Actinoplanes italicus, a New Red-Pigmented Species

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A new species of *Actinoplanes* is described for which the name *A. italicus* is proposed. The organism is characterized by the cherry-red color of its vegetative mycelium and by the production of soluble pigments. It differs significantly, therefore, from the species of *Actinoplanes* so far named and described, which generally produce a yellow or orange vegetative mycelium and which ordinarily do not produce soluble pigments. The type strain of *A. italicus* is A 5221 (=ATCC 27366).

During the course of a screening program for antibiotic producers from uncommon genera of the order *Actinomycetales*, an actinoplanete was isolated which did not produce any antibiotic activity against either gram-positive or gram-negative bacteria but which differed significantly from the previously described species of *Actinoplanes* in its morphological, cultural, and physiological characteristics. Studies were undertaken, therefore, to determine whether this organism represented a new species.

MATERIALS AND METHODS

Organism. A single strain, A 5221 of our collection (=ATCC 27366), was isolated from a soil sample collected in an orchard at Pontelongo, Italy.

Methods. For the examination of growth characteristics, the organism was cultivated on various standard media suggested by Shirling and Gottlieb (9) with the addition of several media recommended by Waksman (11).

The inoculated media were incubated in the dark at 28 C and examined after 1 and 2 weeks. To determine the effect of light on pigment production, the incubated plates were exposed to daylight and at night to an electric lamp.

Stock slant cultures were maintained on oatmeal agar. Colony characteristics were observed from petri dish cultures of starch agar after 8 to 16 days of incubation.

Color determination was made, whenever necessary, by the method of Maerz and Paul (7).

The ability of the organism to utilize different carbon sources was investigated by the method described by Shirling and Gottlieb (9). For this purpose, the media containing the carbon sources were inoculated with mycelium from 72-h-old broth cultures, washed twice, and suspended in distilled water.

The effect of temperature on growth was investigated by incubating inoculated slants of oatmeal agar at 15, 20, 28, 40, and 50 C.

RESULTS

Macroscopic examination. Colonies on starch agar are 3.5 to 4.0 mm in diameter and have well defined and regular contours, a smooth surface, and a cherry-red color. They have a shallow dome shape with a small central protuberance of a deeper color. Some colonies show a poorly accentuated split across the center.

Cherry-red vegetative mycelium is produced on most media. However on International Streptomyces Project media no. 2 and no. 7 and on calcium-malate agar, the color of the vegetative mycelium is orange, while on Hickey and Tresner, Bennett, peptone-iron, and nutrient agar, the vegetative mycelium is amber brown to brown.

A soluble pigment ranging from cherry red to amber yellow or to orange yellow, depending on the medium, is produced. To compare our strain with the previously described species of *Actinoplanes*, *Actinoplanes philippinensis*, *A. missouriensis*, *A. utahensis* (1-2), *A. armeniacus* (6), and *A. brasiliensis* (10) were cultured on the same solid media used for strain A 5221 characterization, and their macroscopic and microscopic morphologies were examined. Strain A 5221 differs significantly from these five species because of the cherry-red color of its vegetative mycelium and the production of a soluble pigment. Strains of the above mentioned species, on the contrary, produce a vegetative

TABLE 1. *Cultural and physiological characteristics of strain A 5221^a*

Culture medium	Vegetative mycelium	Sporangia	Soluble pigment	Physiological characteristics
Medium ISP no. 2 ^b	Abundant growth with thick and wrinkled surface, orange with a pinkish tinge (4/C/11) ^c	Absent	Yellowish pink	
Oatmeal agar ^d	Abundant growth with smooth surface, cherry (4/H/5)	Absent	Cherry (4/5/4) ^c	
Oatmeal agar (ISP no. 3) ^b	Abundant growth with smooth and thick surface, light cherry	Absent	Old rose (4/I/2) ^c	
Glycerol asparagine agar (ISP no. 5) ^b	Abundant growth with smooth and thick surface, cherry (4/I3/5) ^c	Absent	Pinkish with orange shadow	
Hickey and Tresner agar ^e	Abundant growth, with slightly wrinkled surface, amber brown (14/A/10) ^c	Absent	Amber	
Bennett's agar ^d	Abundant growth, with wrinkled surface, light amber brown (13/B/8) ^c	Absent	Absent	
Czapek glucose agar ^d	Abundant growth, with smooth surface, light strawberry (5/H/10) ^c	Absent	Deep orange yellow (9/4/10) ^c	
Glucose asparagine agar ^d	Very scarce growth, light cherry (2/A/5) ^c	Absent	Light pink in traces (2/B/3) ^c	
Nutrient agar ^d	Abundant growth with smooth surface, deep amber (12/A/11) ^c	Absent	Absent	
Potato agar ^d	Abundant growth with smooth surface, light cherry with amber brown edges	Absent	Pinkish amber (6/D/10) ^c	
Starch agar (ISP no. 4) ^b	Abundant growth with smooth and thick surface, deep cherry-red (4/6/1) ^c	Abundant, light gray	Deep cherry-red (4/F/6) ^c	Starch hydrolysis: scarce
Peptone-yeast extract-iron agar (ISP no. 6) ^b	Abundant growth with wrinkled surface, brown	Absent		Production of H ₂ S: positive (melanoid pigment)
Tyrosine agar (ISP no. 7) ^b	Abundant growth with wrinkled surface, deep orange (1/A/12) ^c with light strawberry edges	Absent	Light pink	Tyrosinase reaction: positive
Calcium-malate agar ^d	Very scarce growth, pale orange	Absent	Absent	Digestion of calcium-malate: negative
Gelatin ^d				Gelatin liquefaction: positive
Nitrate broth ^d	Orange growth		Amber	Reduction of nitrate: slightly positive
Skim milk agar ^d	Abundant growth, with smooth surface, light cherry (5/K/9) ^c	Abundant, light gray	Cherry with amber shadow (5/L/10) ^c	Hydrolysis of casein: strongly positive
Litmus milk ^d				Peptonization without coagulation

^a Incubation in the dark.^b Media recommended by the International Streptomyces Project.^c Figures in paranthesis refer to the color according to the color blocks of Maerz and Paul.^d Media recommended by Waksman (11) for the study of *Actinomycetales*.^e See reference 5.

FIG. 1. *Actinoplanes italicus*: sporangia developing on the surface of starch agar. Optical microscope ($\times 500$).

FIG. 2. *Actinoplanes italicus*: sporangium mounted under a cover slip immediately before the rupture of the sporangial wall. The spores inside appear visible. Optical microscope ($\times 1890$).

mycelium of various shades of orange and yellow but never a red pigment.

The cultural details of strain A 5221 grown on various media are presented in Table 1.

Microscopy examination. The vegetative mycelium of strain A 5221 is composed of thin, twisted, and branched hyphae, with a diameter of 0.5 to 1.0 μm .

The sporangia, light gray in color, are abundantly produced on starch and skim milk

TABLE 2. Carbon utilization by strain A 5221

Carbon source	Growth ^a
Inositol	++
Fructose	++
Rhamnose	++
Mannitol	++
Xylose	++
Raffinose	—
Arabinose	++
Cellulose	—
Sucrose	++
Glucose (positive control)	++
No carbon (negative control)	—

^a ++, Strong utilization; —, no utilization.

agar, have a very wrinkled surface and a rather irregular shape varying from spherical to oval and pyriform (Fig. 1, 2, 3, and 4). Their size varies from 6 to 11 μm . They are supported by straight sporangiophores, 4 to 5 μm long, arising from the vegetative mycelium. When mounted under a cover slip, the sporangia have a very irregular outline, and the spores inside are not easily distinguishable. However, immediately before rupture of the sporangial wall, the shape of the sporangia becomes more regular and spherical and the spores appear more visible. After dehiscence from the sporangium, the spores become highly motile. Their

FIG. 3. *Actinoplanes italicus*: morphology of sporangia. Scanning microscope ($\times 6,200$).

morphology varies from spherical to oval with a diameter of 1 to 2 μm .

Physiological and nutritional characters. The physiological and carbon-utilization characteristics of strain A 5221 are presented in Tables 1 and 2.

Comparative studies of the utilization of carbon compounds were performed by culturing strain A 5221 and the five reference species on different carbon sources. Strain A 5221 differs from *A. utahensis* and *A. missouriensis*, which do not utilize inositol; from *A. philippinensis* and *A. armeniacus*, which utilize raffinose; and from *A. brasiliensis* and *A. philippinensis*, which utilize cellulose.

Temperature relationships. The optimum temperature for strain A 5221 ranges from 28 to 37 C. No growth was observed at 50 C, and there was moderate growth at 20 C and little growth at 15 C.

DISCUSSION

Strain A 5221, because of its abundant formation of globose sporangia containing highly motile sporangiospores, can doubtlessly be assigned to the genus *Actinoplanes* Couch (1).

This isolate is interesting because of the production of an extracellular cherry-red pigment which diffuses throughout the vegetative mycelium conferring upon it the same color.

To our knowledge, the production of soluble pigments by *Actinoplanes* strains is unusual. Among the described species, *A. philippinensis* produces a dark brown pigment in one culture medium only; *A. missouriensis* and *A. utahensis* produce a pale lavender and an amber pigment, respectively, on selected media only. A low-molecular-weight red pigment produced by some *Actinoplanes* strains has been observed,

FIG. 4. *Actinoplanes italicus*: surface of a sporangium. Scanning microscope (X6,200).

TABLE 3. Comparison of the physiological characteristics of strain A 5221 with those of the currently accepted species of *Actinoplanes*^a

Test	<i>Actinoplanes</i> A 5221	<i>A. missouriensis</i>	<i>A. utahensis</i>	<i>A. philippinensis</i>	<i>A. brasiliensis</i>	<i>A. armeniacus</i>
Starch hydrolysis	±	+	+	+++	+++	++
H ₂ S formation	+	—	+	±	—	+
Melanin	+	+	+	—	—	+
Tyrosin hydrolysis	+	+++	±	+	—	++
Casein hydrolysis	+	+	+	+	+	++
Calcium malate hydrolysis	—	±	+	+	+	±
Nitrate reduction	±	+	+	+	+	+
Litmus milk	No coagulation, peptonization	No coagulation, no peptonization	No coagulation, no peptonization	No coagulation, no peptonization	Coagulation, no peptonization	No coagulation, peptonization
Gelatin liquefaction	+	—	±	+	+	+

^a —, Negative response; ±, doubtful response; +, weak positive response; ++, positive response; +++, strong positive response.

however, by A. Domnas (personal communication). In contrast, organisms belonging to other genera of the family *Actinoplanaceae* have often been reported to produce pigments. For instance *Microellobosporia cinerea* produces a red and purple pigment (3), several *Kitasatoa* species produce red-brown pigments (8), and A. Domnas (4) has described a blue pigment produced by *Spirillospora* no. 1655. Domnas noticed that the formation of the pigment is strongly inhibited by light. Also in the case of strain A 5221, when the cultures are exposed to light during growth, the red pigment is not produced; under this condition the vegetative mycelium appears deep orange in color, probably due to the promotion of carotenoid synthesis by light.

Strain A 5221 also differs from the previously named and described species of *Actinoplanes* in its physiological characteristics (Table 3).

On the basis of its overall differences with respect to the currently accepted species of *Actinoplanes*, strain A 5221 is regarded here as sufficiently unique to belong to a new species, for which the name *Actinoplanes italicus* is given. (i·ta'li·cus, L. adj. *italicus* pertaining to Italy). The type strain, A 5221, has been deposited in the American Type Culture Collection (ATCC) under the number 27366. Because only a single strain of this organism has thus far been isolated, the description presented here serves as the description both of the species and of the type strain.

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