

STREPTOMYCES SPECIES COMPRISING THE BLUE-SPORE SERIES

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ABSTRACT

TREJO, W. H. (Squibb Institute for Medical Research, New Brunswick, N.J.) AND R. E. BENNETT. *Streptomyces* species comprising the blue-spore series. *J. Bacteriol.* **85**:676-690. 1963.—The objective of this study was to define and delimit the streptomycetes of the blue-spored (*Viridochromogenes*) series. The series, as defined in this study, includes 11 blue and blue-green species. The green-spored species were excluded on the basis of morphology as well as color. It was proposed that NRRL B-1511 be designated as the neotype strain of *Streptomyces viridochromogenes* (Krainsky) Waksman and Henrici, and that IMRU 3761 be designated as the neotype for *Streptomyces cyaneus* (Krasilnikov) Waksman. Evidence was presented to show that physiological criteria cannot be used to differentiate these organisms below the series level. The major characteristics of the *Viridochromogenes* series are blue to blue-green spores borne in spirals, and chromogenicity (melanin-positive). Reverse color and spore morphology provide a basis for separation below the series level.

In recent years, many classification systems for *Streptomyces* species have been proposed, based on groupings of cultures along morphological or color lines. That organisms should be so grouped is a natural consequence of the species-group concept, which many workers today believe is the only rational approach to speciation in the genus *Streptomyces*.

The blue-spored streptomycetes, typified by *S. viridochromogenes*, were first considered as a species-group by Hesseltine, Benedict, and Pridham (1954). Prior to Hesseltine's system, the blue-spored cultures were described as distinct entities, and no attempt was made to consider them as a group.

The objectives of this paper are to: (i) define and delimit the blue-spored or *Viridochromogenes*

series; (ii) attempt to base species concepts upon the study of a large number of similar or closely related strains from diverse sources; and (iii) establish, where possible, ranges of variation for species within the group.

Any attempt to define the blue-spored series must be preceded by the statement that the spore-color groupings used in this paper are those of Pridham, Hesseltine, and Benedict (1958). The color described is that of the aerial mycelium en masse, and not that of the vegetative mycelium or any soluble pigment.

The blue-spored streptomycetes, as delineated by Hesseltine et al. (1954), included blue-, blue-green-, and green-spored forms. The color series appeared to be fairly homogeneous and centered around the species *S. viridochromogenes*. This species, originally described by Krainsky (1914), proved to be stable throughout the years. The *Viridochromogenes* series, as used in the present work, refers to an aggregate of strains or species either identical with or closely related to *S. viridochromogenes* (Krainsky) Waksman and Henrici, strain NRRL B-1511. Pridham et al. (1958) designated this species as the type for the blue-color series in the morphology section *Spira*. This was a fortunate designation, because it is the most commonly occurring type.

MATERIALS AND METHODS

Strains studied. The 62 strains used in this study represent 11 species described in the literature. Table 1 lists all the strains and their origins.

Morphology of sporophores and spores. The morphology of sporophores was determined by direct microscopic examination of 14-day-old petri dish cultures grown at 28 C on tomato-paste-oatmeal agar, yeast-malt extract agar, and inorganic salts-starch agar (Pridham et al., 1957). Cultures were assigned to the morphology sections of Pridham et al. (1958). The morphology of spores was determined by electron microscopy of impression mounts. For the sake of brevity, all color designations appearing in the descrip-

TABLE 1. *List of cultures studied*

Species	Origin	Strain
<i>Streptomyces viridochromogenes</i>	CBS-Millard	NRRL B-1511
	(Krainsky), Waksman and Henrici	NRRL B-1227 (ATCC 3356)
	Waksman and Curtis <i>A. viridis</i> Duché ex CBS	Kutzner H-46
	H. J. Kutzner soil isolates	K3, B14, 206, K4, K1, W1, K-69, Plotho, K172, G523, K70, W9, W8, K74
	C. W. Hesseltine	NRRL B-1132, S-721
	E. Baldacci nos. 13, 15, 21	NRRL 2192, 2194, 2200
	S. G. Bradley	S-45
	A. Capriotti	No. 5
	A. H. Hendrickson	AH-1101
	W. H. Trejo isolates	2-6, M2737, SC3642
<i>S. chartreusis</i>	Upjohn K-180	NRRL 2287
	J. Berger	4222-4
	E. Baldacci no. 20	NRRL 2199
	C. W. Hesseltine	S-111, S-70, S-160
	H. J. Kutzner	K160, K518
	W. H. Trejo isolate	5-1
<i>S. cyaneus</i>	Todorovic	Kutzner (H-112) IMRU 3761
	Kutzner isolate	K989
	C. W. Hesseltine	S-635
	W. H. Trejo	14-2
<i>S. azureus</i>	Soil isolates	M-3339, R-379
	C. W. Hesseltine	S-700
<i>S. caelestis</i>	A. Dietz	UC-2011
<i>S. curacoi</i>	M. Cataldi	A-5828
<i>S. bellus</i>	P. Margalith	NRRL B-2575
<i>S. coerulescens</i>	G. F. Gauze	4562
<i>S. glaucescens</i>	G. F. Gauze	8731
<i>S. coeruleorubidus</i>	G. F. Gauze	12531/54
<i>S. coeruleofuscus</i>	G. F. Gauze	5051/56
Green-spored species	Kutzner's group X	K666, K412, K924, K1075, K1084, K517, K1029, K1069, E-193, K805
	Soil isolate	M-3103

tions are based on the above media, and therefore media will not be indicated in each description.

Chromogenicity. Chromogenicity was determined by the ability of the organism to produce brown, dark-brown, or black soluble pigment on Shinobu's (1958) modification of Matsumoto's tyrosine agar and the tyrosine agar of Gordon and Mihm (1957).

Hydrogen sulfide production. Cultures were grown on Peptone Iron Agar (Difco) supplemented with 0.1% yeast extract, after the method of Tresner and Danga (1958). Presence of a bluish-black color in the medium within 6 to 24 hr indicated the production of H₂S.

Carbon utilization. The utilization of carbon compounds was determined by the method of Pridham and Gottlieb (1948), as modified by

TABLE 2. *Physiological characteristics of blue series*

Species	Chromogenicity	H ₂ S	Amylase	Protease	NO ₂ reduction	Carbon utilization										
						Mannitol	Inositol	Sorbitol	Xylose	Arabinose	Rhamnose	Fructose	Raffinose	Sucrose	Lactose	Glucose
<i>Streptomyces viridochromogenes</i>	+	+	V*	+	V	+	+	-	+	+	+	+	+	V	+	+
<i>S. cyaneus</i>	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+
<i>S. chartreusis</i>	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
<i>S. curacoi</i>	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+
<i>S. glaucescens</i>	+	+	H	+	+	+	+	-	+	+	+	+	-	H	H	+
<i>S. coeruleescens</i>	+	+	H	+	-	+	+	-	+	+	+	+	-	+	+	+
<i>S. coeruleorubidus</i>	+	+	H	+	+	+	+	-	+	+	+	+	-	+	+	+
<i>S. coeruleofuscus</i>	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+
<i>S. bellus</i>	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
<i>S. caelestis</i>	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
<i>S. azureus</i>	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+

* V = variable, + = positive, - = negative.

TABLE 3. *Morphological characteristics of the blue series*

Species	No. of strains	Color of reverse	Spore morphology
<i>Streptomyces viridochromogenes</i>	28	Bluish-green to greenish-black	Spiny
<i>S. cyaneus</i>	4	Bluish-purple	Spiny
<i>S. chartreusis</i>	9	Cinamon, light, amber to colorless	Spiny
<i>S. curacoi</i>	1	Same as above	Spiny
<i>S. glaucescens</i>	1	Same as above	Spiny
<i>S. coeruleescens</i>	1	Same as above	Spiny
<i>S. coeruleorubidus</i>	1	Reddish-brown	Spiny
<i>S. coeruleofuscus</i>	1	Brown	Spiny
<i>S. bellus</i>	1	Pink to orangish-pink	Spiny
<i>S. caelestis</i>	1	Tan to brown	Smooth
<i>S. azureus</i>	3	Colorless to light amber	Smooth

the Subcommittee on Taxonomy of the Actinomycetes (Gottlieb, 1958). The following carbon sources were tested: glucose, mannitol, inositol, sorbitol, xylose, arabinose, rhamnose, fructose, raffinose, sucrose, and lactose.

Proteolysis. Proteolytic activity was determined by the gelatin-plate method of Frazier (1926), and by the casein-plate method described by Gordon and Smith (1955).

Starch hydrolysis. Hydrolysis of starch was detected by flooding 14-day-old plate cultures of Pridham's inorganic salts-starch agar with Lugol's solution. Unhydrolyzed starch gave the characteristic blue starch-iodine reaction.

Nitrate reduction. Reduction of nitrates was determined by the routine procedure outlined

by the Society of American Bacteriologists Committee on Bacteriological Technic (Pelczar, 1957).

RESULTS

The strains used in this study are tabulated in Table 1 according to the type-species to which they have been assigned. Studies of sporophore morphology showed that all the blue and blue-green cultures were assignable to Pridham's section Spira. The green-spored strains, comprising Kutzner's (1956) group X, clearly differed in morphology, falling into the sections *Rectus flexibilis* (R.F) or *Retinaculum apertum* (R.A). Furthermore, all the blue and blue-green cultures were chromogenic, whereas the green-spored members were nonchromogenic.

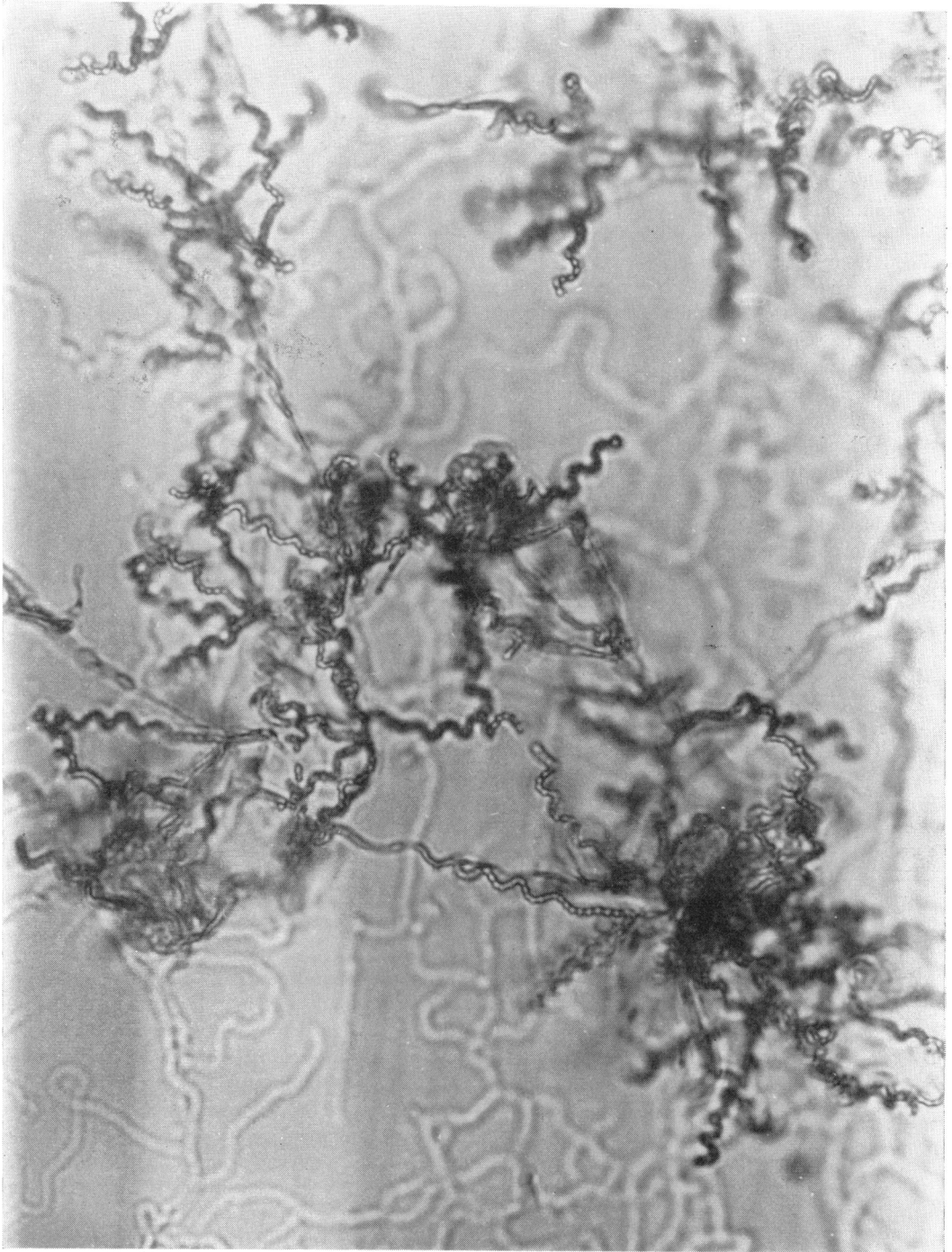


FIG. 1. Characteristic open spirals of *Streptomyces viridochromogenes*. Magnification: 770 X.

The physiological characteristics of the blue series are listed in Table 2. All 11 type-species showed a common pattern of carbon utilization, with only minor variations in the utilization of sucrose, mannitol, and raffinose. No importance can be attached to these variations since they were exhibited by species represented by only one strain each. As more strains are accumulated, it is likely that variations will occur. Our early collections of *S. viridochromogenes* were all sucrose-negative, as were the reference cultures. Currently, 12 of the 28 strains of this species are known to be sucrose-positive. All 11 species were chromogenic, proteolytic, and produced H_2S . The only differences among the 11 species were in their ability to reduce nitrate and hydrolyze starch. Physiologically, these cultures behaved as a homogeneous group. Physiological criteria cannot, therefore, be used to differentiate these organisms below the group level.

The morphological characteristics of the cultures comprising the blue series are summarized in Table 3. Two criteria appeared to provide a

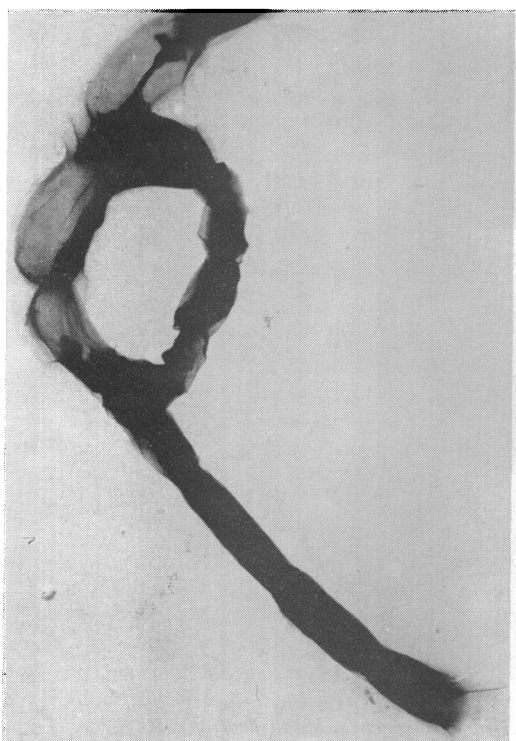


FIG. 2a. Smooth spores of *Streptomyces caelestis*. Magnification: 11,760 \times .

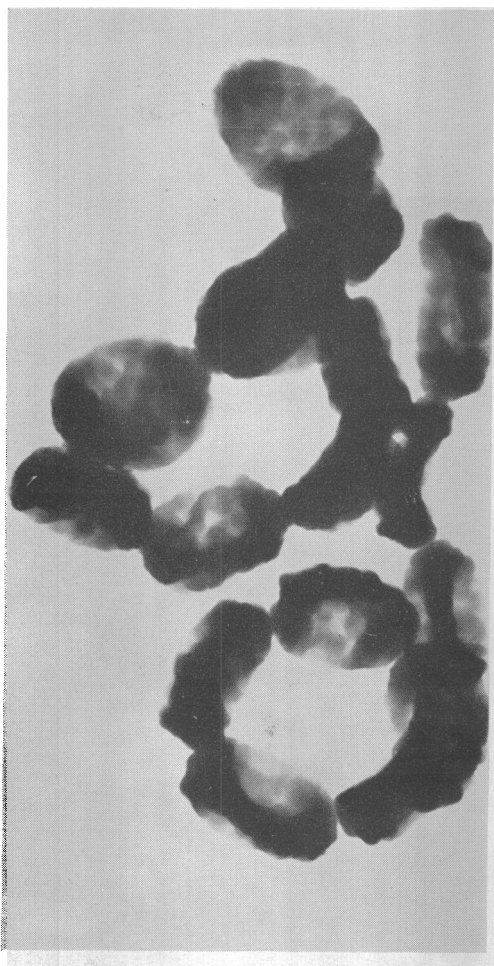


FIG. 2b. Smooth spores of *Streptomyces azureus*. Magnification: 20,400 \times .

basis for separation below the group level: reverse color and spore morphology. All the strains that we assigned to *S. viridochromogenes*, as well as the reference cultures of this series, exhibited characteristic bluish-green or greenish-black reverse color. This color is due to a dark-green insoluble pigment, extractable with acetone, which turns red when acidified. The strains differ quantitatively in color intensity, but the same pigment exists in them all.

Figure 1 shows the characteristic open spirals of the type-strain of *S. viridochromogenes*. The 28 strains assigned to this species exhibited some natural variation in length of spirals and degree of compactness. No importance was attached to rotation of spiralling. The spores were all spiny.

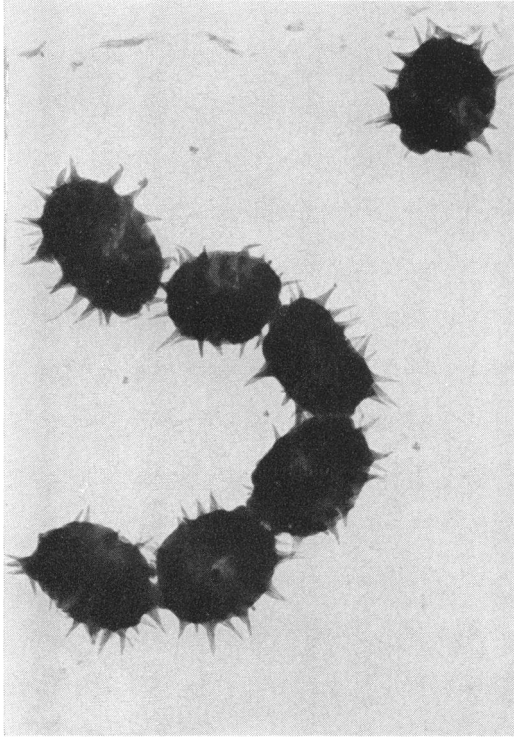


FIG. 2c. Characteristic spiny spores of *Streptomyces chartreusis*, contrasting to smooth-spored streptomycetes shown in Fig. 2a, b. Magnification: 23,290 \times .

S. cyaneus produced a bluish-purple pigment which did not change with alterations in the pH of the medium. The pigment was initially intracellular, but was released (probably by autolysis) into the medium as the culture aged. The spores were borne in compact spirals on short sporophores alternately arranged along the axial hyphae. The spores were all spiny.

The remaining species were characterized by having a reverse color in shades of cinnamon and buff, to almost colorless. They included *S. chartreusis*, *S. caelestis*, *S. azureus*, *S. bellus*, and *S. curacoii*. The new species described by Gauze et al. (1957) in the Coerulescens series also belong in this group. Members of this group differ primarily in spore morphology. As noted in Table 3, the spores of all species in this series were spiny, except those of *S. caelestis* and *S. azureus*, which were smooth (Fig. 2).

Mayama (1959) and Nomi (1960) considered the mode of branching to be taxonomically sig-

nificant. Any attempt to subdivide this subgroup on this basis is at best tenuous, since more than one type of spiral sometimes occurred within the same strain. Figure 3 shows the variations in spiralling in a strain of *S. chartreusis*. The spirals ranged from short tight spirals to loose open spirals. The mode of branching varied also, from single laterals to complex asymmetrical branches attached both laterally and terminally.

DESCRIPTION OF SPECIES

The following descriptions are based on our own study of the species comprising the blue-spored series. The descriptions, although in abbreviated form, contain all the pertinent criteria necessary to make a taxonomic judgment.

As we have already pointed out, the physiological criteria summarized in Table 2 are not of diagnostic value at the species level. Therefore, they are not included in the descriptions. The new species *S. curacoii* is described in more detail, since it has not been published previously, except in the patent literature.

Streptomyces viridochromogenes (Krainsky, 1914)
Waksman and Henrici. 1948

Proposed neotype culture. NRRL B-1511.

Synonymy. *Actinomyces viridochromogenes* Krainsky, 1914. *Actinomyces viridochromogenes* (Krainsky) emend. Waksman, 1919.

Excluded. *Streptothrix viridis* Lombardo-Pellegrino, 1903. *Actinomyces viridis* (Lombardo-Pellegrino, 1903) Sanfelice, 1904. *Actinomyces viridis* Millard and Burr, 1926. *Actinomyces viridis* Duché, 1934. Syn. *Actinomyces baarnensis* Duché, 1934. Hütter (1961) studied the holotype strain 145 obtained from Mme. Nicot in Paris. He considers this to be *Streptomyces griseus* Waksman and Henrici. *Actinomyces viridis* (Lombardo-Pellegrino) Sanfelice emend. Baldacci, 1939.

Morphology of aerial mycelium. Sporophores in open spirals varying in length and degree of compactness. Spores are oval to elliptical, and spiny.

Spore color. Blue to blue-green.

Reverse color. Bluish-green to greenish-black.

Antagonistic properties. Weakly antagonistic. Reported antifungal but activity was not evident in this study.

Ecology. This species is widely distributed in nature.

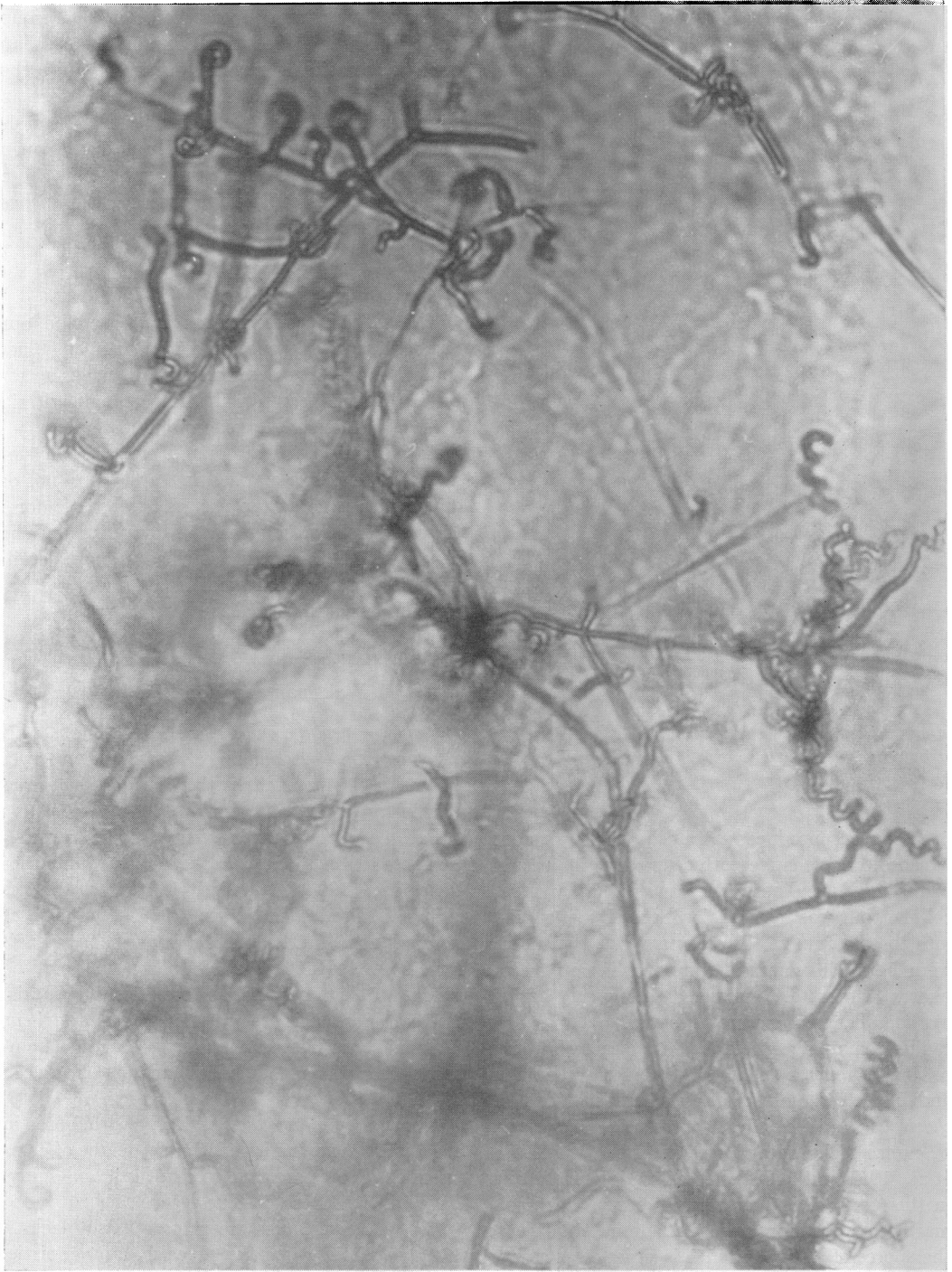


FIG. 3. *Various types of spiralling in Streptomyces chartreusis. Magnification: 820 X.*

Streptomyces cyaneus (Krassilnikov, 1941)
Waksman, 1953

Proposed neotype culture. Waksman's (1961) reference to IMRU 3761 as the type for this species did not refer to the original Krassilnikov strain but rather to the Todorovic strain obtained from Kutzner. Since the original Krassilnikov strain is not extant, we propose the designation of the Todorovic strain as neotype.

Synonymy. *Actinomyces cyaneus* Krassilnikov, 1941.

Morphology of aerial mycelium. Short sporophores producing compact spirals with two to three turns, alternately arranged along the axial hyphae. Spores are spiny.

Spore color. Bluish-gray to blue-green.

Reverse color. Bluish-purple, insoluble pigment not changing with pH of the medium.

Antagonistic properties. No activity.

Streptomyces chartreusis Calhoun and
Johnson, 1956

Type culture. NRRL 2287.

Morphology of aerial mycelium. Sporophores ranging from short tight spirals to loose open spirals. Spores are spiny.

Spore color. Bluish-gray to blue-green.

Reverse color. Shades of cinnamon, buff, cracker, and sudan [Maerz and Paul (1950) plates 12E7, 11K7, 13D6, and 13E4, respectively].

Antagonistic properties. Produces chartreusin.

Streptomyces coerulescens Gauze et al., 1957

Lectotype culture. Strain 4562 distributed by Gauze as representative of this species.

Morphology of aerial mycelium. Sporophores simple, occurring singly, or in pairs, terminating in short spirals of three to four turns. Spores are spiny.

Spore color. Blue to blue-green [light celandine green, Ridgway (1912) plate XLVII].

Reverse color. Brown [Maerz and Paul (1950), plate 16E10].

Antagonistic properties. Produces the antiviral cerulomycin of Braznikova et al. (1957) which is not to be confused with the caerulomycin of Funk and Divekar (1959), produced by *Streptomyces caeruleus* Baldacci.

Streptomyces glaucescens Gauze et al., 1957

Lectotype culture. Gauze strain 8731.

Morphology of aerial mycelium. Sporophores in short tight spirals. Spores are hairy.

Spore color. Blue to blue-green.

Reverse color. Moderate reddish-orange to wood-brown.

Antagonistic properties. None observed.

Streptomyces coeruleorubidus Gauze et al., 1957

Lectotype culture. Gauze strain 12531/54.

Morphology of aerial mycelium. Sporophores in long open spirals borne singly, in opposite pairs, or terminally. Spores are oval and spiny.

Spore color. Blue to blue-green.

Reverse color. Medium reddish-brown.

Antagonistic properties. None observed.

Streptomyces coeruleofuscus Gauze et al., 1957

Lectotype culture. Gauze strain 5051/56.

Morphology of aerial mycelium. Sporophores in short open spirals; some closed spirals present, but the open type are predominant. Spores are oval to elliptical, spiny.

Spore color. Blue to bluish-gray.

Reverse color. Moderate brown [Kelly and Judd (1955), ISCC-NBS 58].

Antagonistic properties. None observed.

Streptomyces bellus Margalith and Beretta, 1959

Type culture. NRRL B-2575.

Morphology of aerial mycelium. Sporophores in tufts terminating in hooks and primitive spirals, with some short closed spirals evident. Spores are oval to spherical, and spiny.

Spore color. Bluish-green.

Reverse color. Light cherry-pink to pinkish-orange.

Antagonistic properties. Produces matamycin.

Remarks. This culture bears close resemblance to *S. coeruleorubidus* and *S. bicolor* (Gauze et al., 1957).

Streptomyces curacoi sp. n. Cataldi, 1962

Type culture. ATCC 13,385.

Morphology of aerial mycelium. Sporophores in compact spirals averaging from five to seven turns, alternately arranged along the axial hyphae. Spores are 0.8 to 1.2 μ , spherical to oval, and spiny.

Spore color. Light greenish-blue.

Reverse color. Cinnamon to tobacco-brown.

Melanoid pigment. Positive.

Hydrogen sulfide production. Positive.

Proteolysis. Rapid.

Starch hydrolysis. Positive.

Nitrate reduction. Negative.

Carbon sources. Utilizes mannitol, inositol, xylose, arabinose, rhamnose, fructose, sucrose, lactose, and glucose; does not utilize sorbitol and raffinose.

Antagonistic properties. Produces curamycin.

Origin. Soil isolate from province of Pampa, Curacoi, Argentina.

Streptomyces caelestis DeBoer et al., 1955

Type culture. NRRL 2418.

Morphology of aerial mycelium. Sporophores in tufts terminating in hooks and simple spirals of one to two turns. Spores are elliptical to cylindrical, and smooth.

Spore color. Pale glaucous-blue to grayish-blue.

Reverse color. Cinnamon.

Antagonistic properties. Produces celesticetin.

Remarks. The similarity of this species to *S. glaucus* sensu Krassilnikov (1949) was suggested by Waksman (1961). However, *S. caelestis* is chromogenic and has primitive spirals of one to two turns, whereas *S. glaucus* is nonchromogenic and has compact spirals of three to five turns.

Streptomyces azureus Kelly, Kutscher, and Tuoti, 1959

Type culture. IMRU 3705.

Morphology of aerial mycelium. Sporophores produced singly, in pairs, and in clusters of spirals resembling verticils. These are not true verticils, since they are not symmetrically arranged along the axial hyphae. Spores are oval and smooth.

Spore color. Porcelain-blue to Medici-blue [Ridgway (1912) plates XLVIII, XXXIV; ISCC-NBS light greenish-blue (Kelly and Judd, 1955)].

Reverse color. Colorless to shades of light amber.

Melanoid pigment. Positive.

Hydrogen sulfide production. Positive.

Proteolysis. Strong.

Starch hydrolysis. Positive.

Nitrate reduction. Variable with strains.

Carbon sources. Utilizes mannitol, inositol, xylose, arabinose, rhamnose, fructose, raffinose, sucrose, lactose, and glucose; does not utilize sorbitol.

Antagonistic properties. Produces thiostrepton.

Remarks. The first-published use of the epithet *azureus* for this species is that of Kelly et al. (1959), and reference to the description of Pagano et al. (1955) is made. The combined specific

epithet and description appears in the patent issued to Donovan, Pagano, and Vandeputte (1961).

The remaining species in Gauze's *Coeruleus* series were not available for this study, but their descriptions indicated quite clearly that they fell into our concept of the *Viridochromogenes* series.

We excluded from the series *Streptomyces caeruleus* (Baldacci) Waksman. Although reported by some to be a member of the blue series, *S. caeruleus* really belongs to the gray series. The intense color saturation of the vegetative mycelium gives the appearance of a blue color but, in fully mature cultures, the gray spore color is evident. Further, our examination of Taber's culture of this species showed it to be in the section RF and nonchromogenic. Ecologically, this species represents a group of alkali-dependent cultures which appear to be characteristic of certain prairie soils in Canada (Taber, 1959, 1960).

We further excluded *S. glaucus* (Lehmann and Schütze, 1912, emend. Krassilnikov, 1941) Waksman, 1953, from the series because it is penicillin-green in spore color and nonchromogenic. This clearly differs from *Actinomyces glaucus* Lehmann and Schütze, 1907, which, according to Hütter (1962), is a monosporous thermophile.

DISCUSSION

The conflict surrounding the nomenclature of the species *S. viridochromogenes* stems from its confusion with *S. viridis*. A brief examination of the history of these species may explain how the same binomial came to be used for three presumably different cultures. Lombardo-Pellegrino (1903) described *Streptothrix viridis*, an actinomycete whose spore color was green to dark-green. Sporulation was by fragmentation, and it is not clear from the description whether spirals were formed. There is no mention of chromogenicity. Sanfelice (1904) emended the classification of this organism to *Actinomyces viridis*.

Krainsky (1914) described *A. viridochromogenes* as a bluish-gray, chromogenic culture. Waksman (1919) amended the original description to include the formation of spirals.

Millard and Burr (1926) described a new species pathogenic to potatoes but unfortunately used the pre-empted name *Actinomyces viridis*. Duché (1934), though cognizant of the work of Lombardo-Pellegrino and Sanfelice, described a

new species to which he, too, assigned the name *Actinomyces viridis*.

Baldacci (1939) studied the early descriptions and examined transfers of *A. viridochromogenes* (Krainsky) Waksman and Curtis and *A. viridis* Millard and Burr, obtained from the Centraalbureau voor Schimmelcultures (CBS), Baarn. Baldacci placed all these cultures in synonymy under *Streptothrix viridis* Lombardo-Pellegrino. He also redescribed the species, based on his study of these two cultures.

In our opinion, this synonymy is unjustified. The early descriptions of *A. viridis* by Lombardo-Pellegrino and Sanfelice are lacking in critical details of morphology and chromogenicity. Thus, they form an inadequate basis for assigning *S. viridochromogenes* to *A. viridis*. Furthermore, in redescribing the species *A. viridis*, Baldacci used two cultures obtained from the CBS, both of which are now known to be *S. viridochromogenes*. The strain of *A. viridis* Millard (ex CBS) which Baldacci examined is the same as *S. viridochromogenes* NRRL B-1511 (Table 1). The origin of this culture is not clear, but it is doubtful that it came from Millard. Further, we examined the *S. viridis* of Millard and Burr (IMRU 3372) and found it to agree with the original description (Millard and Burr, 1926). It is gray, RF, and chromogenic.

The illegitimacy of *Streptothrix* as a generic epithet for actinomycetes has already been established (Lessel, 1960). The uncertain application of the Lombardo-Pellegrino epithet, coupled with a description based on a misidentification, certainly would seem to be grounds for questioning the validity of *A. viridis* sensu Baldacci. Further, we cannot agree with Hütter (1961), who considers *A. viridochromogenes* Krainsky, 1914, a synonym of *S. viridis* (Lombardo-Pellegrino) Waksman, 1953. His synonymy is based on a study of the CBS strain of *S. viridochromogenes* Waksman and Curtis. This strain is identical with Kutzner's H-46 (Table 1), and is a perfectly typical *S. viridochromogenes*. The Waksman and Lechevalier (1953) description of *S. viridis* (Lombardo-Pellegrino) refers to a gray, RF culture with green vegetative mycelium. We have, therefore, excluded these and retained *S. viridochromogenes* sensu (Krainsky) Waksman and Henrici.

In this study, we have attempted to define and delimit our concept of the Viridochromogenes

series. This series encompasses the Viridochromogenes series XIII of Waksman (1961), which is essentially an acceptance of Kutzner's (1956) group IX. It includes the Azureus color group of Ettlenger, Corbaz, and Hütter (1958), the glaucus group of Hütter (1962), Baldacci's (1957) series Viridis and Caeruleus, and Gauze's series Coerulescens. Obviously, the Viridochromogenes series is represented by a multiplicity of names. Regrettably, a good deal of the confusion arising in this group stems from the unfortunate intermingling of species epithets and series designations. It is not in the province of this paper to reject names or to question their validity. It is our expressed hope, however, that, by bringing together all these culture descriptions, certain similarities will become apparent. There is little doubt that some of the species in this group ultimately will have to be relegated to synonymy.

For the sake of simplicity and convenience, we have used the Viridochromogenes-series designation and expanded it to include cultures not included by Waksman (1961) in his latest revision of the actinomycetes. Cognizant of the desirability that each group be designated by a representative species, we followed Pridham in his selection of *S. viridochromogenes* as the type. As we previously noted, *S. viridochromogenes* is not only the most commonly occurring type in the series, but is also the first validly published species and the nomenclatural type.

We propose NRRL B-1511 as neotype for the species *S. viridochromogenes* in preference to NRRL B-1227, which was proposed by Hütter (1962). We base our selection of NRRL B-1511 on the fact that, in our studies, the majority of isolates collected resembled this strain. We found NRRL B-1227 to be atypical in its mode of sporulation. This has been confirmed by Pridham (*personal communication*). Since the origin of these strains is vague, and neither may be claimed to be a holotype, considerations of priority do not enter into the question. Therefore, we submit that, if a neotype is to be designated, it be a typical strain representative of the species.

Hesseltine et al. (1954) included blue-, blue-green-, and green-spored forms in their Viridochromogenes group. Kutzner (1956) and Ettlenger et al. (1958) separated the green-spored forms from the blue and blue-green on the basis of color. We concur with this separation but base our opinion on morphology as well as on color.

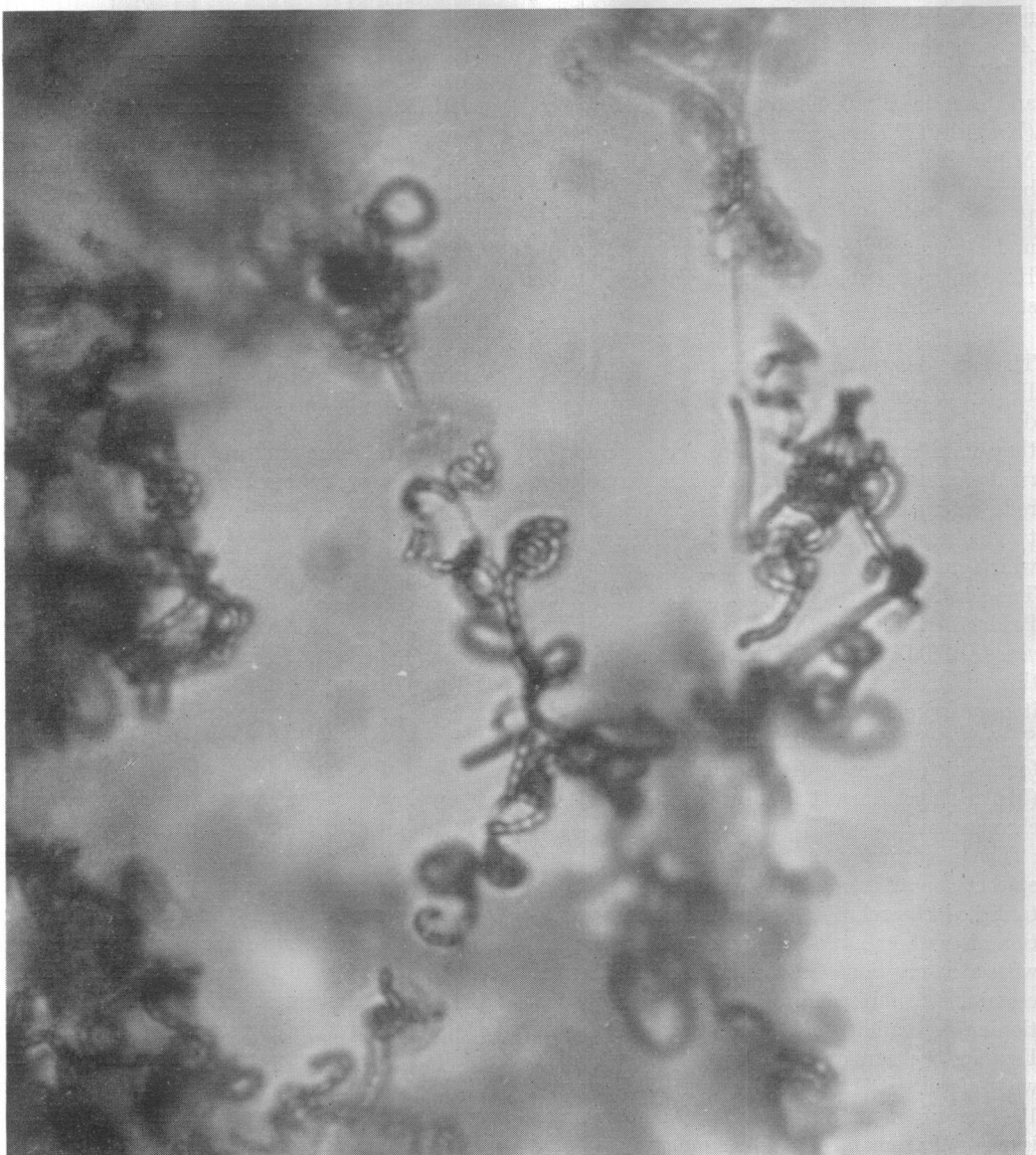


FIG. 4a. Clustered spiralling of *Streptomyces azureus*. Magnification: 640 \times .

All the green-spored cultures we studied were either in the RF or RA morphology sections of Pridham, and were nonchromogenic. This includes the species *S. hirsutus*, *S. prasinus*, and *S. prasinopilosus*, previously described by Ettlinger et al. (1958) and Hütter (1962).

The major characteristics of the Viridochromogenes series are blue to blue-green spores borne in spirals, and chromogenicity (melanin-positive).

Reverse color provides a basis for separation below the group or series level. *S. viridochromogenes* has a dark-green to greenish-black reverse color, whereas that of *S. cyaneus* is bluish-purple, and those of *S. chartreusis* are in shades of light amber, cinnamon, and reddish-orange to brown. Within the *S. chartreusis* subgroup, the species *S. chartreusis*, *S. azureus*, and *S. caelestis* are distinguishable on the basis of spore morphology.



FIG. 4b. Simple sporulation of *Streptomyces caelestis*, contrasting to clustered spiralling of *S. azureus* shown in Fig. 4a. Magnification: 955 \times .

S. chartreusis is characteristically spiny-spored, whereas the other two species are not. *S. azureus* and *S. caelestis*, both smooth-spored, are distinguishable from one another on the basis of

sporophore morphology. Figure 4 shows the abundant cluster of spirals of *S. azureus* in contrast to the simple hooked sporulation of *S. caelestis*.

TABLE 4. Known antibiotics produced by members of the *Streptomyces viridochromogenes* series

Species	Antibiotic
<i>S. chartreusis</i>	Chartreusin
<i>S. bellus</i>	Matamycin
<i>S. curacoi</i>	Curamycin
<i>S. coeruleus</i>	Cerulomycin
<i>S. azureus</i>	Thiostrepton
<i>S. caelestis</i>	Celesticetin

By the criteria employed in this study, *S. bellus*, *S. curacoi*, and the new species in Gauze's Coeruleus series can scarcely be distinguished from *S. chartreusis*. They differ solely in antibiotic production (Table 4). This criterion alone is an inadequate basis for speciation. Ciferri (1959) proposed that species designations be accepted only if they differ in at least two unrelated characteristics. This certainly must be the minimal requirement.

Grouping by color alone is arbitrary and subjective. Color becomes taxonomically significant when it can be correlated with other features or characteristics. Sanchez-Marroquin (1962) proposed the reduction of spore-color ranges to four. He would combine the green-, gray-, and blue-spored forms. This proposal, in our opinion, is an oversimplification that would result in great confusion. It would bring together completely unrelated groups of species and would disregard such significant characters as morphology and chromogenicity. One need only consider the gray-spored streptomycetes to realize the heterogeneous collection of species encompassed. As Waksman (1959) pointed out, the separation of the genus *Streptomyces* into groups or series accomplishes nothing more than the raising of some of the important species to subgeneric status. It becomes apparent, then, that we are no closer to speciation if our series designations are so large and heterogeneous as to be unworkable.

We feel that the concept of the *S. viridochromogenes* series presented here represents a natural group of closely related species from which a rational speciation will eventually emerge. The 28 strains of *S. viridochromogenes* and the 9 strains of *S. chartreusis* exhibit a sufficiently wide range of variability to establish reasonable species concepts. The remaining species are represented by too few isolates to make any definitive

judgment regarding speciation at this time. However, these species have been validly published and represent nomenclatural types which are distinguishable entities, as evidenced by this study. Until more isolates of each of these species appear, it is premature to question their specific status.

The proposed neotype strain of *S. viridochromogenes* has been deposited in the American Type Culture Collection, and is numbered 14920. The neotype *S. cyaneus* strain is numbered ATCC 14923.

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