TOLYPOMYCIN, A NEW ANTIBIOTIC.* I

STREPTOMYCES TOLYPOPHORUS NOV. SP., A NEW ANTIBIOTIC, TOLYPOMYCIN-PRODUCER

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Streptomyces sp. strain No. B2847 producing a new antibiotic, tolypomycin Y, was examined with reference to its taxonomic characteristics. The organism is characterized by the formation of sclerotic granules, which originate from aerial mycelia and are observed on most agar media. Other properties were compared with known species. The strain was judged to be a new species and the name *Streptomyces tolypophorus* nov. sp. proposed.

In the course of our screening program for new broad-spectrum antibiotics, mainly active against Gram-positive bacteria, it was found that *Streptomyces* strain No. B2847, isolated from a soil sample collected in Tokyo, Japan, produced such an antibiotic.

The antibiotic was isolated and named tolypomycin Y.[1,2,3] It is effective against experimental staphylococcal infection in mice[4] with no significant toxicity.

The strain is a mesophilic streptomycete and forms many characteristic sclerotic granules on most agar media. Morphological, cultural and physiological properties were investigated and compared with those of known species. As a result, strain B2847 was considered to represent a new species and the name *Streptomyces tolypophorus* nov. sp. proposed.

In this report the morphological, physiological and cultural characteristics of *Streptomyces tolypophorus* are presented.

Materials and Methods

1. Morphological characteristics

The morphology of spore chains and mycelia, developed on glucose-asparagine agar, glucose-yeast extract-malt extract agar and other media was observed with the light microscope. Electron micrographs of the spores were obtained with a JEM-SS electron microscope (original magnification, ×4,000).

2. Physiological and cultural characteristics

All media were prepared according to methods outlined by WAKSMAN.[5] Each culture was incubated at 28°C for 14 days except those on whole egg (37°C), litmus milk (37°C), LOEFFLER's serum (37°C) and gelatin (24°C). Colors of the cultures were determined by comparison with RIDGWAY's Color Standards and Color Nomenclature.[6] Carbon utiliza-

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tions were determined by the method of PRIDHAM and GOTTLIEB.[7] Optimum pH and temperature were studied using BENNETT's and glucose-asparagine media (liquid and agar). For determination of sugars and diaminopimelic acid the method described by BECKER[8] *et al.* was followed.

3. Antibacterial spectrum

Antibiotic activity of strain B2847 was examined by the cross-streak method. Nutrient and glycerol-nutrient agars in Petri plates were inoculated with the strain. After 4 days incubation at 28°C, the test organisms were closs-streaked. After incubation at 37°C for 18 hours for Gram-positive and Gram-negative bacteria and for 40 hours for acid-fast bacteria, the length of inhibition was measured.

Results and Discussion

Streptomyces sp. strain No. B2847 forms well-developed aerial mycelia with simple branching and straight or flexuous spore-bearing hyphae (Plate 1). Sporangia, flagellated spores and fragmented mycelia are not observed. Spores, occurring in chains, are ellipsoidal to cylindrical, 0.8~1.1 μ by 1.5~2.3 μ, with smooth surfaces (Plate 2). Sclerotic granules are produced on most natural and synthetic agar media, such as glycerol-asparagine agar, glucose-asparagine agar, BENNETT's agar or glucose-yeast extract-malt extract agar, and range in diameter, from 5 to 50 μ (Plates 3 and 4). Cultural characteristics of the strain on various media are presented in Table 1. In general, aerial mycelium of the strain is white. The substrate mycelium varies from colorless to yellowish. An orange yellow to a yellowish brown soluble pigment is

Plate 1. Spore-bearing hyphae of *Streptomyces tolypophorus.* (×3,360)

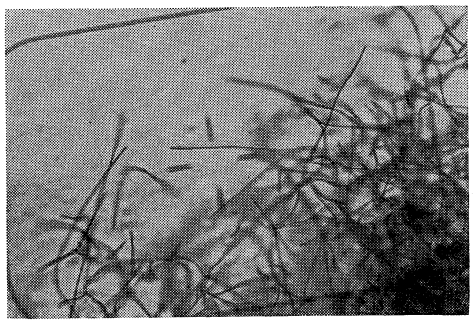

Plate 2. Electron-micrograph of spores of *Streptomyces tolypophorus.* (×22,600)

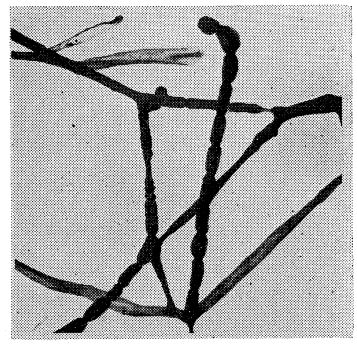

Plate 3. Sclerotic granules, dotting the aerial mycelium of *Streptomyces tolypophorus.* (×1,680)

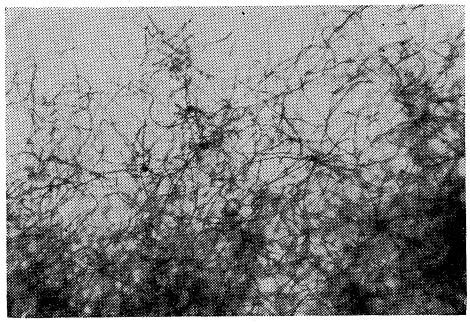

Plate 4. Sclerotic granule formed. (×6,720)

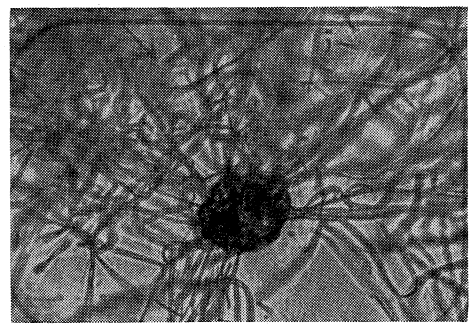

Table 1. Cultural characteristics of *Streptomyces tolypophorus*

Medium	Cultural characteristics
Czapek's agar	G*: abundant, folded and raised with cracked surface at the edge. A*: abundant, powdery, white. R*: Buff Yellow (Rdg.** IV, 19-d) to Light Orange Yellow (Rdg. III, 17-f). S*: Pale Orange Yellow (Rdg. XV, 15'-b).
Glucose-Czapek's agar	Same as on Czapek's agar.
Glycerol-Czapek's agar	Same as on Czapek's agar.
Glucose-asparagine agar	G: abundant, Pale Orange Yellow, penetrating into the medium. A: poor to moderate, white. R: Pale Orange Yellow. S: none.
Nutrient agar	G: poor to moderate, colorless. A: poor, white to Pallid Mouse Gray (Rdg. LI, 15'''''-f). R: colorless to Pale Orange Yellow. S: none.
Glucose-nutrient agar	G: abundant, folded, raised, colorless to Mars Yellow (Rdg. III, 15-i). A: moderate, white. R: Mars Yellow. S: yellowish brown.
Glycerol-nutrient agar	G: abundant, folded, Sudan Brown (Rdg. III, 15-k). A: poor, white. R: colorless. S: yellowish brown.
Glucose-nutrient broth	G: abundant, developing at first on the surface, later producing sediment. A: poor, white to Capulino Buff (Rdg. III, 13-f). S: pale yellow.
Starch agar	G: moderate, colorless. A: poor, white at the margin. R: colorless. S: none.
Whole egg (37°C)	G: moderate, wrinkled, Deep Chrome (Rdg. III, 17-b). A: none. S: none.
Yeast extract agar	G: abundant, folded. A: white at the margin. R: Mars Yellow. S: Mars Yellow to orange yellow.
Potato plug	G: poor, pale yellowish brown to pale brown. A: thin, white. S: none.
Carrot plug	G: poor to moderate. A: white. S: none.
Litmus milk (37°C)	G: poor, ring formed. A: none. S: reddish brown. Slow peptonization without coagulation. Reaction becomes alkaline.
Gelatin (24°C)	G: poor. A: white. S: yellowish brown. Rapid liquefaction.
Loeffler's serum (37°C)	G: poor, orange yellow. A: none. S: none. Weak liquefaction.
Cellulose	G: poor to moderate, colorless to orange yellow. A: white, cottony. Not decomposed.
Ca-malate agar	G: moderate, pale orange yellow, penetrating into the medium. A: thin, white. R: pale orange yellow to Ochraceous Buff (Rdg. XV, 15'-6). S: Chamois (Rdg. XXX, 19''-b).
Tyrosine agar	G: moderate, colorless to yellow. A: poor, white. R: colorless. S: none.
Peptone agar	G: poor to moderate. A: poor, white. R: colorless. S: none.

* G: Growth. A: Aerial mycelium. R: Reverse. S: Soluble pigment.
** Rdg.: R. Ridgway[4], Color Standards and Color Nomenclature.

produced on some media. Based on these results, the strain is assigned to section *Rectus-Flexibilis, White Series* of PRIDHAM *et al.*[9] Growth of strain B2847 was noted between 20 and 43°C. Its optimum temperature was 28°C. Growth and sporulation occur at pH's ranging from 4.0 to 10.0. The optimal pH is at pH 6.0~8.0. A number of physiological properties are summarized in Table 2.

In carbon-source utilization studies good or moderate growth was observed with: *i*-inositol, D-mannitol, dulcitol, D-xylose, L-arabinose, D-galactose, D-glucose, D-mannose, D-fructose, rhamnose, melibiose, maltose, sucrose, lactose, raffinose, trehalose, salicin, starch, inulin, glycerin, sodium acetate, sodium succinate and sodium citrate. There was no growth or poor growth with erythritol, adonitol, D-sorbitol and esculin. Carbon utilization patterns of strain B2847 are summarized in Table 3. Cell-wall preparations of strain B2847 contain the meso isomer of the diaminopimelic acid. Arabinose was found to be not present, but a few amount of galactose was found to be always present in the hydrolysate.

From the results described above, especially morphological characteristics and the cell-wall composition it is satisfactory to consider strain B2847 as a streptomyces.

Table 2. Physiological characteristics of *Streptomyces tolypophorus*

Optimum pH	6.0~8.0 (growth: 4.0~10.0)
Optimum temperature	28°C (growth: 20~43°C)
Starch hydrolysis	(−)
Nitrate reduction	(+)
Chromogenicity	(−)

Table 3. Carbon source utilization of *Streptomyces tolypophorus*

Carbon sources		Carbon sources	
Erythritol	−	Maltose	++
Adonitol	−	Sucrose	++
D-Sorbitol	−	Lactose	+~++
i-Inositol	+++	Raffinose	+~++
D-Mannitol	+~++	Trehalose	+++
Dulcitol	+~++	Salicin	+~++
D-Xylose	++~+++	Esculin	−~±
L-Arabinose	++	Starch	+~++
D-Galactose	+++	Inulin	++
L-Sorbose	+	Glycerol	++
D-Glucose	+~++	Na-acetate	+~++
D-Mannose	++	Na-succinate	+~++
D-Fructose	++	Na-citrate	+~++
Rhamnose	+++	Control	−
Melibiose	++		

+++ : Abundant growth. ± : Poor growth.
++ : Moderate growth. − : No growth.
+ : Growth.

Table 4. Antibacterial spectrum of *Streptomyces tolypophorus* by cross-streak method

Test organisms	Inhibition zone (mm)	
	Nutrient agar	Glycerol-nutrient agar
Escherichia coli	0	0
Proteus vulgaris	0	0
Staphylococcus aureus	35	36.5
Bacillus subtilis	29.5	35.5
Bacillus cereus	17	16.5
Bacillus brevis	32	—
Sarcina lutea	34	36.5
Micrococcus flavus	35	40.5
Mycobacterium avium	3	0

Strain No. B2847 produced antibiotic activity against Gram-positive bacteria. As shown in Table 4, strong activity was noted in the cross-streak tests against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus brevis*, *Sarcina lutea* and *Micrococcus flavus* little or no activity was noted against *Escherichia coli*, *Proteus vulgaris* and *Mycobacterium avium*.

Strain No. B2847 was compared with named cultures maintained at the Institute for Fermentation, Osaka and its characteristics with those cited in published descriptions in BERGEY's Manual[10] of Determinative Bacteriology and the publications of

WAKSMAN[5], ETTLINGER *et al.*[11], HÜTTER[12], *etc.* Among the known species of *Streptomyces*, *Streptomyces globisporus* (KRASSILNIKOV, 1941) WAKSMAN, 1953[5], *Streptomyces autotrophicus* TAKAMIYA and TUBAKI, 1956[13], *Streptomyces fulvissimus* (JENSEN, 1930) WAKSMAN and HENRICI, 1948[5], *Actinomyces griseoloalbus* KUDRINA, 1957[14] were found to be some similarity to the strain No. B2847. However, those strains were easily distinguished from the strain by the following descriptions of their morphological and cultural characteristics.

The sporophores of *Streptomyces globisporus* are arranged in bloom-shaped clusters. Aerial mycelium on potato shows a greenish yellow color. *Streptomyces autotrophicus* grows poorly on starch agar and do not liquefy gelatin and nitrate reduction is negative, and *Streptomyces fulvissimus* shows more resemblance with the strain No. B2847 on the morphological properties, but on CZAPEK's agar or glucose-asparagine agar *Streptomyces fulvissimus* produces golden to orange soluble pigment and furthermore develops in deep red brown growth on nutrient agar. *Actinomyces griseoloalbus* shows good or moderate growth on gelatin, milk and potato, and the hydrolysis of starch is moderate or good, however, strain No. B2847 shows poor growth on these media respectively and does not hydrolyze starch.

A formation of sclerotic granules of the Actinomycetaceae has been reported on some strains of *Chainia*[15], *Streptomyces griseus*[16], and several species of *Actinomyces*[17]. It has been reported that sclerotic granules of these organisms are originated from vegetative mycelium. Especially it was reported that in case of *Streptomyces* (*Actinomyces*) a granule formation depended upon the composition of the medium. On the contrary, the granules of the strain No. B2847 are very similar to those of *Streptomyces aerocolonigenes* SHINOBU and KAWATO, 1960[18]. But the strain is obviously different from *Streptomyces aerocolonigenes* on the next physiological and cultural characteristics, that is, the strain produces abundant aerial mycelia, a slightly yellow soluble pigment on glycerol-CZAPEK's agar, and non-soluble pigment on glucose-asparagine agar and the growth on starch agar is colorless. Furthermore, the tyrosinase and diastase reactions of the strain are negative. On the utilization of carbon sources, the strain utilizes rhamnose well and utilizes raffinose moderately. On the other hand the aerial mycelia of *Streptomyces aerocolonigenes* develop poorly and brownish soluble pigment is produced on glycerol-CZAPEK's agar, and on glucose-asparagine agar a pale brownish soluble pigment is produced and the color of the growth on starch agar is an apricot yellow. Also the tyrosinase and diastase reactions of *Streptomyces aerocolonigenes* are positive, and raffinose and rhamnose utilization is negative or doubtful, respectively.

Judging from the above-mentioned properties, including the fact of the production of a new antibiotic, tolypomycin Y, the strain No. B2847 was recognized as a new species of *Streptomyces*, and the name, *Streptomyces tolypophorus* nov. sp. is proposed.

The proposed species epithets "tolypé" and "phoréo" are the Greek noun and verb meaning "granule" and "to carry" respectively.

A detailed characterization of the new taxon follows:

Streptomyces tolypophorus nov. sp.

Spore chain morphology: Long straight or flexuous spore bearing hyphae simply branched. Globular sporangia, flagellated spores or fragmented mycelia not observed. Spores (0.8~1.1 μ by 1.5~2.3 μ in size) arranged in chains, ellipsoidal to cylindrical with smooth surfaces. Many sclerotic granules (5~50 μ) are formed on various agar media.

Spore surface: Smooth.

Color of colonies: Aerial mass color, white on CZAPEK's agar, glucose-asparagine agar, Ca-malate agar, nutrient agar.

Color in medium: Non-chromogenic. Pale orange yellow or pale yellowish brown diffusible pigment forms in CZAPEK's agar, glucose and glycerol nutrient agars.

Physiological properties: Starch not hydrolyzed, gelatin liquefied, milk slowly peptonized. A variety of carbon sources such as D-glucose, D-xylose, D-fructose, rhamnose, L-arabinose, sucrose, *i*-inositol, raffinose and D-mannitol are utilized for growth.

Mesophilic.

Aerobic.

Habitat: Soil.

Antagonistic properties: Produces tolypomycin Y, rifamycins B and O[19].

Type strain: Strain No. B2847 is designated as the type of this species and has been deposited in the Institute for Fermentation, Osaka and assigned accession number IFO 12554.

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