We determined almost complete 16S ribosomal DNA sequences for 12 actinomycete strains which were either previously classified as Kitasatospora strains or defined as Streptomyces strains but shown to contain major amounts of meso-diaminopimelic acid in their whole-cell hydrolysates. These sequences were subjected to phylogenetic analyses together with the sequences of 34 Streptomyces species. Phylogenetic trees were reconstructed by using both neighbor-joining and maximum-parsimony methods. The Kitasatospora species always formed a stable monophyletic clade. However, the genus Kitasatospora appeared to be either a sister taxon of the genus Streptomyces or a lineage that originated from within Streptomyces species, depending on the outgroup used. Phylogenetic trees were also constructed by using the sequences of the 16S-23S rRNA gene spacers. Streptomyces and Kitasatospora species were consistently recovered as two distinct clades independent of the outgroup used. On the basis of phylogenetic, chemotaxonomic, and phenotypic evidence, we propose that the genus Kitasatospora Omura et al. 1982 should be revived.

The genus Kitasatospora was proposed by Omura et al. in 1982 for actinomycete strains which were phenotypically similar to Streptomyces strains but contained major amounts of the meso isomers of diaminopimelic acid (DAP) and galactose in whole-cell hydrolysates (15, 16). Takahashi et al. (24) studied the distribution of the two isomers of DAP in cells at different stages of differentiation and growth and found that Streptomyces species contained only LL-DAP in both aerial and vegetative mycelia, while Kitasatospora species contained LL-DAP in the aerial mycelium and meso-DAP in the vegetative mycelium. Although the relative amounts of the two isomers seemed to vary in different experiments and some Streptomyces species were also found to contain various amounts of meso-DAP, Kitasatospora species consistently had a much higher ratio of meso-DAP to LL-DAP than Streptomyces species (29). The Kitasatospora species are also characterized by their resistance to polyvalent Streptomyces phages (29) and the formation of submerged spores in liquid culture (15, 16, 24), which are features rarely observed in Streptomyces species.

Wellington et al. (29) reported that the 16S rRNA sequence of Kitasatospora setae showed 91.6% similarity to the 16S rRNA sequence of Streptomyces baldeceii and that a Streptomyces-specific oligonucleotide probe could recognize all four valid Kitasatospora species. On the basis of these observations and phenotypic properties shared by Kitasatospora and Streptomyces species, these authors proposed that the name Kitasatospora should be reduced to synonymy with the name Streptomyces. Ochi and Hiranuma (14) later supported this proposal on the basis of the results of an analysis of the N-terminal sequences of ribosomal protein AT-L30. However, the unification of these two genera is not unequivocal. For instance, Nakagaito et al. (12), using the results of DNA-DNA reassocation and phenetic studies, found that the Kitasatospora species and the original Streptomyces species formed two distinct clusters. Recently, Kim et al. (8) conducted a 16S rRNA sequence-based phylogenetic analysis of a large number of Streptomyces species and observed that three Kitasatospora species formed a distant and stable clade outside the clade comprising Streptomyces species.

In order to further clarify the phylogenetic relationship between the genera Kitasatospora and Streptomyces, we determined the nucleotide sequences of 16S rRNA genes and 16S-23S rRNA gene spacers of 12 actinomycete strains which were either previously classified as Kitasatospora strains or defined as Streptomyces strains but shown to contain major amounts of meso-DAP in their whole-cell hydrolysates. Here, we report the results of phylogenetic analyses in which we used sequences of both 16S ribosomal DNAs (rDNAs) and 16S-23S rRNA gene spacers, and we propose that the genus Kitasatospora Omura et al. 1982 (15) should be revived.

**MATERIALS AND METHODS**

Organisms and culture conditions. The actinomycete strains used in this study were purchased from the Japan Collection of Microorganisms (Wako, Japan) and the Institute for Fermentation (Osaka, Japan). Strain designations are listed in Table 1. Cells were cultured in media as described by the suppliers.

Preparation of genomic DNAs. The genomic DNAs were prepared as previously described (27, 28).

PCR amplifications. The pair of oligonucleotides and PCR conditions used for amplification of the nearly complete 16S rRNA genes have been described previously (27, 28). For amplification of the 16S-23S rRNA gene spacers, one primer was designed to target the conserved sequence at the end of the 16S rRNA gene, and the second primer was specific for a conserved block at the beginning of the 23S rRNA gene. The sequences of the two oligonucleotides are as follows: 5' CCG GGA TCC GGT TGG ATC CAC CTC CTG (nucleotides 1525 to 1542; Escherichia coli numbering) [1] and 5' AAG GGA TCC TGC CAA GGC ATC CAC C3' (nucleotides 33 to 48; E. coli numbering). The restriction site for BamHI (underlined nucleotides) was added to each primer for convenient cloning of the PCR-amplified fragments. The PCR conditions were basically the same as those used for amplifying 16S rRNA genes (27, 28), except that the elongation time was 20 s.

Cloning and sequencing analysis. Cloning and sequencing of the PCR-amplified rDNA and spacer fragments were carried out as described previously (27, 28).

Sequence alignment and phylogenetic analysis. Multiple alignment of sequences and computation of sequence similarities were carried out by using the Clustal method of the DNASTAR program (DNASTAR, Inc., Madison, Wis.). Evolutionary distance matrices were generated by the method of Jukes and Cantor (6). Phylogenetic trees were constructed by using both the maximum-parsimony method of the PAUP program (23) and the neighbor-joining method described by Saitou and Nei (15). The confidence level of phylogenetic tree topology was evaluated by performing 1,000 bootstrap replications and using the bootstrap program contained in the ClustalW package (4).
organism was used in this study. The organisms used in this study are listed in Table 1. The original names of these organisms are used below. There are four valid Kitasatospora species (Kitasatospora setae [15], Kitasatospora grisea [25], Kitasatospora phosacalinea [25], and Kitasatospora mediocidica [10]) and four invalid Kitasatospora species ("Kitasatospora melanogenes" [20], "Kitasatospora brunnea" [21], "Kitasatospora cystarginea" [9], and "Kitasatospora kifinense" [5]). "Streptomycetes cochleatus" and "Streptomyces paracochleatus" (12) were defined after the proposed unification of the genera Kitasatospora with Streptomyces. These two species and the previously defined taxon "Streptomyces azaticus" (3, 12) were shown to contain major amounts of meso-DAP in their cell walls and to exhibit closer DNA-DNA relatedness with Kitasatospora species than with members of the original genus Streptomyces (12). "Nocardiosis streptosporus" (11) was combined, by Nakagaito et al. (12), with K. phosacalinea and "K. brunnea" in one species. For convenience, we refer to the species mentioned above as members of the original genus Streptomyces throughout this paper. We also included "Kitasatospora papulosa" (13) and "Kitasatospora grisea" (13), which were originally classified as Kitasatospora species but later were found to belong to the original genus Streptomyces because no meso-DAP was detected in their cell walls (12).

**Analysis of 16S rDNA sequences.** We obtained almost complete 16S rDNA sequences (nucleotides 7 to 1507; E. coli numbering [1]) from all of the organisms described above and subjected them to phylogenetic analyses. The initial analysis, which included sequences from representative species of most actinomycete groups, confirmed that the kitasatosporae and streptomycetes were most closely related to each other in the order Actinomycetales (data not shown) (8, 29). We then carried out more detailed analyses in which we focused on sequences from members of the two groups of organisms. Figure 1 presents the phylogenetic trees inferred when the neighbor-joining method (18) was used. When the alignment gaps were included as a fifth base and Nocardia asteroides and Bacillus subtilis were used as outgroups (Fig. 1A), all of the Kitasatospora species aggregated, with a bootstrap value of 100%, in one clade separate from the clade containing Streptomyces species. This clade contained "S. coelicus," "S. paracochleatus," "S. azaticus," and "Nocardiosis streptosporus," which confirmed the close relationships of these species with members of the genus Kitasatospora determined by Nakagaito et al. (12).

The misclassified taxa "K. papulosa" and "K. grisea" aggregated closely with six Streptomyces species. When the nucleotide positions corresponding to the alignment gaps were excluded from the analysis (Fig. 1B), the Kitasatospora clade was shown to originate from within the radiation of the genus Streptomyces and to form a sister group of the genus containing several Streptomyces species, represented by Streptomyces bikiiniensis. Similar trees were also obtained when Streptosporangium and Micromonospora species were used as outgroups with or without inclusion of the alignment gaps in the analysis and when the maximum-parsimony method (23) was used (data not shown). Kim et al. (8) also noticed the effect of different outgroups on the relationship between kitasatosporae and streptomycetes. However, in no case were Kitasatospora species intermixed with Streptomyces species, and the bootstrap value for the Kitasatospora clade was always more than 95%. The integrity of the Kitasatospora group was also demonstrated by the results of a pairwise analysis of the levels of nucleotide sequence similarity (data not shown). The average level of sequence similarity between members of the genus Kitasatospora was 97.65%, and the levels of sequence similarity ranged from 95.9% (between "S. azaticus" and "K. brunnea") to 99.5% (between "S. coelicus" and "S. paracochleatus"), whereas the average level of sequence similarity between Kitasatospora and Streptomyces species was much lower, 93% (range, 90.7 to 95.6%).

**Analysis of 16S-23S rRNA gene spacers.** The 16S rRNA sequence analysis described above could not distinguish between the following two possible relationships of kitasatosporae to streptomycetes: (i) both groups are monophyletic, and the two taxa are sister taxa; and (ii) the kitasatosporae represent a lineage that originated from within the radiation of the genus Streptomyces, in which case the genus Streptomyces would be paraphyletic. An example of the second type of relationship is the evolutionary origin of the genus Nocardia from within the radiation of Rhodococcus species (17).

16S rRNA sequences are highly conserved in evolution (31), and closely related species are often found to have identical or nearly identical sequences (22). Thus, 16S rRNA sequence-based phylogenetic analysis may not be powerful enough to reliably resolve close phylogenetic relationships, such as the relationship between the kitasatosporae and the streptomycetes (22). Nucleotide sequences of genes that are evolutionarily more variable than the rRNA genes may provide useful information for the resolution of close relationships. To further investigate the phylogenetic relationship between the two groups of organisms, we analyzed the internally transcribed spacers between the 16S and 23S rRNA genes, a region known to be more variable than 16S rRNA genes (2). We cloned and sequenced the 16S-23S rRNA gene spacers of all of the strains listed in Table 1 and of species belonging to the genera Streptosporangium, Micromonospora, Microtetraspora, and Microbispora for use as outgroups in phylogenetic analyses. Figure 2 shows the phylogenetic trees inferred when the spacer sequences were used. Although considerable gaps had to be introduced in some regions in the alignment (Fig. 3), the kitasatosporae and streptomycetes were consistently separated into two distinct clades irrespective of the inclusion (Fig. 2A) or exclusion (Fig. 2B) of the gaps. The separation of the two groups was substantiated by high bootstrap values and was not affected by the different outgroups used. Spacers from more distant groups of actinomycetes could not be used as outgroups, because they exhibit very low levels of homology with the spacers of streptomycetes and kitasatosporae. These results strongly suggest that the genera Kitasatospora and Streptomyces are sister taxa.

**TABLE 1. Actinomycete strains used**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Strain</th>
<th>GenBank accession no.</th>
<th>16S rDNA Spacer region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitasatospora kifinense</td>
<td>JCM9081T</td>
<td>U93322 U93323</td>
<td></td>
</tr>
<tr>
<td>&quot;Kitasatospora brunnea&quot;</td>
<td>IOFI4627</td>
<td>U93314 U93315</td>
<td></td>
</tr>
<tr>
<td>&quot;Kitasatospora cystarginea&quot;</td>
<td>JCM7356T</td>
<td>U93318 U93319</td>
<td></td>
</tr>
<tr>
<td>Kitasatospora grisea</td>
<td>JCM3339</td>
<td>U93320 U93321</td>
<td></td>
</tr>
<tr>
<td>Kitasatospora mediocidica</td>
<td>IOFI4879</td>
<td>U93324 U93325</td>
<td></td>
</tr>
<tr>
<td>&quot;Kitasatospora melanogenes&quot;</td>
<td>JCM3337</td>
<td>U93326 U93327</td>
<td></td>
</tr>
<tr>
<td>Kitasatospora phosacalinea</td>
<td>JCM3340</td>
<td>U93330 U93331</td>
<td></td>
</tr>
<tr>
<td>Kitasatospora setae</td>
<td>JCM3304</td>
<td>U93332 U93333</td>
<td></td>
</tr>
<tr>
<td>&quot;Nocardiosis streptosporus&quot;</td>
<td>IOFI4362</td>
<td>U93334 U93335</td>
<td></td>
</tr>
<tr>
<td>&quot;Streptomyces azaticus&quot;</td>
<td>IOFI3803+</td>
<td>U93312 U93313</td>
<td></td>
</tr>
<tr>
<td>&quot;Streptomyces coelicus&quot;</td>
<td>IOFI4768+</td>
<td>U93316 U93317</td>
<td></td>
</tr>
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<td>&quot;Streptomyces paracochleatus&quot;</td>
<td>IOFI4769+</td>
<td>U93328 U93329</td>
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</tr>
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<td>&quot;Kitasatospora grisea&quot;</td>
<td>JCM7249</td>
<td>U93336 U93337</td>
<td></td>
</tr>
<tr>
<td>&quot;Kitasatospora papulosa&quot;</td>
<td>JCM7250</td>
<td>U93338 U93339</td>
<td></td>
</tr>
</tbody>
</table>
FIG. 1. Neighbor-joining trees generated by using 16S rDNA sequences from members of the genera *Kitasatospora* and *Streptomyces*. The numbers at the nodes indicate the levels of bootstrap support based on 1,000 resamplings. Bootstrap values lower than 500 are not shown. The bars indicate the numbers of inferred substitutions per nucleotide. The sequences for *Streptomyces* species were retrieved from the GenBank database. (A) The gaps in the multiple-sequence alignment were treated as a fifth base. The position of the root was determined by using *Nocardia asteroides* and *Bacillus subtilis* as outgroups. (B) The nucleotide positions corresponding to the alignment gaps were excluded from the analysis. The arrows indicate the positions of the root when *Nocardia asteroides* (N), *Bacillus subtilis* (B), *Micromonospora* species (M), and *Streptosporangium* species (S) were used as outgroups.
FIG. 2. Neighbor-joining trees generated by using the sequences of 16S-23S rRNA gene spacers. For details, see the legend to Fig. 1. The GenBank accession numbers for the spacer sequences of non-Kitasatospora species and subspecies are as follows: Micromonospora carbonacea subsp. carbonacea, AF004000; Micromonospora carbonacea subsp. aurantica, AF004001; Micromonospora echinospora subsp. hylotechna, AF004004; Micromonospora echinospora subsp. ferruginea, AF004005; Micromonospora echinospora subsp. pallida, AF004006; Nocardiosis streptosporus, AF004007; Microtetraspora africana, AF004008; Microtetraspora carbonacea subsp. carbonacea, AF004009; Streptosporangium amethystogenum, AF004010; Streptosporangium argillaceum, AF004011; Streptosporangium fragilis, AF004012; and Streptosporangium pseudovulgare, AF004013.
Specific nucleotide signatures in the spacer region.

The spacer sequence alignment revealed nucleotide signatures specific for each group of organisms (Fig. 3) unique to members of the genus *Kitasatospora*. Such signatures can be targeted by oligonucleotides for convenient differentiation between kitasatosporae and streptomycetes by hybridization or PCR. Figure 4 shows that a pair of oligonucleotide primers designed to be specific for the *Kitasatospora* species amplified an expected fragment only from members of the genus *Kitasatospora*. For example, the results of our phylogenetic analyses, based on the sequences of both 16S rRNA genes and 16S-23S rRNA gene spacers, strongly suggest that the genus *Kitasatospora* is a taxon in that the two groups of *Kitasatospora* species amplified an expected fragment only from members of the genus *Kitasatospora*.
organisms are indeed closely related. However, in the study of Wellington et al. (29), which was based on partial 16S rRNA sequences from only one Kitasatospora species, K. setae, and four Streptomyces species, the authors could not possibly discover the phylogenetic separation of the two groups of actinomyces. In addition to the phylogenetic evidence, the chemotaxonomic differences between the members of the genus Kitasatospora and the members of the genus Streptomyces are also substantial. It is well accepted that both DAP and galactose have great discriminatory value in classification and identification of actinomycetes at the generic and suprageneric levels (7, 19). The presence of major amounts of meso-DAP and galactose in the members of the genus Kitasatospora (12, 15, 25) but not in the members of the genus Streptomyces reflects the phylogenetic integrity of each group and the distance between the two groups of organisms. The formation of submerged spores in liquid culture and resistance to polyvalent Streptomyces phages are other characteristics that may distinguish the members of the genus Kitasatospora from at least the majority of Streptomyces species.

On the basis of the phylogenetic, chemotaxonomic, and phenotypic evidence described above, we propose that the genus Kitasatospora Omura, Takahashi, Iwai, and Tanaka 1982 (15) should be revived.

**Nomenclature considerations.** As a result of the revival of the genus Kitasatospora, the description of the genus Streptomyces Waksman and Henrici 1948 (26) should follow the description given by Witt and Stackebrandt (30). On the basis of chemotaxonomic and phenotypic properties determined by Nakagaito et al. (12) and the result of this study, the Streptomyces species “S. azaticus,” “S. cocheleatus,” and “S. paracocheleatus” should be transferred to the genus Kitasatospora.

**Emended description of the genus Kitasatospora Omura, Takahashi, Iwai and Tanaka 1982.** Kitasatospora (K. ta.ta.so.to.spo'ra. Jpn. n. Kitasato, a Japanese microbiologist; M.L. fem. n. spora spore; M.L. fem. n. Kitasatospora, Kitasato spore). The description below is based on data from this study and previous studies (12, 15, 29).

The substrate mycelium is as well-developed as Streptomyces substrate mycelium, and the aerial mycelium bears long spore chains containing more than 20 spores. No fragmentation of substrate mycelium occurs. No sporangia are formed. The major constituents of the cell wall are glycine, galactose, and meso-DAP or LL-DAP, depending on the type of cells analyzed. When cells are grown on agar media, the aerial spores contain LL-DAP, whereas the substrate mycelium contains meso-DAP. When cells are grown in liquid media, submerged spores are formed, and the spores contain LL-DAP and the filamentous mycelia contain meso-DAP. Whole-cell hydrolysates contain galactose, but lack arabinose, madurose, and xylose. The phospholipid type is type II. The glycolate test is negative. The organism is gram positive, aerobic, and chemoorganotrophic. The growth temperature range is 15 to 42°C, and the pH range is 5.5 to 9.0. The G+C content is 66 to 73 mol%.

The genus Kitasatospora can be distinguished from the genus Streptomyces by the ratio of meso-DAP to LL-DAP in whole-cell hydrolysates. The meso-DAP content is 49 to 89% in Kitasatospora strains and 1 to 16% in Streptomyces strains. Galactose is present in the whole-cell hydrolysates of Kitasatospora strains but not in the whole-cell hydrolysates of Streptomyces strains. In the dendrogram constructed by using 16S rRNA sequences of actinomycete species, Kitasatospora species form a tight clade that excludes all Streptomyces species; and in the dendrogram based on the 16S-23S rRNA gene spacers, the genera Kitasatospora and Streptomyces form distinct clades. The genus Kitasatospora can be readily distinguished from the genus Streptomyces by specific nucleotide signatures in the sequences of both the 16S rRNA and the 16S-23S rRNA gene spacers.

The type species of the genus is Kitasatospora setae Omura, Takahashi, Iwai, and Tanaka 1982 (15).

**Descriptions of new combinations.** The description of Kitasatospora azatica comb. nov. (a.za'ti.ca. L. adj. azatica, referring to the product azu amino acid, an antitumor agent) is the same as that given by Nakagaito et al. (12).

The description of Kitasatospora cocheleata comb. nov. (coh'le.e-a.ta. L. adj. cocheleata, spiral, referring to the formation of spiral aerial mycelium) is the same as that given by Nakagaito et al. (12).

The description of Kitasatospora paracocheleata comb. nov. (pa.ru.co.ch'e.l.e-a.ta. L. pron. para, along side of, resembling; L. adj. paracocheleata, a species like K. cocheleata) is the same as that given by Nakagaito et al. (12).

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**REFERENCES**


