STREPTOMYCES AURANTICOLOR SP. NOV., A NEW ANTICOCCIDIAL ANTIBIOTICS PRODUCER

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A new species of *Streptomyces* is described and designated *Streptomyces auranticolor* (FERM-P No. 5365) which produces new anticoccidial antibiotics, designated as WS-5995 A and WS-5995 B[1]. The organism is characterized by gray spore mass color, spiral spore chain with smooth spores, non-chromogenic reaction, soluble pigment, and carbon utilization characteristics. It differs from previously described streptomycetes on the basis of carbon utilization, and pigment production.

In the course of screening for new antibiotics, the interesting anticoccidial antibiotics, WS-5995 A and WS-5995 B, were discovered in a culture broth of strain No. 5995 which was recently isolated from a soil sample collected at Mt. Takao, Tokyo Prefecture, Japan. This report contains a description of this organism and a discussion of its taxonomic position.

Materials and Methods

The organism was selected from a series of soil isolations that were plated at dilutions of $1:10^2$ to $1:10^4$ on media for isolation.

The methods and media recommended by the International *Streptomyces* Project (ISP)[2] were used primarily, along with several supplementary tests. All tests were carried out at 28°C.

Microscopic observations were made on cultures that were grown for 7~21 days on sucrose-nitrate, glycerin-asparagine, starch-inorganic salts, yeast-malt extract and oatmeal agar media. Sporophore morphology was observed on undisturbed plate cultures and carbon shadowing technique was used to obtain electron micrograph.

Colony characteristics were observed on slant cultures after 7 and 21 days of incubation using 12 kinds of media. The formulas for BENNETT's, glucose-asparagine, sucrose-nitrate and nutrient agars are those described by WAKSMAN[3].

Temperature range for growth was determined on BENNETT's agar slants using a temperature gradient bio-autorecorder (Toyo Kagaku Sangyo Co. Ltd.). Gelatin liquefaction was examined at 21 days on a medium composed of 20% gelatin, 2% glucose and 0.5% peptone. The medium was refrigerated after incubation to detect liquefaction. Starch hydrolysis was observed by the starch-iodine reaction after incubation on starch-inorganic salts agar plates at 28°C for 7 days. Melanin production was determined on agar slants of tyrosine, peptone-yeast iron, and other organic media, especially tryptone-yeast extract broth. Carbon utilization tests were made according to the PRIDHAM-GOTTLIEB method[2].

The procedure of BECKER *et al.*[4] was used for preparation of cells and for the chromatographic detection of the isomers of diaminopimelic acid.

Results

Strain No. 5995 produces vegetative mycelium, which does not fragment into spores, and aerial mycelium, which later forms spore chains. After 10 days of incubation at 28°C, the spore chains appear

Table 1. Cultural characteristics of strain No. 5995.

Medium	Characteristics
Sucrose-nitrate agar	AM: none VG: pale yellow, small colonies SP: pink
Glucose-asparagine agar	AM: light gray to slightly greenish, cottony VG: pale yellowish brown, small colonies SP: orange
Glycerin-asparagine agar	AM: light gray, powdery VG: pale yellow to pale yellowish brown, wrinkled colonies SP: pale orange
Starch-inorganic salts agar	AM: light gray, powdery to short cottony VG: yellowish brown to olive gray, small colonies SP: none
Tyrosine agar	AM: light gray to whitish gray, powdery to short cottony VG: yellowish brown, wrinkled colonies SP: reddish orange
Nutrient agar	AM: none VG: colorless to pale yellow, small colonies SP: none
Yeast-malt extract agar	AM: white to whitish gray, powdery VG: pale yellow to pale yellowish brown, wrinkled colonies SP: none
Oatmeal agar	AM: light gray to gray, powdery to short cottony VG: colorless to pale yellow, small colonies SP: dull orange
Peptone-yeast iron agar	AM: none VG: colorless to cream, small colonies SP: none
BENNETT's agar	AM: gray, powdery to short cottony VG: pale yellowish brown, slightly wrinkled colonies SP: dull reddish orange
Glucose-peptone gelatin	AM: none VG: colorless to pale yellow, wrinkled colonies SP: none
Milk	AM: none VG: weak growth SP: none

Symbols: AM, aerial mycelium. VG, vegetative growth. SP, soluble pigment.

spiral in form with 5~50 spores per chain. The spore chain morphology is classified in the Spirales Section (Fig. 1). The spores are ellipsoidal to short cylindrical, averaging 0.5~1.1 by 0.9~1.7 μm in size. The spore surface is smooth (Fig. 2).

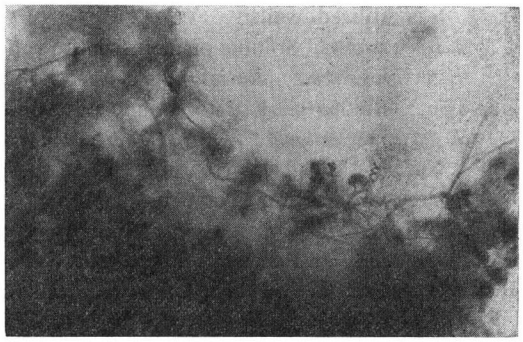
The cultural characteristics and physiological properties are shown in Tables 1 and 2, respectively. On most media, strain No. 5995 develops pale yellow to yellowish brown moderate vegetative growth and the aerial mycelium is powdery to short cottony and light gray. The reverse color of colonies is not pH sensitive. Melanoid pigment is not produced in tyrosine agar or peptone-yeast iron agar. An orange pigment is found in the medium in glycerin-asparagine agar, oatmeal agar and in certain other media. The

Table 2. Physiological properties of strain No. 5995.

Property observed	Characteristics
Temperature requirement	growth from 15°C to 40°C good sporulation at 28°C
Gelatin liquefaction	no liquefaction
Action on milk	no coagulation, no peptonization
Starch hydrolysis	hydrolyzed
Melanin production	none
Cell wall type	I (containing LL-DAP)
Carbon utilization	
L-Arabinose	+
D-Xylose	±
D-Glucose	+
D-Fructose	+
Sucrose	+
Inositol	−
L-Rhamnose	+
Raffinose	±
D-Mannitol	+
Mannose	+
Salicin	−
Galactose	+
Lactose	−
Maltose	+
Glycerin	+

Symbols: +, good utilization. ±, doubtful utilization. −, no utilization.

Fig. 1. Spore chain morphology of strain No. 5995 grown on yeast-malt extract agar: optical micrograph (×300).

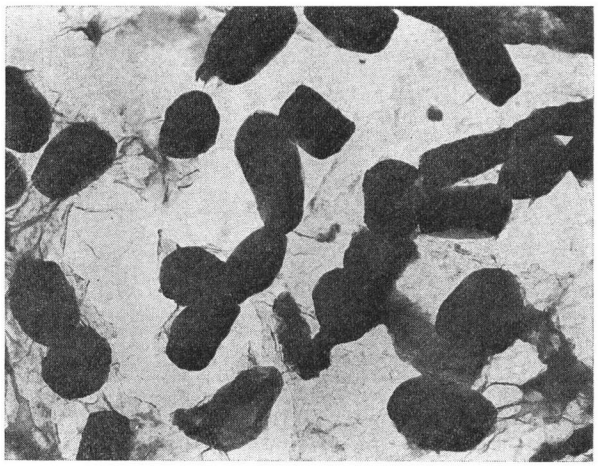


pigment is pH sensitive when tested with 0.05 N NaOH or HCl. The orange pigment is changed to yellow by 0.05 N HCl, and to red by 0.05 N NaOH. Strain No. 5995 hydrolyzes starch well, but the hydrolytic activity is not detected on gelatin or milk. Inositol, salicin and lactose are not utilized for growth. Utilization of D-xylose and D-mannitol is doubtful and whole cell hydrolysates contain LL-diaminopimelic acid.

Discussion

Microscopic studies and cell wall type indicate that strain No. 5995 belongs to the genus *Streptomyces*. Accordingly, a comparison of the organism was made with those of *Streptomyces* species described in BERGEY's Manual[5] and NONOMURA's key for classification[6]. From the above-mentioned information, strain No. 5995 is considered to be closely related to *Streptomyces minoensis*,

Fig. 2. Spores of strain No. 5995 grown on yeast-malt extract agar: electron micrograph (× 9,000).



S. saraceticus, *S. erythrogriseus*, and *S. griseoaurantiacus*. The above species can be differentiated as follows:

S. minoensis: Aerial mycelium is often flexous to hooked. Soluble pigment is seldom produced. Inositol is utilized well.

S. saraceticus: Soluble pigment is not produced. L-Rhamnose is not utilized for growth.

S. erythrogriseus: Aerial mass color is not only gray, but also red and white on glycerin-asparagine agar. Neither sucrose nor raffinose is utilized. Inositol is utilized well.

S. griseoaurantiacus: Orange pigment in medium is changed to red by 0.05 N HCl, to brown by 0.05 N NaOH. Inositol is utilized well and sucrose is not.

On the basis of the above comparisons, strain No. 5995 is considered a new species of genus *Streptomyces*. *Streptomyces auranticolor* is proposed for strain No. 5995, referring to the distinctive orange pigment in medium. The type strain has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, as FERM-P No. 5365.

References

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