

NEW BASIC WATER-SOLUBLE ANTIBIOTICS BD-12 AND BY-81. I*

TAXONOMY OF THE PRODUCING ORGANISMS AND ANTIBIOTIC PRODUCTION

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In taxonomic studies on two streptomycetes, the strain MCRL-0357 which produces the antibiotic BD-12 was recognized to be a new species of streptomycetes and named *Streptomyces luteocolor* nov. sp. The strain MCRL-0358 which produces antibiotic BY-81 was concluded to be a new variety of *Streptomyces olivoreticuli* ARAI *et al.* The production of both antibiotics were described.

In the course of screening antibiotics, two basic antibiotics, tentatively named BD-12 and BY-81, were found. These antibiotics were not identical with any other known antibiotics and seemed to be closely related to each other chemically. Antibiotics BD-12 and BY-81 were produced by the strains indexed as MCRL-0357 and MCRL-0358 respectively. These strains belong to the Genus *Streptomyces*, and, according to the classification proposed by PRIDHAM *et al.*¹⁾, the strain MCRL-0357 may be classified into *Rectus-Flexibilis*, the Yellow series of streptomycetes and the strain MCRL-0358 to *Biverticillus*, the Olive-buff series. The former was not identical with any streptomycetes species previously described and, therefore, was named *Streptomyces luteocolor* nov. sp., while the latter was determined to be a new variety of *S. olivoreticuli* ARAI *et al.*²⁾

The present paper concerns the taxonomic study on both strains and also deals with the production of the antibiotics. Isolation, purification and properties of the antibiotics BD-12 and BY-81 will be reported in the succeeding paper.³⁾

Taxonomic Study

The strains MCRL-0357 and MCRL-0358 were isolated from a soil sample collected at Fukiage, Saitama Prefecture and Kanzaki, Hyogo Prefecture, respectively. Micro-

Table 1. Morphological characteristics of *Streptomyces* spp. MCRL-0357 and MCRL-0358.

Strain	Morphology
MCRL-0357 (Plates 1~3)	Sporophores sympodially branched, forming tufts. No spirals, no whorls. Spores, cylindrical, 0.6~1.0×0.8~1.0 μ, phalangiiform, smooth-surfaced. Unidentified bodies observed on the top of sporophores.
MCRL-0358 (Plates 4~6)	Sporophores straight, branching primary and secondary whorls. No spirals. Spores cylindrical, 0.4~0.6×1.2~2.0 μ, phalangiiform, smooth-surfaced. Ball-like structure formed.

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Plate 1. Aerial mycelium of strain MCRL-0357 (Sucrose nitrate agar, $\times 400$)



Plate 2. Electron micrograph of strain MCRL-0357 (Sucrose nitrate agar, $\times 6,667$)



Plate 3. Electron micrographs of unidentified bodies. (Sucrose nitrate agar, $\times 6,667$)

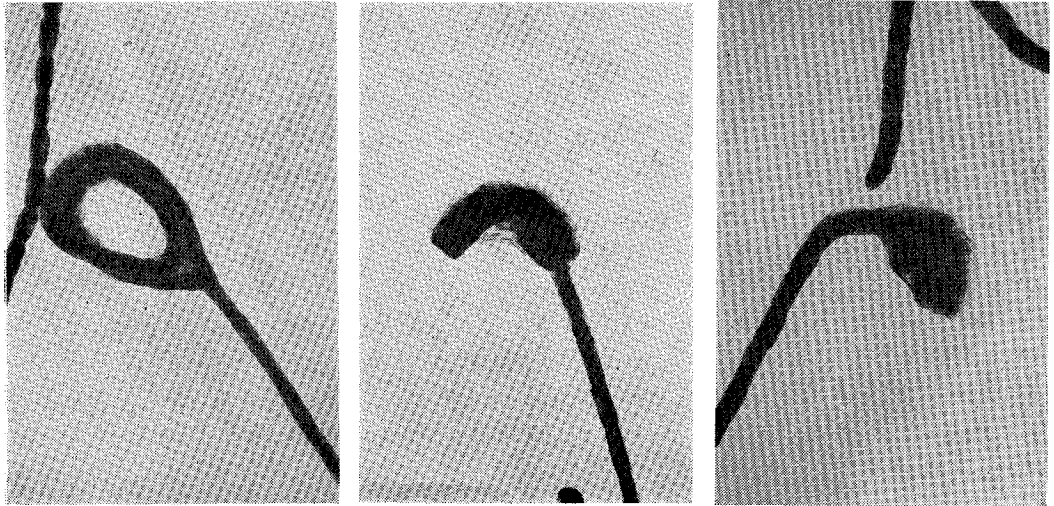


Plate 4. Aerial mycelium of strain MCRL-0358 (BENNETT's agar, $\times 400$)

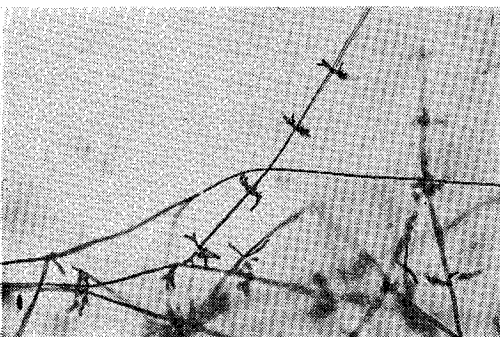


Plate 5. Electron micrograph of strain MCRL-0358 (BENNETT's agar, $\times 6,667$)

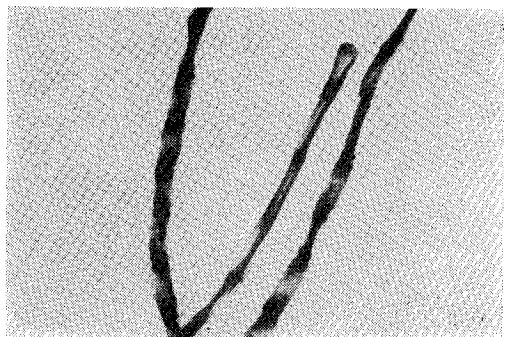


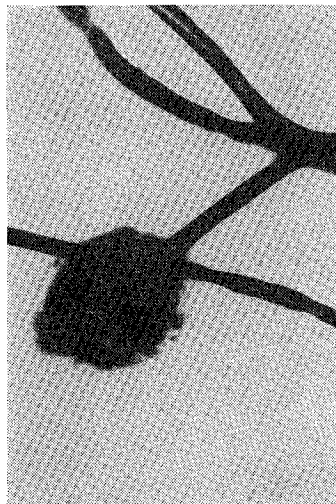
Table 2. Cultural characteristics of *Streptomyces* spp. MCRL-0357 and MCRL-0358.

G : growth, Rev : reverse, AM : aerial mycelium
 SP : soluble pigment

Medium	MCRL-0357	MCRL-0358
Glucose nitrate agar	G : gold (2 lc) Rev : bright yellow (3 na) AM : powdery, light ivory (2 ca) SP : none	G : pale yellow (1 ca) Rev : light amber (3 ic) AM : powdery, white (13 ba) SP : none
Glycerol nitrate agar	G : gold (1½ pc) Rev : amber (3 nc) AM : powdery, cream (1½ ca) SP : none	G : pale yellow (1 ca) Rev : light yellow (1 ea) AM : powdery, white (13 ba) SP : none
Sucrose nitrate agar	G : cream (1½ la) Rev : butter yellow (1½ ga) AM : cottony, light ivory (2 ca) SP : none	G : colorless Rev : colorless AM : cottony, light olive gray (1 ge) SP : none
Calcium malate agar	G : colorless to light ivory (2 ca) Rev : gold (2 lc) AM : cottony, white to light ivory (2 ca) SP : none	G : colorless Rev : colorless AM : cottony, putty (1 ec) SP : none
Starch agar	G : light ivory (2 ca) Rev : gold (2 lc) AM : cottony, light ivory (2 ca) SP : none	G : lemon yellow (1 ia) Rev : pale yellow (1 ca) AM : cottony, parchment (1 cb) SP : none
Tyrosine agar	G : colorless Rev : squash yellow (2 ia) AM : cottony, white SP : none	G : light brown (3 lg) Rev : clove brown (3 ni) AM : cottony, parchment (1 cb) SP : dark brown (3 nl)
Glucose-asparagine agar	G : gold (2 lc) Rev : light gold (2 ic) AM : cottony, pearl pink (3 ca) SP : none	G : bamboo (2 gc) Rev : golden brown (3 pi) AM : cottony, scant, white (13 ba) SP : none
Glucose nitrate solution	G : ring, old gold (2 le) AM : cottony, light ivory (2 ca) SP : none	G : colorless, pellicle AM : none SP : none
BENNETT'S agar	G : amber (3 pc) Rev : amber (3 lc) AM : white to cream (1½ ca) SP : none	G : gold (1½ pc) Rev : gold (1½ lc) AM : cottony, cream (1½ ca) SP : none
Nutrient agar	G : topaz (3 ne) Rev : old gold (2 nc) AM : none SP : none	G : bright gold (2 nc) AM : none SP : none

(To be continued)

Plate 6. Electron micrograph of the ball-like structure (BENNETT'S agar, ×6,667)



scopical and electron-microscopical observations of both strains are shown in Table 1 and Plates 1~6. The unidentified body (Plate 3) formed on top of the sporophores of the strain MCRL-0357 was unique—some looked like a loop and others appeared to be covered with cottony material. Functions of these bodies are unknown.

The cultural characteristics of the strains grown on various media are shown in Table 2. The color terms and color codes used in the culture description are those adopted in "Color Harmony Manual" (3rd Ed. 1950. Container Corporation of America). Physiological properties and utilization of carbon sources⁴⁾ of both strains are summarized in Tables 3 and 4 respectively.

Table 2 (Continued)

Medium	MCRL-0357	MCRL-0358
Glucose nutrient agar	G : amber (3 pe) AM : none SP : none	G : russet orange (4 nc) AM : none SP : maple (4 le)
Potato plug	G : cinnamone yellow (3 le) AM : cottony, pearl (2 ba) Color : none	G : camel (3 ie) AM : none Color : none
Peptone glucose agar	G : bright yellow (2 na) AM : powdery, light ivory (2 ca) SP : none	G : mustard gold (2 pl) AM : none SP : none
Blood agar	G : olive gray (1 ig) AM : none SP : none	G : pastel green (23 ec) AM : none SP : none
Whole egg	G : amber (3 nc) AM : powdery, pearl (2 ba) SP : bamboo (2 gc)	G : wrinkled, colonial yellow (2 gc) AM : none SP : none
LOEFFLER'S coagulated serum	G : light gold (2 ic) AM : powdery, white SP : none	G : camel (3 ie) AM : none SP : dark brown (5 nl)
Gelatin stab	G : bright yellow (3 na) AM : none SP : none	G : colorless AM : powdery, white (13 ba) SP : golden brown (3 pi)
Litmus milk	G : ring, amber (3 nc) AM : none Color : none	G : ring, pearl (3 ba) AM : none Color : ash rose (7 ie)
Cellulose medium	G : none	G : none

Table 3. Physiological properties of *Streptomyces* spp. MCRL-0357 and MCRL-0358

	MCRL-0357	MCRL-0358
Solubilization of Ca-malate	positive	negative
Nitrate reduction	negative	negative
Hydrolysis of starch	strong(44/5)	positive
Cellulose decomposition	negative	negative
Tyrosinase reaction	negative	positive
Coagulation of milk	positive	positive
Peptonization of milk	positive	limited
Hemolysis	negative	positive
Liquefaction of gelatin	positive	positive
Liquefaction of coagulated serum	strong	negative
Melanin formation	negative	positive

that the strain showed some resemblance in property to *S. kanamyceticus* OKAMI *et* UMEZAWA⁷⁾, *S. flavus* (KRAINSKY) WAKSMAN *et* HENRICI⁸⁾ and *S. alboflavus* (WAKSMAN *et* CURTIS) WAKSMAN *et* HENRICI⁹⁾. However, the latter three strains are different from the strain MCRL-0357 in the following properties. *S. kanamyceticus* forms flexible, hook-shaped aerial mycelia, produces a light brown soluble pigment on

The microbiological properties of the strain MCRL-0357 and MCRL-0358 are summarized as follows: the strain MCRL-0357 belongs to the non-chromogenic type of streptomyces and can be further classified into the section *Rectus-Flexibilis* according to the PRIDHAM'S classification¹⁾. The spores are phalangiiform⁵⁾ and smooth-surfaced. It grows with a gold color and produces light ivory-colored aerial mycelia on synthetic media. It hydrolyzes starch and protein, while it is unable to reduce nitrate to nitrite. The strain MCRL-0358 belongs to the chromogenic type of streptomyces with smooth, phalangiiform spores. Morphologically it is classified into the section *Biverticillus*. It grows colorless to yellowish and produces white to light olive gray aerial mycelia on synthetic media. It hydrolyzes starch, liquefies protein, and reduces nitrate.

According to the classification system proposed by WAKSMAN⁶⁾, the strain MCRL-0357 might be classified into the section A-I-1 or A-I-10, and it was found

glucose-asparagine agar, and gives white aerial mycelia when grown on a potato plug. Coagulation and peptonization of milk by *S. kanamyceticus* are doubtful. Differences are also observed in utilization of the following carbon sources: arabinose, xylose and inositol. *S. flavus* grows in pinkish cream color on starch agar and gray on nutrient agar, and produces white to gray aerial mycelia on glucose-asparagine agar and gray aerial mycelia on the potato plug. Data are not available about utilization of carbon sources by *S. flavus*. *S. alboflavus* does not produce aerial mycelia on either glucose-asparagine agar or starch agar. It grows colorless on the gelatin stab with slow liquefaction, and peptonizes milk weakly without coagulation. The above consideration led to the conclusion that the strain MCRL-0357 is a new species of streptomycetes, and therefore it is named *Streptomyces luteocolor* FURUMAI *et* OKUDA nov. sp.* after the color of basal mycelium on synthetic media.

The strain MCRL-0358 should be classified into the section B-II-5 of the WAKSMAN's classification system. Among the streptomycetes belonging to the same section, *S. olivoreticuli*²⁾ was recognized to be quite similar to the strain MCRL-0358. Thus, taxonomic work was attempted on the type culture of *S. olivoreticuli*. Differences observed between both microorganisms are shown in Table 5. However, in other

Table 4. Utilization of carbon sources by *Streptomyces* spp. MCRL-0357 and MCRL-0358.

Carbon sources	MCRL-0357	MCRL-0358
Arabinose	—	—
Fructose	+	—
Galactose	+	++
Glycerol	+	++
Glucose	+	++
Inositol	+	—
Lactose	+	—
Maltose	++	+
Mannitol	++	—
Mannose	+	+
Raffinose	++	—
Rhamnose	—	—
Salicin	+	—
Starch	++	++
Sucrose	+	±
Xylose	+	—

++ : strongly positive utilization
 + : positive utilization
 ± : doubtful utilization
 — : negative utilization

Table 5. Differences in cultural properties between *S. sp.* MCRL-0358 and *S. olivoreticuli* ARAI *et al.*

Medium	MCRL-0358	<i>S. olivoreticuli</i> **
Sucrose nitrate agar	G : colorless Rev : colorless AM : light olive gray (1 ge) SP : none	G : colorless Rev : colorless AM : pink tint (7 ba) SP : none
Calcium malate agar	G : colorless Rev : colorless AM : putty (1 ec) SP : none, negative solubilization	G : butter yellow (1½ ga) Rev : yellow tint (1 ba) AM : pearl (2 ba) SP : none, positive solubilization
Starch agar	G : lemon yellow (1 ia) Rev : pale yellow (1 ca) AM : parchment (1 cb) SP : none	G : dusty yellow (1½ gc) Rev : mustard gold (2 pg) AM : pastel yellow (1 bd) SP : none
BENNETT'S agar	G : gold (1½ pc) Rev : gold (1½ lc) AM : cream (1½ ca) SP : none	G : chestnut brown (4 ni) Rev : deep brown (4 pl) AM : pearl (2 ba) SP : maple (4 lc)
Potato plug	G : camel (3 ie) AM : none Color : none	G : dark brown (5 nl) AM : none Color : brown mahogany (6 pl)

** Reported to produce a viomycin-like substance.

* At the meeting of J.A.R.A., we proposed naming the strain *S. luteus*. However, this name was abandoned to avoid confusion with *Actinomyces luteus* and *S. diastatochromogenes* var. *luteus*.

properties including morphological, physiological properties and carbon source utilization, the strain MCRL-0358 showed good agreement with *S. olivoreticuli*. Therefore, the strain MCRL-0358 was concluded to be a new variety of *S. olivoreticuli*.

Antibiotic Production

Fermentation pattern was examined in 200-liter fermentor. Antibiotic production was checked by the cup plate method using *B. subtilis* PCI 219 as a test organism. The fermentation medium was composed of glucose, glycerol, starch (each 1%), sodium chloride (0.3%), calcium carbonate (0.1%) and a nitrogen source (1.5%) which was Prorich (soy bean meal; Ajinomoto Co., Ltd.) in the case of the strain MCRL-0357 and Gotoku (soy bean meal; Yoshihara Oil Mill Ltd.) in the case of the strain MCRL-0358. In a 200-liter fermentor, 120 liters of medium was prepared and pH of the medium was adjusted to 7.0. After autoclaving and inoculation of 4.8~5.0 liters of seed culture, fermentation was carried out under the following conditions: temperature $27 \pm 1^\circ\text{C}$, inner pressure 0.5 kg/cm², aeration 60 liters/min., agitation 262 r.p.m. (MCRL-0357) or 150~262 r.p.m. (MCRL-0358). As an antifoam agent, Klearol-Nikkol PBC-4I (9:1) was used.

As shown in Figs. 1 and 2, the maximum production of antibiotic BD-12 by the strain MCRL-0357 was observed after 30~35 hours, and that of BY-81 by the strain MCRL-0358 after 42~48 hours. pH changes during fermentation are quite opposite between both strains. In the fermentation of MCRL-0357, pH tends to rise gradually, while in the case of MCRL-0358 pH tends to decrease during fermentation. These phenomena are ascribed to difference in physiological properties of the strains. The fermentation broths obtained were used for the isolations of each antibiotic as reported in the succeeding paper.

Fig. 1. Fermentation pattern of antibiotic BD-12

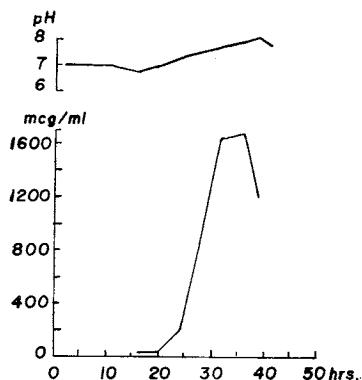
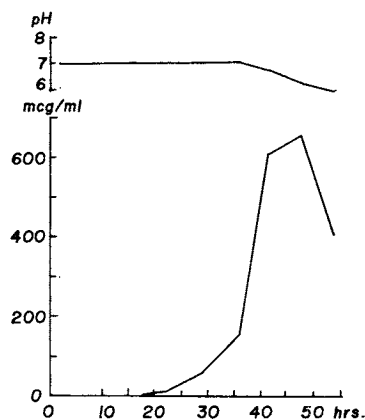


Fig. 2. Fermentation pattern of antibiotic BY-81



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