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STREPTOMYCES WISTARIOPSIS SP. NOV.
A NEW VIOLET-SPORED SPECIES

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ABSTRACT. In a taxonomic study of 35 "violet-spored" streptomycetes certain essential differences were encountered among the different members. When the organisms were classified according to their sporophore morphology and spore surface configuration, they formed two distinct groupings. One of the groups was already represented by the species Streptomyces violascens Gauze; however, the other group contained organisms heretofore undescribed in the taxonomic literature. A new species Streptomyces wistariopsis is, therefore, proposed and described to represent this group.

The violet spore color series of the streptomycetes is comprised of notably colorful and unusual species having spore shades ranging from violet to lilac (Tresner and Backus 1963). In our experience, members have been isolated mostly from high latitude soils under forest cover. As far as can be determined only two species have been adequately described in the taxonomic literature—Streptomyces violascens Gauze 1957 and S. mauvecolor Okami et Umezawa (in Murase et al. 1961). Based on their published descriptions, these two species appear to be synonymous, in which case the name S. violascens should have priority.

A considerable wealth of violet-spored specimens has been accumulated over the years in our antibiotic screening program. More than half of our isolates are strains of S. violascens. In the remainder, certain significant taxonomic features are found which cannot be encompassed by this species. The present work was undertaken with the aim of establishing the new specific category necessary for accommodating these organisms and distinguishing them from the already established species.

MATERIALS AND METHODS

The cultures used in the present study were comprised of both soil isolates and standard reference cultures. The cultural and morphological features of the organisms were observed after they were grown at 28°C for 14 days on the following agar media recommended for taxonomic study by Pridham et al. (1957): potato dextrose, Bennett's, tomato paste oatmeal, Kuster's oatflake, yeast extract, asparagine dextrose, Czapek's solution, and Hickey and Tresner's. Additional observations were made on Pridham and Gottlieb's (1948) basal medium supplemented with various carbon sources. The color terminology used for descriptive purposes was taken from the Color Harmony Manual (Jacobson et al. 1948).

Micromorphology of the spore-bearing appendages was determined by light microscopy, whereas that of the spores was observed by electron microscopy at 8000X.

Utilization of carbon sources was assessed according to the methods of Pridham and Gottlieb (1948); nitrate reduction was measured according to procedures described in "A Manual of Methods of Pure Culture Study of Bacteria" (1946); chromogenic responses were determined on peptone-iron agar (Tresner and Danga 1958); and tolerance to a graded series of NaCl concentrations (4-13%) was determined in a yeast extract agar medium, the formula of which is given by Pridham et al. (1957).

RESULTS AND DISCUSSION

Thirty-five violet-spored streptomycetes were studied with respect to several of the more useful diagnostic criteria. When classified according to the morphology of their sporiferous appendages and their spore surface configuration, two distinct groups were evident, I) cultures with straight to flexuous chains of smooth-walled spores (Fig. 1a and Fig. 2), and II) cultures having spore chains as hooks, loops, coils or spirals with spiny spores (Fig. 1b and Fig. 3).

In Group I there were 14 cultures, all of which had been isolated from soils originating in the northeastern United States (Maine, New Hampshire, New York); they were typified by isolate AT-628 which was obtained from New York State. The specific binomial *Streptomyces wistariopsis* n. s. was proposed for members of this group to denote the similarity of their spore color to that of the inflorescences of

Figure 1. a. Sporiferous appendages of S. wistariopsis NRRL B-3433 by light microscopy.
b. Sporiferous appendages of S. violascens NRRL B-3434 by light microscopy.

Figure 2. Smooth-walled spores of *S. wistariopsis* NRRL B-3433 by electron microscopy.

Figure 3. Spiny-walled spores of *S. violascens* NRRL B-3434 by electron microscopy.

the higher plant genus Wistaria. The type strain (AT-628) was deposited as NRRL B-3443 in the permanent collection of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

In addition to the major differences indicated above between the organisms in the two groups, members of Group I tend to be poorer in sporulation and they usually produce darker vegetative mycelium and diffusible pigments. On media which promote good sporophore development, the spore chains arise as long and rather straight appendages from stoloniferous hyphae. Frequently, verticils of simple or branched chains appear along the aerial hyphae at the periphery of colonies; however, the verticillate feature is not sufficiently well founded as to characterize the cultures. Although chromogenicity on peptone-iron agar was found in all strains of *S. wistariopsis*, some gave an extremely weak and sometimes erratic response. Detailed characteristics of this organism are given in Tables I, III, IV and V.

The 21 cultures in Group II were all identified as members of the species Streptomyces violascens Gauze. This species appears to be widely distributed; strains were iso-

lated from soils collected in Iowa, Maine, New York, Ohio and Wisconsin, as well as from Australia, Finland and Nova Scotia. As might be expected, strains from widely separated regions showed variability in some cultural characteristics. Also noteworthy, certain of the strains were not typically of the Spira sporophore type (Pridham et al. 1958) found in S. violascens, but tended to produce an abundance of coils and rudimentary spirals suggestive of the Retinaculum-Apertum type (Pridham et al. 1958).

Chromogenicity on peptone-iron agar was also variable with this species, an occasional strain giving only a very weak and sometimes erratic response. Illustrative of this was the streptomycete NRRL B-2256 which has been variously referred to as Streptomyces sp. on two occasions (Moore and Chapman 1959; Lyons and Pridham 1965) and in a third instance (Pridham 1965) it was assigned to the species S. violascens. Aside from its erratic chromogenic reaction on peptone-iron agar, it appeared to be reasonably typical of the species S. violascens.

The culture S. mauvecolor (ATCC 14911) expresses the same basic characteristics as S. violascens, and it appears to be very similar to a culture we have isolated from Australian soil.

A strain of S. violascens (Lederle AB-864), isolated from Wisconsin soil, was especially typical of the species and sporulated much better than the type culture (Gauze 3959/54). The Wisconsin strain is on deposit as NRRL B-3434. Detailed characteristics of this culture are given in Tables II, III, IV and V.

An examination was made of the growth responses of S. wistariopsis NRRL B-3433 and S. violascens NRRL B-3434 on the Pridham and Gottlieb (1948) basal medium supplemented with 19 individual carbon sources. Table V displays the patterns of utilization observed. However, we regard these as of limited utility in the differentiation of these organisms, since considerable variation in response to individual C-sources may be observed among different strains of each of these species.

Pridham and Gottlieb basal medium enriched with certain carbon sources enhanced sporulation and intensified spore pigmentation. In general, l-arabinose, dextrose, d(+) galactose, d(+) melibiose, and d(+) trehalose were the most favorable sources for both species. Sporulation in some strains of S. violascens was greatly enhanced by d(+) trehalose, whereas d(+) raffinose, d(+) melibiose, i-inositol,

Table I Cultural Characteristics of *Streptomyces wistariopsis* NRRL B-3433
 [Underscored descriptive colors taken from Color Harmony Manual, Jacobson et al (1948)]

Medium	Amount of Growth	Aerial Mycelium and/or Spores	Soluble Pigment	Reverse Color	Remarks
Potato Dextrose Agar	Good	Aerial growth yellowish-white. Sporulation very light.	Brownish; heavy	<u>Deep Brown</u> <u>Mahogany</u> (6 pl)	
Bennett's Agar	Moderate	Aerial growth whitish, becoming tinted with <u>Pale Lilac</u> (11 ca) in sporulation zones. Sporulation light.	Brownish; moderate	<u>Chestnut Brown</u> (4 ni)	
Tomato Paste Agar	Moderate	Aerial growth whitish, becoming tinted with <u>Pale Lilac</u> (11 ca) in sporulation zones. Sporulation light.	Brownish; heavy	<u>Oak Brown</u> (4 pl)	Forming sectors with better sporulation.
Kuster's Oatflake Agar	Moderate	Aerial growth whitish, becoming <u>Pale Lilac</u> (11 ca) to <u>Wistaria</u> (11 ec) in sporulation zones. Sporulation moderate	Yellowish-brown; light	<u>Topaz</u> (3 ne)	
Yeast Extract Agar	Moderate	Aerial growth whitish, becoming <u>Pale Lilac</u> (11 ca) to <u>Wistaria</u> (11 ec) in sporulation zones. Sporulation light.	Brownish; moderate	<u>Coca Brown</u> (5 ni)	
Asparagine Dextrose Agar	Moderate	Aerial growth whitish, with tint of <u>Pale Lilac</u> (11 ca) in sporulation zones. Sporulation very light.	Yellowish-brown; light	<u>Chestnut Brown</u> (4 ni)	
Czapek's Solution Agar	Very light	No aerial growth	None	Whitish	
Carvajal's Oatmeal Agar	Good	Aerial growth whitish, becoming <u>Wistaria</u> (11 ec) in sporulation zones. Sporulation good.	Brownish; heavy	<u>Chocolate</u> (4 ni)	Moderate yellowish exudate.

Table II Cultural Characteristics of Streptomyces violascens NRRL B-3434
 [Underscored descriptive colors taken from Color Harmony Manual, Jacobson et al (1948)]

Medium	Amount of Growth	Aerial Mycelium and/or Spores	Soluble Pigment	Reverse Color	Remarks
Potato Dextrose Agar	Good	Aerial growth yellowish-white, fluffy. Sporulation very light. Colonies raised, spreading.	Yellowish-brown; light	<u>Lt. Brown</u> (4 ng)	
Bennett's Agar	Moderate	Aerial growth whitish, becoming <u>Pale Lilac</u> (11 ca) in sporulating zones. Sporulation light.	None	<u>Yellow Maple</u> (3 ng)	
Tomato Paste Oatmeal Agar	Good	Aerial growth whitish, fluffy, becoming <u>Pale Lilac</u> (11 ca) in sporulation zones. Sporulation moderate.	Brownish-yellow; moderate	<u>Topaz</u> (3 ne)	
Kuster's Oatflake Agar	Good	Aerial growth whitish, fluffy, becoming <u>Orchid Mist</u> (10 ec) in sporulation zones. Sporulation good.	None	<u>Cinnamon</u> (3 le)	Colonies raised, spreading.
Yeast Extract Agar	Good	Aerial growth whitish, fluffy, becoming <u>Pale Lilac</u> (11 ca) in sporulation zones. Sporulation moderate.	None	<u>Lt. Spice Brown</u> (4 lg)	
Asparagine Dextrose Agar	Moderate	Aerial growth whitish, fluffy, becoming <u>Pale Lilac</u> (11 ca) in sporulation zones. Sporulation light.	None	<u>Lt. Wheat</u> (2 ea)	
Czapek's Solution Agar	Light	Aerial mycelium whitish, sparse. Tinted with <u>Pale Lilac</u> (11 ca). Sporulation very light.	None	Pinkish-white	
Hickey and Tresner's Agar	Good	Aerial mycelium whitish, fluffy, becoming <u>Pale Lilac</u> (11 ca) in sporulation zones. Sporulation moderate.	Brownish; light	<u>Dk. Spice Brown</u> (4 pl)	Moderate colorless exudate.

Table III Micromorphology of Spores and Spore-bearing Appendages

Culture	Aerial Mycelium and/or Sporiferous Structures	Spore Shape	Spore Size	Spore Surface
<u>S. wistariopsis</u> NRRL B-3433	Thin cobwebby aerial mycelium gives rise to straight sporiferous side branches which often appear somewhat verticillate.	Elliptical to elongate	Range 1.2-1.5 μ x 0.4-0.6 μ . Average 1.4 μ x 0.5 μ .	Smooth as determined by electron microscopy.
<u>S. violascens</u> NRRL B-3434	Long chains of spores arise from aerial mycelium; chains frequently branched. Distal portion of spore chains terminate in tight coils and well-formed spirals of several turns.	Elliptical to elongate	Range 0.8-1.1 μ x 0.3-0.5 μ . Average 1.0 μ x 0.4 μ .	Spiny as determined by electron microscopy.

Table IV Miscellaneous Physiological Tests

Culture	Medium	Incubation	Growth	Physiological Reaction
<u>S. wistariopsis</u> NRRL B-3433	Organic Nitrate Broth	14 days	Moderate	Moderate reduction of nitrate to nitrite.
	Peptone-Iron Agar	24 hours	Moderate	Melanoid pigments produced
	4-13% NaCl in Yeast Extract Agar	10 days		Tolerated a maximum of 4% NaCl
<u>S. violascens</u> NRRL B-3434	Organic Nitrate Broth	14 days	Good	Good reduction of nitrate to nitrite
	Peptone-Iron Agar	24 hours	Moderate	Melanoid pigments produced
	4-13% NaCl in Yeast Extract Agar	10 days		Tolerated a maximum of 7% NaCl

Table V Carbon Source Utilization* Patterns
(after 10 days incubation at 28°C)

Carbon Source	<u>S. wistariopsis</u> NRRL B-3433	<u>S. violascens</u> NRRL B-3434
Negative control	0	0
dextrose	3	3
l-arabinose	2	3
dulcitol	0	0
fructose	2	2
d(+) galactose	3	3
glycerol	3	3
l-inositol	3	1
lactose	3	3
d-mannitol	0	0
d(+) melizitose	0	0
d(+) melibiose	3	3
d(+) raffinose	3	3
salicin	0	3
Na-acetate	0	1
sucrose	0	0
d(+) trehalose	3	3
d(+) xylose	0	2
adonitol	0	0
l(+) rhamnose	1	0

*3 Good utilization

2 Fair "

1 Poor "

0 No "

lactose and d(+) galactose promoted sporulation best in individual cultures of *S. wistariopsis*.

It is hoped that the division of the violet-spored streptomycetes into two sections, and the proposal of the new species name will aid in the future classification of new isolates belonging to this spore color series.

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