

STUDIES ON A NEW ANTIBIOTIC, ALBOCYCLINE. II

TAXONOMIC STUDIES ON ALBOCYCLINE-PRODUCING STRAINS

TAMOTSU FURUMAI, NOBORU NAGAHAMA and TOMOHARU OKUDA

Microbial Chemistry Research Laboratory,
Tanabe Seiyaku Co., Ltd., Toda, Saitama

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Three different *Streptomyces* producing a new antibiotic albocycline were examined in reference to their taxonomic characteristics. It was concluded that two of the strains, *S. MCRL-0129* and *S. MCRL-0356*, are new species of *Streptomyces* and they were named *S. brunneogriseus* nov. sp. and *S. roseocinereus* nov. sp., respectively. The third strain, *S. MCRL-0355*, was recognized to be a variety of *S. roseochromogenes* and named *S. roseochromogenes* var. *albocyclini* var. nov.

In the previous paper¹⁾, production, isolation and properties of a new antibiotic named albocycline was reported. Introduction of gas chromatographic technique to the fermentation metabolites of this *Streptomyces* made it easy to distinguish the albocycline-producing strains from others. Thus, three albocycline-producing *Streptomyces* were isolated in our laboratory from soils collected at various places throughout Japan.

The present paper concerns the taxonomic studies on these albocycline-producing strains, *S. MCRL-0129*, *S. MCRL-0356* and *S. MCRL-0355*, which were named *Streptomyces brunneogriseus*, *S. roseocitreus* and *S. roseochromogenes* var. *albocyclini*, respectively.

Taxonomic Studies

Three microorganisms producing albocycline, *S. MCRL-0129*, *S. MCRL-0356* and *S. MCRL-0355*, were isolated from soil samples respectively collected at Matsushima, Miyagi Prefecture, Seta, Gumma Prefecture and Shiozu, Kagawa Prefecture. The taxonomic studies on these strains were carried out according to accepted methods of

Table 1. Morphology of albocycline-producing *Streptomyces*

Strain	Morphology
<i>S. MCRL-0129</i>	Aerial mycelium long, straight or slightly wavy, forming sporophores with numerous spirals with 2 to 5 turns. Spores oblong, 0.9~1.0 by 1.3~1.6 μ , smooth with phalangoform structure. (Figs. 1, 2)
<i>S. MCRL-0356</i>	Sporophores producing open spirals as side branches on sterile aerial mycelium. Spores oval, 0.6 by 1.1 μ , spiny. (Figs. 3, 4)
<i>S. MCRL-0355</i>	Sporophores monopodially branched, producing irregular open spirals at the end of long hyphae. Spores cylindrical, 0.4~0.5 by 1.0~1.2 μ , smooth with phalangoform structure. (Figs. 5, 6)

Table 2. Cultural characteristics of albocycline-producing *Streptomyces**¹⁾

Medium* ²⁾		S. MCRL-0129	S. MCRL-0356	S. MCRL-0355
Glucose nitrate agar	G	camel (3 ie)	luggage tan (4 ne)	cinnamon (3 le)
	R	amber (3 nc)	dark luggage tan (4 pq)	dark luggage tan (4 pq)
	AM	powdery, white	powdery, white, scant	powdery, white
	SP	maple (4 le)	colonial yellow (2 ga)	amber (3 lc)
Glycerol nitrate agar	G	amber (3 nc)	dark luggage tan (4 pq)	colorless to bright yellow (2 na)
	R	amber (3 nc)	russet orange (4 nc)	bright yellow (3 na)
	AM	powdery, white	powdery, white, scant	powdery, white
	SP	maple (4 le)	colonial yellow (2 ga)	none or bright gold (2 nc)
Sucrose nitrate agar	G	colorless	dark luggage tan (4 pq)	colorless to bright yellow (2 na)
	R	colorless	russet orange (4 pc)	bright gold (2 nc)
	AM	powdery, beaver (4 li)	powdery, light ivory (2 ca), scant	powdery, white, later powder rose (6 ec)
	SP	none	light yellow (1½ ea)	none or honey gold (2 ic)
Glucose- asparagine agar	G	bamboo (2 gc)	amber (3 pe)	colorless to bright yellow (2 na)
	R	colonial yellow (2 ga)	dark luggage tan (4 pq)	russet orange (4 pc)
	AM	powdery, beige (3 ge), later slate tan (2 ig)	velvety, rose gray (6 ge), with white patches	powdery, rose gray (6 ge)
	SP	bright maize (3 la)	none to colonial yellow (2 ga)	none or light wheat (2 ea)
Glucose CZAPEK'S solution	G	light ivory (2 ca)	colorless	light ivory (2 ca)
	AM	none	none	none
	SP	light mustard tan (2 ie)	none	none
Calcium malate agar	G	colorless	colorless	colorless
	R	colorless	colorless	colorless
	AM	powdery, white	powdery, white, scant	powdery, white
	SP	none	none	none
Starch agar	G	light brown (4 ng)	colorless	colorless to honey gold (2 ic)
	R	oak brown (4 pi)	colorless	mustard gold (2 pe)
	AM	powdery, rose gray (6 ge), later taupe brown (5 li)	velvety, beige brown (3 ig), with white patches	powdery, powder rose (6 ec)
	SP	none	none	cream (1½ ca)
Tyrosin agar	G	dark brown (4 pn)	colorless	chestnut brown (4 ni)
	R	dark brown (4 pn)	colorless	fawn (4 ig)
	AM	powdery, white, scant	powdery, white, scant	powdery, white
	SP	dark brown (4 nl)	none	dark brown (3 pn)
Nutrient agar	G	colorless to ivory tint (2 cb)	yellow tint (1 ba)	yellow tint (1 ba)
	AM	none	none	none
	SP	dark brown (3 nl)	none	deep brown (3 pl)
Glucose nutrient agar	G	ivory tint (2 cb)	colorless	yellow tint (1 ba)
	AM	powdery, white	powdery, white, later pearl (3 ba)	powdery, white, scant
	SP	persimmon (5 lc)	light amber (3 ie)	maple (4 le)

(To be continued)

Table 2 (Continued)

Medium*2)		S. MCRL-0129	S. MCRL-0356	S. MCRL-0355
Glucose peptone agar	G	light brown (3 lg)	colorless	yellow tint (1 ba)
	AM	powdery, white, scant	powdery, white	powdery, white
	SP	dark brown (3 nl)	none	maple (4 le)
BENNETT'S agar	G	light brown (4 ng)	colorless	colorless
	R	chestnut brown (4 ni)	dusty orange (4 lc)	colorless
	AM	at first white, rose gray (6 ge) to olive gray (1½ ig), later beige gray (3 ih)	velvety, rose taupe (5 ig) with white patches	powdery, rose gray (6 ge)
	SP	yellow maple (3 ng)	none	natural (3 dc)
Potato plug	G	golden brown (3 pi)	colorless	golden brown (3 pi)
	AM	powdery, white, later pearl (3 ba)	powdery, white to bright yellow (1½ na)	powdery, white, later pearl (3 ba)
	SP	dark brown (6 pn)	none	deep brown (4 pl)
Egg medium	G	mustard tan (2 lg)	colorless	mustard tan (2 lg)
	AM	powdery, white	powdery, white to bright yellow (1½ na)	powdery, white
	SP	dark brown (3 pn)	none	dark brown (3 pn)
Blood agar	G	tile red (5 ne)	pale aqua green (19 ca)	cork tan (4 ie)
	AM	none	none	none
	SP	beige (3 ge)	none	deep brown (3 pl)
Coagulated serum	G	camel (3 ie) to cinnamon brown (3 lg)	colorless	none
	AM	none	powdery, white, scant	none
	SP	clove brown (3 ni)	none	none
Gelatin stab	G	colorless	colorless, scant	beige brown (3 ig)
	AM	powdery, beige brown (3 ig)	none	powdery, beige (3 ge)
	SP	dark brown (3 nl)	none	dark brown (3 pn)
Litmus milk	G	dark brown (3 nl)	cream (1½ ca)	maple (4 le)
	AM	none	none	none
	SP	dark purple wine (11 pn)	none	dark purple wine (11 pn)
Cellulose	G	light ivory (2 ca)	colorless	none
	AM	none	none	none
	SP	biscuit (2 ec)	none	none

*1) Color names accord to Color Harmony Manual, 4th Ed. 1963. Container Corporation of America.

*2) G : growth, R : reverse, AM : aerial mycelium, SP : soluble pigment.

Fig. 1. Sporophores of *S. MCRL-0129* on BENNETT'S agar.

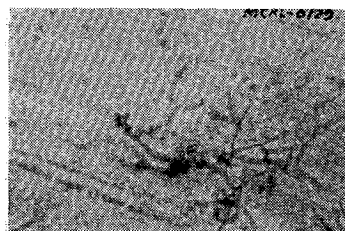


Fig. 2. Electronmicrogram of *S. MCRL-0129* on BENNETT'S agar. (×5000)

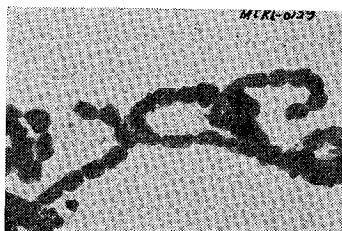


Fig. 3. Sporophores of *S. MCRL-0356* on glucose-asparagine agar.

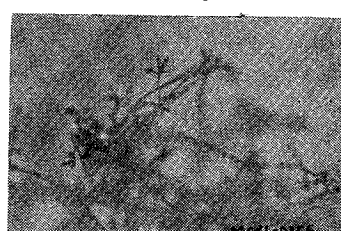


Fig. 4. Electronmicrogram of *S. MCRL-0356* on BENNETT's agar. ($\times 6000$)

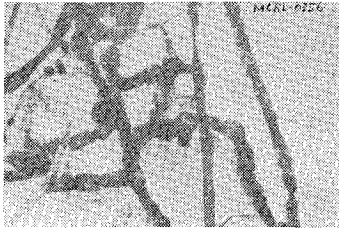


Fig. 5. Sporophores of *S. MCRL-0355* on starch agar.

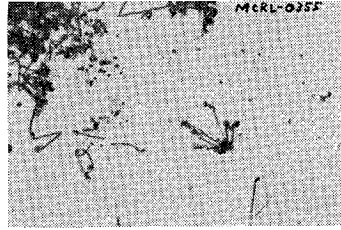


Fig. 6. Electronmicrogram of *S. MCRL-0355* on starch agar. ($\times 6000$)

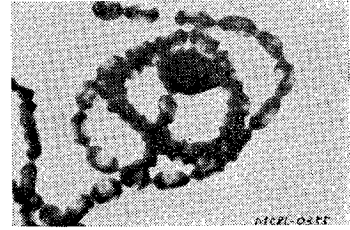


Table 3. Physiological characteristics of albocycline-producing strains

	<i>S. MCRL-0129</i>	<i>S. MCRL-0356</i>	<i>S. MCRL-0355</i>
Solubilization of calcium malate	positive	negative	negative
Hydrolysis of starch	positive	weak	strong
Liquefaction of gelatin	strong	negative	negative
Hemolysis	positive	positive	weak
Milk coagulation	positive	positive	negative
Milk peptonization	positive	positive	positive
Milk reaction	acidic	acidic	acidic
Liquefaction of serum	negative	negative	negative
Reduction of nitrate	positive	negative	negative
Melanin formation	positive	negative	positive
H ₂ S production	positive	negative	positive
Tyrosinase reaction	positive	negative	positive
Cellulose decomposition	negative	negative	negative

identification for *Streptomyces*, particularly with regard to their taxonomic positions.

Morphology, cultural characteristics and physiological properties of these strains are illustrated in Tables 1, 2 and 3 respectively. Utilization of carbohydrates by these strains tested according to PRIDHAM and GOTTLIEB²⁾ is summarized in Table 4. These strains grew well at 27°C as well as 37°C in the pH ranges of 5 to 9, but they showed no growth at 5° and 45°C in any pH ranges.

(1) *S. MCRL-0129*: *S. MCRL-0129* grows colorless to pale yellowish on various agar media. In early stage aerial mycelium is white, but turns later to brownish white to brownish gray. Aerial mycelium on BENNETT's agar changes its color notably with time: brownish white

Table 4. Utilization of carbohydrates by albocycline-producing *Streptomyces*

†† : good utilization + : moderate utilization
± : little utilization - : no utilization

	<i>S. MCRL-0129</i>	<i>S. MCRL-0356</i>	<i>S. MCRL-0355</i>
Arabinose	+	+	+
Dextrin	±	+	+
Fructose	††	+	+
Galactose	+	+	+
Glucose	††	+	+
Glycerol	+	+	+
Inositol	-	+	+
Lactose	+	+	+
Maltose	+	+	+
Mannitol	+	+	-
Mannose	+	††	††
Raffinose	††	+	-
Rhamnose	-	+	-
Salicin	-	+	-
Starch	+	††	††
Sucrose	+	+	+
Xylose	+	+	+
Control	-	-	-

at first, then turning to grayish red, further to olive gray and finally to brownish gray. As shown in tables, this culture belongs to Spira-gray series according to the classification of PRIDHAM *et al.*^{3,4}) and it is further characterized by producing phalangoform-spores⁵) with smooth surface and by its chromogenic nature and strong proteolytic activity. Based on these characteristics, there is a suggestion that S. MCRL-0129 is related to *S. aureus* (WAKSMAN *et* CURTIS) WAKSMAN *et* HENRICI⁶), *S. filipinensis* AMMANN *et al.*⁷), *S. flavochromogenes* (KRAINSKY) WAKSMAN *et* HENRICI⁸) and *S. cinerochromogenes* MIYAIRI *et al.*⁹). The latest strain produces cineromycin B which is quite similar to albocycline. However, *S. aureus* differs from S. MCRL-0129 by dark brown growth and mouse gray aerial mycelium on CZAPEK's agar, gray growth on nutrient agar, production of brown soluble pigment on gelatin stab, negative hemolytic activity and no utilization of arabinose. According to AMMANN *et al.*⁷) *S. filipinensis* grows best at 37°C and at 23~26°C it produces no aerial mycelium. It grows light yellowish, produces yellow soluble pigment on CZAPEK's agar, shows weak tyrosinase reactions, and little nitrate-reduction activity. These properties are different from those of S. MCRL-0129. *S. flavochromogenes* is differentiated from S. MCRL-0129 by its light brownish gray growth and production of brownish yellow soluble pigment on CZAPEK's agar, formation of gray aerial mycelium and brown soluble pigment on glucose-asparagine agar, yellow growth and gray aerial mycelium formation on calcium malate agar, and formation of spores without phalangoform structure. *S. cinerochromogenes* differs from S. MCRL-0129 in the respects that the former forms white aerial mycelium on CZAPEK's agar, produces no soluble pigment on glucose-asparagine agar, lacks ability to liquefy gelatin and to reduce nitrate, and hydrolyses starch slightly. *S. cinerochromogenes* and S. MCRL-0129 are also different in the utilization pattern of the following carbohydrates: inositol, mannitol, raffinose, rhamnose, and salicin.

In view of the above characteristics, S. MCRL-0129 was considered to be a new species and named *Streptomyces brunneogriseus* FURUMAI *et* OKUDA nov. sp. after the color of aerial mycelium on BENNETT's agar.

(2) S. MCRL-0356: The strain grows yellowish brown on synthetic agars. Spore formation was well observed on glucose-asparagine agar and starch agar. On these media the color of aerial mycelium was at first white, but, on maturity it turned beige brown to rose gray with white patches. It was noted that spores bear fairly long spine. The strain belongs to Spira-red series, and to non-chromogenic type. Proteolytic activity is rather weak.

The above properties suggested that S. MCRL-0356 is closely related to *S. calvus* BACKUS *et al.*¹⁰) However, *S. calvus* forms white, gray to mouse gray aerial mycelium on synthetic agars including glucose-asparagine agar and starch agar and never produces aerial mycelium of rose to reddish color. *S. calvus* has been reported to produce nucleocidin, but S. MCRL-0356 does not produce nucleocidin or any similar antibiotics. Thus, we concluded that S. MCRL-0356 is different from *S. calvus*, and should be assigned to a new species for which we propose the name of *S. roseocinereus* FURUMAI *et* OKUDA nov. sp. based on the color of its aerial mycelium on starch agar

or on glucose-asparagine agar.

(3) *S. MCRL-0355*: On synthetic agars, *S. MCRL-0355* grows colorless to yellowish brown and produces aerial mycelium which is at first white and later turns to rose gray. Sucrose nitrate agar, starch agar, glucose-asparagine agar and BENNETT's agar are suitable for spore formation. Surface of spore is smooth and phalangiiform. *S. MCRL-0355* belongs to chromogenic type and Spira-red series. Proteolytic action of the strain is very limited.

It is suggested that *S. MCRL-0355* is closely related to *S. roseochromogenes* (JENSEN) WAKSMAN and HENRICI¹¹⁾. The difference between both strains is small: *S. roseochromogenes* grows yellowish gray to brown-red and produces white to rose-gray aerial mycelium on nutrient agar and liquefies gelatin moderately. However, these differences are insufficient to assign *S. MCRL-0355* to the rank of a new species of *Streptomyces*. Thus, we propose to call *S. MCRL-0355* a variant strain of *S. roseochromogenes* and to name *S. roseochromogenes* (JENSEN) WAKSMAN and HENRICI var. *albocyclini* FURUMAI et OKUDA var. nov.

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