Kineosporia, a New Genus of the Order *Actinomycetales*

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The morphological, cultural, physiological, and biochemical characteristics of a new actinomycete are described. The organism is distinctive because of the absence of aerial mycelium and the presence of glycine and LL-diaminopimelic acid in its cell wall. The mycelium bore numerous round-to-pyriform sporangia, each of which contained a single zoospore. The strain could not be classified in any of the previously named genera of the *Actinomycetales*, and it is therefore considered to be a member of a new genus, for which the name *Kineosporia* is proposed. The type species (monotype) of this genus is *K. aurantiaca* sp. nov., so named because of its orange color when grown on agar media. The type strain of *K. aurantiaca* is A/10312 (=ATCC 29727).

In the course of an extensive screening program for new species of *Actinomycetales*, an organism was isolated which had characteristics which precluded its placement in any of the previously described genera. In this paper, a detailed description of this organism is presented. The organism was found to differ sufficiently from the members of all other described genera to warrant its being regarded as belonging to a new genus, for which the name *Kineosporia* (Ki.ne.o.spó.ri.a. Gr. n. *kinesis* motion; Gr. n. *sporus* a seed; M. L. n. *spora* a spore; M. L. fem. n. *Kineosporia* motile spore) is proposed. The type species of *Kineosporia* is *K. aurantiaca* sp. nov.

MATERIALS AND METHODS

Bacterial strain. The new organism was isolated by plating a water suspension of a soil sample from St. Raphael (France) on Czapek-glucose agar and incubating it at 30°C. It was given the designation A/10312.

Media. The media used were the International *Streptomyces* Project media recommended by Shirling and Gottlieb (7) and those recommended by Waksman (12). The cultures were grown on media in petri dishes incubated at 30°C for 6 to 17 days.

Gram and acid-fast stains. The procedures used for determining the Gram reaction and acid fastness are described in the *Manual of Methods* published by the Society of American Bacteriologists (8).

Examination of spore germination. Spores were carefully scraped off a mature agar-slant culture with a sterile loop and streaked onto the surface of Czapek-glucose agar in petri dishes. The inoculated plates were incubated at 30°C for 7 to 14 days and examined directly under the microscope for the appearance of germ tubes.

Photographs. Photomicrographs were taken on Agfapan 25 professional film with a Zeiss photomicroscope equipped with Optovar.

Antibiotic susceptibility. Inocula were streaked onto the surfaces of plates of yeast extract-malt agar containing antibiotics diluted according to a twofold

dilution program. The minimal inhibitory concentration, defined as the minimal concentration (in micrograms per milliliter) of an antibiotic that completely inhibits growth after 6 days of incubation at 30°C, was determined for each antibiotic.

Determination of pathogenicity. A heavy suspension of spores was injected either intravenously or subcutaneously into CF₁ mice of both sexes. The mice were observed for 10 days for the possible appearance of ill effects.

Analysis for cell wall components. For cell wall analysis, the organism was grown in flasks of Emerson medium (12) which were placed on a rotary shaker (250 rpm) at 30°C for 72 h. The growth was collected by centrifugation and was thoroughly washed with distilled water. The washed mycelium was then treated as described by Becker et al. (1), and the diaminopimelic acid and sugars were chromatographed and visualized as described by the same authors.

RESULTS

The salient characteristics of *Kineosporia* gen. nov. and of *K. aurantiaca* sp. nov. are as follows.

Small, orange-colored colonies are formed by a fine, branched substrate mycelium (about 1.0 µm in diameter). Numerous small sporangia are borne at the distal ends of the hyphae; aerial mycelium is never formed. Each sporangium contains a single planospore. The cell wall contains LL-diaminopimelic acid, glycine, and traces of lysine, but not aspartic acid.

Colonial morphology. A great variety of colonial topography was observed even on the same agar plate, particularly on yeast extract-malt agar, where two basic colony types were observed (Fig. 1): conical-crateriform and cerebriform. When each type was carefully removed from the plate and inoculated onto a fresh plate, both types reappeared. The dynamics of colony growth on yeast extract-malt agar were as fol-

FIG. 1. Colonies formed on oatmeal agar. (A) Conical-crateriform colony; (B) cerebriform colony. Magnification, $\times 9$.

lows: 3 days after streaking, the colonies had diameters of 0.35 mm; after 4 days, their diameters were 0.5 mm; after 7 days, their diameters were 1 mm; and after 10 days, their diameters were 2 mm, at which size they remained thereafter. The colonies were colorless for the first few days of growth and then turned cream to orange, depending on the medium. Upon prolonged incubation, the whole surface of the streak became moist and acquired a glossy appearance.

Vegetative mycelium. The vegetative mycelium consisted of fine ($1\text{-}\mu\text{m}$ diameter), slightly branched hyphae which grew into the agar surface and formed a compact layer above it (Fig. 2).

Aerial mycelium. Aerial mycelium was not observed on any of the media tested.

Sporangia. Sporangia were formed on all media and were particularly abundant on oatmeal agar, where they conferred a glossy appearance to the streak. They appeared as elongated, club-shaped vesicles at the terminal end of the vegetative hyphae, from which they were separated by what appeared to be a transverse septum. The tip of the sporangium was slightly enlarged (Fig. 2). The sporangial membrane was not visible in the intact sporangium; however, it became visible after the sporangium ruptured

(Fig. 3). Each sporangium contained only one spore.

Sporangium dehiscence. Dehiscence of the sporangium occurred soon after the sporangium-bearing mycelium was placed into water. The sporangium always ruptured at the terminal, or upper, end. The spore immediately swam about and remained motile for several hours.

Spore morphology. The spores were pleomorphic, their shapes ranging from nearly spherical to oval or pyriform. The long axes of the spores varied between 1 and $2\text{ }\mu\text{m}$.

Spore germination. Germination of the spores began with the terminal formation of a yeastlike bud, which developed into a highly branched mycelium. In some cases, several germ tubes originated from a single spore, usually in a bipolar fashion.

Cultural characteristics. The cultural characteristics are presented in Table 1.

Carbon-source utilization and physiological properties. The simple carbohydrates (glucose, fructose, and rhamnose) which enter the carbohydrate metabolism pattern at the level of hexose-phosphate, with the exception of mannose and mannitol, are readily utilized. The pentoses xylose and arabinose are also readily utilized. However, the glycosidic bonds of lactose, salicin, raffinose, and cellulose are not hy-

FIG. 2. Vegetative mycelium on oatmeal agar. Magnification, $\times 1,200$.

drolyzed, and growth is very poor with sucrose. However, growth is abundant with starch. Protein (litmus milk, casein, and gelatin) are not hydrolyzed, indicating an inability to synthesize extracellular proteolytic enzymes under the conditions of the test. Tyrosine is not hydrolyzed, H_2S is not produced, calcium malate is very slightly solubilized, and nitrate is not reduced.

Cell wall and whole-cell sugar determinations. The cell wall contains LL-diaminopimelic acid, and the whole-cell sugars are xylose, arabinose, and galactose (6).

Staining reactions. The young mycelium grown under submerged conditions was used for determining the staining reactions. The mycelium was gram positive and was not acid fast.

Effect of temperature on growth. The effect of temperature on growth was investigated by streaking the inoculum over the surfaces of Bennett, oatmeal, and yeast extract-malt agars (pH 7) and incubating at 20, 30, 37, 42, and 50°C. No growth was observed on any medium at temperatures equal to or higher than 37°C. Growth was as abundant at 20 as at 30°C in all three media; sporulation was more conspicuous in oatmeal agar than in the other media at both temperatures.

Effect of pH on growth. The effect of pH on growth was determined by streaking the inoculum onto the media used for the temperature studies but at pH values of 4, 5, 6, 7, 8, 9, and 10

and incubating at 30°C. The best growth on all media occurred at pH 7; some growth was observed at pH 6 and 5, but none was evident at pH 4 or at pH 8 or higher.

Susceptibility to antibiotics. The minimum inhibitory concentrations (expressed in micrograms per milliliter) of several antibiotics were tested in yeast extract-malt agar. *K. aurantiaca* is susceptible to streptomycin ($<0.1 \mu\text{g/ml}$), bacitracin ($0.2 \mu\text{g/ml}$), tetracycline ($0.2 \mu\text{g/ml}$), rifampin ($1.0 \mu\text{g/ml}$), to the sulfur-containing polypeptide termotiocin ($6.0 \mu\text{g/ml}$), an antibiotic active only against gram-positive bacteria (3), and to chloramphenicol ($2.0 \mu\text{g/ml}$). As expected, it is resistant to the antifungal antibiotics cycloheximide ($>500 \mu\text{g/ml}$) and amphotericin B ($>500 \mu\text{g/ml}$). It is moderately susceptible to penicillin G ($20 \mu\text{g/ml}$).

Pathogenicity. No sign of illness was observed in mice treated subcutaneously or intravenously with heavy suspensions of mycelium.

Type strain. The type strain is *K. aurantiaca* A/10312 (= American Type Culture Collection 29727). Because the species description is based on a single strain—the type strain—the species description given here also serves as the description of the type strain.

DISCUSSION

The absence in *K. aurantiaca* of an aerial mycelium confers to colonies of this organism

FIG. 3. Rupture of sporangia. The upper-left arrow points to a sporangium before (A) and the same sporangium after (B) rupture and spore dehiscence. The sporangium wall is clearly visible after rupture. A ruptured sporangium is also evident (lower-right arrow). Magnification, $\times 1,900$.

the gross morphological appearance of colonies of members of the genera *Actinoplanes* and *Micromonospora*. However, the colonies of *K. aurantiaca* are more complex (cerebriform to

crateriform surface) and have a glossy appearance due to the large number of highly refractile sporangia.

Sporangia-forming actinomycetes may be di-

TABLE 1. *Cultural characteristics of Kineosporia aurantiaca* sp. nov.

Medium	Cultural characteristics at 30°C
ISP no. 2 ^a	Abundant growth, wrinkled surface, opaque, hard, amber-orange
ISP no. 3 ^a	Moderate growth, smooth surface, moist, bright, light orange
ISP no. 4 ^a	Scarce growth, crusty surface, opaque, hyaline
ISP no. 5 ^a	Scarce growth, crusty surface, opaque, hyaline
ISP no. 6 ^a	Moderate growth, wrinkled surface, opaque, hard, amber-orange
ISP no. 7 ^a	Scarce growth, smooth surface, opaque, hyaline to cream
Oatmeal agar ^b	Abundant growth, smooth surface, moist, bright, soft, deep orange, slightly yellowish pigment
Bennett agar ^b	Abundant growth, wrinkled surface, opaque, amber-orange
Czapek-glucose agar ^b	Scarce growth, smooth surface, opaque, hyaline
Glucose asparagine agar ^b	Scarce growth, smooth surface, white, waxy
Nutrient agar ^b	Moderate growth, smooth surface, opaque, hyaline to cream
Potato agar ^b	Moderate growth, smooth surface, opaque, cream to orange
Calcium malate agar ^b	Scarce growth, smooth surface, opaque, cream to light orange
Skim milk agar ^b	Abundant growth, wrinkled surface, opaque, deep orange
Potato plug	Moderate growth, wrinkled surface, opaque, deep orange
Carrot plug	Scarce growth, wrinkled surface, opaque, deep orange

^a Media recommended by International *Streptomyces* Project.

^b Media recommended by S. A. Waksman.

vided into two groups with respect to the location of the sporangium on the mycelium: (i) organisms with sporangia on the substrate (primary) mycelium, the sporangia growing either directly on the hyphae or supported on a sporangiophore, and (ii) organisms with sporangia on a well-developed secondary or aerial mycelium. *Actinoplanes*, *Ampullariella*, *Dactylosporangium*, *Elytrosporangium*, and *Kineosporia* belong to the former group and *Microellobosporia*, *Planobispora*, *Planomonospora*, *Kitasatoa*, and *Streptosporangium* belong to the latter.

The sporangium of *Kineosporia* is, however, clearly distinguishable from those of other genera of the former group on the basis of shape, size, connection to the substrate mycelium, and number of spores. Members of *Actinoplanes* have round to globose sporangia which are carried at the distal end of a long sporangiophore stemming from the substrate mycelium into the air and which contain several motile spores. Their diameters range from 5 to 25 μ m, depending on the species.

Ampullariella has large bottle- to fan-shaped sporangia which contain several motile spores (2). *Dactylosporangium* bears long (up to 6 μ m) finger-like sporangia which are produced in dense clusters and which contain a short chain of elongated motile spores (11). *Elytrosporangium* has pod-shaped sporangia containing a short row of one to six nonmotile spores (5).

Kineosporia, on the other hand, has numerous very small (1 to 2 μ m) sporangia, each containing only one round motile spore. They are borne directly on the vegetative hyphae, a feature that distinguishes them from the sporangia of *Microellobosporia* (4), *Planobispora* (9), and *Planomonospora* (10), which are formed on the aerial mycelium. The amino acid composition of the cell wall and the whole-cell sugar pattern are considered to be two important characteristics in *Actinomycetales* taxonomy (1). The cell wall composition of *Kineosporia*, like that of the *Streptomycetaceae* and the genus *Sporichthya*, is of type I (presence of LL-diaminopimelic acid and glycine).

The whole-cell sugar pattern is characterized by the presence of arabinose, xylose, and galactose.

It is evident that the new isolate cannot be included in any of the currently known genera of the *Actinomycetales*, and for this reason it is placed in a new genus.

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REPRINT REQUESTS

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