Taxonomic Status of Kitasatospora, and Proposed Unification with Streptomyces on the Basis of Phenotypic and 16S rRNA Analysis and Emendation of Streptomyces Waksman and Henrici 1943, 339AL

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Species classified within the genus Kitasatospora share many of the phenotypic characteristics typical of streptomycetes. By using a probabilistic identification scheme, they were identified with Streptomyces exfoliatus cluster 5, a species group within Streptomyces. The four species studied hybridized with a 16S rRNA genus probe for Streptomyces spp., indicating a close relationship between the two genera. The kitasatosporias were resistant to selected polyvalent streptomycete phages tested. Quantitative analysis showed that meso-diaminopimelic acid varied from 49 to 89% in Kitasatospora species and from 1 to 16% in Streptomyces species depending on growth conditions. On the basis of 16S rRNA analysis, it is proposed to reduce Kitasatospora to synonymy with Streptomyces. As a result, the new names proposed are Streptomyces mediocidicus comb. nov., Streptomyces phosalacineus comb. nov., Streptomyces setae comb. nov., and Streptomyces griseolosporeus comb. nov., nom. nov.

The genus Kitasatospora was proposed to include newly isolated actinomycete strains which contained similar amounts of LL and meso isomers of diaminopimelic acid (DAP) and also glycine and galactose in their cell walls (9). Further analysis revealed that most of the LL-DAP was in the aerial mycelium, and the meso-DAP was in the vegetative setae. The sugars galactose and rhamnose were also detected. Further species were described but they had more LL- than meso-DAP (ratio of 3:1) (7). The cell wall typical of Streptomyces spp. contains major amounts of LL-DAP with glycine, wall chemotype 1 (1), although minor amounts of meso-DAP can be detected.

The aim of the present study was to clarify the relationship between the genera Kitasatospora and Streptomyces and determine if the former should be grouped with members of the family Streptomycetaceae on the basis of 16S rRNA, phage susceptibility, chemotype, and phenotype.

MATERIALS AND METHODS

Strains. K. griseola NRRL B-16229, K. mediocidica NRRL 16109T, K. phosalacineus NRRL B-16230, and K. setae NRRL B-16185 (representing the type species) were kindly supplied by D. Labeda and preserved in 10% glycerol at −20°C. Other actinomycetes used for cell wall analysis are listed in Table 2.

Morphology and physiology. Strains were examined by using the methods of Williams et al. (19), and their phenotypes were compared with those of Streptomyces spp. by use of a probabilistic identification scheme for the major species groups (20).

Cell wall analysis. Strains were grown in shake flasks at 30°C for 3 days in tryptone soy broth (Oxoid) (25 ml in 250-ml flasks), harvested by centrifugation, and washed with distilled water and ethanol before being dried. Whole-cell hydrolysates were prepared by using 60 mg of dried cells, and cell wall type was determined by thin-layer chromatography (14). Cells were also harvested from plate cultures grown for 2 days at 30°C on peptone agar containing (weight/volume) peptone, 0.6%; Bacto Casitone, 0.4%; yeast extract, 0.3%; glucose, 0.1%; beef extract, 0.15%; and agar, 2.0%. For high-pressure liquid chromatography (HPLC) analysis, dried hydrolyzed samples were suspended in 10 ml of Milli-Q water and diluted 1:3 before injection into the HPLC. LL- and meso-DAP were measured as the fluorescent o-phthaldialdehyde derivatives (3).

Phage typing. The polyvalent phage DP9, 102, 93, 35, and 34, known to be specific for the Streptomyctetaceae with cell wall chemotype 1 (17), were tested for lysis on lawns of the four Kitasatospora species by using the methods of Wellington and Williams (17).

Isolation of crude RNA, probe synthesis, and hybridization conditions. Cells grown in shake flasks containing tryptone soy broth (30 ml in 100-ml flasks) were harvested after 12 h by centrifugation. Isolation of crude RNA, labeling of probes, and dot blot hybridization were done as described by Stackebrandt and Charfreitag (12). Three hundred nanograms of crude RNA from each of the four Kitasatospora strains and the 16 streptomycete and nonstreptomycete reference strains was hybridized with 10⁶ cpm of 32P-labeled probes (depending on the specific activity, between 20 and 50 ng of RNA). The probes were directed against specific stretches of the 16S rRNA. Hybridization was done in 6x SSC (1x SSC is 0.15 M NaCl, 0.015 M trisodium citrate, and 0.001 M EDTA, pH 7)-0.1% sodium dodecyl sulfate (pH 7.2) at 28°C for the eubacterial consensus probe (5' CTACCA
RESULTS

Probabilistic identification. The phenotypes of the four Kitasatospora species, all identified with the Streptomyces cluster group 5 of Williams et al. (20) (Streptomyces exfoliatus), and the identification scores show a close similarity between all four species and S. exfoliatus (Table 1). The results also indicate that there is phenotypic similarity among the Kitasatosporia species. The strains had a number of atypical characters; they failed to degrade xanthine or allantoin, and K. setae and K. phosalacinea were resistant to rifampin (test method of Williams et al. [20] was used). The taxonomic distance scores (Table 1) indicate that the species are not close to the centroid of the group.

Cell wall analysis. Cell wall analysis of submerged growth (3-day culture) by thin-layer chromatography revealed significant amounts of LL-DAP in all Kitasatosporia species except K. mediocidica. Quantification of the relative amounts of each isomer present in biomass from both liquid and solid cultures was achieved by HPLC analysis (Table 2). By using HPLC, meso-DAP was also detected in Streptomyces species at levels between 1 and 16%, depending on the species analyzed. The level of meso-DAP detected in Kitasatosporia strains varied from 49 to 89%, where variation was dependent on the amount of sporulation in the cultures, as previously reported for this group (15). Except for S. griseola, which produce sporelike bodies, the Streptomyces species did not produce submerged spores. It can be concluded that under any of the conditions tested, the Kitasatosporia species always had at least three times more meso-DAP in the cells than Streptomyces species had. Within the Streptomyces genus, variations in the amounts of meso-DAP were recorded, but no clear correlation was seen with the morphological type of biomass analyzed.

The ratios of meso- to LL-DAP for Kitasatospora species (submerged growth, 3 days) varied from 9:1 to 1:1. Only Kitasatosporia strains contained galactose in the hydrolysate.

Phage typing. All species of Kitasatospora tested were resistant to polyvalent Streptomyces phage. These phage lyse some members of S. exfoliatus cluster 5 and a wide range of Streptomyces species, although some species, such as S. aureofaciens, were resistant to all phage (17).

rRNA probing. The results of the dot blot hybridization are shown in Fig. 1. The autoradiogram illustrates hybridization between crude RNA of Kitasatosporia species, selected streptomycetes, other actinomycetes, and the 32P-labeled probes. The universal probe (Fig. 1A) was used to show the presence and availability of RNA at each dot. The Streptomyces probe (Fig. 1B and C) has been shown to detect all members of the genera Streptomyces and Streptovorticillium (13) but fails to bind to RNA from all other members of the order Actinomycetales. Nonactinomycetes so far investigated also do not bind with the probe. The specificity of the probe is due to a single but highly unique base pair (C at position 955 [E. coli numbering]). The washing temperature therefore has to be raised to 52°C (autorigram C in Fig. 1), which is only 2°C below the melting temperature of the homologous hybrid. The RNAs from all the Kitasatosporia strains, even at the highly stringent washing temperature (Fig. 1C), gave strong hybridization signals with the Streptomyces probe. RNAs from Streptomyces and Streptovorticillium species hybridized with the genus probe. It is evident that the 16S rRNAs from the Kitasatospora species contain the Streptomyces-specific signature nucleotide at position 955.

16S rRNA homologies. The sequence homology between the type species K. setae, S. albus, and Streptovorticillium baldacci (Table 3) shows that there is a high degree of homology between K. setae and S. baldacci. The latter
species has a higher degree of homology to K. setae than to other streptomycetes such as S. brasiliensis. K. setae is also closely related to S. lavendulae.

**DISCUSSION**

The taxonomic status of the genus *Kitasatosporia* is uncertain. The data presented here strongly indicate that members of this genus are closely related to members of the genus *Streptomyces*. We have provided definitive evidence that *Kitasatosporia* species can be grouped with *Streptomyces* and *Streptoverticillium* species on the basis of rRNA probe analysis. However, *Kitasatosporia* species identify phenotypically with *Streptomyces* and not with *Streptoverticillium* species. Conversely, streptoverticillia are susceptible to phage used to type the *Streptomyces* genus, but the species of *Kitasatosporia* tested were resistant. This may reflect differences in restriction enzymes or receptors. The cell wall analysis of *Kitasatosporia* species is confused but does indicate more meso-DAP than occurs in *Streptomyces* and *Streptoverticillium* species. Takahashi et al. (16) made a detailed study of the distribution of meso- and LL-DAP in the different phases of growth. *Streptomyces* species contained only LL-DAP in aerial and vegetative mycelia, whereas *K. setae*, *K. phosalacineus*, and *K. griseola* contained LL-DAP in the aerial mycelium and meso-DAP in the vegetative mycelium. Only LL-DAP was detected in submerged and aerial spores. Nakamura et al. (7) reported differences in the ratio of LL- to meso-DAP when they analyzed submerged growth. *K. setae* was found to contain the isomers in a ratio of 1:3, while two new species were described with ratios of 3:1. Other analyses of submerged growth have simply quoted the isomers to be present in equal amounts (5, 9). Obviously, the amounts of meso- and LL-DAP are variable and depend on the growth conditions and extent of sporulation. In the current study, meso-DAP was detected in significant amounts only by HPLC of extracts from submerged mycelium grown in tryptone soy broth. However, the results were variable and depended on the species examined and the extent of sporulation in the culture, as spores contained LL-DAP but mycelium contained predominantly meso-DAP. Differing amounts (1 to 16%) of meso-DAP can be detected by HPLC in the whole-cell hydrolysates of mycelia from *Streptomyces* species. This suggests that the relative amounts of meso-DAP may not be a good criterion for the delimitation of a genus. *Kitasatosporia* species are also identified by the presence of galactose in whole-cell hydrolysates (9), which appears to be unaffected by the form of growth. This is the only distinguishing feature serving to separate *Kitasatosporia* from *Streptomyces* species. One of the other characteristics cited is the formation of submerged spores, but there have been reports clearly illustrating the formation of spores in submerged cultures of *S. griseus* (2).

**Nomenclatural considerations.** On the basis of 16S rRNA homology, *K. setae* shows a high degree of homology to *S. lavendulae* and *S. baldaccii*. *Streptoverticillium* has been proposed as a synonym of *Streptomyces* species. This suggests that the relative amounts of meso-DAP may not be a good criterion for the delimitation of a genus. *Kitasatosporia* species are also identified by the presence of galactose in whole-cell hydrolysates (9), which appears to be unaffected by the form of growth. This is the only distinguishing feature serving to separate *Kitasatosporia* from *Streptomyces* species. One of the other characteristics cited is the formation of submerged spores, but there have been reports clearly illustrating the formation of spores in submerged cultures of *S. griseus* (2).

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**TABLE 3. Homology of stretches of 16S rRNA (excluding all absolutely conserved positions) of selected Streptomyces species and *K. setae***

<table>
<thead>
<tr>
<th>Organism</th>
<th>Collection no.</th>
<th>% Homology with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>K. setae</em></td>
</tr>
<tr>
<td><em>K. setae</em></td>
<td>ATCC 33774</td>
<td>91.6</td>
</tr>
<tr>
<td><em>S. baldaccii</em></td>
<td>DPDU 0819</td>
<td>91.0</td>
</tr>
<tr>
<td><em>S. lavendulae</em></td>
<td>ISP 5069</td>
<td>89.3</td>
</tr>
<tr>
<td><em>S. albus</em></td>
<td>ISP 5313</td>
<td>89.0</td>
</tr>
<tr>
<td><em>S. brasiliensis</em></td>
<td>ATCC 23727</td>
<td>88.2</td>
</tr>
</tbody>
</table>

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A few species are pathogenic for animals and humans; others utilize only a limited number of carbon sources. Substrate ricini 1943, 339.

diglycerol, phosphatidylinositol, and phosphatidylmannosides. They are widely distributed in soil, including compost. The aerial mycelium consists of long straight filaments bearing branches (3 to 6) at regular intervals which are arranged in whorls (verticils). The appearance at ca. ×100 magnification is of barbed wire. Each branch of the verticil produces at its apex an umbel of two or more spore chains, with spores lacking ornaments. A few species produce spores on the substrate mycelium or in liquid culture. Sclerotia, pycnidia, sporangiumlike bodies, and synnemalike structures may be formed. Form discrete and lichenoid, leathery or butyrous colonies. Initially, colonies are smooth surfaced but later develop a weft of aerial mycelium that may appear floccose, cottony, granular, powdery, or velvety.

Produce a wide variety of pigments responsible for the color of the vegetative and aerial mycelia. Colored diffusible pigments may also be formed. Many strains produce one or other genera. Amino acids, contain major amounts of saturated, iso-, and anteiso-fatty acids, possess either hexa- or octahydrogenated menaquinones with nine isoprene units as the predominant isoprenolog, and have complex polar lipid patterns that typically contain diphasatidyethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol. They are widely distributed in soil, including compost. A few species are pathogenic for animals and humans; others are phytopathogens. The G+C content of the DNA is 66 to 78 mol% (at melting temperature).

Type species: S. albida (Rossi-Doria) Waksman and Henrici 1943, 339.

Description of S. griseolosporeus nom. nov. grí′se.o.lo. spo′re.us. M.L. adj. griseolus, somewhat grey; M.L. n. spora, a spore; griseolosporeus, somewhat grey spored.

The species has been described as K. griseola by Takashashi et al. (16) and by Omura et al. (8). The new name is substituted as a result of homonymy (Rule 34a).

REFERENCES


