, 30%, and 40% aqueous acetone as eluent. Final purification of the enriched material was achieved by HPLC on a reverse phase ultrasphere-ODS (10 mm × 25 cm) column using 20–100% MeOH/H_2O gradient to yield 200 μg of a bioconvertible red substance. The red substance was identified as vitamin B_{12} by proton NMR spectroscopy and FAB mass spectroscopy ($[M-CN]^+$ *m/z* 1329, $[M-CN+Na]^+$ *m/z* 1352). The IR and UV absorption spectra confirmed this.

The bioconvertible activity of vitamin B_{12} is catalytic. The minimum concentration required for THM synthesis (1 mg ml^{-1}) by MA 5953 was determined to be 0.1 μg ml^{-1}. *Streptomyces cattleya* MA 5953 is not a vitamin B_{12} auxotroph and cobalt cannot substitute for vitamin B_{12}, suggesting that this vitamin may be involved in the biosynthesis of THM. However, more experiments are needed to define the role of vitamin B_{12} in THM biosynthesis.

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