

STREPTOMYCES PROPURPURATUS NOV. SP.,
A NEW STREPTOMYCETE WHICH PRODUCES A SOLUBLE,
DEEP PURPLISH-RED PIGMENT IN MIXED CULTURE
WITH THE OTHER MICROORGANISMS

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(Received for publication September 26, 1969)

A *Streptomyces* species, originally designated as No. 751, isolated from soil, was carefully studied from the standpoint of its morphological, physiological, and cultural characteristics. Because it produces soluble, deep purplish-red pigment in mixed culture with various microorganisms, but does not produce such pigment when cultivated independently, it is proposed as a new species and is designated *Streptomyces propurpuratus* nov. sp. SHINOBU *et* KANDA. The type strain is designated as OEU No. 751. It is not difficult to imagine that, in nature, a product of metabolism of a microorganism might be changed by the effect of a product of metabolism of another microorganism, whether the reactions are chemical or enzymatic. The authors believe that such phenomena occur not only with regard to pigment formation, but also with regard to production of antibiotics. An example of such a phenomenon is reported herein.

Several strains of a streptomycete (originally designated as No. 751) were isolated from soil samples collected at Koyadera, Itami City, Hyogo Prefecture, July 1966* ; Shinge-cho, Sennan-gun, Osaka Prefecture, Oct. 1967** ; Kuala Lumpur, Oct. 1968 ; and Takebe-cho, Okayama Prefecture, Jan. 1969*** ; and Satsukiyama, Ikeda City, Osaka Prefecture, Mar. 1969***.

Because strains of streptomycete No. 751 have been isolated from soils geographically dispersed, the authors believe that this organism is of broad distribution and not uncommon. This organism, which we propose to name *Streptomyces propurpuratus* nov. sp. SHINOBU *et* KANDA (type strain OEU 751), has not been reported previously, because it and other microorganism were associated with one another in such a way that it could not be separated with ease as pure cultures from the other members of the association. Also, strains of streptomycete No. 751 produce such a faint soluble purplish-red pigment under ordinary condition that this characteristic did not attract our attention. We successfully isolated strains of streptomycete No. 751 in pure culture by using a mixer with 9,500~10,000 rotations per minute.

The strains of streptomycete No. 751 produce soluble, purplish-red pigment in mixed culture with other microorganisms. The pigment is characteristically very

* TOMOKO ISHIYAMA, ** TERUMI OKUDA and *** KENJI MATSUDA (Biological Laboratory, Ikeda Branch, Osaka Kyoiku University) isolated these strains.

intense. Moreover, strains of this species inhibit the growth of *Micrococcus flavus*, *Aspergillus* sp., *Piricularia oryzae*, etc.

Production, isolation, purification, and characterization of the antibiotics of a strain of streptomycete No. 751 will be reported in another paper.

Taxonomy*

I. Morphological Characteristics

1. Macrocolony: Chrysanthemum pattern on glycerol starch glutamate agar (Fig. 2, A).
2. Electron microscopy of conidium: Spiny type on ammonium Czapek agar (Fig. 2, B, a, b, c).

Fig. 1. Aerial mycelium of *Streptomyces propurpuratus* nov. sp.
 A. Glycerol starch glutamate agar (10-day culture) B. Ammonium Czapek agar (10-day culture)

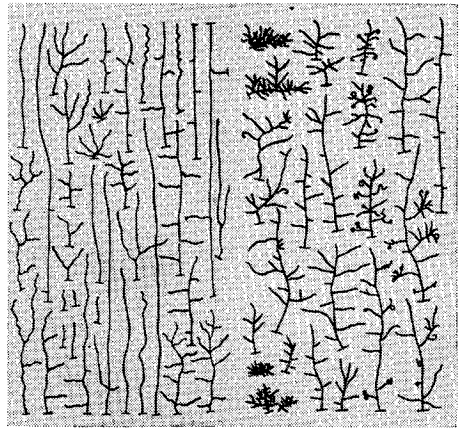
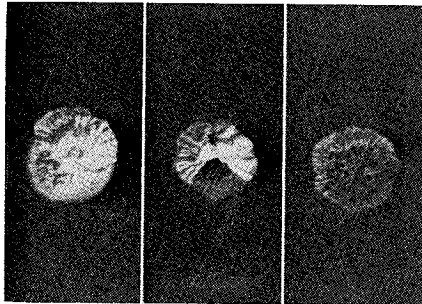


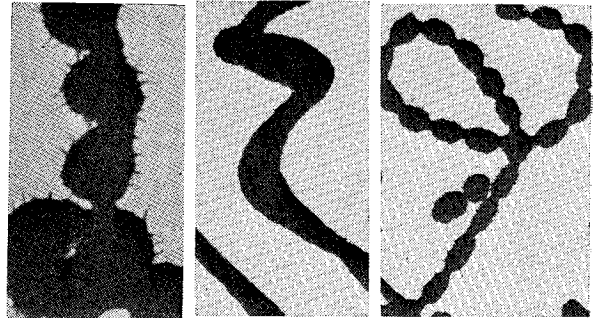
Fig. 2. Morphology of *Streptomyces propurpuratus*

A. Macrocolony on glycerol starch glutamate agar (20-day culture)



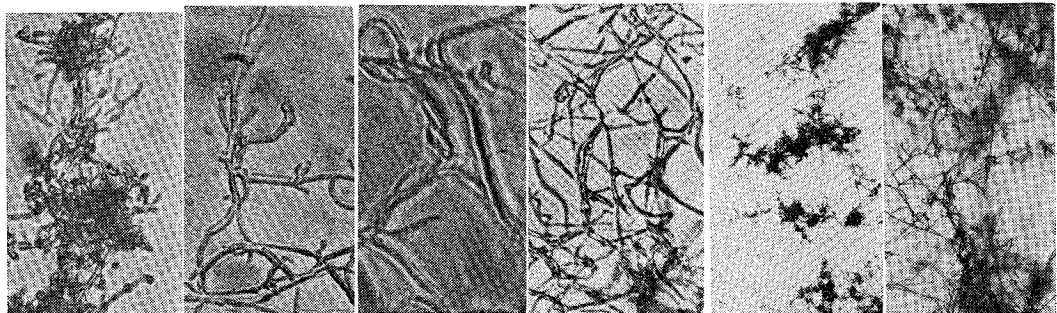
B. Electron-microphotographs

- a. Conidium formation on ammonium Czapek agar (12-day culture) × 20,000
- b. Conidiophore on ammonium Czapek agar (12-day culture) × 10,000
- c. Spiral on ammonium Czapek agar (12-day culture) × 5,000



C. Microphotographs

- a. Conidium formation on ammonium Czapek agar (8-day culture; impression method)
- b. Aerial mycelium on glycerol starch glutamate agar (15-day culture; impression method)
- c. Aerial mycelium on SHINOBU's tyrosine agar (15-day culture; impression method)
- d. Aerial mycelium on ammonium Czapek agar (15-day culture)



* SHINOBU's descriptive method was used.

3. Light-microscopy: On glycerol starch glutamate agar, there were abundant aerial mycelia but few conidia (Fig. 1, A; Fig. 2, C, b). Glucose asparagine agar allowed only sparse development of aerial mycelium and no conidia were observed. Ammonium CZAPEK agar was most suitable for the light-microscopic observations, although the aerial mycelia were sparse.

The following data were obtained from observations of cultures growing on ammonium CZAPEK agar (Fig. 1, B; Fig. 2, C, a, d). Observations were made after 8~15 days incubation at 30°C.

- (1) Aerial mycelium: Straight~flexuous; 0.6~0.7 μ in width.
- (2) Spiral: Many; conidia masses rare; 1~2 volutions, sometimes 3 volutions; often hook- or loop-like spirals; most spirals sinistrorse.
(No spiral on CZAPEK agar.)
- (3) Conidium: Spherical~oval; *ca.* 0.6 μ in width; 0.6~0.8 μ in length.
- (4) Substrate mycelium: Flexuous; no fragmentation or segmentation; 0.4~0.5 μ in width.

II. Physiological Characteristics

1. Tyrosinase reaction: Negative.
2. Nitrite production: Negative: rarely positive; weak.
3. Diastase reaction (iodine test): Clear zone; 14~16 mm with cultures incubated on glycerol starch glutamate agar for 10 days at 30°C; 10~12 mm with cultures incubated on starch agar for 10 days (Growth: Trace on WAKSMAN's starch agar).
4. Carbon utilization (Basal agar: CZAPEK solution with sucrose omitted): D-galactose, trehalose, and L-inositol were utilized; D-xylose, L-rhamnose, D-fructose, sucrose, lactose, and raffinose were not utilized; utilization of D-mannitol was doubtful.

III. Cultural Characteristics

The cultural characteristics of streptomycete No. 751 are shown in Table 1.

Table 1. Cultural characteristics of Streptomycete No. 751.

	Growth ¹⁾	Aerial mycelium ²⁾	Color of the substrate mycelium	Soluble pigment
1. Ammonium CZAPEK agar	Poor; thin	Poor; white	Pale buff~pale dull yellow orange	None
2. Glycerol CZAPEK agar	Good~moderate	Moderate; white	Buff~pale dull yellow orange	None
3. Glucose asparagine agar	Moderate~poor; thin	Poor~trace; white	Pale buff~pale dull yellow orange	None
4. Calcium malate agar	Moderate~poor	Poor; white~yellowish white~brownish white	Pale buff~pale dull yellow orange~pale brown	None
5. Starch agar (WAKSMAN'S A)	Poor~trace	Trace~none; if any, white	Pale buff	None
6. Urea glycerol agar	Moderate~poor	None~trace; if any, white	Colorless~pale dull yellow	None
7. Tyrosine agar (Shinobu's)	Moderate~good	Moderate~good; white	Pale buff~pale dull yellow orange	None
8. Glycerol starch glutamate agar	Good	Good; white	Pale buff~pale brown	None

1) Growth of the colony and its nature.

2) Growth and nature of the aerial mycelium and its color.

Table 2. The differences of characteristics between some strains and No. 751.

		KRASSILNIKOV (1957)	BERGEY (1957)	WAKSMAN (1961)	No. 751
<i>S. alboflavus</i>	Spiral		Aerial mycelium : straight, branching mycelium, with very little tendency to form spirals	Morphology : sporophores straight, branching, with very little tendency to produce spirals	On ammonium CZAPEK agar : spiral, many; rarely conidia masses; On CZAPEK agar : no spiral
	Synthetic agar		Aerial mycelium : white, powdery, with yellow tinge	Aerial mycelium : powdery, white, with yellowish tinge	Aerial mycelium : trace; white
	Potato		Moist, cream-colored, wrinkled growth	Growth : wrinkled, moist, cream-colored	Growth : Good; no moist Aerial mycelium : Good; white
	Milk	Does not coagulate milk	Litmus milk : pinkish ring No coagulation	Surface : ring, pinkish No coagulation	Coagulation occurs in 2~3 days Substrate mycelium : colorless~pale buff
<i>S. candidus</i>	Spiral	Sporophores long, straight or undulatory never twist into spirals	Aerial mycelium : sporophores long, straight or wavy, but never forming spirals	Morphology : sporophores long, straight or wavy, but never forming spirals; occasionally arranged in broom-shaped bodies of fascicles	On ammonium CZAPEK agar : spiral, many; rarely conidia masses On CZAPEK agar : no spiral
	Synthetic agar	Develops satisfactorily on synthetic agar	Well developed, colorless colonies		On ammonium CZAPEK agar and CZAPEK agar : growth poor~trace
	On cellulose	Grows satisfactorily on cellulose	Good growth on cellulose	Good growth	Cellulase reaction : negative (no growth)
	Potato			Aerial mycelium : poorly developed	Aerial mycelium : good; white
<i>S. albus</i>	Glucose asparagine agar		Gray aerial mycelium becoming brownish	Aerial mycelium : gray, becoming brownish	Aerial mycelium : poor~trace; white
	Ca-malate agar		Aerial mycelium : abundant, all over surface, pale mouse gray		Aerial mycelium : poor; white~yellowish white~brownish white
(WAKSMAN 1919)	Potato		Growth : abundant, much wrinkled, cream-colored, with a faint greenish tinge developing in 15 days. Color of plug : purplish with age		Growth : good. Substrate mycelium : pale buff~dull yellow orange. Soluble pigment : none
	Milk		Coagulation : none		Coagulation : strong
	Conidium type on ammonium CZAPEK agar		Smooth		Spiny

IV. Comparison of the Strain of Streptomycete No. 751 with other Streptomycetes

In order to compare the characteristics of No. 751 with those of other *Streptomyces* species described so far, the cultures were grown on additional media to study their morphological and cultural characteristics: CZAPEK agar, potato peptone glycerol agar, bouillon agar, glucose bouillon agar, glycerol bouillon agar, glucose broth, peptone water, potato plug, carrot plug, egg, milk, etc. Moreover, milky, gelatinase, and cellulase reactions were carried out for comparative purposes.

No. 751 belongs to the 'Albus series' ^{2,3,4,13}, based on our interpretation of the

Table 3. Soluble, purplish-red pigment formation in mixed cultures
Medium: Glycerol starch glutamate agar

Mixed cultures with No. 751	Pigment formation
<i>Streptomyces albus</i> (IFO 3418)	+
<i>S. albus</i> (IFO 3195)	+
<i>S. griseus</i> (OEU No. 24)	+
<i>S. griseus</i> (RIMD Y-41)	+
<i>Mycobacterium tuberculosis</i>	-
<i>Escherichia coli</i>	+
<i>Bacillus subtilis</i>	+
<i>Candida albicans</i>	-

Table 4. Soluble, purplish-red pigment formation in mixed cultures
Medium: Potato peptone glycerol agar

Mixed cultures with No. 751	Pigment formation
<i>Myc. tuberculosis</i>	-
<i>Esch. coli</i>	+
<i>Bac. subtilis</i>	+
<i>Can. albicans</i>	-
<i>Staphylococcus aureus</i>	-
b	+
d	+

b: Bacteria-like microorganism, growing with No. 751

d: Yeast-like microorganism, growing with No. 751

Table 5. Soluble, purplish-red pigment formation in mixed cultures on various synthetic media

	Pigment formation		
	No. 751 +b*	No. 751 +d*	No. 751 +f*
Ammonium CZAPEK agar	-	-	-
Glycerol CZAPEK agar	+	+	-
Glucose asparagine agar	+	+	-
Calcium malate agar	-	-	-
Starch agar (WAKSMAN'S A)	-	-	-
Urea glycerol agar	-	-	-
Tyrosine agar (SHINOBU'S)	+	+	-
Glycerol starch glutamate agar	+	+	-

b: Bacteria-like microorganism growing with No. 751

d: Yeast-like microorganism growing with No. 751

f: Sterile type of No. 751

Table 6. Soluble, purplish-red pigment formation in mixed cultures on various synthetic media
(Other species of *Streptomyces* + b, d)

	Pigment formation	
	<i>S. alboflavus</i> <i>S. candidus</i> + b*	<i>S. alboflavus</i> <i>S. candidus</i> + d*
CZAPEK agar	-	-
Ammonium CZAPEK agar	-	-
Glycerol CZAPEK agar	-	-
Glucose asparagine agar	-	-
Urea glycerol agar	-	-
Glycerol starch glutamate agar	-	-

results of these studies. Unfortunately there are so many species which resemble No. 751 that it is difficult to compare characteristics. Nevertheless the species which appear to closely resemble No. 751 are:

- (1) *Streptomyces spheroides*¹¹,
- (2) *S. No. 160*⁵ (KUROYA *et al.* 1958),
- (3) *S. mitakaensis*¹¹,
- (4) *S. siyoaensis* (H-690)⁷,
- (5) *S. flocculus*^{2,13},
- (6) *S. alboflavus*^{2,4,13},
- (7) *S. candidus*^{2,4,11,13},
- (8) *S. albus*^{2,3,4,12,13}.

As the former four are different from No. 751 in many points of the cultural characteristics, No. 751 is one species and the former four are another.

We can find no morphological descriptions of *S. flocculus*, and the physiological and cultural descriptions of it are too brief to compare the characteristics of *S. flocculus* with those of No. 751.

The differences of the characteristics between *S. alboflavus*, *S. candidus*, *S. albus*, and No. 751 are as follows (Table 2): The most distinct characteristic of No. 751 is that it produces a soluble, deep purplish-red pigment when it is cultivated on some media conjointly with certain microorganisms. The results of our studies on this phenomenon are given in Tables 3, 4, 5 and 6. *S. alboflavus*, *S. candidus*, and *S. albus* have no such characteristics. Therefore, we propose *Streptomyces propurpuratus* is a new species because of its production of the soluble, deep purplish-red pigment conjointly with other microorganisms.

The species epithet is derived from Greek prefix (pro-, in front of *propurpuratus*, prior to; Latin adj. *purpuratus*, provided with purple; M. L. adj. prior to providing with purple).

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