Actinomadura atramentaria, a New Species of the Actinomycetales

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A new species of Actinomadura, for which we propose the name Actinomadura atramentaria, was isolated from a soil sample collected in Japan. Whole-cell hydrolysates contain meso-diaminopimelic acid and a trace of madurose. MK-9(H6) is the major menaquinone, and tuberculostearic acid is the major fatty acid. No nitrogenous phospholipids or mycolic acids are present. A. atramentaria is characterized by its white aerial masses of thickly tufted short chains of two to five spores (mostly two to three spores) with smooth surfaces and by the formation of inky brown diffusible pigments. The type strain of A. atramentaria is strain SF2197 (= JCM 6250).

In the course of screening for new antibiotics, we isolated an actinomycete designated strain SF2197T (T = type strain). This organism produces the antibiotic SF2197 (Japan Kokai patents 85-83585, May 1985, and 86-141889, June 1986; assignee, Meiji Seika Kaisha, Ltd.), which is strongly active against anaerobic bacteria. Strain SF2197T belongs to the genus Actinomadura Lechevalier and Lechevalier (9) because of its morphological and chemotaxonomic properties. In this paper we describe the morphological and physiological properties and the results of a chemical analysis of the strain, for which we propose the name Actinomadura atramentaria sp. nov.

MATERIALS AND METHODS

The methods used were generally similar to those used previously (13).

Bacterial strains. Strain SF2197T was isolated in August 1981 in our laboratory by the dry heating method developed by Nonomura and Ohara (14). The soil sample was collected from Yaizu City, Shizuoka Prefecture, Japan. For comparative purposes, the type strains of some species of the genera Microbispora, Microtetraspora, and Actinomadura were also studied (see Table 2). These strains were obtained from the American Type Culture Collection, Rockville, Md., or the Japan Collection of Microorganisms, Saitama, Japan.

Morphological characteristics. Morphological observations were made with a light microscope on cultures grown on oatmeal agar (ISP medium 3) and inorganic salts-starch agar (ISP medium 4) (18) at 28°C for 2 to 4 weeks. Spore morphology was studied with a model JEM 100C electron microscope and a scanning electron microscope (model ASID-4D; Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan).

Cultural and physiological characteristics. The media and procedures used for cultural and physiological characterization of SF2197T were those described by Shirling and Gottlieb (18), by Waksman (22), and by Berd (4). Each culture was incubated at 28°C for 2 to 4 weeks. Color determinations were made by comparing the cultures with color chips from the Color Harmony Manual (8). The temperature range for growth was determined on yeast extract-malt extract agar (ISP medium 2) (18). Carbohydrate utilization was investigated by using the procedure of Pridham and Gottlieb (17).

Chemical analysis of cells. The presence and form of diaminopimelic acid and the presence of carbohydrates in purified cell wall and whole-cell hydrolysates were determined by the methods of Becker et al. (3) and Lechevalier (10). Phospholipids and mycolic acids were analyzed by the procedures of Lechevalier et al. (11) and Hecht and Causey (7), respectively. Menaquinones were prepared by extraction with chloroform-methanol (2:1) from dried cells, were purified by using thin-layer chromatography, and were analyzed by using mass spectrometry and high-performance liquid chromatography (5, 20). The fatty acid composition was determined by the method of Okami et al. (15). The guanine-plus-cytosine content of deoxyribonucleic acid (DNA) was calculated from its thermal denaturation temperature (12).

RESULTS AND DISCUSSION

Morphological characteristics. The vegetative mycelia of strain SF2197T were fine, 0.3 to 0.5 μm diameter, long, and irregularly branched. Fragmentation of hyphae did not occur either on agar or under submerged growth conditions. The
aerial hyphae branched monopodially and were 0.4 to 0.6 μm in diameter. Strain SF2197T formed thickly tufted chains of arthrospores on the aerial mycelia only. Longitudinally paired spores or straight chains of three (or rarely four) spores were closely arranged along the main axes of the sporogenous hyphae and their branches (Fig. 1 and 2). Straight chains of three to five spores were occasionally observed at the tips of sporulating aerial hyphae. Spores were oval to ellipsoidal in shape and smooth surfaced and measured 0.6 to 0.8 by 0.8 to 1.5 μm. Sporophores were extremely short or nonexistent. We observed that protrusions from the spore sheath acted as connections between the individual spores in a longitudinal direction (Fig. 2c). Spores were not motile, and sclerotia or sporangia (including pseudosporangia) were not observed. Cells were gram positive and not acid fast.

Cultural characteristics. The cultural characteristics of strain SF2197T are summarized in Table 1. Mature aerial masses were flat, powdery, and white on all of the media tested. The vegetative mycelium often seemed to be darkened by diffusible pigments. These pigments, which were pale brown to inky brown, were produced on various media, especially in submerged cultures. Under alkaline conditions, the pigments turned dark greenish brown and were found to be more soluble in water than in organic solvents. In contrast, they turned dark violet and were more soluble in organic solvents under acidic conditions.

Physiological characteristics. The physiological characteristics of strain SF2197T are given below. Gelatin liquefaction, hydrolysis of starch, and coagulation of milk were negative. Reduction of nitrate, peptonization of milk, and formation of melanoid pigment in peptone-yeast extract-iron agar, tyrosine agar, and tryptone-yeast extract media were positive. Strain SF2197T grew in media containing NaCl
### TABLE 1. Cultural characteristics of strain SF2197T

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth</th>
<th>Reverse color</th>
<th>Aerial mycelium</th>
<th>Soluble pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose-nitrate agar</td>
<td>Poor</td>
<td>Colorless</td>
<td>Thin, white (a)</td>
<td>None</td>
</tr>
<tr>
<td>Glucose-asparagine agar</td>
<td>Moderate</td>
<td>Colorless</td>
<td>Moderate, white (a)</td>
<td>Pale brown</td>
</tr>
<tr>
<td>Glycerol-asparagine agar (ISP medium 5)</td>
<td>Good</td>
<td>Dark brown</td>
<td>Moderate, white (a)</td>
<td>Pale brown</td>
</tr>
<tr>
<td>Inorganic salts-starch agar (ISP medium 4)</td>
<td>Poor</td>
<td>Colorless</td>
<td>Moderate, white (a)</td>
<td>None</td>
</tr>
<tr>
<td>Oatmeal agar</td>
<td>Poor</td>
<td>Colorless</td>
<td>Thin, white (a)</td>
<td>None</td>
</tr>
<tr>
<td>Yeast extract-malt extract agar (ISP medium 2)</td>
<td>Good</td>
<td>Dark brown</td>
<td>Moderate, white (a)</td>
<td>Inky brown</td>
</tr>
<tr>
<td>Tyrosine agar (ISP medium 7)</td>
<td>Good</td>
<td>Dark brown</td>
<td>Moderate, white (a)</td>
<td>Inky brown</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>Moderate</td>
<td>Colorless</td>
<td>Moderate, white (a)</td>
<td>Pale brown</td>
</tr>
<tr>
<td>Calcium malate agar</td>
<td>Poor</td>
<td>Colorless</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Bennett agar</td>
<td>Good</td>
<td>Dark brown</td>
<td>Moderate, white (a)</td>
<td>Inky brown</td>
</tr>
</tbody>
</table>

* The color codes (in parentheses) were taken from reference 8.

Concentrations up to 4%, but 5% NaCl was inhibitory. Cultures were susceptible to lysozyme. B vitamins were not essential for growth. The temperature range for growth was 15 to 42°C, with the best growth occurring between 28 and 37°C. On ISP medium 9, good growth was obtained with D-glucose and glycerol. No growth or only a trace of growth was observed with D-fructose, D-xylose, D-mannitol, L-arabinose, L-rhamnose, sucrose, raffinose, and myo-inositol.

**Chemical analysis of cells.** The cell wall hydrolysates contained meso-diaminopimelic acid, glutamic acid, alanine, muramic acid, and glucosamine. Whole-cell hydrolysates contained meso-diaminopimelic acid, glucose, mannose, galactose, ribose, and a trace of madurose (3-O-methyl-D-galactose). These data indicate that strain SF2197T has a type III cell wall and a type B whole-cell sugar pattern. A type PI phospholipid pattern (no nitrogenous phospholipid) was found. Mycolic acids were not present. A MK-9(H₆) menaquinone was detected as the major component. A quantitative analysis of the menaquinone composition of the cells revealed 69% MK-9(H₆), 14% MK-9(H₈), 13% MK-9(H₁₀), and 4% others. The whole-cell fatty acids consisted of 44% 10-methyloctadecanoic acid (10Me-19:0, tuberculostearic acid), 19% hexadecanoic acid (16:0, palmitic acid), 8% 10-methylheptadecanoic acid (10Me-18:0), 7% octadecanoic acid (18:1, oleic acid), 6% 14-methylpentadecanoic acid (iso-16:0), 6% 2-hydroxyhexadecanoic acid (2OH-16:0), and other minor components. The guanine-plus-cytosine content of the DNA was 72 mol%.

**Identity of strain SF2197T.** There are three recognized genera of sporoactinomycetes (Actinomadura, Microbispora, and Microtetraspora) that form short spore chains on the aerial mycelia only and have wall chemotype IIIB (19). Our strain was therefore compared with representative species of Microbispora, Microtetraspora, and Actinomadura (Table 2). The chemotaxonomic properties of strain SF2197T coincide with those of Actinomadura citrea ATCC 27887T, Actinomadura malachitica ATCC 27888T, Actinomadura verrucospora JCM 3147T, and Microbispora echinospora ATCC 27888T, but clearly differ from those of Microbispora rosea ATCC 12950T and Microtetraspora gauca JCM 3300T. Moreover, the morphological properties of strain SF2197T, which bears short chains of two to four spores vertically and...
The chains develop into thick tufts on the aerial hyphae. 0.8 liquefaction, hydrolysis of starch, and coagulation of milk mycelium. The aerial mass color is white. Inky brown dinal pairs or in straight chains of three to five spores are occasionally formed only on aerial mycelia, but this genus has not been validated. According to these reports, strain SF2197T, which forms aerial mycelia with short spore chains and contains a type IIIB cell wall, a type PI phospholipid, MK-9(H4) as the major menaquinone, and tuberculostearic acid as the major fatty acid, should be identified as a member of the genus Actinomadura. Strain SF2197T was also compared with previously described Actinomadura species. There are no previously described species characterized by white aerial masses of thickly tufted short chains of two to five (mostly two to three) spores with smooth surfaces and by the formation of inky brown diffuse pigments. Also, the spore-bearing style and the spore chain morphology (Fig. 2) differ from those of other species in this genus. From these facts, we believe that strain SF2197T represents a new species in the genus Actinomadura. The new taxon is described below.

**Actinomadura atramentaria** sp. nov. Actinomadura atramentaria (a-tra-men.ta-ri-a. L. adj. atramentaria, inky, referring to the inky brown diffusible pigments) forms branching vegetative and aerial mycelia. Spores are borne in longitudinal pairs or in straight chains of three or rarely four spores. The chains develop into thick tufts on the aerial hyphae. Straight chains of three to five spores are occasionally observed at the tips of sporulating aerial hyphae. The spores are smooth and oval to ellipsoidal and measure 0.6 to 0.8 by 1.5 μm. No spores are borne on the vegetative mycelium. The aerial mass color is white. Inky brown diffuse pigments are formed on yeast extract-malt extract agar and Bennett agar. Sporangia, sclerotia, flagellated spores, and fragmented hyphae are not formed. Gelatin liquefaction, hydrolysis of starch, and coagulation of milk are negative. Reduction of nitrate, peptonization of milk, and formation of melanoid pigment are positive. Utilizes D-glucose and glycerol, but not D-fructose, D-xylene, D-mannitol, L-arabinose, L-rhamnose, sucrose, raffinose, or myo-inositol. Cell wall type IIIB. Phospholipid type PI.

MK-9(H4) is the major menaquinone. Tuberculostearic acid is the major fatty acid. The DNA guanine-plus-cytosine content is 72 mol%. Aerobic. Mesophilic. Antagonistic properties: produces antibiotic SF2197. Habitat: soil. Type strain: strain SF2197 (JCM 6250).

**LITERATURE CITED**


