Notes

BMY-28438 (3,7-DIHYDROXYTROPOLONE), A NEW ANTITUMOR ANTIBIOTIC ACTIVE AGAINST B16 MELANOMA

II. TAXONOMY OF PRODUCING ORGANISM

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In the course of our screening program, a new antibiotic, BMY-28438, was found to show antitumor and weak antibacterial activities, and to be an analog of tropolone[1]. This paper describes the taxonomic characterization of the producing organism.

Materials and Methods

Source and Isolation Procedures of Producing Organism

Strain K611-97 was isolated from a soil sample collected in Uttar Pradesh State, India, and obtained on a diluted nutrient agar, supplemented with xanthine at 0.4% (w/v) and rifampicin at 2 μg/ml. After inoculation of soil sample onto the agar plate, the plate was incubated at 47°C for 4 weeks.

Results

Morphology

Both vegetative and aerial mycelia were long, well-branched and not fragmented into short elements. Chains of arthrospores were born on the aerial hyphae. The spore-chain morphology was as follows:

1) Straight or hooked and short chains containing 5 to 10 spores per chain, mainly on natural organic media.

Fig. 1. Photograph of spore chains of 3,7-dihydroxytropolone-producing strain K611-97 on inorganic salts - starch agar.

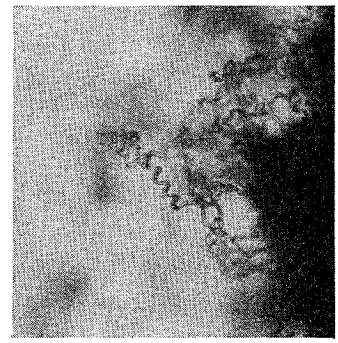
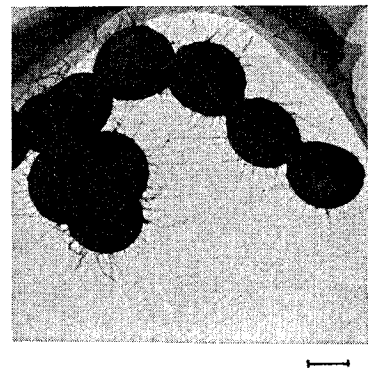


Fig. 2. Transmission electron micrograph of hairy spores of strain K611-97.

Bar represents 0.5 μm.



2) Chains of open or compact spirals, containing 10 to 50 spores per chain in chemically defined media such as ISP medium No. 4.

3) Sporophores with monopodial or pseudoverticillate branching.

The spores were spherical to oblong in shape (0.5 to 0.7 by 0.6 to 1.2 μm) and had a hairy surface. Large numbers of long hairs were formed in the spores of compact spirals (subgroup A[2]), while no hairs or fewer and shorter hairs than the subgroup A were seen in the spores of straight or open-spiral chains (subgroups C and B). Sporangium, motile spores and sclerotium were not observed.

Table 1. Cultural characteristics of strain No. K611-97[a].

Sucrose - nitrate agar (CZAPEK - DOX agar)	G: Abundant
	R: Light grayish red (18)[b]
	A: Abundant; light gray (264)
	D: Pale yellowish pink (31)
Tryptone - yeast extract broth (ISP medium No. 1)	G: Abundant; floccose and not turbid
	D: None
Yeast extract - malt extract agar (ISP medium No. 2)	G: Abundant
	R: Grayish reddish orange (39)
	A: Abundant; pinkish gray (10)
	D: None
Oatmeal agar (ISP medium No. 3)	G: Moderate
	R: Colorless
	A: Moderate; light gray (264)
	D: None
Inorganic salts - starch agar (ISP medium No. 4)	G: Abundant
	R: Light reddish brown (42)
	A: Abundant; medium gray (265)
	D: None
Glycerol - asparagine agar (ISP medium No. 5)	G: Abundant
	R: Colorless or strong orange (50)
	A: Abundant; medium gray (265)
	D: None
Peptone - yeast extract - iron agar (ISP medium No. 6)	G: Abundant
	R: Strong orange yellow (68)
	A: Poor; white (263)
	D: Strong yellow (84)
Tyrosine agar (ISP medium No. 7)	G: Abundant
	R: Dark red (16)
	A: Abundant; light gray (264)
	D: None
Glucose - asparagine agar	G: Abundant
	R: Brilliant orange yellow (67)
	A: Abundant; white (263)
	D: None
Nutrient agar	G: Moderate
	R: Brilliant orange yellow (67)
	A: Poor; white (263)
	D: Moderate yellow (87)
BENNETT's agar	G: Abundant
	R: Brilliant orange yellow (67)
	A: Moderate; light gray (264)
	D: None

[a] Observation after incubation at 28°C for 2 to 3 weeks.
[b] Color and number follow ISCC-NBC designation.

Cultural (Table 1) and Physiological Characteristics (Table 2)

Strain K611-97 grew well and sporulated both in nutritionally rich media and chemically defined media including CZAPEK's sucrose - nitrate agar. The aerial mass color was in the gray series. A red pigment in the substrate mycelia was formed in CZAPEK's agar and in ISP media Nos. 2, 4 and 7. An orange yellow pigment in the substrate mycelium was also observed, mainly in organic media. Both pigments were not pH-indicators. Melanin and other diffusible pigments were not formed. Tyrosinase activity was negative. The culture tolerated NaCl at 6% or less, but not at 8%. The temperature range for growth was 15 to 49°C, no growth was observed at 52°C. Among the diagnostic sugars, which were described in the characterization of

Table 2. Physiological characteristics of strain K611-97.

Hydrolysis of:	
Gelatin	−
Starch	+
Milk peptonization	+
Milk coagulation	−
Production of:	
Nitrate reductase[a]	+, −
Tyrosinase	−
Tolerance to:	
0.01% (w/v) lysozyme	+(w)
6% (w/v) NaCl	+(w)
8% (w/v) NaCl	−
Growth at:	
pH 5.5 to 12.0	+
15 to 49°C	+
13 and 52°C	−
Utilization of[b]:	
Glycerol	+
D-Arabinose	+(w)
L-Arabinose	+
D-Xylose	+
D-Ribose	+
L-Rhamnose	+
D-Glucose	+
D-Galactose	+
D-Fructose	+
D-Mannose	+
L-Sorbose	−
Sucrose	+
Lactose	+
Cellobiose	+
Melibiose	+
Trehalose	+
Raffinose	−
D-Melezitose	−
Soluble starch	+
Cellulose	−
Dulcitol	−
Inositol	+
D-Mannitol	+
D-Sorbitol	−
Salicin	+
Keratin	−

[a] Positive in CZAPEK's sucrose - nitrate broth, and negative in peptone - nitrate broth.
[b] Basal medium: PRIDHAM-GOTTLIEB medium (ISP medium No. 9).
(w): Weak.

species of the genus *Streptomyces*[3], all sugars were utilized for growth except raffinose.

Cell Chemistry

The whole cell hydrolysate contained LL-diaminopimelic acid and none of the diagnostic sugars.

Determination of Taxonomic Position

The above-mentioned characteristics indicated that strain K611-97 belongs to the genus *Streptomyces*. According to the description of the genus *Streptomyces* by PRIDHAM and TRESNER[3], the major characteristics of strain K611-97 are summarized as follows: 1) Aerial mycelium, gray (GY), 2) Spore chain morphology, *Spira* (S), 3) Melanoid pigments, none (C-), 4) Spore wall ornamentation, hairy (H).

Comparisons of strain K611-97 to eleven known species of this species group are shown in Table 3. A red pigment in the vegetative mycelium which is seen in strain K611-97, is not produced by any of the eleven known species. In addition, strain K611-97 is differentiated from these known species in the carbohydrate utilization profile, the maximum growth temperature and other cultural and physiological characteristics. Based on the comparative studies of strain K611-97 to the relevant species, the strain is proposed as a new species, *Streptomyces tropolofaciens*. The type strain is K611-97 (ATCC 53548), which is a single isolate.

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Table 3. Differential characteristics of strain K611-97 and eleven relevant *Streptomyces* species, which have gray aerial mycelium, spiral spore chain, hairy spore and negative melanin.

Organism	Distinct color of vegetative mycelium	Utilization					Growth at 47.5°C	Additional differences
		Xyl	Rham	Suc	Raf	Inos		
Strain K611-97	Red and orange yellow	+	+	+	−	+	+	
Streptomyces calvus ATCC 13382[4]	Brownish black	+	+	+	+	+	+	No aerial mycelium in ISP medium No. 5 and CZAPEK's agar; negative starch hydrolysis
S. cyanoalbus ATCC 23902[3,5]	Blue or green	+	+	+	+	−		Greenish aerial mycelium on ISP media Nos. 2 and 4
S. finalayi ATCC 23906[3,5]	Green to yellowish green	+	+	±	−	−		—
S. flaveolus ATCC 3319[3,4]	None	+	+	+	+	+	−	No or trace aerial mycelium in ISP media Nos. 2 and 5, BENNETT's agar and CZAPEK's agar; negative starch hydrolysis
S. herbiferis INMI 10[3]	Grass-green	+	+	+	+	−		—
S. pactum NRRL 2939[3]	None	−	−	−	−	−		Poor growth on CZAPEK's agar
S. conglobatus ATCC 31005[6]	Light green	+	−	−	+	−		A salt and pepper appearance on surface of ISP medium No. 2, because of areas of dense and no sporulation
S. heimi ATCC 25460[4]	None	+	+	+	−	+	−	Fair growth in ISP media Nos. 2 and 4; whitish pink aerial mycelium on BENNETT's agar; negative starch hydrolysis
S. spadicogriseus ATCC 31179[7]	None	+	−	−	−	−	−	Poor growth on CZAPEK's agar; gelatin liquefaction
S. capillispiralis NRRL 12279[4]	Dark brown	+	+	−	−	+	−	Reddish gray aerial mycelium on ISP medium No. 2; no aerial mycelium on BENNETT's agar; negative starch hydrolysis
S. calvus TM-521[8]	None	+	−	±	±	−	−	White, scant aerial mycelium on CZAPEK's agar; gelatin liquefaction.

Abbreviations: Xyl, D-Xylose; Rham, L-rhamnose; Suc, sucrose; Inos, inositol.

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