STUDIES ON A NEW ANTIBIOTIC SF-2330

I. TAXONOMY, ISOLATION AND CHARACTERIZATION

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A new antibiotic SF-2330 active against Gram-positive bacteria has been isolated from the culture broth of *Streptomyces* sp. SF-2330. The antibiotic was obtained as orange needle crystals and its molecular formula was $C_{22}H_{14}O_7$. This is a new member of the pluramycin group antibiotics.

In the course of our screening for new antibiotics, a new antibiotic SF-2330 has been isolated from the fermentation broth of *Streptomyces* sp. SF-2330. The UV spectrum of SF-2330 is similar to those of pluramycin[1], rubiflavin[2], hedamycin[3], neopluramycin[4], kidamycin[5], griseorubin[6] and largomycin[7], but SF-2330 was differentiated from those antibiotics by other physico-chemical properties.

In this paper, the taxonomy of the producing organism, the production, isolation, characterization and biological properties of the antibiotic are described.

Taxonomy of the Producing Organism

The antibiotic-producing organism, strain SF-2330, was isolated from a soil sample collected at Nihondaira, Shizuoka Prefecture, Japan. For the taxonomic characterization of strain SF-2330, the methods and media recommended by the International Streptomyces Project[8] and those recommended by WAKSMAN[9] were used.

Morphological observation were made on the cultures grown at 28°C for 14 days on oatmeal agar (ISP medium 3) and yeast extract - malt extract agar (ISP medium 2). Vegetative mycelium is well developed and branched. The hyphae does not fragment into coccoid or bacillary elements. Strain SF-2330 produces aerial mycelia with straight, flexuous or very loose spiral spore chains. The spore chains have more than 10 spores per chain. The spores are cylindrical ($0.5\sim0.8\times0.7\sim1.5$ μm) with smooth surfaces as shown in Plate 1. Sclerotic granules, sporangia and flagellated spores are not observed. The cultural and summarized physiological properties are shown in Tables 1 and 2, respectively. The results were recorded after 7, 14 and 21 days of incubation. Strain SF-2330 shows moderate to abundant growth on oatmeal agar, yeast extract - malt extract agar and Bennett agar, but no or very poor growth on synthetic agar, tyrosine agar (ISP medium 7) and nutrient agar. Aerial mass color was difficult to determine because of very scant formation of aerial mycelium, but it might be in the white, yellow or gray color series of the PRIDHAM and TRESNER grouping[10]. Strain SF-2330 produces many yellow crystalline substances around its colony (Plate 2). These are seen on oatmeal agar, yeast extract - malt extract agar and Bennett agar. LL-Diaminopimelic acid was detected in the whole-cell hydrolysates.

Based on the taxonomic properties described above, strain SF-2330 is considered to belong to the

Plate 1. Scanning electron micrograph of spore chains of *Streptomyces* sp. SF-2330 (×6,000).

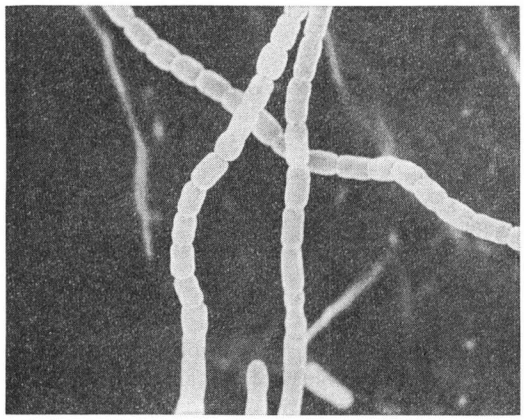

Plate 2. Yellow crystalline substances around the colony of strain SF-2330 (×150).

Table 1. Cultural characteristics of strain SF-2330.

Medium	Growth	Aerial mycelium*	Reverse	Soluble pigment
Sucrose - nitrate agar	None	—	—	—
Glucose - asparagine agar	Very poor	None	Colorless	None
Glycerol - asparagine agar (ISP medium 5)	None	—	—	—
Inorganic salts - starch agar (ISP medium 4)	Very poor	None	Colorless	None
Oatmeal agar (ISP medium 3)	Moderate	Scant, white~ natural (2dc)~ ivory (2db)	Light gold (2ic) ~dull gold (2ng)	Pale yellow
Yeast extract - malt extract agar (ISP medium 2)	Moderate~ good	Scant, white~ natural (2dc)~ ivory (2db)	Amber (3pe)	Pale yellow
Tyrosine agar (ISP medium 7)	Very poor	None	Colorless	None
Nutrient agar	None	—	—	—
Bennett agar	Moderate	None	Mustard gold (2pg)	Pale yellow

* The color scheme used was Color Harmony Manual, 4th Ed. 1958 (Container Corporation of America, Chicago).

genus *Streptomyces*. Strain SF-2330 has been deposited in Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the name *Streptomyces* sp. SF-2330 and the accession number FERM P-7919.

Production and Isolation

A well-grown agar slant of strain SF-2330 was inoculated into 20 ml of a seed culture medium consisting of soluble starch 2.0%, glucose 1.0%, wheat germ 0.6%, Polypeptone 0.5%, yeast extract 0.3%, soybean meal 0.2% and $CaCO_3$ 0.1% (pH 7.0) in a 100-ml Erlenmeyer flask. The flask was shaken on a rotary shaker (220 rpm) at 28°C for 5 days. Four milliliters of the first seed culture was inoculated into 80 ml of the same medium in a 500-ml Erlenmeyer flask. After shaking at 28°C for 2 days, 80 ml of the second seed was transferred into 1,000 ml of the same medium in a 5-liter Erlenmeyer

flask. The flask was shaken at 28°C for 2 days. The third seed culture was added to a 50-liter jar fermentor containing 35 liters of a production medium composed of maltose syrup 3.5%, corn steep liquor 1.5%, soluble vegetable protein 1.0%, cotton seed meal 0.5%, $CaCO_3$ 0.3% and $CoCl_2 \cdot 6H_2O$ 0.001%. The medium was adjusted to pH 7.0 before sterilization. The fermentation was carried out at 28°C for 4 days with an airflow rate of 35 liters per minute and an agitation rate of 250 rpm. The antimicrobial activity was assayed by a paper disc agar diffusion method using *Bacillus subtilis* ATCC 6633 as a test organism.

Table 2. Physiological characteristics of strain SF-2330.

Temperature range for growth		15~35°C	
Melanin formation		Negative	
Liquefaction of gelatin		Negative	
Hydrolysis of starch		Negative	
Reduction of nitrate		Negative	
Peptonization of milk		Positive (weak)	
Coagulation of milk		Positive	
NaCl (%) tolerance		<1.5%	
Carbon utilization*			
D-Glucose	+	D-Fructose	−
L-Arabinose	−	D-Mannitol	−
D-Xylose	−	L-Rhamnose	+
i-Inositol	−	Raffinose	−
Sucrose	−		

+; Utilized, −; not utilized.

* Carbon utilization test was performed in LUEDEMANN's basal medium[12], because the strain showed no growth on ISP medium 9 and other synthetic agar media.

The fermentation broth was filtered at pH 7.5 with the aid of Celite (Manville Products Corp.). The antibiotic in the filtrate (30 liters) and mycelial cake were extracted separately with ethyl acetate (30 liters) and 50% aqueous acetone (30 liters), respectively. The latter extract was concentrated to remove acetone. The antibiotic in the concentrate was extracted with ethyl acetate (30 liters) and the extract was combined with the former one. The combined extract was evaporated to dryness. The residue was dissolved in methanol, and chromatographed on a silica gel column (300 g). After washing the column with chloroform, the antibiotic was eluted with chloroform - methanol (200: 1). The active fractions were combined and concentrated. The residue was rechromatographed on a silica gel column (170 g) using the same solvent mixture. Evaporation of the active fractions gave 115 mg of the crude substance, which was recrystallized from chloroform to give 75 mg of orange needles of the antibiotic.

Fig. 1. The UV spectrum of SF-2330.

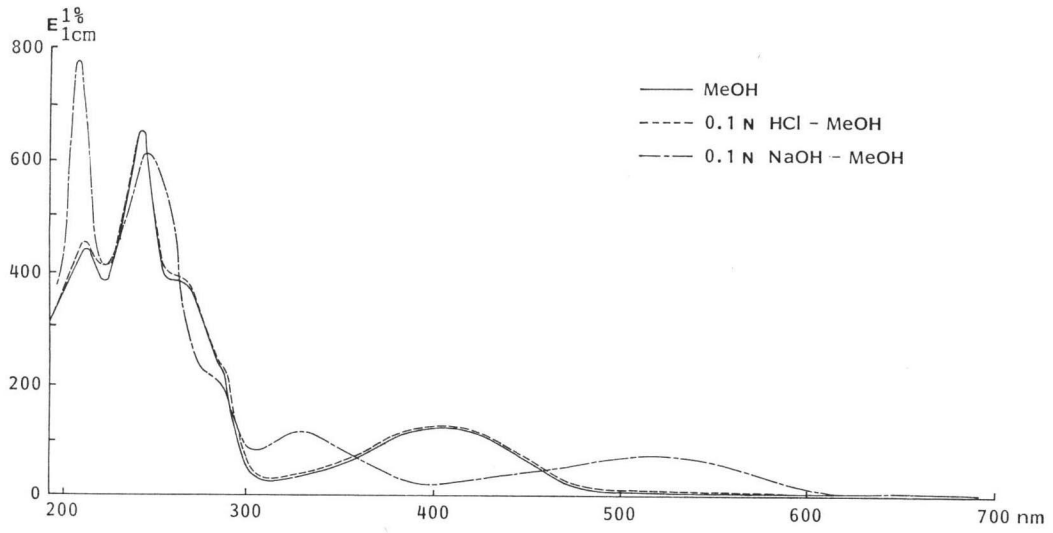

Fig. 2. The IR spectrum of SF-2330 (KBr).

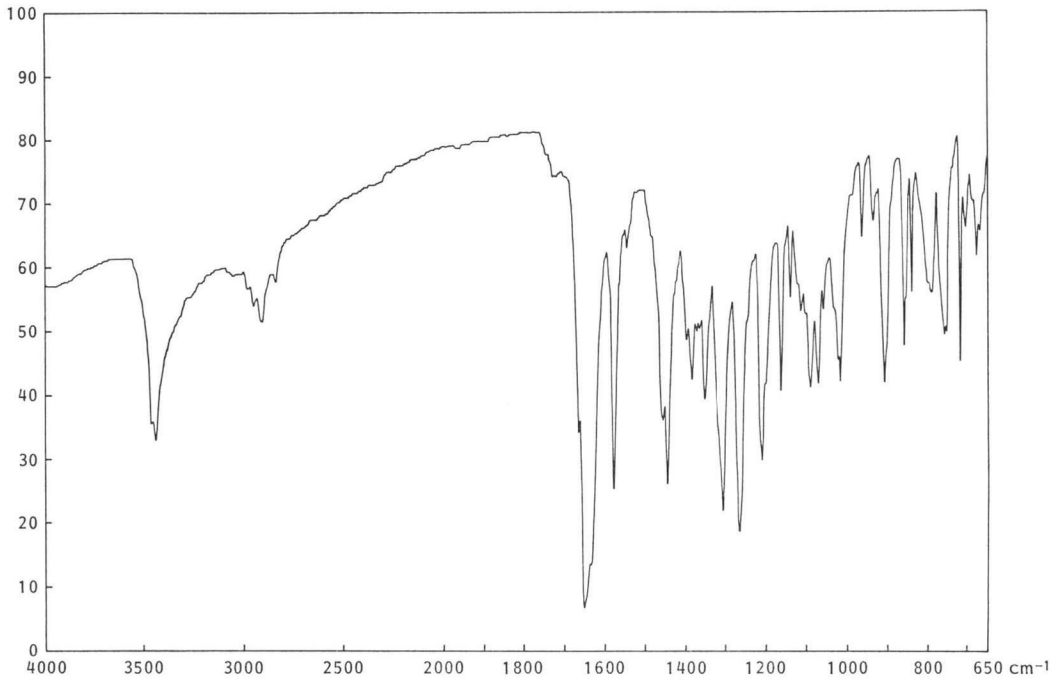

Table 3. The antimicrobial spectrum of SF-2330.

Organism	MIC (μg/ml)	Organism	MIC (μg/ml)
Staphylococcus aureus 606	1.56	*B. anthracis*	3.13
S. aureus 209P JC-1	1.56	*B. cereus* ATCC 10702	3.13
S. aureus Smith (I)	1.56	*Micrococcus flavus* FDA 16	0.78
S. aureus No. 26	1.56	*M. luteus* PCI 1001	0.39
S. aureus Apo-1	3.13	*Corynebacterium bovis* 1810	1.56
S. aureus N-0089	1.56	*Escherichia coli* NIHJ JC-2	>100
S. epidermidis ATCC 14990	3.13	*Pseudomonas aeruginosa* E-2	100
S. epidermidis 109	1.56	*Klebsiella pneumoniae* PCI 602	>100
Enterococcus faecalis ATCC 8043	3.13	*Proteus vulgaris* OX 19	>100
Bacillus subtilis ATCC 6633	0.78	*Salmonella typhi* O-901-W	>100
B. subtilis PCI 219	1.56	*Serratia marcescens* No. 1	>100
B. subtilis NRRL B-558	1.56	*Enterobacter cloacae* GN 7471	>100

Physico-chemical Properties

Antibiotic SF-2330 melted at 233~235°C with decomposition. It is soluble in alkaline water, slightly soluble in chloroform and almost insoluble in methanol, acetone and water. It turns to yellow from orange in acidic solution and to purple in alkaline solution. It gives a reddish purple color with magnesium acetate in methanol. Fehling, Molisch, Sakaguchi and Ninhydrin reactions are negative. No optical rotation was observed. The molecular formula was determined on the basis of the mass spectrum and elemental analysis. The FD-MS spectrum showed the molecular ion peak at m/z 390. Elemental analysis; Calcd for $C_{22}H_{14}O_7$: C 67.69, H 3.62. Found: C 67.35, H 3.65.

It showed the following UV absorption maxima: nm (ε) 240 (25,400), 262 (15,100) and 415 (4,900) in methanol; 240 (25,400), 262 (15,400) and 415 (4,800) in 0.1 N HCl - methanol; 243 (24,200), 280 (sh,

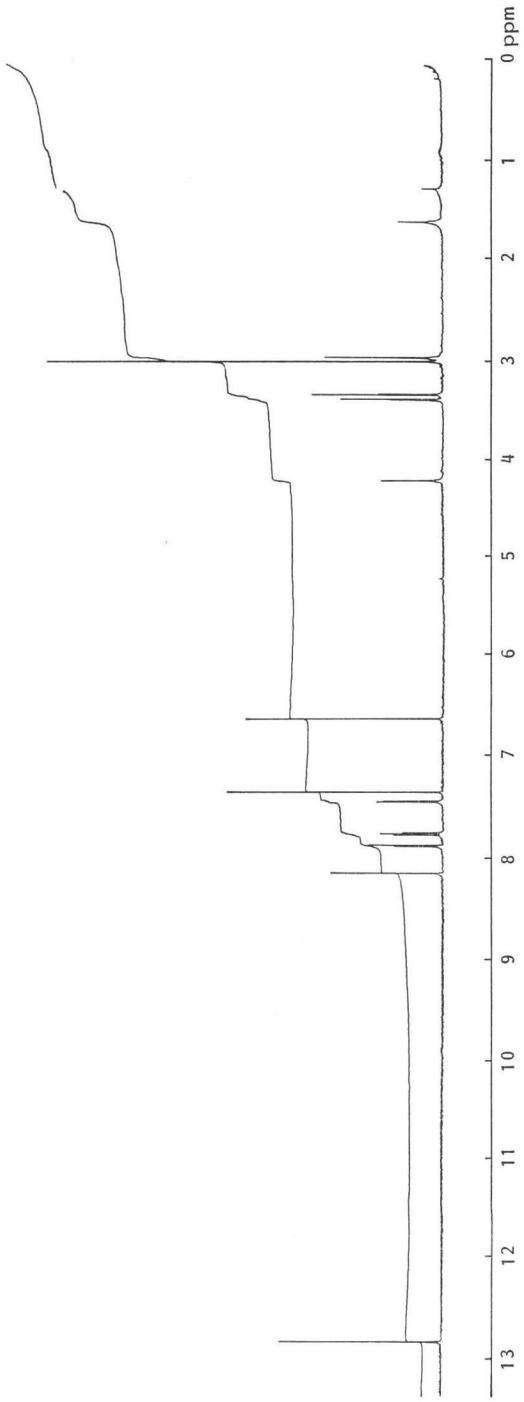

Fig. 3. The ^1H NMR spectrum of SF-2330 ($CDCl_3$, 400 MHz).

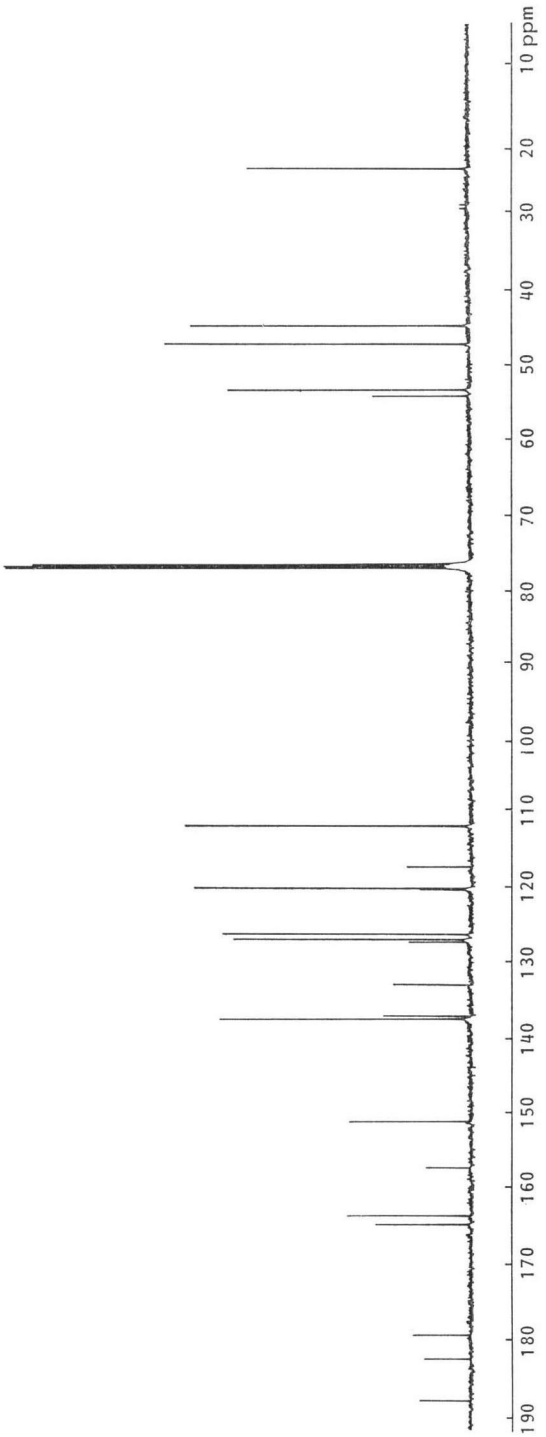

Fig. 4. The ^{13}C NMR spectrum of SF-2330 ($CDCl_3$, 100.7 MHz).

8,600), 328 (4,600) and 514 (2,800) in 0.1 N NaOH - methanol as shown in Fig. 1. The IR spectrum in KBr showed the characteristic bands at 1660, 1630 and 1580 cm^{-1} as illustrated in Fig. 2. ^{1}H NMR at 400 MHz and ^{13}C NMR at 100.7 MHz spectra in $CDCl_3$ are shown in Figs. 3 and 4, respectively.

From the physico-chemical properties mentioned above, it was concluded that SF-2330 was a new antibiotic related to the pluramycin group antibiotics. Details of the structural elucidation will be reported in a separate paper[11].

Biological Properties

The antimicrobial activity of antibiotic SF-2330 against various microorganisms is shown in Table 3. The antibiotic is active against Gram-positive bacteria, but is not effective against Gram-negative bacteria.

The acute toxicity in mice was LD_{50} 18.8 mg/kg by intraperitoneal route.

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