

BBM-928, A NEW ANTITUMOR ANTIBIOTIC COMPLEX

II. TAXONOMIC STUDIES ON THE PRODUCING ORGANISM

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An actinomycete strain No. G455-101 isolated from a soil sample collected in Luzon Island, Philippines produced a new antitumor antibiotic complex BBM-928. The organism was determined to be a new species of the genus *Actinomadura* and designated *Actinomadura luzonensis* nov. sp. The type strain, No. G455-101, has been deposited under the number ATCC 31491.

A new antitumor antibiotic complex, BBM-928¹⁾, has been isolated from the fermentation broth of an actinomycete strain, No. G455-101, isolated from a soil sample collected in Luzon Island, Philippines. The strain G455-101 belongs to the genus *Actinomadura* and has been designated *Actinomadura luzonensis* nov. sp. This paper reports the morphological, cultural, physiological and biochemical characteristics of strain No. G455-101. The procedures described by SHIRLING and GOTTLIEB²⁾ and LECHEVALIER and LECHEVALIER³⁾ were generally followed for the characterization.

Micromorphology

Strain No. G455-101 forms both substrate and aerial mycelia, and the substrate mycelium is well-developed, long and branched ($0.5\sim 0.8\ \mu$ in width). Distinct fragmentation of the substrate mycelium is not seen. Unlike ordinary species of *Streptomyces*, strain G455-101 bears only short or rudimental aerial mycelia, or does not form any in some agar media. Short or long spore-chains are produced at the tip of aerial hyphae which contain 2~30 spores in a chain (mostly 5~20 spores). Spore-chains are straight, flexuous or looped in shape (Fig. 1). The spores are spherical ($0.3\sim 0.4\ \mu$), oval or cylindrical ($0.3\times 1.5\sim 3.0\ \mu$) in shape and have a smooth surface (Fig. 2). Spores are often separated by empty

Fig. 1. Conidiospore-chains of strain No. G455-101 (ISP No. 2 medium, 37°C, 2 weeks; $\times 600$, bar: 10 μ m).

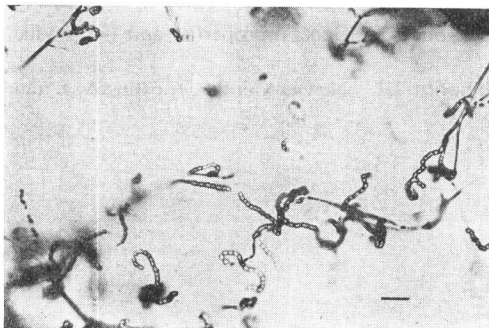


Fig. 2. Transmission electron-micrograph of conidiospores (ISP No. 2 medium, 37°C, 3 weeks, bar: 1 μ m).

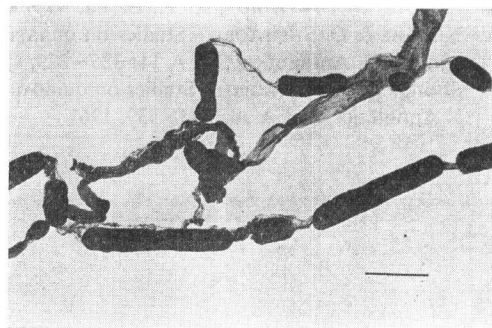


Table 1. Cultural characteristics.

		<i>Actinomadura luzonensis</i> strain No. G 455-101*	<i>Actinomadura roseoviolacea</i> KCC A-0145
CZAPEK's agar (Sucrose-nitrate agar)	G** R A D	no or scant growth dark red (16)*** white to pale pink (7) none	poor, thin dark pink (6)~reddish brown (43) rudimental, white none or pale pink (7)
Tryptone-yeast extract broth (ISP No. 1)		moderate growth, floccose, sedimented and not pigmented	moderate growth, floccose, sedimented and not pigmented
Yeast extract-malt extract agar (ISP No. 2)	G R A D	abundant deep red (13) to strong reddish brown (40) abundant, greyish pink (8) to dark pink (6) none	abundant, raised, plicate dark red (16) to dark wine partially formed, white to pale pink (7) vivid orange yellow (66)
Oat meal agar (ISP No. 3)	G R A D	abundant deep yellowish pink (27) moderate, pink (5) greyish yellow (90)	moderate moderate pink (5) scant, white to pale pink (7) pale yellowish pink (31)
Inorganic salts-starch agar (ISP No. 4)	G R A D	poor light yellowish brown (76) to dark greyish red (20) scant, white to brownish pink (33) none	poor light yellowish brown (76) to dark greyish red (20) moderate, white later pale pink (7) none
Glycerol-asparagine agar (ISP No. 5)	G R A D	poor yellowish pink (29) to reddish brown (43) scant, white none	poor colorless to brown (58) none none
Peptone-yeast extract- iron agar (ISP No. 6)	G R A D	poor, plicate strong yellowish pink (26) none pale orange yellow (73)	moderate or poor reddish black (24) none none
Tyrosine agar (ISP No. 7)	G R A D	poor dark red (16) scant, white none	poor light brown (57) to dark brown (59) none none
Glucose-ammonium salts agar	G R A D	poor reddish brown (43) scant, light grey (264) none	no or scant
BENNETT's agar	G R A D	moderate reddish brown (43) restricted, greyish pink (8) none	abundant dark red (16) to dark wine poor, white to pale pink (7) light orange (52)

* observed after incubation at 37°C for 3 weeks.

** Abbreviation: G—Growth; R—Reverse color of substrate mycelium; A—Aerial mycelium; D—Diffusible pigment.

*** Color and number in parenthesis follow the color standard in Ref. 8.

hyphae. An amorphous sporangium-like vesicle which envelops short coiled spore-chains is observed occasionally on the aerial mycelium.

Cell Wall Composition and Whole Cell Sugar Components

The cell wall of strain G455-101 contains *meso*-diaminopimelic acid but lacks glycine. A whole cell hydrolyzate shows the presence of glucose, mannose and madurose (3-O-methyl-D-galactose). The aforementioned cell wall composition and whole cell sugar components indicate the strain G455-101 is an actinomycete species of cell wall type IIIB.

Cultural and Physiological Characteristics

Strain G455-101 grows abundantly, forms pink or greyish pink aerial mycelium, and produces a reddish water-insoluble pigment in nutritionally rich agar media, such as yeast extract-malt extract agar and oat meal agar. However, in inorganic salts-starch agar, glycerol-asparagine agar and tyrosine agar, it shows poor growth, forms white or beige rudimental aerial mycelium, and produces a small amount of reddish pigment. Melanoid pigment is not produced in peptone-yeast-iron agar and tyrosine agar. Nitrate is reduced to nitrite. It grows abundantly at 28°C, 37°C and 45°C, but does not grow at 10°C or at 50°C. Pentoses and hexoses are well utilized by the strain. Cultural and physiological characteristics of strain G455-101 are shown in Tables 1 and 2, respectively. Carbon source utilization is shown in Table 3.

Taxonomy

Strain G455-101 forms short, pale pink aerial mycelium, aerial spore-chains and occasionally amor-

Table 2. Physiological characteristics of strain No. G 455-101.

Test	Response	Method and medium
Nitrite from nitrate	Positive	Inorganic medium: CZAPEK's sucrose nitrate broth
Nitrite from nitrate	Positive	Organic medium: 0.5 % yeast extract, 1.0 %, glucose, 0.5 % KNO ₃ , 0.1% CaCO ₃
Casein hydrolysis in agar medium	Weakly positive	LUEDEMANN's agar medium
Skimmed milk coagulation	Positive	
Gelatin liquefaction	Negative	15 % gelatin in tryptone-yeast extract broth (ISP No. 1 medium)
H ₂ S production from L-cysteine	Positive	L-Cysteine (0.1 %) added to tryptone-yeast extract broth (ISP No. 1 medium) plus agar. H ₂ S detected with a paper strip containing 10 % aq. lead-acetate solution.
Formation of melanoid	Negative	Peptone-yeast-iron agar (ISP No. 6) and tyrosine agar (ISP No. 7)
Catalase reaction	Positive	H ₂ O ₂ aq. solution
Oxidase reaction	Positive	KOVACS' reagent
Growth temperature	Abundant growth at 28, 37 and 45°C. Poor growth at 20°C. No growth at 10°C and 50°C.	BENNETT's agar

Table 3. Utilization of carbon sources.

	<i>Actinomadura luzonensis</i> strain No. G 455-101		<i>Actinomadura roseoviolacea</i> KCC-A-0145
	PG	Lm	Lm
1. Glycerol	++	+	+
2. D(-)-Arabinose	+	+	-
3. L(+)-Arabinose	+	+	+
4. D-Xylose	++	+	-
5. D-Ribose	++	+	+
6. L-Rhamnose	++	+	+
7. D-Glucose	+	+	+
8. D-Galactose	++	+	+
9. D-Fructose	++	+	+
10. D-Mannose	++	+	+
11. L(-)-Sorbose	-	-	-
12. Sucrose	-	-	-
13. Lactose	-, ±	-	-
14. Cellobiose	+	+	+
15. Melibiose	-, ±	-	-
16. Trehalose	+	+	+
17. Raffinose	-	-	-
18. D(+)-Melezitose	-	-	-
19. Soluble starch	+	-	+
20. Dulcitol	-	-	-
21. Inositol	+	-	-
22. D-Mannitol	++	+	-
23. D-Sorbitol	-	-	-
24. Salicin	-	-	-
25. Cellulose	+	+	+
26. Chitin	+	+	-
27. Keratin	+	+	+

Basal medium PG: PRIDHAM-GOTTLIEB's inorganic medium, supplemented with 0.1 % yeast extract.

Lm: LUEDEMANN's organic medium

Incubation for 2 weeks at 37°C.

phous pseudosporangia. The conidiospores have a smooth surface. The color of the substrate mycelium is dark red or yellowish pink, and neither melanin nor any other diffusible pigment is produced. The cell wall type is IIIB.

The above-mentioned morphological, cultural or physiological characteristics and the cell wall composition of strain G455-101 are similar to those described for the genus *Actinomadura* LECHEVALIER *et* LECHEVALIER 1970⁴⁾. In 1971 NONOMURA and OHARA⁵⁾ reported five saprophytic species of the *Actinomadura*. In 1977 PREOBRAZHENSKAYA *et al.*⁶⁾ published a paper on the identification key for the species of *Actinomadura*, which included 21 species of the genus.

Among the known species of the genus *Actinomadura*, strain G455-101 bears some resemblance to *Actinomadura roseoviolacea* NONOMURA *et* OHARA 1971 (KCC A-0145) as shown in Table 1. They have the following characteristics in common: (1) Formation of pseudosporangia and spore-chains, (2)

smooth spore surface, (3) pinkish aerial mycelium, (4) reddish mass color of substrate mycelium, (5) no melanoid pigment production, and (6) good growth on organic media and poor growth on chemically defined media. However, strain G455-101 was differentiated from *A. roseoviolacea* in spore-chain morphology and the cultural and physiological characteristics described below: Strain G455-101 forms predominantly straight or flexuous spore-chains, while the spore-chains of *A. roseoviolacea* are short, hooked or spiraled on a long aerial mycelium. The color of substrate mycelium of *A. roseoviolacea* is dark purplish red in yeast extract-malt extract agar and BENNETT's agar, and reddish black in peptone-yeast extract-iron agar, while that of strain G455-101 is reddish brown without purplish shade in the former media, and yellowish pink in the latter medium. Liquefaction of gelatin is negative for strain G455-101 but positive for *A. roseoviolacea*. D-Arabinose, D-mannitol and chitin are utilized by strain G455-101 but not by *A. roseoviolacea*⁷⁾.

A. carminata GAUSE *et* SVESHNIKOVA 1973 and *A. rubra* MEYER *et* SVESHNIKOVA 1974 also have some similarities to strain G455-101. According to the descriptions of PREOBRAZHENSKAYA *et al*⁸⁾, however, *A. carminata* is differentiated from strain G455-101 in that its spore-chains form tightly closed spirals, and the substrate mycelium has a purplish tinge. *A. rubra* is differentiated from the present strain in its spiraled spore-chains, its lack of pseudosporangium and its warty as well as smooth surface structure of spores.

Strain G455-101 is thus determined to be a new species of the genus *Actinomadura*, for which the name *Actinomadura luzonensis* nov. sp. is proposed in view of the origin of the soil sample. The type strain is No. G455-101 (single isolate) and has been deposited in the American Type Culture Collection with an accession number ATCC 31491.

Acknowledgement

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