

A NEW INDOLE *N*-GLYCOSIDE ANTIBIOTIC SF-2140
FROM AN *ACTINOMADURA*

I. TAXONOMY AND FERMENTATION OF PRODUCING MICROORGANISM

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A new indole *N*-glycoside antibiotic SF-2140 which shows antiviral and weak antibacterial activity has been obtained from the cultured broth of an actinomycete strain. Strain SF-2140, designated *Actinomadura albolutea* sp. nov., was isolated from a soil sample collected in Hyogo Prefecture, Japan.

In the course of our screening program for new antibiotics from so-called rare actinomycetes, we have isolated a strain of *Actinomadura* which produces methyl (3-cyanomethyl-4-methoxyindol-1-yl-4-deoxy- α -D-lyxo-hexopyranosid)uronate¹⁾. Taxonomic studies indicate the producing microorganism belongs to the genus *Actinomadura* Lechevalier and Lechevalier, 1970²⁾.

In this paper, taxonomy and fermentation of the producing microorganism are described.

Taxonomy

The methods for characterization of strain SF-2140 were based on those described by SHIRLING and GOTTlieb³⁾. Additional media recommended by WAKSMAN⁴⁾ were also used. Diaminopimelic acid and sugars in whole cell hydrolysates were determined by the methods of BECKER *et al.*⁵⁾ and LECHEVALIER⁶⁾, respectively. Phospholipids and menaquinones were analyzed by the procedure of LECHEVALIER *et al.*⁷⁾ and COLLINS *et al.*⁸⁾, respectively.

Morphology

Aerial mycelia of strain SF-2140 were well developed, long, straight to wavy and monopodially branched on yeast extract - malt extract agar (ISP medium 2), oatmeal agar (ISP medium 3), inorganic salts - starch agar (ISP medium 4) and glycerol - asparagine agar (ISP medium 5). They then divided into many spores (Fig. 1), giving an appearance of total sporulation which is observed commonly in *Nocardiosis* strains. Spores were non-motile, $0.4\sim 0.6\times 0.6\sim 1.3\ \mu\text{m}$ in size, oval to cylindrical in shape and with

Fig. 1. Total sporulation of aerial mycelia of strain SF-2140. ($\times 600$)

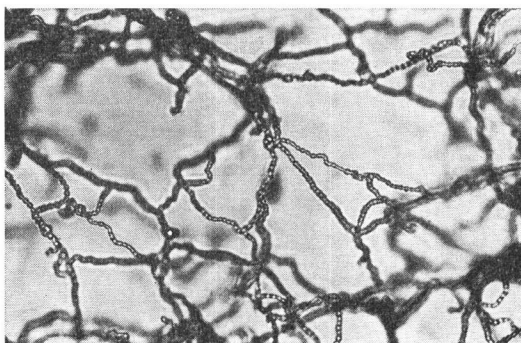


Fig. 2. Scanning electron micrograph of strain SF-2140.

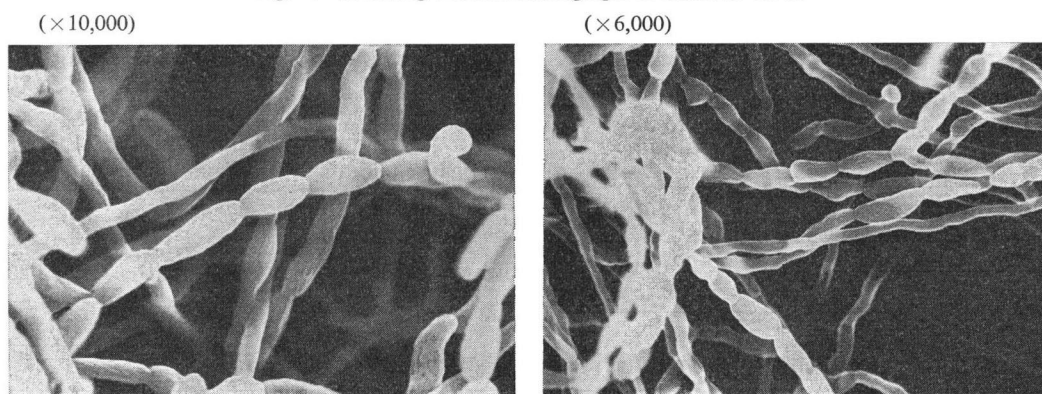


Table 1. Cultural characteristics of strain SF-2140.

Medium	Strain SF-2140	
Sucrose - nitrate agar	GR: Good RC: Golden brown (3pg)	AM: Poor, pearl pink (3ca) SP: None
Glucose - asparagine agar	GR: Good RC: Golden brown (3pg)	AM: Poor, pearl pink (3ca) SP: None
Glycerol - asparagine agar (ISP No. 5)	GR: Good RC: Yellow maple (3le)	AM: Good, light tan (3ge) SP: None
Inorganic salts - starch agar (ISP No. 4)	GR: Good RC: Oak brown (4pi)	AM: Good, pearl pink (3ca) SP: None
Oatmeal agar (ISP No. 3)	GR: Good RC: Topaz (3ne)	AM: Good, light melon yellow (3ea) SP: None
Yeast extract - malt extract agar (ISP No. 2)	GR: Good RC: Oak brown (4pi)	AM: Good, pussywillow gray (5dc) SP: None
Tyrosine agar (ISP No. 7)	GR: Good RC: Chocolate brown (4pn)	AM: Good, melon yellow (3ga) SP: None
Nutrient agar	GR: Moderate RC: Amber (3nc)	AM: Poor, white (a) SP: None
Ca-malate agar	GR: Good RC: Topaz (3ne)	AM: Good, pale peach pink (5cb) SP: None
Bennett agar	GR: Good RC: Oak brown (4pi)	AM: Good, pearl pink (3ca) SP: None

GR: Growth, RC: reverse color, AM: aerial mycelium, SP: soluble pigment.

() : Color number designated from Color Harmony Manual, 4th edition, Container Corporation of America, Chicago, Illinois, 1958.

smooth surfaces (Fig. 2). Fragmentation of substrate mycelia of strain SF-2140 was not observed on agar media or in liquid culture media. Sporangia were not formed.

Cultural Characteristics

Cultural characteristics of strain SF-2140 are shown in Table 1.

Physiological Characteristics

Physiological characteristics of strain SF-2140 are shown in Table 2 and the pattern of carbohydrate utilization in Table 3.

Table 2. Physiological characteristics of strain SF-2140.

Characteristics	Strain SF-2140
Temperature for growth	20~45°C
Liquefaction of gelatin	Positive
Hydrolysis of starch	Positive
Milk peptonization	Negative
Milk coagulation	Positive
Melanoid pigment formation	Negative
Nitrate reduction	Negative
Tolerance of NaCl	Up to 5%

Table 3. Carbohydrate utilization of strain SF-2140.

Positive utilization	D-Glucose, D-fructose, D-xylose, D-mannitol, L-rhamnose, sucrose, L-arabinose
Negative utilization	<i>i</i> -Inositol, raffinose
Basal medium: ISP No. 9.	

Table 4. Comparison of strain SF-2140 with related genera.

	<i>Actinomadura</i>	Strain SF-2140	<i>Nocardioopsis</i>
Fragmentation of substrate mycelium	—	—	+
Total sporulation of aerial mycelium	—	+	+
Madurose	+	+	—
Phospholipid-type ¹⁴⁾	PI/PIV	PIV	PIII
Major menaquinones	MK-9	MK-9	MK-10

Chemical Analysis

Whole cell hydrolysates contained *meso*-diaminopimelic acid and a small amount of madurose, but no arabinose or xylose. This indicates that the strain is an actinomycete of cell wall type III B according to the classification of LECHEVALIER *et al.*⁹⁾ The strain had phospholipids of type PIV and contained MK-9 (the main component is H₃) as its major menaquinones.

Comparison with Related Organisms

The characteristics mentioned above place strain SF-2140 between the genus *Actinomadura* and *Nocardioopsis* Mayer, 1976¹⁰⁾. Strain SF-2140 morphologically resembles *Nocardioopsis* rather than *Actinomadura* because total sporulation of aerial mycelia was observed. But the presence of madurose in whole cell hydrolysates and other chemical characteristics indicate the strain is clearly differentiated from the genus *Nocardioopsis* (Table 4). Therefore, strain SF-2140 should be identified as a member of the genus *Actinomadura*.

Three species of *Actinomadura*, *A. kijaniata*¹¹⁾, *A. coeruleofusca*¹²⁾ and *A. flava*¹³⁾, were previously reported to form long spore chains (total sporulation) like strain SF-2140. However, strain SF-2140 is obviously differentiated from the three: *A. kijaniata* has a type PI phospholipid pattern and dark green reverse side color, whereas strain SF-2140 has a type PIV phospholipid pattern and yellowish brown to dark brown reverse side color; *A. coeruleofusca* has blue to bluish gray aerial mycelia, while those of strain SF-2140 are white to whitish yellow; *A. flava* is the strain most similar to strain SF-2140. A direct comparison of strain SF-2140 with *A. flava* ATCC 29533 was carried out: *A. flava* has light amber to light bamboo reverse side color, rarely forms white aerial mycelia and produces madumycin, while strain SF-2140 is yellowish brown to dark brown in reverse side color, forms whitish yellow aerial mycelia abundantly and produces antibiotic SF-2140.

Therefore, strain SF-2140 is a new species of *Actinomadura*, for which we propose the name *Actinomadura albolutea* (albo L. adj. white, lutea L. adj. yellow, referring to the color of aerial mycelia). The type strain of *Actinomadura albolutea* is strain SF-2140.

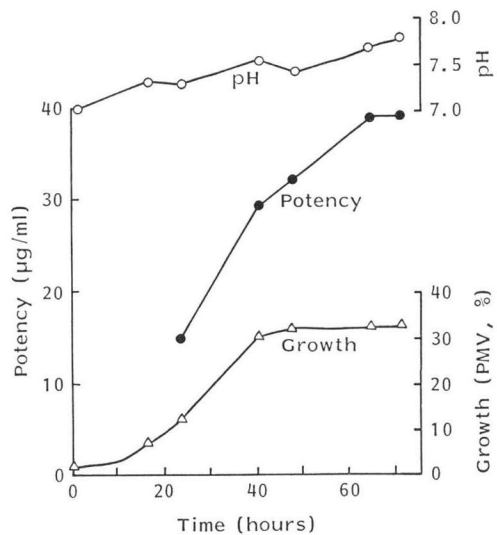
Strain SF-2140 has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, with accession number of FERM-BP 386.

Fermentation

The production of antibiotic SF-2140 was carried out as follows: Several loops of spores of strain SF-2140 were inoculated into 20 ml of a seed culture medium consisting of soluble starch 1.0%, glucose 1.0%, Polypeptone (Daigo-eiyo Chemical Co., Osaka) 0.5%, meat extract 0.2%, yeast extract 0.3%, soybean meal 0.2% and CaCO_3 0.1% (pH 7.0) in a 100-ml Erlenmeyer flask. The inoculated flask was shaken on a rotary shaker (220 rpm) at 28°C for 3 days. Four milliliters of the first seed were inoculated into 80 ml of the same medium in a 500-ml Erlenmeyer flask. The inoculated flask was shaken at 28°C

for 2 days. Fifty milliliters of the second seed were re-inoculated into 1 liter of the same medium in a 5-liter Erlenmeyer flask. After shaking at 28°C for 2 days, 1 liter of the third seed was transferred to a 50-liter fermentor containing 35 liters of the following production medium: maltose syrup 2.0%, soybean oil 0.15%, soybean meal 0.5%, distiller's solubles (Sun-grain Co., Aichi) 0.125%, Pharmamedia (Traders Oil Mill Co., Texas) 0.25%, Polypeptone 0.4%, K_2HPO_4 0.1% and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0005% in a tap water, and adjusted to pH 7.0 before sterilization. Fermentation was maintained at 28°C for 65 hours with an air-flow rate of 35 liters per minute and an agitation at 270 rpm. The progress of fermentation was monitored by determination of the growth measured as packed mycelial volume (PMV, %), pH and potency of substance SF-2140 by a microbial paper-disc agar diffusion assay using *Comamonas terrigena* ATCC 8461 as the test organism (Fig. 3). The production of antibiotic SF-2140 was maximum at 65 hours after inoculation, reaching 40 $\mu\text{g}/\text{ml}$.

Fig. 3. Time course of fermentation of strain SF-2140 illustrating pH, growth and antibiotic production.



References

- 1) ITO, T.; K. OHBA, M. KOYAMA, M. SEZAKI, H. TOHYAMA, T. SHOMURA, H. FUKUYASU, Y. KAZUNO, T. NIWA, M. KOJIMA & T. NIIDA: A new antiviral antibiotic SF-2140 produced by *Actinomadura*. J. Antibiotics 37: 931~934, 1984
- 2) LECHEVALIER, H. A. & M. P. LECHEVALIER: A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetes*. pp. 393~405. Gustav Fischer Verlag, Jena, 1970
- 3) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
- 4) WAKSMAN, S. A.: The Actinomycetes. Vol. II. The Williams & Wilkins Company, Baltimore, 1961
- 5) BECKER, B.; M. P. LECHEVALIER, R. E. GORDON & H. A. LECHEVALIER: Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. Appl. Microbiol. 12: 421~423, 1964
- 6) LECHEVALIER, M. P.: Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. 71: 934~944, 1968
- 7) LECHEVALIER, M. P.; C. DEBIEVRE & H. A. LECHEVALIER: Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochem. Syst. Ecol. 5: 249~260, 1977

- 8) COLLINS, M. D.; T. PIROUS & M. GOODFELLOW: Distribution of menaquinones in actinomycetes and corynebacteria. *J. Gen. Microbiol.* 100: 221~230, 1977
- 9) LECHEVALIER, M. P. & H. A. LECHEVALIER: Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int. J. Syst. Bacteriol.* 20: 435~443, 1970
- 10) MAYER, J.: *Nocardiopsis*, a new genus of the order *Actinomycetales*. *Int. J. Syst. Bacteriol.* 26: 487~493, 1976
- 11) HORAN, A. C. & B. C. BRODSKY: A novel antibiotic-producing *Actinomadura*, *Actinomadura kijaniata* sp. nov. *Int. J. Syst. Bacteriol.* 32: 195~200, 1982
- 12) PREOBRAZHENSKAYA, T. P. & M. A. SVESHNIKOVA: New species of the genus *Actinomadura*. *Mikrobiologiya* 43: 864~868, 1974
- 13) GAUSE, G. F.; T. S. MAKSIMOVA, O. L. OLKHOVATOVA, M. A. SVESHNIKOVA, G. V. KOCHETKOVA & G. B. ILCHENKO: Production of madumycin, an antibacterial antibiotic, by *Actinomadura flava* sp. nov. *Antibiotiki* 9: 771~775, 1974
- 14) LECHEVALIER, M. P.; A. E. STERN & H. A. LECHEVALIER: Phospholipids in the taxonomy of actinomycetes. *In Actinomycetes*. Gustav Fischer Verlag, Stuttgart, 1981