**Streptomyces spinoverrucosus**, a New Species from the Air of Kuwait

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We describe a new *Streptomyces* species, for which the name *Streptomyces spinoverrucosus* is proposed. This species is characterized as follows: green, yellow, red, and gray aerial mass colors; spiral spore chains; spiny and warty spore surfaces; reverse of the colony colorless or faint yellow on salts starch agar and brownish red on other media (the red pigment is a pH indicator); melanoid pigments formed in International *Streptomyces* Project media 1, 6, and 7: brown, red, or reddish brown water-soluble pigments; and a characteristic carbon utilization pattern. This species differs from all *Streptomyces* species described previously on the basis of its aerial mass color, color of reverse of colonies, and color of water-soluble pigments. The type strain of *S. spinoverrucosus* is Diab 163MA (= NCIB 11666).

The identification of aerobic *Actinomycetales* is not easy. Many attempts have been made to find stable characteristics for genera and species. The International Subcommittee on the Taxonomy of the *Actinomycetales* established an internationally accepted system for characterizing *Streptomyces* species and placed cultures of all available type strains in four major culture collections. In the first phase of this work (1958 through 1961), a critical examination of the criteria previously used to characterize *Streptomyces* species was made. As a rule, a number of reliable, stable criteria were obtained. These criteria, with some modifications, were then used in the International *Streptomyces* Project (ISP) to describe the type strains of *Streptomyces* species. This project involved description of the available type strains by a number of workers who used the same criteria under standardized conditions. Each strain was examined independently by three experts in different countries.

The methods used in the ISP have been described in detail by Shirling and Gottlieb (6). The following characters were considered important and are now commonly used in keys for classification of the species of the genus *Streptomyces*: morphology of spore chains, morphology of spore surfaces, color of aerial and vegetative mycelia, melanin production, water-soluble pigment production, and utilization of carbon sources.

Küster (2) offered a simple working key for classification of the named taxa included in the ISP. In this work, oatmeal agar was used for morphology and color determinations, and peptone yeast extract iron agar was used for melanin production determinations. In the key of Küster, in the key of Pridham and Tresner in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (5), and in the keys of Nonomura (4) and Szabo et al. (13), utilization of sugars and other carbon-containing compounds is used to delineate species further. These keys, as well as the ISP descriptions of the *Streptomyces* type strains published by Shirling and Gottlieb (7–10), are useful in identifying *Streptomyces* isolates and in recognizing and characterizing new species of the genus.

In this paper we report the isolation and description of a new species isolated from the air during a study of the distribution of aerobic *Actinomycetales* strains in the atmosphere of Kuwait.

**MATERIALS AND METHODS**

Air from a crowded section of Kuwait City was collected with a Casella slit sampler, as described by Diab et al. (1). Oatmeal agar and inorganic salts starch agar (ISP media 3 and 4) plates were used in collecting the air samples. The plates were incubated at 30°C for 14 days, after which *Actinomycetales* strains were isolated, purified, and subcultured on slants of inorganic salts starch agar.

One of the isolates (strain 163MA) was characterized by its green, yellow, red and gray aerial mass colors and by its formation of brownish red reverse of colony and red to reddish brown water-soluble pigments on oatmeal agar. Because of these characteristics strain 163MA was selected for study.

The generic identification of this isolate was made on the basis of morphological characteristics and a chemical analysis of a whole-cell hydrolysate (4). Subsequently, the tests used to characterize strain 163MA were those described by Shirling and Gottlieb (6). The keys of Küster (2), Pridham and Tresner (5), Nonomura (4), and Szabo et al. (13), together with the
ISP descriptions of the *Streptomyces* type strains (7–10), were used to identify the isolate.

**RESULTS**

The results of the characterization of strain 163MA by the methods mentioned above are as follows.

**Cell wall analysis.** A whole-cell hydrolysate analysis revealed the presence of L,L-diaminopimelic acid.

**Spore chain morphology.** The spore chains were in the form of terminal, closed, or compact spirals with two or more turns (Fig. 1 and 2). There were 10 to 20 or more spores per chain.

FIG. 1. *S. spinoverrucosus*. Photomicrographs showing spiral spore chains. ×1,200.
Spore surface. The spore surfaces were spiny; some spores had spiny and warty surfaces (Fig. 2).

Color of colony. The mature aerial mass colors in the green color series on salts starch agar, yeast malt agar, oatmeal agar, and glycerol asparagine agar (Tresner-Backus color series tabs) were matched to the red, yellow, green, and gray color wheels. T. G. Pridham (who confirmed the color series) stated that strain 163MA represents a good example of the problem involved in use of color as a major criterion in characterization and classification of streptomycetes and streptoverticillia.

Color of reverse side of colony. The reverse sides of the colonies were colorless or faint yellow on salts starch agar and brownish red on yeast malt agar, oatmeal agar, and glycerol asparagine agar. The red color was pH sensitive.

Color in medium. Melanoid pigments were formed in peptone yeast iron agar, tyrosine agar, and tryptone yeast broth. The water-soluble pigments produced were brown on yeast malt agar and glycerol asparagine agar and red or reddish brown on oatmeal agar. An orange water-soluble pigment may be formed in salts starch agar.

Carbon sources utilized. D-Glucose, D-xylose, L-arabinose, D-xylose, D-mannitol, fructose, rhamnose, sucrose, and raffinose were utilized for growth.

Growth on Czapek solution. Growth on Czapek solution was good.

Identification of strain 163MA. (i) Key of Kütaster. The entry characteristics were green aerial mycelium, distinctive reverse pigments, and melanin positive. At this point, further use of the key was blocked because of the absence of any dichotomy below "soluble pigments positive." Also, all melanin-positive species have a hairy spore surfaces. The same results were obtained when the entry characteristic "no distinctive reverse pigment" was used. With the entry characteristic "gray aerial mycelium," use of the key was blocked at "distinctive reverse pigments."
ments” because of the absence of dichotomies after “spiny and warty spore surfaces,” “soluble pigments positive,” and “melanin positive.” On the other hand, when yellow aerial mycelium was used as an entry characteristic, it was difficult to identify strain 163MA because there is no “melanin positive” listed in the table of “distinctive reverse pigments” and no “melanin positive with spirals or with spiny spore surfaces” listed in the table of “no distinctive reverse pigments.” The entry characteristic “red aerial mycelium” allowed identification of strain 163MA as Streptomyces purpurascens, Streptomyces roseoviolaceus, Streptomyces violaceus, Streptomyces violatus, or Streptomyces arenae.

(ii) Keys of Pridham and Tresner. Strain 163MA could not be identified in the green series because there is no entry for the following set of four characteristics: green, spira (spore chains in form of hooks, open loops, and coils), melanoid pigments produced, spiny. When the gray series set of characteristics (gray, spira, melanoid pigments produced, spiny) was checked, the suggested identifications were Streptomyces afghanensis, S. arenae, Streptomyces fimbriatus, and Streptomyces iakyrus. When the yellow series was considered, only one species (Streptomyces hawaiienis) had the characters melanin positive and spiny spore surface. In the red series (red, spira, melanoid pigments produced, spiny), the suggested identifications were S. purpurascens, S. roseovio- laeus, S. violaceus, S. violatus, Streptomyces violatus, Streptomyces yokosukansis, Streptomyces janthinus, “Streptomyces roseospinus,” and “Streptomyces flavovariabilis” (names in quotation marks are not on the Approved Lists of Bacterial Names [11], have not been validly published since 1 January 1980, and hence have no standing in bacterial nomenclature).

(iii) Key of Nonomura. The tables of the green series and the yellow series were blocked for the same reasons found in the key of Küster, where-as the table of the gray series led to S. iakyrus, Streptomyces cyaneus, and “Streptomyces indigocolor.” In the red series, the table led to S. purpurascens, S. roseoviolaceus, S. violaceus, S. violatus, and S. arenae.

(iv) Key of Szabo et al. With the initial characteristic “spiny spore surface,” the suggested identifications were Streptomyces griseoruber and Streptomyces erythraeus. With the initial characteristic “warty spore surface,” the key led to Streptomyces cinnabarinus and “Streptomyces coralus.”

(v) ISP descriptions. Each of the keys mentioned above suggested more than one species for strain 163MA. Some of these species (S. fimbriatus, “S. flavovariabilis,” and “S. roseospinus”) are not described in the ISP publications, and for this reason it was difficult to compare strain 163MA with them. On the basis of their ISP descriptions, the other species differ from strain 163MA with regard to the color of the aerial mycelium, the color of the reverse sides of colonies, and the color of the water-soluble pigments produced.

**DISCUSSION**

A comparison of the description of strain 163MA with the descriptions of Streptomyces species in the above-mentioned keys for identification and with the ISP descriptions showed that no known Streptomyces species in the green series is melanin positive and has a spiny or warty spore surface. All melanin-positive species in this series have hairy spore surfaces. On the other hand, in the key of Küster none of the species with green aerial mycelia produces a water-soluble pigment. In the key of Nonomura, two species produce water-soluble pigments, but these species are melanin negative and differ significantly from strain 163MA. In the yellow series, no species is melanin positive, has a distinctive reverse pigment, and produces a water-soluble pigment, except “Streptomyces flavofungi,” which has Rectiflexibles spore chain morphology and smooth spore surfaces and is unable to utilize sucrose for growth. In the gray series there are no melanin-positive species with warty spore surfaces. Three species (S. iakyrus, S. cyaneus, and “S. indigocolor”) have spiny spore surfaces, are melanin positive, and produce distinctive reverse pigments and water-soluble pigments. However, these species differ from strain 163MA. In the red series, only one species has a warty spore surface (Streptomyces lateritius); this species has Rectiflexibles- Retinaculapierti spore chain morphology, produces a blue or violet water-soluble pigment and a grayish yellow reverse side of colony modified by violet or blue, and is unable to utilize sucrose, mannitol, and raffinose. On the other hand, the following species produce soluble pigments and have spiny spore surfaces: S. purpurascens, S. roseoviolaceus, S. violaceus, S. violatus, S. violinus, and S. arenae. All of these species differ from strain 163MA in characters mentioned above.

According to the data given in the ISP descriptions, Szabo and Marton (12) reported that of the 426 strains identified, 54% fall within the range delineated by one Tresner-Backus color wheel, whereas 46% fall into two, three, and four different color series when they are examined on three different media. Only two species fall into four color series: one is in the red, gray, yellow, and blue series, and the other in the yellow,
gray, green, and red series. According to the ISP descriptions, the latter (Streptomyces olivaceoviridis) has smooth spore surfaces and is melanin negative. It produces no, or only a trace of, yellow water-soluble pigments and no distinctive reverse pigment. Hence, it differs from strain 163MA.

To the best of our knowledge, there has not yet been reported a Streptomyces species with an aerial mass color which allows placement of the organism in the green, red, yellow, and gray color series along with the following characters: melanin positive; spiral spore chains; spiny spore surfaces; brownish red reverse sides of colonies; brown, red, or reddish brown water-soluble pigments; and utilization of D-glucose, D-xylene, L-arabinose, D-ribose, D-mannitol, fructose, rhamnose, sucrose, and raffinose for growth.

Therefore, on the basis of the results presented above, we believe that strain 163MA represents a new species of Streptomyces, for which we propose the name Streptomyces spinoverrucosus (spi.no.ve.ru.co'sus). L. adj. spinosus thorny; L. adj. verrucosus warty; M.L. adj. spinoverrucosus spiny and warty, referring to the spiny and warty spore surface.

The type strain is 163MA (= NCIB 11666). Since the description of the species is based on a single strain, the description given above for the type strain also serves for the present as the species description.

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REPRINT REQUESTS

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LITERATURE CITED