STREPTOMYCES PRASINOSPORUS SP. NOV.
A NEW GREEN-SPORED SPECIES

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SUMMARY. An unusual green-spored streptomycete has been isolated repeatedly from soils collected in India. The organism differs sufficiently from existing green-spored members of the genus to be considered a new species. The name Streptomyces prasinosporus is proposed for the new organism. The specific epithet was chosen to be descriptive of its spores.

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The green-spored series of the streptomycetes, as delimited colorwise by Tresner and Backus (1963), comprises but a small segment of the genus in terms of number of species. Less than a dozen have been described with sufficient clarity as to definitely ally them with this color series. Hütter (1962) has proposed that, for reasons of synonymy, the number of species be reduced to two.

Members of this series can be delineated, for the most part, on the basis of spore morphology and on cultural differences. Sporophore morphology is not especially helpful, since all of the presently described species exhibit some degree of spiralling of spore chains.

It has been our observation that certain green-spored isolates, derived from soils studied in our antibiotic research program, differ from any presently defined species. Interestingly enough, when the sources of these cultures were traced, it was discovered that all had been obtained from soils collected in various geographical areas in India. Since these isolates appear to occupy a unique niche in the systematics of this color series, in that they are all chromogenic, it is our opinion that they should constitute a new species.

MATERIALS AND METHODS

Six of the green-spored Indian soil isolates, for which the descriptive epithet S. prasinosporus is proposed, were selected for taxonomic study and for comparison with the reference species S. hirsutus ETC 16660, S. prasinus ETH 13815, S. acrimycini Gause 7699 and S. prasinopilosus ETH 13765.

The media employed for the study of cultural and morphological features were those recommended by Pridham et al. (1957) for taxonomic study of Streptomyces. Cultures were incubated at 28°C for 14 days. Determinations of color characteristics were made with the aid of the Color Harmony Manual (Jacobson et al. 1948).

Micromorphology of the sporophores was determined by the system of Pridham et al. (1958), and spore surface morphology was studied by electron microscopy according to the methods of Tresner et al. (1961).

Among the physiological measurements made, carbon source utilization was assessed by the Pridham and Gottlieb (1948) technique; nitrate reduction was measured according to "A Manual of Methods of Pure Culture Study of Bacteria" (1946); and the chromogenicity was determined on peptone-iron agar by the Tresner et al. (1958, 1964) methods.

RESULTS AND DISCUSSION

Comparative studies of the S. prasinosporus isolates revealed a marked similarity among them with respect to the taxonomic features observed. Consequently, it was feasible to prepare a composite species description that encompassed any strain differences. Lederle strain BD-278, appearing to be highly representative of the species, was chosen as the type strain and has been deposited with the American Type Culture Collection as ATCC 17918.

Cultural characteristics, micromorphology, carbon source utilization and miscellaneous physiological reactions of the proposed species are presented in Tables I-IV, respectively. This group of isolates was found to be characterized by a greater tendency to form a reddish vegetative mycelium (reverse color) on various media than is typical for previously described species in this color series. A second distinguishing characteristic common to this group of isolates was their "chromogenicity" or capacity to produce melanoid pigments on peptone-iron agar (Table III).

Table I. Cultural Characteristics of *Streptomyces prasinosporus*
 [Underscored descriptive colors taken from Color Harmony Manual, Jacobson et al (1948)]

Medium	Growth	Sporulation (amount/color)	Soluble Pigment	Reverse Color
Asparagine Dextrose Agar	Light; restricted	Aerial mycelium scanty to none. No sporulation.	None	Various yellowish shades from Lt. <u>Melon Yellow</u> (3 ea) to <u>Parchment</u> (1½ db)
Hickey & Tresner's Agar	Moderate	Aerial mycelium whitish, becoming Lt. <u>Olive Drab</u> (1 li)(1½ li) to <u>Mistletoe Green</u> (2½ li) to <u>Olive Gray</u> (1½ ig) to <u>Mistletoe Gray</u> (2½ ih). Sporulation moderate to good.	None	From <u>Mustard</u> (2 ie) to <u>Rose Wine</u> (7½ le) to <u>Topaz</u> (3 ne) to <u>Beaver</u> (3 li) to <u>Cork Tan</u> (4 ie)
Potato Dextrose Agar	Light; restricted	Aerial mycelium and spores absent with most strains. When present, white, becoming <u>Olive Gray</u> (1½ ig) in sporulation zones. Sporulation light.	None	Yellowish white to <u>Rust Tan</u> (5 le)
Kuster's Oatflake Agar	Moderate	Aerial mycelium whitish, becoming Lt. <u>Olive Drab</u> (1 li)(1½ li) to <u>Mistletoe Green</u> (2½ li) in sporulating areas. Sporulation light to moderate.	Pinkish; light	Greenish gray with <u>Dusty Coral</u> (6 gc) to <u>Colonial Rose</u> (7 ic) in central areas.
Bennett's Agar	Light to moderate	Aerial mycelium absent with some strains. When present, white, becoming Lt. <u>Olive Drab</u> (1½ li) to <u>Olive Gray</u> (1½ ig) in sporulation zones. Sporulation light to moderate.	None	Various yellowish shades from <u>Mustard</u> (2 ie) to <u>Honey Gold</u> (2 ic)
Czapek's Solution Agar	Light	Aerial mycelium whitish, becoming Lt. <u>Olive Drab</u> (1 li)(1½ li) to <u>Olive Gray</u> (1½ ig) in sporulation zones. Sporulation light.	None	Greenish gray to grayish white shades.
Yeast Extract Agar	Moderate	Aerial mycelium whitish, becoming Lt. <u>Olive Drab</u> (1 li)(1½ li) to <u>Mistletoe Gray</u> (2½ ih) in sporulation zones. Sporulation good to heavy.	None	<u>Mustard</u> (2 ie) to <u>Rose Wine</u> (7½ le) to <u>Old Wine</u> (7½ ng) to <u>Oak Brown</u> (4 pi)
Tomato Paste Oatmeal Agar	Good	Aerial mycelium whitish, becoming Lt. <u>Olive Drab</u> (1 li) to <u>Mistletoe Green</u> (2½ li) to <u>Ivy</u> (24 ml) to <u>Olive Gray</u> (1½ ig) to <u>Mistletoe Gray</u> (2½ ih) to <u>Sage Gray</u> (24 ih) in sporulation areas. Sporulation heavy.	None	<u>Mustard</u> (2 ie)

Table II

Micromorphology of Streptomyces prasinosporus

Morphology of Spore Chains	Spore Shape	Spore Size	Spore Surface
Spores in contorted loops, coils and spirals of a few turns, often clumping or clustering together.	Elliptical to globose	0.8 - 1.3 μ x 0.6 - 0.8 μ	Mostly short hairs (0.1 - 0.5 μ); occasionally a suggestion of a few spines.

Table III

Miscellaneous Physiological Reactions of Streptomyces prasinosporus

Medium	Incubation	Physiological Reaction
Organic Nitrate Broth	7 days	Nitrate reduced to nitrite.
" " "	14 days	" " " "
Gelatin	7 days	Light liquefaction
"	14 days	Variable degrees of liquefaction.
Peptone Iron Agar	24 hours	Chromogenic

Table IV
Carbon Source Utilization Pattern of
Streptomyces prasinosporus

Carbon Source	Utilization
l-Arabinose	Fair to good
d-Fructose	Fair to good
i-Inositol	Good
d-Mannitol	Good
Sucrose	Poor to none
d-Xylose	Fair to good
Dextrose	Fair to good
l-Rhamnose	Fair to good
Salicin	Fair to good

Spore chains of the species were in the form of contorted loops, coils, and spirals of a few turns (Fig. 1). The spore surfaces when viewed at the magnifications afforded by electron microscopy, were observed to be ornamented with short hairs (0.1-0. μ) (Fig. 2). Comparison of the spores of this organism with those of the other hairy-spored species in this color series revealed closest resemblance to S. prasinopilus ETH 13765. The hairy spore-appendages of S. acrimycini Gause 7699, however, were considerably longer, ranging up to 3.0 μ . In a scheme based upon the three criteria—sporophore morphology, spore surface ornamentation and chromogenicity on peptone-iron agar—S. prasinosporus occupies a keystone position in the systematics of the green-spored streptomycetes. This is exemplified in the following key:

Figure 1. Spore chains of S. prasinusporus by light microscopy.

Figure 2. Spores of S. prasinusporus by electron microscopy.

I. Spore chains as hooks, coils or spirals (Spira division)

A. Cultures chromogenic

1. Spores hairy (Section I)

S. prasinosporus (ATCC 17918)

B. Cultures nonchromogenic

1. Spores spiny (Section II)

S. hirsutus ETH 16660

S. prasinus ETH 13815

2. Spores hairy (Section III)

S. acrimycini Gause 7699

S. prasinopilosus ETH13765

It may be noted that the chromogenicity exhibited by S. prasinosporus on peptone-iron agar sets this organism quite apart from the other nonchromogenic species. Since the measurement of chromogenicity on peptone-iron agar has been a reliable criterion in the systematics of most streptomycetes, it is believed that it should also bear prime importance in the present instance.

All the S. prasinosporus strains gave relatively uniform reactions in the physiological tests performed. Nitrates were readily reduced to nitrites; gelatin was partially liquefied and each gave a reasonably similar carbon source utilization pattern. It was observed that some of the other green-spored species exhibited different reactions on these tests. For example, S. acrimycini showed no nitrate reduction; S. hirsutus failed to liquefy gelatin; and S. hirsutus and S. prasinus were able to utilize sucrose readily.

In view of the uniformity with which the Indian soil isolates reacted in these tests and their homogeneity in other respects, the authors have concluded that new species status among the green-spored streptomycetes should be given these organisms.

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