STREPTOMYCES COELICOLOR MÜLLER AND *STREPTOMYCES VIOLACEORUBER* WAKSMAN AND CURTIS, TWO DISTINCTLY DIFFERENT ORGANISMS

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In 1908, Müller described a culture of an actinomycete, isolated as a dust contaminant on a potato plug. This organism, which produced a blue soluble pigment on this medium, was designated *Streptothrix coelicolor*.

In 1916, Waksman and Curtis described a culture of an actinomycete freshly isolated from the soil, which produced a red and blue pigment on several media, both synthetic and organic in nature, as *Actinomyces violaceus ruber*. Due to an unfortunate error, the word "ruber" was left out in the description (but not in the tables and in the key given in this publication). As a result the name *Actinomyces violaceus* was used for this species in the first and second editions of *Bergey's Manual of Determinative Bacteriology*. In the third edition of the *Manual*, Bergey personally changed the name of the organism to *Actinomyces waksmanii*. In the meantime, attention was called to the similarity of this organism to that described by Müller, resulting in a change in name to *Actinomyces coelicolor* (Müller) Lieske, recorded in the fourth and fifth editions of the *Manual*. Finally, with the establishment of the genus *Streptomyces*, the name was changed to *Streptomyces coelicolor* (Müller) Waksman and Henrici in the sixth and seventh editions, and in Waksman and Lechevalier (1953).

Meanwhile, numerous other species and names were proposed for what appeared to be the same or similar organisms, all producing a blue pigment, some of them only on special media and under certain conditions of culture. In several cases, isolation and characterization of the pigment was made, and in some cases, it was actually obtained in a crystalline state.

A list of all the described species of *Streptomyces* producing a blue pigment is given in table 1. The nature of the pigments isolated from these species and from various unnamed strains belonging to this group is given in table 2.

Most of the pigmented preparations have indicator properties, blue at an alkaline reaction and red at an acid reaction. Amylocyanin and a second unnamed and uninvestigated substance isolated from *Nocardia cyanea* are said to become red at an acid reaction and green at an alkaline reaction; in addition, these two pigments show some similarity with respect to their solubility. The pigment produced by some strains of Streptomyces, such as *S. cyaneus*, does not change in color with increasing or decreasing H-ion concentration.

As a result of the indicator properties of the pigments, the same culture may show a very different appearance on different media; this may be due either to different initial pH values or to different concentrations of acid produced by the organism (Conn and Conn, 1940*a*, *b*, 1941; Conn, 1943; Cochrane and Conn, 1947). This fact, however, did not seem to result in too much confusion within the group as a whole. Rather it sometimes rendered more difficult the differentiation between this group and the many strains and species of actinomycetes which show a blue-violet to blue-red substrate growth, and sometimes excrete the same type of pigment, but do not produce soluble pigments which are distinctly blue. Several strains named *S. violaceus* (Rossi-Doria) Gasperini appear to belong to *S. violaceoruber*; this is true, for example of Ciferri's strain of *S. violaceus* deposited in the Centraalbureau voor Schimmelcultures (CBS) (Kutzner, 1956). A similar case is also seen with the species listed in table 1. A strain received from Baldacci, under the name *A. novaecaesareae*, and regarded

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TABLE 1

Streptomyces species, producing a blue pigment

Organism	Color of Aerial Mycelium	Spirals	Spore Surface	Melanoid Pigment	Author
S. caeruleus	Light grayish blue	−			Baldacci, 1944
S. coelicolor[a]	Grayish yellow	−	Smooth	−	Müller, 1908
S. cyaneofuscatus[b]	Greenish-grayish yellow	−		−	Gause *et al.*, *1957*
S. cyaneus[a,c]	Bluish gray to blue	+	Spiny	+	Krassil'nikov, 1949
S. cyanoflavus	Greenish brownish-gray	−		+	Funaki *et al.*, 1958
S. litmocidini[b]	Gray, sometimes with brownish tinge	− (seldom +)			Gause *et al.*, 1957
S. novaecaesareae (= *S. violaceus caesari*)	White with purple tinge due to substrate mycelium	+		−	Waksman and Curtis, 1916; Waksman, 1919
S. pluricolor[d]	Whitish gray	+			Berestnev, 1897; Krassil'nikov, 1949
S. tricolor	Light brown to light gray	+			Wollenweber, 1920, 1921
S. violaceoruber[e]	Ash gray	+	Smooth	−	Waksman and Curtis, 1916; Waksman, 1919
S. olivaceus	Ash gray	−	Smooth	−	Corbaz *et al.*, 1957
Streptomyces sp., no. 169	Mouse gray			+	Kurosawa, 1951

[a] These data are taken from Kutzner (1956).

[b] Designated as *Actinomyces cyanofuscus* and *A. litmocidini*, respectively.

[c] The description is based on two strains, one of them was obtained from Todorovic and regarded by him as *S. cyaneus*, the other isolated by Kutzner.

[d] *Actinomyces pluricolor diffundens* Berestnev (1897) = *Act. pluricolor* (Berestnev) Krassil'nikov (1941).

[e] According to recent practice the name *S. violaceus ruber* is changed to *S. violaceoruber*.

by him as belonging to the *S. violaceus* series, proved to be identical with *S. violaceoruber* (Kutzner, 1956). One of the strains of Conn and Conn (1941), "R_1," probably does not belong to *S. violaceoruber*, since the pigment changed from yellow in acid to violet in an alkaline solution.

It often cannot be decided from the data reported whether there are other duplications under the species listed in table 1. *S. tricolor*, for example, seems to be closely related to *S. violaceoruber*, and this seems to be also true of *S. pluricolor*. *A. cyaneofuscatus*, recently described by Gause *et al.* (1957) seems to be closely related to *S. coelicolor* Müller; *Streptomyces* sp., strain 169 (Kurosawa, 1951), shows some similarity to *S. cyanoflavus*. No final decision can be made without a direct comparison of the cultures. All the other named species seem to be more or less distinct.

Corbaz *et al.* (1957) reported on the differences between several substances isolated from blue pigmented cultures (table 2). These authors state that, in spite of their differences in chemical and antibiotic properties, "the composition and absorption spectra are, as far as known, very similar, so that we have to do with chemically similar compounds or even with different substances originating from the same precursor." The results of Kriss (1936*a*) who regarded the pigment of one of his cultures as an anthocyanin, were rigorously criticized by Erikson *et al.* (1938), who did not believe in the occurrence of anthocyanins in microorganisms. However, the cultures used by these two investigators were not the same. An anthocyanin, containing glucose and rhamnose as sugar components, was found by Frampton and Taylor (1938) in a culture regarded as *S. violaceoruber*. Further attention may, there-

TABLE 2

Blue pigmented substances produced by actinomycetes

Preparation	Organism	Melting Point	Solubility	Author
Amylocyanin	*Streptomyces coelicolor*		In water and in dimethylformamide; insoluble in other solvents[a]	Müller, 1908
Litmocidin	*Nocardia cyanea*[b]	144–146	Slightly soluble in water at an acid reaction and extracted from it by ethanol, ether or amylacetate	Gause, 1946; Brazhnikova, 1946
Coelicolorin	*S. coelicolor*[c]	142–146	Very soluble in acetone, ethylacetate, or chloroform; soluble in ethanol, methanol, benzene, or ether; insoluble in petroleum ether	Kominami, 1949; Hatsuta, 1949
Cyanomycin	*Streptomyces cyanoflavus*	128	Extracted from water at an alkaline reaction by chloroform or methylenechloride	Funaki *et al.*, 1958
Granatacin	*Streptomyces olivaceus*	204–206	Extracted from water at an acid reaction by acetone; soluble in ethylacetate, and dimethylsulfoxyd; insoluble in petroleum ether	Corbaz *et al.*, 1957
Actinorhodin	*S. coelicolor*[c]	270 (decomposes)	Soluble in pyridine, piperedine or phenol; weakly soluble in dioxane or acetone; insoluble in ether, CS_2, CCl_4 or petroleum ether	Brockmann *et al.*, 1947; 1950; 1955*a*, *b*
Streptocyanin	*Streptomyces* sp.[c]	290–300 (decomposes)	Soluble in acetone, dioxane, or pyridine	Tonolo *et al.*, 1954
Anthocyanin	*Streptomyces violaceoruber*		Extracted with hot or cold water and dilute alcohol	Kriss, 1936*a*
Anthocyanin	*S. coelicolor*[d]		Extracted with hot or cold water and dilute alcohol	Kriss, 1937
Anthocyanin	*S. violaceoruber*			Frampton and Taylor, 1938
Hydroactinochrome	*Streptomyces* sp., producing violet growth and pigment		Soluble in water	Kriss, 1936*b*
Lipoactinochrome			Insoluble in water	Kriss, 1936*b*

[a] Flaig and Kutzner, (*unpublished data*).

[b] Not identical with *Actinomyces litmocidini*; Gause *et al.*, 1957, (see table 1).

[c] Probably *S. violaceoruber*.

[d] The change in color to green on addition of alkali and the insolubility of pigment in organic solvents suggests the relationship of at least one of Kriss' cultures to Müller's organism.

fore, be given to this group of chemical substances produced by the streptomycetes under consideration.

There seems to be no doubt that several chemical substances listed in table 2 (coelicolorin, actinorhodin, streptocyanin, and the substance isolated by Sanchez-Marroquin and Zapata (1954) active only against species of Rhizobium) are

produced by closely related strains, perhaps all belonging to *S. violaceoruber*. On the other hand, there are several cases known in which two or three pigmented substances could be isolated from one culture. As mentioned above, the litmocidin-producing culture forms a second blue pigment; the actinorhodin-producing culture also forms a second substance regarded as a derivative of prodigiosin. Kriss (1936*b*) could differentiate three different pigments in the red-colored strains and two different pigments in the violet strains, these being identical with two of the three pigments found in the red cultures.

This information tends to prove that there are several *Streptomyces* species which produce a blue pigment, and that there are several different chemical compounds responsible for these blue pigments. In some cases, the same compound may be produced by different species; in other cases, different compounds may be formed by strains of the same species or even by the same culture. At present, however, the results do not allow general conclusions as to whether a certain species produces a certain chemical substance and another species another compound.

EVIDENCE FOR DIVERSITY OF THE TWO SPECIES

As shown in table 1, *S. coelicolor* and *S. violaceoruber* may be regarded as two different species. This may be surprising to most investigators, since the species have usually been regarded as synonyms. The differences between these two species, especially with respect to the solubility of the pigments (the other data given by Müller were too scanty to allow any comparison) have been given, however, definite consideration. Thus, Conn (1943) pointed out that Müller's *S. coelicolor* and Waksman's *S. violaceoruber* appeared to be different species; unfortunately, she came to the right conclusion on the basis of a wrong statement; namely, she regarded her strains as more closely related to *S. coelicolor* than to *S. violaceoruber*. An examination of these cultures proved that they are definitely not *S. coelicolor*, but can be regarded as *S. violaceoruber* strains.

As previously mentioned, it is difficult to obtain, from Müller's description alone, an idea about the characteristics of *S. coelicolor*. When one compares the original strain of Müller, which is still available at CBS, with *S. violaceoruber*, it is quite clear that the species are different. Unfortunately, such a comparison was not undertaken until 1956, by Kutzner, on the one hand, and by Zähner and Ettlinger (1957), on the other. But even Kutzner, although recognizing the differences, was not aware of Müller's paper. He, therefore, did not regard the original strain as the true *S. coelicolor*, but considered *S. violaceoruber* as *S. coelicolor*; this error was corrected later (Flaig and Kutzner, 1959*a*). Zähner and Ettlinger (1957) found the same differences in the color of the aerial mycelium between the two species; they pointed out that the strains called *S. violaceoruber* should, according to *Bergey's Manual*, be properly named *S. coelicolor*; however, they hesitated to do so because they observed these important differences. As these authors never found any blue pigment in the *S. coelicolor* cultures, they left open the question as to whether *S. coelicolor* and *S. violaceoruber* were synonymous or represented different species. Furthermore, Zähner and Ettlinger found differences in the utilization of carbon compounds by these two species, as shown in table 3, where, in addition, the results of other investigations are summarized.

S. coelicolor and *S. violaceoruber* differ in the utilization of L-rhamnose and raffinose, the two sugars regarded as most suitable for species differentiation (Pridham and Gottlieb, 1948; Kurosawa, 1951; Zähner and Ettlinger, 1957), as well as in the utilization of *meso*-inositol. The granaticin-producing strain has a "carbon source spectrum" very similar to that of *S. coelicolor* Müller. Some similarities can be detected between strain 169 of Kurosawa (1951) and *S. cyanoflavus*.

On the basis of the differences in color and morphology of the aerial mycelium (table 1), properties of the pigment (table 2), and utilization of carbon sources (table 3), the two species are readily separated. *S. coelicolor* belongs, on the basis of its spore color and morphology, to the *S. griseus* group.

S. violaceoruber, known to most people under the wrong name *S. coelicolor*, is a distinct, well characterized species. New descriptions of both organisms based on the reports of Waksman and Curtis (1916) and Waksman (1919), as well as on the data obtained in a study carried out by Kutzner (1956), are presented below.

SEPARATION OF *S. COELICOLOR* FROM OTHER SPECIES OF THE *S. GRISEUS* GROUP

As will be pointed out elsewhere (Waksman, 1959), the "griseus group" comprises all Strep-

TABLE 3

Utilization of carbon compounds by certain blue pigment producing Streptomyces species

Carbohydrate	*S. violaceoruber* Zähner and Ettlinger, 1957			*S. coelicolor* [b] Benedict *et al.*, 1955			*S. coelicolor* Kurosawa, 1951		*S. cyanoflavus* Funaki *et al.*, 1958	*S. coelicolor* Müller. Zähner and Ettlinger, 1957			*S. olivaceus* Corbaz *et al.*, 1957
	3030[a]	ETH 9447; ATTC 3355	ETH 9448; ATTC 10147	B-122	B-1257	B-1260	O-168[b]	169[c]		Beijerinck	Müller	Müller	
L-Xylose	+	+	+	+	+	+	+	−	−	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+	(+)	+	(+)	+	+
L-Rhamnose	+	+	+	+	+	+	+	−	(+)	−	−	−	−
D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+				+	+		+	+	+	+
Sucrose	?	(+)	(+)	−	(−)	−	+	+	+	−	−	−	(−)
Maltose	+	+	+	+	+	+	(−)	+	+	(+)	−	+	+
Lactose	+	+	(+)	+	+	+	+	−	(+)	(−)	+	(+)	+
Raffinose	+	(+)	+	(+)	+	(−)	+	+	+	−	−	−	−
Inulin	−	−	−	−	(−)	(−)			(+)	−	−	+	−
D-Mannitol	+	+	+	+	+	+	+	−	(+)	+	−	+	−
D-Sorbitol	+	(+)	−	−	(−)	−	+	−	(+)	(−)	(−)	(−)	−
Dulcitol	+	(−)	−				−	−	(+)	−	−	−	−
meso-Inositol	+	+	+	+	+	+	+	−	+	−	−	−	−
Salicin	+	+	+	(+)	+	(+)	+	−	(+)	+	(+)	+	+

+ = Good growth, positive utilization; (+) = poor to fair growth, utilization uncertain; (−) = faint growth, probably no utilization; − = no growth, no utilization; ? = results different at times.

[a] These data were originally reported by Pridham and Gottlieb (1948); DL-inositol was used instead of *meso*-inositol.

[b] Probably *S. violaceoruber*.

[c] Probably *S. cyanoflavus* (?).

tomyces strains and species which produce a grayish-greenish-yellow aerial mycelium, with the sporophores arranged in tufts and without spirals, and not producing a melanoid pigment on protein-containing media. The species description of *S. griseus* given by Ettlinger *et al.* (1958) includes in addition, the smooth surface of the spores, thus corresponding to what may be considered as the characteristics of the *S. griseus* group. There seems to be also agreement between this group and Krassil'nikov's *A. globisporus* group.

S. coelicolor cannot always be separated from all the other members of the *S. griseus* group by the production of a blue pigment. Unfortunately, not all strains belonging to this species are able to form such a pigment; some form a blue pigment at one time and not at another; there are still certain obscure questions concerning the best conditions for pigment production. Strains of *S. coelicolor* may be recognized by the following properties.

1. Cultural characteristics. In a study of 1800 Streptomyces cultures, carried out by Kutzner (1956), a subgroup which included the *S. coelicolor* strain from CBS was separated from the other subgroups of the *S. griseus* group on the basis of very poor growth on glycerol-nitrate agar. There were only 3 among the 62 cultures of this subgroup which showed good growth and heavy sporulation on this medium; they were placed in this subgroup on the basis of other similarities with the remaining cultures.

2. Phage specificity.[2] A specific phage was found capable of lysing strains of *S. coelicolor* as well as strains of two other subgroups probably

[2] The results relative to phage specificity have been briefly reported by Kutzner and Waksman (1959); a detailed study will be presented elsewhere.

closely related to *S. coelicolor*. About 120 other cultures producing an aerial mycelium similar to *S. griseus*, including strains forming streptomycin, grisein, and actinomycin, were not attacked by this phage. In addition to the original culture of *S. coelicolor* and our own isolates of this species, this phage lysed two other "species," which had been placed into the *S. coelicolor* subgroup on the basis of their morphological and cultural characteristics; these were *A. alni* (CBS, strain v. Plotho) and *A. albidoflavus* (CBS, strain Höhle); both cultures were regarded as related to *S. coelicolor* also by Dr. G. A. deVries (CBS, *personal communication*, 1959). When this phage was tested against several strains of the culture collection of the Institute of Microbiology, it was found that the candicidin-producing strain 3570 (Lechevalier *et al.*, 1953) was lysed by this phage. This strain was found to produce a blue pigment on potato plug (not observed in earlier experiments). This suggested the probability that the candicidin-producing strain is really *S. coelicolor*. Recent experiments have shown that the ascosin-producing species *S. canescus* (NRRL 2419) is also lysed by this phage. Since this organism shows similarities to *S. coelicolor* in other properties as well (see below), this species should be regarded as synonymous with *S. coelicolor* Müller.

3. Antibiotic properties. Müller reported just fifty years ago an antagonistic effect of his culture against *Oidium lactis*; this antifungal activity can still be observed with his culture. The sensitivity of the candicidin-producing culture and the ascosin-producing *S. canescus* to the specific phage of *S. coelicolor* suggested the possibility that the antifungal antibiotic produced by Müller's culture was also a polyene. Indeed, Müller's original culture as well as seven other strains, including *S. albidoflavus* (CBS) and the *S. coelicolor* strain of Heymer (Heymer, 1957) were found to produce antibiotics of the heptaene type.

It might be of interest to note here that *A. levoris*, belonging to Krassil'nikov's *A. globisporus* group, showed antagonistic properties against yeasts and fungi (Krassil'nikov, 1958). It is not improbable that *A. levoris* represents a strain of *S. coelicolor*, since the author pointed out that some strains "acquired a blue coloration" on glucose-containing media, when he attempted to obtain mutants of all the species within this group. It is uncertain, however, whether or not all these mutants originated only from *A. levoris*. Further, *A. levoris*, like the candicidin producing strain (Lechevalier *et al.*, 1953) and other strains of *S. coelicolor*, proved (*unpublished data*) to be highly sensitive to the streptomycin-producing *S. griseus*.

4. Utilization of carbon sources. It can be seen from table 3 that *S. coelicolor* Müller utilizes L-arabinose, but not L-rhamnose and raffinose. It differs in this regard from closely related strains of the *S. griseus* group, for instance the streptomycin-, grisein-, and actinomycin-producing cultures. This can be seen by comparison with the carbon source spectra of these strains given by Kurosawa (1951), Benedict *et al.* (1955), and Zähner and Ettlinger (1957); the same results were obtained in our experiments with several strains of each type. It remains to be seen, however, whether strains of *S. coelicolor* can be separated on this basis from the numerous other strains producing an aerial mycelium similar to *S. griseus*. *S. canescus* shows similarity in this property with the other heptaene-producing organisms (Hickey *et al.*, 1952).

5. Production of a soluble blue pigment. The name of *S. coelicolor* was derived from the fact that the original culture produced a blue, soluble pigment. Unfortunately, this property seems to be unsuitable for the recognition of this species, since only a few of the many cultures which are regarded as belonging to *S. coelicolor* with regard to the characteristics mentioned above produce such a pigment; moreover, a great inconstancy of this property can be observed. Müller (1908) reported that he obtained the blue pigment only on potato plugs and on media prepared with macerated potatoes plus agar, or on agar media containing potato or wheat starch, but not on other media, not even on a medium containing potato juice and glucose. This led him to name the pigment "amylocyanin." Beijerinck (1913) pointed out that his culture of *S. coelicolor* was colorless on most media, producing some pigment on two calcium malate media. Kutzner (1956) found that only 15 (including Beijerinck's strain) of the 62 cultures of *S. coelicolor* produced a blue pigment on potato plug, but not on any of the other media used; the actual production of the pigment by several strains was very irregular; an observation also made by Müller (1908). Heymer (1957) obtained good pigment production with her strain on Sabouraud's glucose-peptone agar.

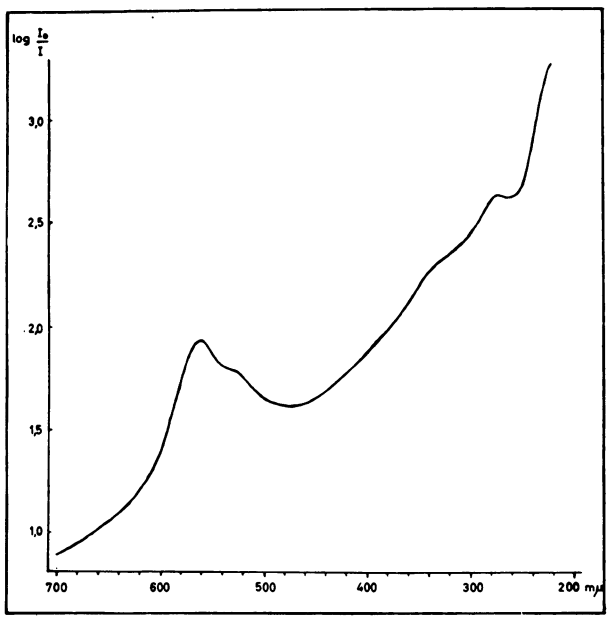

Figure 1. Ultraviolet absorption spectrum of the pigment of *Streptomyces coelicolor* in aqueous solution.

When eight of our strains of *S. coelicolor* which were known to produce the pigment on potato plug were cultivated on the two calcium malate media of Beijerinck (1913) and on Sabouraud's agar used by Heymer (1957), only two strains were found to produce a deep blue pigment on the mannitol-Ca-malate-peptone agar and a very weak pigment on the glucose-Ca-malate-NH_4NO_3-agar; two other cultures showed a very weak green-blue pigment only at the tops of the slants on Sabouraud's medium. In conformity with the results of Müller (1908) and Heymer (1957), we observed only a weak pigment or none at all at temperatures above 30 to 32 C.

As mentioned above, *S. coelicolor* Müller is a species of the *S. griseus* group. Moreover, from a "lumping" point of view one could consider the whole group as one species (Ettlinger *et al.*, 1958), in which case, however, the name *S. coelicolor* would have priority. Since we consider the characteristics of *S. coelicolor* mentioned above as distinct enough to separate it from the other species of this group, we regard *S. coelicolor* Müller as an independent species. Using the name *S. griseus*, created later, for the whole group does not seem to us against the rules of nomenclature. In addition, changing the name now of this "species-group" to "*S. coelicolor* group" would be not only confusing but also improper, because the name "griseus" expresses the color of the aerial mycelium which is common for all members of the *S. griseus* group, whereas the name "coelicolor" is derived from a property of only some strains of one species of this group.

THE PIGMENT OF *S. COELICOLOR* MULLER

Of all the blue pigments listed in table 2, least is known about amylocyanin produced by *S. coelicolor*. Müller (1908) characterized the pigment on the basis of solubility, changes of color at acid and alkaline reactions, or at addition of several salts to the water extract, and precipitation. Amylocyanin could be extracted from potato plugs by water, but it was insoluble in all the organic solvents. When the water-extract was acidified it became red; addition of alkali made it green. The pigment was precipitated in the form of green flakes by $Hg_2(NO_3)_2$, $HgSO_4$, and Pb-acetate; as red flakes by $ZnCl_2$ and Na_2SnCl_4.

Flaig and Kutzner (*unpublished data*) also found the pigment to be soluble in water; the extract from potato plugs or agar media was colored deep blue with a violet tinge to sky blue to light green-blue depending upon the pigment production on these substrates. On addition of acid the pigment changed to dark red-violet to pink;

on addition of alkali it changed to dark green to light green. These colors disappeared after some hours. The pigment could not be extracted from the acid or the alkaline solution by numerous solvents. On evaporating the blue water-extract *in vacuo*, a dark red to violet extract could be obtained from the blue residue with dimethylformamide. When this solution was concentrated and finally dried, the residue was again dissolved in water, giving a red-colored solution, the absorption spectrum of which is shown in figure 1. Heymer (1957) reports a change of the pigment color to red at an acid reaction, and dark blue (not green) at an alkaline reaction.

CHARACTERISTICS OF ORGANISMS

Streptomyces coelicolor (Müller) Kutzner and Waksman (Müller, 1908). Centr. Bakteriol. Parasitenk. Abt. I, **46,** 195, 1908)

Type culture. Müller's original strain deposited in the CBS.

Synonyms. Streptothrix coelicolor Müller (Müller, 1908); *A. albidoflavus* (strain Höhle, CBS); *A. alni* (strain v. Plotho, CBS) and *S. canescus* Hickey *et al.* (Hickey *et al.*, 1952, NRRL 2419).

Possible synonyms. A. cyaneofuscatus Gause *et al.* (Gause *et al.*, 1957) and *A. levoris* Krassil'nikov (Krassil'nikov, 1958).

Morphology of aerial mycelium. Sporophores of most strains short, arranged in small tufts, wavy; never spirals; morphology of spores: spherical to short ellipsoidical, surface smooth.

Color of aerial mycelium. Grayish yellow, often with a greenish or pinkish shade.

Melanoid pigment. Negative.

Color of substrate mycelium. On most media colorless or untypical yellowish-brownish; sometimes pinkish red, especially in the lower part of the slants.

Soluble pigment. On most media, no pigment, or yellowish-brown. Blue pigment is produced by some strains on potato plug, glucose-Ca-malate-NH_4NO_3-agar, mannitol-Ca-malate-peptone-agar, and glucose-peptone-agar.

Starch hydrolysis. Strong.

Nitrate reduction. Positive (none reported for *S. canescus* by Hickey *et al.*, 1952).

Milk. Rapid peptonization, complete within 15 days at 22 to 27 C; coagulation within 3 to 5 days, followed by peptonization at 36 C.

Gelatin. Rapid liquefaction.

Carbon sources. Utilizes L-xylose, L-arabinose, D-fructose, D-galactose, D-mannitol, salicin; does not utilize L-rhamnose and raffinose; most strains do not utilize sucrose.

Antagonistic properties. Active upon several fungi and yeasts; all strains as far as tested produce polyene antibiotics.

Ecology. S. coelicolor is widely distributed in nature. In a search for polyene producing organisms, Pledger and Lechevalier (1955–1956) found 26 strains among 93 isolates which produced polyenes and which can be regarded as belonging to this species. Among the 382 subgroups of Kutzner (1956), the *S. coelicolor* subgroup was the one which comprised most strains (Flaig and Kutzner, 1959*b*). Heymer (1957) found this organism strikingly often on the skin and in the tonsils of men. The first culture of this species isolated by Müller (1908) as well as the ascosin-producing organism (*S. canescus*) were found as chance contaminants; this indicates the wide distribution of the organism in air.

Streptomyces violaceoruber (Waksman and Curtis) Waksman (Waksman and Curtis, Soil Sci., **1,** 110–111, 1916; **8,** 160–163, 1919)

Type culture. Waksman and Curtis strain no. 3030 available in culture collection of Institute of Microbiology, Rutgers University.

Synonyms. A. violaceus Waksman and Curtis (Waksman and Curtis, 1916). *A. waksmanii* Bergey (*Bergey's Manual,* 3rd ed., 1930). *A. coelicolor* (Müller) Lieske (*Bergey's Manual,* 4th and 5th ed., 1934, 1939). *Str. coelicolor* (Müller) Waksman and Henrici (*Bergey's Manual* 6th and 7th ed., 1948, 1957). *A. coelicolor* (Müller) Krassil'nikov (Krassil'nikov, 1949).

Morphology. Aerial mycelium monopodially branched; abundant formation of spirals with 3 to 8 turns, sinistrorse. Surface of spores smooth.

Color of aerial mycelium. Ash gray; on some media light pink to cinnamon; sometimes blue drops can be observed on the surface of the aerial mycelium.

Pigment production. Blue pigment on many media, both of synthetic and complex nature.

Melanoid pigment. Negative.

Starch hydrolysis. Medium.

Cellulose. Good growth.

Nitrate reduction. Excellent.

Milk. Limited coagulation; rapid peptonization.

Gelatin liquefaction. Slow to medium.

Sucrose inversion. None to strong.

Carbon sources. Utilizes L-xylose, L-arabinose, L-rhamnose, D-fructose, raffinose (some strains only faintly), D-mannitol; none or poor utilization by most strains: sucrose, inulin.

Antagonistic activities. Most strains do not show any strong antagonistic effect; several cultures described in the literature as possessing antagonistic properties, which seem to belong or are closely related to *S. violaceoruber*, produce coelicolorin, actinorhodin, and streptocyanin.

Ecology. Very common, especially in field soils.

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SUMMARY

A brief survey of the *Streptomyces* species which produce a blue pigment is presented. Two Streptomyces species, *Streptomyces coelicolor* Müller and *Streptomyces violaceoruber* Waksman and Curtis, considered for a long time as synonyms and designated as *S. coelicolor* (Müller) Waksman and Henrici, are now shown to be distinctly different species.

S. violaceoruber Waksman and Curtis is a species with gray aerial mycelium, forming spirals and smooth spores, nonchromogenic, and producing a blue pigment on many media. *S. coelicolor* Müller is a species with grayish-yellow aerial mycelium, forming no spirals, producing smooth spores, and nonchromogenic. Some strains produce a blue, soluble pigment on potato plugs, glucose-Ca-malate-NH_4NO_3-agar, mannitol-Ca-malate-peptone-agar, and glucose-peptone-agar. The blue pigment of *S. coelicolor* can be extracted in water; it changes to red in an acid reaction and to green in an alkaline reaction. The pigment is insoluble in most organic solvents. When the water-extract is evaporated, a red-violet substance can be extracted from the blue residue by dimethylformamide.

S. coelicolor Müller is a species of the *Streptomyces griseus* group. It can be separated from the other species of this group on the basis of poor growth on glycerol-nitrate-agar, sensitivity to a particular phage, carbon utilization, and antifungal properties; an antifungal antibiotic of the polyene type is produced. *Streptomyces canescus* Hickey *et al.* is considered to be synonymous with *S. coelicolor* Müller.

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