Analysis of Thermophilic Clades within the Genus *Streptomyces* by 16S Ribosomal DNA Sequence Comparisons

DAEJIN KIM,^{1,2} JONGSIK CHUN,^{1,2} NEVZAT SAHIN,^{2,3} YUNG CHIL HAH,¹ AND MICHAEL GOODFELLOW^{2*}

Department of Microbiology and Research Center for Molecular Microbiology, Seoul National University, Seoul 151-742, Republic of Korea¹; Department of Microbiology, The Medical School, Newcastle upon Tyne, NE2 4HH, United Kingdom²; and O.M.Ü. Egitim Fakültesi, Biyoloji Bölümü, 55200 Atakum-Samsun 55200, Turkey³

Almost complete sequences of the 16S rRNA genes of eight representative thermophilic streptomycetes were determined following the isolation and cloning of the amplified genes. These sequences were aligned with those of representative mesophilic streptomycetes, and phylogenetic trees were inferred by using four tree-making algorithms. The thermophilic streptomycetes formed two distinct clades that were supported by high bootstrap values based on 1,000 resamplings. One clade encompassed *Streptomyces macrosporus* and related species, and the second clade included *Streptomyces thermodiastaticus* and allied taxa. The relationships between the organisms were not markedly influenced by the different tree-making methods, but the rooting of the tree was sensitive to the choice of the outgroup strain. It is evident from this study that the thermophilic streptomycetes do not merit recognition as a distinct taxon within the genus *Streptomyces*.

Most of the emphasis in streptomycete systematics has been focused on mesophilic strains, which grow at temperatures between 15 and 37°C and have optimum temperatures around 25°C. Thermophilic streptomycetes have growth temperature ranges between 28 and 55°C, although cultures of “*Streptomyces thermofuscus*” and “*Streptomyces thermophilus*” have been reported to grow at 65°C (45). There is some controversy about whether streptomycetes that grow at 45°C or above should be assigned to distinct taxa or should be considered thermotolerant variants of mesophilic species. Craveri and Pagani (6) proposed the subgenus *Thermostreptomyces* for thermophilic strains, but other workers (5, 21) have considered such organisms thermotolerant rather than thermophilic streptomycetes. The name *Thermostreptomyces* was listed under genera incertae sedis in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (33).

The only comprehensive taxonomic study of thermophilic streptomycetes was carried out by Goodfellow et al. (12), who examined 50 thermophilic, neutrophilic streptomycetes isolated from diverse habitats and representative mesophilic marker strains that had been included in the extensive numerical taxonomic survey of Williams et al. (48). The test strains were screened for 135 unit characters, and the resultant data were analyzed by using three resemblance coefficients and an average-linkage algorithm. In all three analyses, the thermophilic streptomycetes formed a distinct aggregate taxon that encompassed three major clusters (7 to 19 strains), five minor clusters (2 to 3 strains), and two single-member clusters.

The three major clusters were equated with *Streptomyces megasporus*, *Streptomyces thermoviolaceus*, and *Streptomyces thermovulgaris*, and one of the minor clusters was equated with *Streptomyces macrosporus*. The name *Streptomyces thermolineatus* was proposed for one of the remaining minor clusters. Emended descriptions were given for *Streptomyces macrosporus* (Krassilnikov et al. 1968) Agre 1983 (1, 20), *Streptomyces thermoviolaceus* Henssen 1957 (13), and *Streptomyces thermovulgaris* Henssen 1957 (13). It was also proposed that the name *Streptomyces macrosporus* Krassilnikov et al. 1968 should be revived. The mesophilic streptomycetes were recovered in the

major and minor clusters recognized by Williams et al. (48), and, with the exception of the *Streptomyces albus* strains, they formed a second aggregate group.

Molecular systematic methods, notably the methods used to determine taxonomic relationships on the basis of nucleic acid sequence and DNA-DNA relatedness data (36), are having an increasingly important impact on streptomycete systematics (31, 37, 38, 40, 46, 49). In this investigation, the almost complete sequences of the 16S rRNA genes (rDNAs) of representative thermophilic streptomycetes were determined and compared, by using several algorithms (14, 18, 23), with corresponding sequences of mesophilic streptomycetes held in the public domain in order to further clarify the taxonomy of organisms classified in the genus *Streptomyces*.

MATERIALS AND METHODS

Organisms and culture conditions. Cultures of the test strains (Table 1) were grown on modified Bennett's agar plates (15) at 45°C for 3 days. After incubation, biomass was scraped from the plates and washed twice with T.E. buffer (pH 8) (35). The streptomycetes were maintained on modified Bennett's agar slopes and as glycerol suspensions (20%, vol/vol) at –20°C.

DNA extraction. Chromosomal DNA was isolated by using a procedure (4) slightly modified from that of Pitcher et al. (32).

PCR amplification of 16S rDNA. The 16S rDNAs were enzymatically amplified by using two universal primers, p27f and p1525r (22), and then further purified by using an Ultrafree-MC filter unit (pore size, 0.45 µm; Millipore, Ltd., Watford, England) and the protocol recommended by the manufacturer. The PCR procedure which we used has been described previously (4).

Isolation and cloning of amplified 16S rDNA. Purified 16S rDNA fragments were ligated into the pGEM-T vector (Promega Co., Southampton, England) by following the manufacturer's instructions. Ligated plasmids were then transformed into *Escherichia coli* JM109, and transformants were selected on the basis of the results of the blue-white screening procedure (35).

Sequencing of 16S rDNA. Plasmids containing the 16S rDNA fragment were isolated by using the standard purification method recommended by the manufacturer (Promega Co.). Purified plasmids were sequenced by using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.). Sequencing gel electrophoresis was carried out and nucleotide sequences were automatically obtained by using an Applied Biosystems model 373A DNA sequencer and software provided by the manufacturer. The oligonucleotide primers used in the sequencing reaction have been described previously (4).

Phylogenetic analysis. The 16S rDNA sequences obtained were aligned manually with streptomycete nucleotide sequences (Table 1) obtained from the Ribosomal Database Project (24) and EMBL-GenBank databases. Two data sets were generated and analyzed separately as some of the nucleotide sequences obtained from the databases were partial sequences (that is, they consisted of less than 1,300 nucleotides). The first data set included sequences containing more than 1,300 nucleotides, and the second data set consisted of partial sequences

* Corresponding author. Mailing address: Department of Microbiology, The Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, United Kingdom. Fax: 44-91-222-7736.

TABLE 1. Strains used and their nucleotide sequence accession numbers

Species or subspecies	Strain ^a	Source ^b	Accession no.	Reference
Organisms whose complete sequences (>1,300 nucleotides) were used				
<i>Kitasatosporia griseola</i>		DSM 43859 ^T	M55221	46
<i>Kitasatosporia phosalacinea</i>		DSM 43860 ^T	M55223	46
<i>Kitasatosporia setae</i>		DSM 43861 ^T	M55220	46
<i>Streptomyces ambofaciens</i>	ISP 5053 ^T	ATCC 23877 ^T	M27245	31
<i>Streptomyces bikiniensis</i>	ISP 5581 ^T	DSM 40581 ^T	X79851	25
<i>Streptomyces bluensis</i>	ISP 5564 ^T		X79324	25
<i>Streptomyces caelestis</i>	ISP 5084	NRRL 2418 ^T	X80824	25
<i>Streptomyces violaceoruber</i> (<i>Streptomyces coelicolor</i>)		A3(2)	X60514	40
" <i>Streptomyces espinosus</i> "		NRRL 5729	X80826	25
<i>Streptomyces galbus</i>	ISP 5089 ^T	DSM 40089 ^T	X79852	25
<i>Streptomyces glaucescens</i>		DSM 40716	X79322	25
<i>Streptomyces griseus</i> subsp. <i>griseus</i>	ISP 5236 ^T	KCTC 9080 ^T	X61478	17
" <i>Streptomyces hygrosopicus</i> subsp. <i>limoneus</i> "		ATCC 21431	X79853	25
<i>Streptomyces lincolnensis</i>	ISP 5355 ^T	NRRL 2936 ^T	X79854	25
" <i>Streptomyces ornatus</i> "	ISP 5307 ^T	DSM 40307 ^T	X79326	25
<i>Streptomyces pseudogriseolus</i>		NRRL 3985	X80827	25
<i>Streptomyces subutilus</i>	ISP 5445 ^T	DSM 40445 ^T	X80825	25
<i>Streptomyces mashuensis</i>	ISP 5221 ^T	DSM 40221 ^T	X79323	25
<i>Streptomyces thermodiastaticus</i>	ISP 5573 ^T	DSM 40573 ^T	Z68101	This study
<i>Streptomyces thermolineatus</i>		DSM 41451 ^T	Z68097	This study
<i>Streptomyces megasporus</i>		DSM 41476 ^T	Z68100	This study
<i>Streptomyces macrosporus</i>		DSM 41449 ^T	Z68099	This study
<i>Streptomyces thermonitrificans</i>	ISP 5579 ^T	DSM 40579 ^T	Z68098	This study
<i>Streptomyces thermovulgaris</i>	ISP 5444 ^T	DSM 40444 ^T	Z68094	This study
<i>Streptomyces thermoviolaceus</i> subsp. <i>apingens</i>		DSM 41392 ^T	Z68095	This study
<i>Streptomyces thermoviolaceus</i> subsp. <i>thermoviolaceus</i>	ISP 5443 ^T	DSM 40443 ^T	Z68096	This study
Organisms whose partial sequences (<1,300 nucleotides) were used				
<i>Streptomyces albus</i>	ISP 5313 ^T	DSM 40313 ^T	X53163	37
<i>Streptomyces brasiliensis</i>		DSM 43159 ^T	X53162	37
<i>Streptomyces diastaticus</i>	ISP 5496 ^T	DSM 40496 ^T	X53161	37
<i>Streptomyces lavendulae</i>	ISP 5069 ^T	DSM 2014 ^T	X53173	37
<i>Streptomyces purpureus</i>		DSM 43460 ^T	X53170	37
<i>Streptomyces abikoensis</i>		DSM 40831 ^T	X53168	37
<i>Streptomyces baldacii</i>		DPDU 0819 ^T	X53164	37
" <i>Streptomyces cinnamoneus</i> subsp. <i>azocolutum</i> "		DPDU 0074 ^T	X53165	37
<i>Streptomyces cinnamoneus</i> subsp. <i>cinnamoneus</i>	ISP 5005 ^T	DPDU 0093 ^T	X53171	37
<i>Streptomyces ladakanum</i>	ISP 5587 ^T	DSM 40587 ^T	X53167	37
<i>Streptomyces luteoreticuli</i>	ISP 5509	ATCC 27446	X53172	37
<i>Streptomyces olivoreticuli</i> subsp. <i>cellulophilus</i>		DPDU 0278 ^T	X53166	37
<i>Streptomyces salmonis</i>		DPDU 00098 ^T	X53169	37

^a ISP, International *Streptomyces* project.

^b ATCC, American Type Culture Collection, Rockville, Md.; DPDU, Instituto di difesa delle Plante, Universita degli Studi di Udine, Udine, Italy; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; KCTC, Korean Collection of Type Cultures, Korean Research Institute of Bioscience and Biotechnology, Taejeon, Republic of Korea; NRRL, Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill.

(Table 1). Data set 1 contained information on 1,438 positions for 26 streptomycetes, and data set 2 contained information on 1,028 nucleotide positions for 39 streptomycetes.

Evolutionary trees for the two data sets were inferred by using the following four algorithms: the neighbor-joining (34), least-squares (11), maximum-parsimony (19), and maximum-likelihood (8) methods. Evolutionary distance matrices for the neighbor-joining and least-squares methods were generated as described by Jukes and Cantor (16). The PHYLIP package (10) was used for the neighbor-joining, Fitch-Margoliash, and maximum-parsimony analyses, and the fastDNAmI program (29) was used for the maximum-likelihood method. The resultant unrooted tree topologies were evaluated by performing bootstrap analyses (9) of the neighbor-joining method data based on 1,000 resamplings. The root positions of the unrooted trees were estimated by using the neighbor-joining method and five outgroup organisms (*Arthrobacter globiformis* DSM 20214^T [T = type strain] [accession number M23411], *Bacillus subtilis* [K00637], *E. coli* [J01695], *Nocardia asteroides* ATCC 19247^T [Z36934], and *Streptosporangium roseum* DSM 43021^T [X70425]) as described by Swofford and Olsen (39).

RESULTS AND DISCUSSION

The almost complete 16S rDNA sequences (1,481 to 1,490 nucleotides between *E. coli* positions 28 and 1524 [3]) obtained

for eight representative thermophilic streptomycetes were compared with the corresponding sequences of 18 mesophilic streptomycetes (including *Kitasatosporia* strains) by using four tree-making algorithms. It is evident from the phylogenetic tree and the sequence similarity values (Table 2) that the thermophilic streptomycetes formed two phyletic lines (Fig. 1). The taxonomic integrity of the two clades was supported by relatively high bootstrap values based on 1,000 resamplings. The integrity of the phyletic line consisting of *Streptomyces macrosporus* and related taxa was supported by the results obtained with all four algorithms (i.e., the neighbor-joining, least-squares, maximum-parsimony, and maximum-likelihood methods). The phyletic line that included *Streptomyces thermodiastaticus* and related taxa was also recovered in all four analyses. The two phyletic lines were consistently recovered in analyses based on the partial nucleotide sequences of the 8 thermophilic and 31 mesophilic streptomycetes (Fig. 2). It is evident from these data that the thermophilic streptomycetes

TABLE 2. Levels of similarity based on 16S rRNA sequences of representative thermophilic and mesophilic streptomycetes

Organism ^a	No. of nucleotide differences or % 16S rRNA sequence similarity ^b																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>Streptomyces macrosporus</i>	100	28	44	41	49	45	41	48	69	72	71	58	57	50	53	46	64	52	67	66	65	53	69	49	71					
<i>Streptomyces megasporus</i>	98.0	100	60	53	62	57	57	61	76	79	79	71	74	64	62	58	76	64	80	78	79	65	81	66	84					
<i>Streptomyces thermodiastaticus</i>	96.9	95.8	100	51	32	13	9	31	72	76	75	36	50	25	34	31	49	27	64	50	47	41	65	33	60					
<i>Streptomyces thermolineatus</i>	97.1	96.3	96.4	100	62	49	48	61	59	70	57	54	53	57	52	51	59	56	60	61	58	46	62	48	62					
<i>Streptomyces thermotritificans</i>	96.6	95.7	97.8	95.7	100	22	26	1	74	80	77	49	60	41	46	38	58	40	64	63	62	47	65	40	66					
<i>Streptomyces thermoviolaceus</i> subsp. <i>apigenens</i>	96.9	96.0	99.1	96.6	98.5	100	8	21	66	70	67	32	47	26	29	31	49	23	56	50	47	29	57	29	52					
<i>Streptomyces thermoviolaceus</i> subsp. <i>thermoviolaceus</i>	97.1	96.0	99.4	96.6	98.2	99.4	100	25	71	74	71	29	45	24	27	23	46	21	59	47	46	33	60	25	56					
<i>Streptomyces thermovulgaris</i>																														
<i>Kitasatospora griseola</i>																														
<i>Kitasatospora phosalacinea</i>																														
<i>Kitasatospora setae</i>																														
<i>Streptomyces ambofaciens</i>																														
<i>Streptomyces bikiniensis</i>																														
<i>Streptomyces bluensis</i>																														
<i>Streptomyces caelestis</i>																														
" <i>Streptomyces</i> <i>espinosus</i> " NRRL 5729																														
<i>Streptomyces galbus</i>																														
<i>Streptomyces glaucescens</i> DSM 40716																														
<i>Streptomyces griseus</i>																														
" <i>Streptomyces hygroscopicus</i> subsp. <i>limoneus</i> " ATCC 21431																														
<i>Streptomyces lincolnensis</i>																														
<i>Streptomyces mashuensis</i>																														
" <i>Streptomyces ornatus</i> "																														
<i>Streptomyces pseudogriseolus</i> NRRL 3985																														
<i>Streptomyces subtritus</i>																														
<i>Streptomyces violaceoalbescens</i> A3(2)																														

^a The type strain of each taxon was used unless otherwise indicated.

^b Calculations were based on 1,438 nucleotide positions. The numbers on the upper right are the numbers of nucleotide differences, and the numbers on the lower left are the percentages of 16S rRNA sequence similarity.

FIG. 1. Neighbor-joining tree (34) based on nearly complete 16S rRNA sequences of 26 streptomycetes. f, p, and m indicate branches that were also found when we used the Fitch-Margoliash (11), maximum-parsimony (19), and maximum-likelihood methods (8), respectively; the asterisks indicate branches that were recovered with all four methods. The numbers at the nodes indicate the levels of bootstrap support based on a neighbor-joining analysis of 1,000 resampled data sets; only values that are more than 40% are given. The estimated positions of the root organisms are indicated by the circled letters, as follows: A, *A. globiformis*; B, *B. subtilis*; E, *E. coli*; N, *N. asteroides*; S, *Streptosporangium roseum*. The test strains were type cultures unless otherwise indicated.

should not be assigned to a distinct subgenus within the genus *Streptomyces* as proposed by Craveri and Pagani (6).

The average nucleotide sequence similarity value obtained when the *Streptomyces macrosporus*, *Streptomyces megasporus*, and *Streptomyces thermolineatus* strains were compared was $97.1\% \pm 0.7\%$, and the closest relationship found was the relationship between the type strains of *Streptomyces macrosporus* and *Streptomyces megasporus* (level of similarity, 98.0%). The relatively large number of nucleotide differences found in the 16S rRNA sequences of the type strains of these three species provides further support for the taxonomic integrity of these species. Members of all three species formed distinct clusters within a recognizable aggregate group in the numerical phenetic survey of Goodfellow et al. (12). The average level of similarity between members of the *Streptomyces macrosporus* clade and the mesophilic streptomycetes was $95.5\% \pm 0.7\%$. The closest relationships were the relationships between *Streptomyces macrosporus* DSM 41449^T and "*Streptomyces espinosus*" NRRL 5729 (level of similarity, 96.8%) and between *Streptomyces thermolineatus* DSM 41451^T and *Streptomyces mashuensis* DSM 40221^T (level of similarity, 96.8%). The *Streptomyces macrosporus* clade was also well separated from the mesophilic streptomycetes as determined by the broader analysis based on partial nucleotide sequences.

The second phyletic line included the type strains of *Streptomyces thermodiastaticus*, *Streptomyces thermolineatus*, *Streptomyces thermoviolaceus* subsp. *apingens*, *Streptomyces thermoviolaceus* subsp. *thermoviolaceus*, and *Streptomyces thermovulgaris*. The average level of nucleotide similarity found for these strains was high ($98.7\% \pm 0.7\%$). The highest level of similar-

ity was the level of similarity between the *Streptomyces thermovulgaris* and *Streptomyces thermovulgaris* strains (99.9%). This result is consistent with the proposal of Williams et al. (48) that *Streptomyces thermovulgaris* Desai and Dhala 1967 (7) should become a subjective synonym of *Streptomyces thermovulgaris* Henssen 1957 (13). However, on the basis of phenotypic properties, Goodfellow et al. (12) concluded that *Streptomyces thermovulgaris* should be reduced to a synonym of *Streptomyces thermoviolaceus* Henssen 1957 (13) emend. Goodfellow et al. 1987 (12). AT-L30 ribosomal protein analyses also revealed that *Streptomyces thermovulgaris*, *Streptomyces thermoviolaceus*, and *Streptomyces thermovulgaris* are closely related (27). DNA-DNA relatedness studies are needed to resolve the finer taxonomic relationships among *Streptomyces thermovulgaris*, *Streptomyces thermoviolaceus*, and *Streptomyces thermovulgaris*.

The type strains of *Streptomyces thermoviolaceus* subsp. *apingens* and *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* did not show monophyly even though they were closely related in terms of nucleotide sequence similarity (level of similarity, 99.4%). This result can be attributed to the tree-making algorithms which considered the close relationships between *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* and *Streptomyces thermodiastaticus* (level of similarity, 99.4%) and between *Streptomyces thermoviolaceus* subsp. *apingens* and *Streptomyces thermovulgaris* or *Streptomyces thermovulgaris* (level of similarity, 98.5%). Clearly, representatives of these taxa should be included in DNA-DNA pairing studies. The average level of similarity between members of the *Streptomyces macrosporus* and *Streptomyces thermodiastaticus* clades was $96.3\% \pm 0.5\%$.

FIG. 2. Phylogenetic tree based on partial 16S rRNA sequences of 39 *Streptomyces* strains. For details see the legend to Fig. 1.

The G+C contents of the 16S rDNAs of the eight thermophilic streptomycetes were 60.05 ± 0.63 mol%, and the corresponding value for the 15 mesophilic strains was 58.83 ± 0.61 mol%; the differences between these two sets of data were too small to affect the outcome of the phylogenetic analyses. Members of the *Streptomyces thermodiastaticus* clade showed a closer relationship to some of the mesophilic streptomycetes than to *Streptomyces macrosporus* and related strains (Table 2; Fig. 1). Particularly high levels of nucleotide similarity were found between *Streptomyces thermodiastaticus* DSM 40573^T and *Streptomyces bluensis* ISP 5564^T (98.3%), between *Streptomyces thermoviolaceus* subsp. *apingens* DSM 41392^T and *Streptomyces glaucescens* DSM 40716 (98.4%), and between *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* DSM 40443^T and *Streptomyces glaucescens* DSM 40716 (98.5%). Although the relationships which we found between the members of the *Streptomyces thermodiastaticus* subgroup and the associated mesophilic streptomycetes were not supported by the product of the maximum-likelihood analysis, this clade was

apparent in the trees based on both nearly complete (Fig. 1) and partial (Fig. 2) nucleotide sequences; the bootstrap values obtained for members of the clade were more or less similar to those found in the *Streptomyces thermodiastaticus* subgroup.

It is likely that *Streptomyces thermodiastaticus* and allied mesophilic streptomycetes form a monophyletic group. This conclusion is supported by the results of AT-L30 protein analyses, which show that *Streptomyces bluensis*, *Streptomyces diastaticus*, *Streptomyces glaucescens*, and *Streptomyces violaceoruber* A3(2) are closely related to *Streptomyces thermotrophicus*, *Streptomyces thermoviolaceus*, and *Streptomyces thermovulgaris* (27). Consequently, it seems likely that the close relationship proposed on the basis of phenotypic properties between *Streptomyces thermodiastaticus* (Bergey et al. 1923) Waksman 1953 (2, 41) and *Streptomyces halstedii* (Waksman and Curtis 1916) Waksman and Henrici 1948 (42, 44, 47) is more apparent than real. The position of *Streptomyces mashuensis* DSM 40221^T is equivocal (Fig. 1 and 2).

The relationships among the organisms were not markedly

influenced by the different algorithms used to analyze the almost complete nucleotide sequences. However, the classification was affected by the nature of the outgroup strains used. The tree was rooted between the kitasatosporiae and the streptomycetes when *A. globiformis*, *B. subtilis*, *E. coli*, and *N. asteroides* strains were used as the outgroup strains (Fig. 1). However, when *Streptosporangium roseum* DSM 43021^T was used as the outgroup strain, the root more or less bisected the tree. The position of the root in the corresponding tree based on the partial nucleotide data set was also influenced by the outgroup strain (Fig. 2). However, once again it is interesting that the kitasatosporiae were separated from the streptomycetes when the *B. subtilis*, *E. coli*, and *N. asteroides* strains were used as the outgroup strains. It is clear from these results that the position of the root in the *Streptomyces* clade cannot be estimated unequivocally.

Wellington et al. (46) proposed, mainly on the basis of 16S rRNA sequence data, that the name *Kitasatosporia* Omura et al. 1982 (30) should be reduced to a synonym of the name *Streptomyces* Waksman and Henrici 1943 (43). In addition, Ochi and Hiranuma (28) found that *Kitasatosporia griseola*, *Kitasatosporia mediocidica*, *Kitasatosporia phosalacinea*, and *Kitasatosporia setae* are closely related to *Streptomyces exfoliatus* by using data derived from analyses of ribosomal protein AT-L30. It has also been proposed that *Kitasatosporia cystarginea* should be transferred to the genus *Streptomyces* on the basis of chemotaxonomic, physiological, and DNA-DNA relatedness data (26). Nevertheless, kitasatosporiae can be distinguished from streptomycetes because major amounts of galactose and meso-diaminopimelic acid are found in their whole-organism hydrolysates (30). They also consistently produce submerged spores in liquid culture, a trait rare among streptomycetes.

In this investigation, the kitasatosporiae were recovered as a deeply rooted phyletic line when the first data set was analyzed with *A. globiformis*, *B. subtilis*, *E. coli*, and *N. asteroides* strains as outgroup strains (Fig. 1). The overall average level of nucleotide sequence similarity for the 16S rRNA molecules of the kitasatosporiae and mesophilic and thermophilic streptomycetes was 95.1% ± 0.6%. The detailed taxonomic relationships of the kitasatosporiae need to be reevaluated in light of additional molecular and chemosystematic data.

The results of this study provide further evidence of the value of 16S rRNA sequencing in the circumscription of actinomycete genera and in the elucidation of the subgeneric structure of complex taxa, such as the genus *Streptomyces*. It is evident from our results that thermophilic streptomycetes form at least two phyletic lines which can be distinguished from clades composed of mesophilic streptomycetes.

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