A NEW ANTIBIOTIC, LIPOPEPTIN A

Sir:

We wish to describe here isolation and characterization of a new antibiotic from cultures of *Streptomyces* sp. No. AC-69, which resembles *Streptomyces violaceochromogenes*. This strain was isolated from a soil sample collected in Futaba-gun, Fukushima Prefecture, Japan.

The strain was cultured at 27°C for 72 hours in a jar fermentor containing 30 liters of a medium, which is composed of 2% glucose, 1% soluble starch, 0.1% meat extract, 0.4% dry yeast, 2.5% soybean flour, 0.2% NaCl and 0.005% K_2HPO_4. Fermentation broth was adjusted to pH 2.0 with 10% HCl, heated for 10 minutes at 60°C and filtered. The filtrate was extracted twice with 30 liters of ethyl acetate. Mycelium was extracted with 15 liters of 80% acetone, the extract was concentrated *in vacuo* to give an aqueous solution (4 liters), and this was then extracted twice with 4 liters of ethyl acetate. All the ethyl acetate extracts were combined and concentrated *in vacuo* to dryness. The residue was purified with the following successive chromatographies: (1) Silicic acid (isopropanol-0.1 N NH_4OH, 5: 0.2→5: 1), (2) Sephadex LH-20 (methanol), (3) DEAE-sepharose (carbonate form, 60% methanol→0.5 M NH_4HCO_3-methanol, 4: 6), (4) Diaion HP-20 (water→methanol).

The antibiotic was obtained as purified white powder (600 mg) from methanol - ether. H.p.l.c. analysis showed that it contained a major component and a minor component. Separation was achieved by repeated preparative chromatography using a Hitachi RP-18 column with methanol containing 0.5% H_3PO_4 and 30% H_2O. Main fraction was collected and neutralized with dilute NH_4OH. After adsorption and elution from Diaion HP-20, 390 mg of lipopeptin A was obtained as white powder from methanol - ether.

Lipopeptin A free acid melted at 206~208°C with decomposition. It is optically active, $[\alpha]_D^{20}$ −45.4° (*c* 1.06, methanol).

Anal. Calcd. for $C_{54}H_{84}N_{10}O_{19}\cdot 2H_2O$:
C 53.47, H 7.26, N 11.55, O 27.72.
Found: C 53.55, H 7.22, N 11.55, O 26.39.

FD mass spectrum showed $(M+Na)^+$, 1,199. Electrometric titration showed it is a dibasic acid with a pKa′ of 4.6 and titration equivalent of 587. It is soluble in lower alcohols, sparingly soluble in acetone, chloroform, ethyl acetate, and hardly soluble in benzene, ether and petroleum ether. It is positive to DRAGENDORFF and RYDON-SMITH tests but negative to a ninhydrin test. It showed end absorption in UV region. The IR absorption spectrum is shown in Fig. 1. The presence of amide (1650 and 1525 cm^{-1}) and ester (1735 cm^{-1}) was suggested. On acid hydrolysis, the following amino acids were identified; serine, threonine, aspartic acid, glutamic acid, *N*-methylaspartic acid, *N*-methylphenylalanine, and *threo*-β-hydroxyglutamic acid. In addition, saturated C_{15}-fatty acid was isolated and charac-

Fig. 1. IR absorption spectrum of lipopeptin A (in KBr).

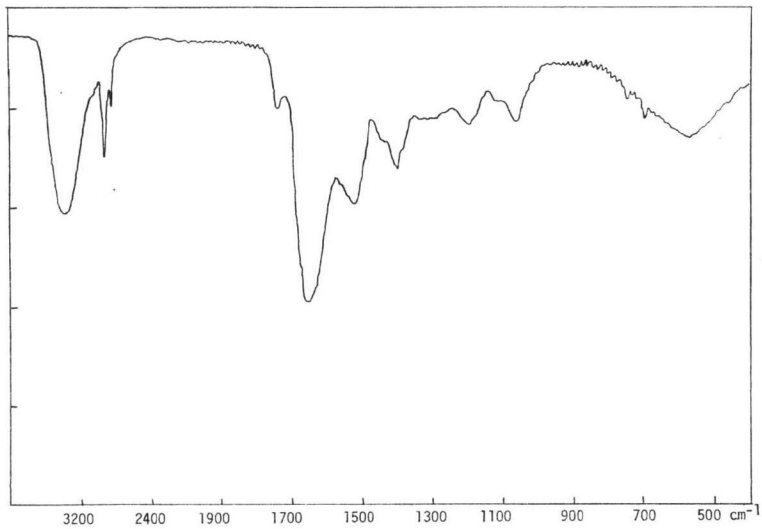

Table 1. Antifungal activity of lipopeptin A.

Microorganism	MIC (μg/ml)
Piricularia oryzae	150
Colletotrichum lagenarium	150
Alternaria mali	>300*
Botrytis cinerea	>300*
Cochliobolus miyabeanus	>300*

Conventional agar dilution method was employed using potato-sucrose medium.

* Partial inhibition was observed at this concentration.

terized by mass spectroscopy.

The antibiotic showed weak inhibitory activity against some species of phytopathogenic fungi (Table 1). It caused swelling of fungal cells. It also inhibited peptidoglycan synthesis of *Escherichia coli* Y-10 *in vitro* (I_{50} 150 μg/ml). However, it showed only a very weak inhibitory activity against bacteria cells. Mice tolerated 250 mg per kg of body weight of intravenous injection of lipopeptin A.

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