Notes

F-244 (1233A), A SPECIFIC INHIBITOR OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A SYNTHASE: TAXONOMY OF PRODUCING STRAIN, FERMENTATION, ISOLATION AND BIOLOGICAL PROPERTIES

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In the course of screening for physiologically active compounds from microbial metabolites, a fungal β-lactone termed F-244 was independently isolated from *Scopulariopsis* sp. by ŌMURA's group and from *Fusarium* sp. by GREENSPAN's group. It inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase [EC 4.1.3.5], an enzyme involved in an early step of cholesterol biosynthesis.[1] F-244 with the structure of 3,5,7-trimethyl-12-hydroxy-13-hydroxymethyl-2,4-tetradecadienedioic acid 12,14-lactone (for structure, see Fig. 3), was proved identical with 1233A originally isolated by ALDRIDGE *et al.*[2,3] as an fungal metabolite, for which biological properties have not been reported. We describe here taxonomy of the producing strain, fermentative production, and biological properties of F-244.

The taxonomic characterization of strain F-244 was carried out by the methods described by MCGINNIS *et al.*[4] and TUBAKI.[5]

For morphological study, sporulating mycelia of strain F-244, previously grown on yeast extract-soluble starch (YpSs) agar, were fixed overnight in the vapor of OsO_4, coated with platinum-palladium, and then were observed under a Hitachi scanning electron microscope (model S-430). YpSs agar contained soluble starch 1.5%, yeast extract 0.4%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.005% and agar 2.0%, pH 6.0.

As shown in Table 1(A) and Fig. 1, sporulation was abundant on YpSs agar. Conidia are formed as an aleuriospore, and annellophore is observed (Fig. 1(b), arrowed). Table 1(B) and (C) show that aerial hyphae were ivory or cream in color on agar media after 14 days at 27°C. The arsenic reaction[5] was positive. The above characteristics of strain F-244 are consistent with the generic designation of *Scopulariopsis* Bainier.

Table 1. Morphological (A), cultural (B) and physiological (C) properties of strain F-244.

(A)

Mycelia:	Branched, mycelial size of width; 2.1~2.3 μm
Conidiophore:	Stard erect to mycelia, not branched
Annellide:	Observed
Conidia:	Aleuriospore, ovalate, smooth surface, spore size; 5.9~7.2×4.2~5.5 μm

(B)

Medium	Colony size (mm)	Aerial hyphae
YpSs agar	60~63	Velvety, pastel yellow
Potato dextrose agar	18~20	Lanose, light ivory
Malt extract agar	21~23	Velvety, ivory
CZAPECK agar	18~20	Lanose, cream

(C)

Temperature range for growth:	15~37°C
pH range for growth:	pH 5.0~10.0
Arsenic reaction:	Positive

Strain F-244 was cultivated in 5-liter Erlenmeyer flasks containing 1 liter of production medium (glycerol 3%, glucose 1%, peptone 0.5%, NaCl 0.2%, natural zeolite 1.0% and agar 0.1%, pH 7.0), on a rotary shaker at 27°C for 10~14 days. Fig. 2 illustrates F-244 appeared at day 3 after inoculation, then gradually increased to reached a maximum (42 μg/ml) at day 8~9, as detected by HPLC. Addition of natural zeolite, an ammonium-trapping agent known to increase antibiotic yields in other fermentation systems, resulted in steady production and yield improvement.

Fig. 1. Scanning electron micrographs of spore (a) and annellide (b) of strain F-244 on YpSs agar after 7 days at 27°C.

Bars represent 10 μm. Arrow indicates annellide.

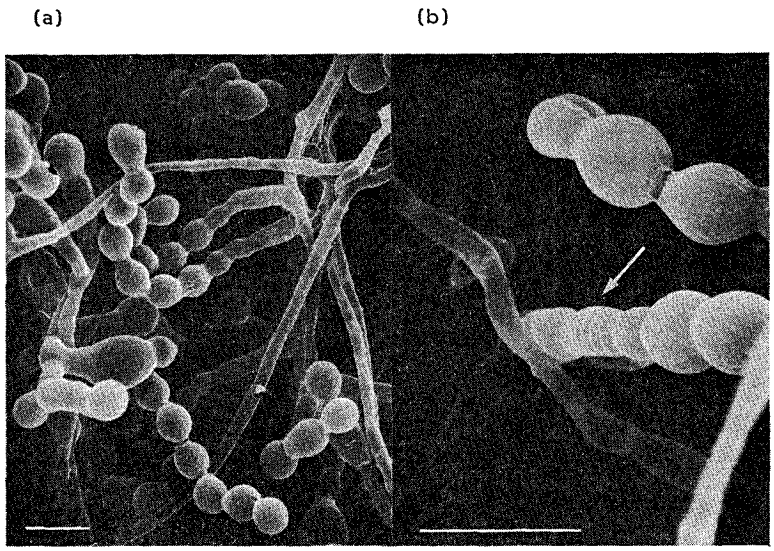
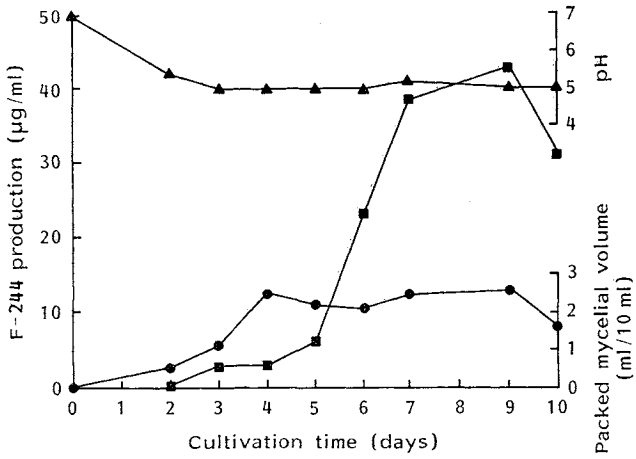


Fig. 2. A typical time course of F-244 production.

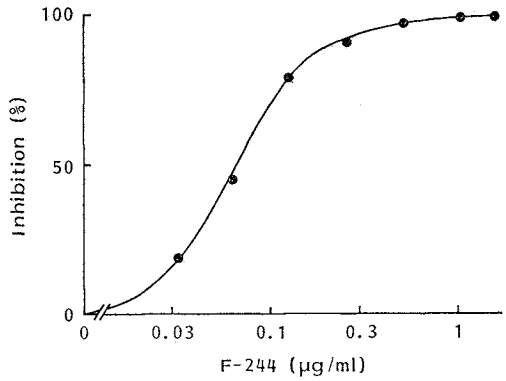
▲ pH, ■ F-244, ● growth.

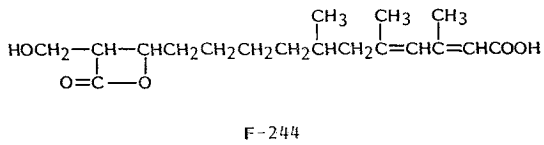


The cultured broth (21 liters) was centrifuged to obtain about 20 liters of supernatant fluid. The mycelial cake was extracted with 1 liter of 80% aq acetone. After removal of acetone by evaporation under reduced pressure, the aqueous solution was combined with the supernatant fluid. The mixture, adjusted to pH 3.0 with conc HCl, was extracted with 20 liters of ethyl acetate. The extract was concd *in vacuo* to yield a brown paste (5.7 g). The active principle in the paste, dissolved in 6 ml of ethanol, was purified by HPLC. The operation conditions for HPLC were as follows: Jasco Tri Rotar V system; column, YMC Pack A-343 (ODS, 20×250 mm); solvent, 60% CH_3CN in 0.1% aq H_3PO_4; flow rate, 8.0 ml/minute; detection, UV at 270 nm. The fractions eluted with retention time at 12.3 minutes were combined, evaporated, and extracted with ethyl acetate. The extracts were evaporated to yield a white powder of pure F-244 (600 mg).

Results of elemental analysis (C 66.48, H 8.87

Fig. 3. Inhibition of HMG-CoA synthase by F-244 in a rat liver enzyme system.





F-244

and O 24.55), field desorption (FD) mass (*m/z* 325, $M^+ + 1$), UV ($\lambda_{max}^{CH_3CN}$ 270 nm), IR (ν_{max}^{KBr} cm^{-1} 3530, 3200~2800, 1830, 1800, 1700, 1680), ^1H and ^{13}C NMR revealed that F-244 was identical with 1233A.[2,3]

Antimicrobial activity of pure F-244 was assayed by a conventional agar dilution method using Mueller-Hinton agar for bacteria incubated at 37°C for 20 hours and potato - glucose agar for fungi incubated at 27°C for 96 hours. F-244 was active against *Candida albicans* (MIC in μg/ml, 12.5), *Penicillium herquei* (25), *Piricularia oryzae* (6.25) and *Staphylococcus aureus* (50).

It was inactive against other yeasts, filamentous fungi and bacteria tested. The growth inhibition of *P. oryzae* and *S. aureus* was reversed when 1 mM mevalonate was added to the medium. It is suggested that the growth inhibition is due to a blockade of mevalonate biosynthesis by F-244 in these microorganisms.

Fig. 3 shows inhibition of HMG-CoA synthase by F-244 in a rat liver enzyme system, as assayed by the method described previously.[1] The apparent IC_{50} value (concentration required for 50% inhibition) was 0.065 μg/ml. Mode of action of F-244 will be reported elsewhere.[6]

Intraperitoneal injection to mice at 100 mg/kg and oral administration to mice at 500 mg/kg of F-244 did not have any toxic effect.

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