A Taxonomic Study of the Genus *Streptomyces* by Analysis of Ribosomal Protein AT-L30

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The ribosomal AT-L30 proteins from 81 species of the genus *Streptomyces* as listed by Williams et al. in *Bergey's Manual of Systematic Bacteriology* were analyzed. My results provided further evidence that the genus *Streptomyces* is well circumscribed. On the basis of levels of AT-L30 N-terminal amino acid sequence homology, the strains were classified into four groups (groups I to IV) and a nongrouped category, whose members contained amino acid sequences characteristic of each species. A phylogenetic tree constructed on the basis of the levels of similarity of the amino acid sequences revealed the existence of six clusters within the genus. The first cluster contains the members of groups I and II together with several other species; the second cluster contains the members of groups III and IV and several other species; the third cluster contains *Streptomyces ramulosus* and *Streptomyces ochraceiscleroticus*; the fourth cluster contains only *Streptomyces rimosus*; the fifth cluster contains *Streptomyces aurantiacus* and *Streptomyces tubercidicus*; and the sixth cluster contains *Streptomyces albus* and *Streptomyces sulphureus*. Considerable agreement between the results of the AT-L30 analyses and the results of numerical phenetic classification was found, although there were numerous disagreements in details. For example, four groups (groups I to IV) defined by the AT-L30 analysis data did not correlate with the aggregate groups defined by numerical classification. In general, but not always, the species classified in a particular cluster in the numerical classification system had the same or similar AT-L30 terminal amino acid sequences. The AT-L30 analysis data were more consistent with the 16S rRNA analysis data than with the numerical classification data, indicating that there was a good correlation between the four groups defined by AT-L30 analysis data and the aggregate groups defined by 16S rRNA analysis data. I stress that discrepancies between results of phenetic analyses and results of phylogenetic analyses should be taxonomically significant and can be resolved by other taxonomic approaches, such as DNA relatedness analysis.

The genus *Streptomyces* of the family *Streptomycetaceae* contains the largest number of species among the genera of the *Actinomycetales* and can be separated from other actinomycete genera with wall chemotype I by using a combination of chemical and morphological properties (reviewed in references 2, 10, and 33). Traditionally, streptomycete systematics has been based mainly on morphology, pigmentation, and physiological properties, but increasing weight is now given to chemical and genetic features (2, 3, 9, 11, 15, 28), especially for generic circumspection. As mentioned by O'Donnell (27), analysis of quantitative data by appropriate cluster analysis techniques may be essential for the characterization of streptomycetes at the subgeneric level. Because of importance of the genus *Streptomyces* as a source of novel antibiotics, the number of proposed streptomycete species, including those only cited in the patent literature, is more than 3,000. The names of 378 validly described streptomycete species are listed on the Approved Lists of Bacterial Names (29).

Many attempts to delimit *Streptomyces* species have been made. In particular, a large-scale numerical phenetic survey of the genus *Streptomyces* and related taxa was performed by Williams et al. (34) to clarify the infrastructure of the genus; 394 *Streptomyces* type cultures were examined for 139 unit characters, and the data were analyzed statistically. The resulting classification indicated that the type strains of *Streptomyces* species were distributed in 23 major clusters (containing four or more strains), 20 minor clusters (containing two or three strains), and 25 clusters containing a single member. The minor clusters and the single-member clusters were considered species by Williams et al., and the major clusters were consid-

ered species complexes. These results of this study are summarized in *Bergey's Manual of Systematic Bacteriology* (33).

A numerical phenetic survey of *Streptomyces* species was also performed by Kämpfer et al. (8). The phenetic data of these authors in most cases confirmed the existence of the major phenons found in the study of Williams et al. (34), although only some of the cluster groups defined in the study of Williams et al. were detected by Kämpfer et al. Data which describe DNA relatedness among strains are also valuable for taxonomy, especially at the species level, and such data have been applied to streptomycetes by Labeda and Lyons (12–14). The phenetic clustering of these organisms actually reflects their genomic relationships as determined by DNA relatedness analyses and rRNA-based analyses (1, 35).

On the basis of the heterogeneity of the ribosomal proteins of *Streptomyces* species, I (17) developed a novel method for identifying and classifying actinomycetes. Ribosomal protein patterns determined by two-dimensional polyacrylamide gel electrophoresis (PAGE) could be used for *Streptomyces* taxonomy at the species level, while analysis of ribosomal AT-L30 proteins (homologous to *Escherichia coli* L30 protein) could be used to classify actinomycetes at the genus level (18, 19, 21, 23, 25, 26). The latter method is based on the electrophoretic mobilities of the AT-L30 proteins and N-terminal amino acid sequences. L30 protein analysis has been proven to be effective in the taxonomy of not only actinomycetes but also other eubacteria (20, 22). Witt and Stackebrandt (35) have proposed that the genera *Streptoverticillium* and *Streptomyces* should be united on the basis of their high levels of phylogenetic and phenetic similarity. Wellington et al. (32) have proposed that the genus *Kitasatosporia*, whose members' cell walls contain similar amounts of the LL and meso isomers of diaminopimelic acid, as well as glycine and galactose, is a synonym of the genus *Streptomyces*. Recent AT-L30 sequence analysis data have con-

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TABLE 1. Strains used in this study

Species or subspecies	Strain	Cluster of Williams et al. ^a
<i>Streptomyces aburaviensis</i>	JCM 4613 ^T (= ATCC 23869 ^T) ^b	A-2
<i>Streptomyces albidoflavus</i>	JCM 4446 ^T (= ATCC 25422 ^T)	A-1A
<i>Streptomyces alboflavus</i>	JCM 4615 ^T (= ATCC 12626 ^T)	E-54
<i>Streptomyces albus</i> subsp. <i>albus</i>	JCM 4450 ^T (= ATCC 25426 ^T)	A-16
<i>Streptomyces amakusaensis</i>	JCM 4617 ^T (= ATCC 23876 ^T)	B ^c
<i>Streptomyces aminophilus</i>	JCM 4619 ^T (= ATCC 13558 ^T)	A-16 ^d
<i>Streptomyces antibioticus</i>	JCM 4620 ^T (= ATCC 23879 ^T)	A-31
<i>Streptomyces anulatus</i>	JCM 4721 ^T (= ATCC 27416 ^T)	A-1B
<i>Streptomyces atroolivaceus</i>	JCM 4345 ^T (= ATCC 19725 ^T)	A-3
<i>Streptomyces aurantiacus</i>	JCM 4453 ^T (= ATCC 19822 ^T)	C-45
<i>Streptomyces aurantiogriseus</i>	JCM 4346 ^T (= ATCC 19887 ^T)	A ^c
<i>Streptomyces aureofaciens</i>	JCM 4624 ^T (= ATCC 23884 ^T)	A-14
<i>Streptomyces badius</i>	JCM 4350 ^T (= ATCC 19888 ^T)	C ^c
<i>Streptomyces bambergiensis</i>	JCM 4728 ^T (= ATCC 13879 ^T)	A ^c
<i>Streptomyces bikiniensis</i>	JCM 4011 ^T (= ATCC 11062 ^T)	F-64
<i>Streptomyces bluensis</i>	JCM 4729 ^T (= ATCC 27420 ^T)	A-39 ^e
<i>Streptomyces cacaioi</i>	JCM 4352 ^T (= ATCC 19732 ^T)	A-16 ^d
<i>Streptomyces californicus</i>	JCM 4567 ^T (= ATCC 19734 ^T)	A-9
<i>Streptomyces canus</i>	JCM 4569 ^T (= ATCC 19737 ^T)	A-25
<i>Streptomyces cellulosa</i>	JCM 4462 ^T (= ATCC 25439 ^T)	A-13
<i>Streptomyces chattanoogensis</i>	JCM 4571 ^T (= ATCC 19739 ^T)	A-35
<i>Streptomyces chromofuscus</i>	JCM 4354 ^T (= ATCC 23896 ^T)	A-15
<i>Streptomyces cyanoalbus</i>	JCM 4363 ^T (= ATCC 23902 ^T)	A-37 ^d
<i>Streptomyces diastaticus</i> subsp. <i>diastaticus</i>	JCM 4745 ^T (= ATCC 3315 ^T)	A-19
<i>Streptomyces exfoliatus</i>	JCM 4366 ^T (= ATCC 19750 ^T)	A-5
<i>Streptomyces filipinensis</i>	JCM 4369 ^T (= ATCC 23905 ^T)	A-30
<i>Streptomyces finlayi</i>	JCM 4637 ^T (= ATCC 23906 ^T)	I ^c
<i>Streptomyces flaveolus</i>	JCM 4577 ^T (= ATCC 19754 ^T)	A-24
<i>Streptomyces flaveus</i>	JCM 3035 ^T (= ATCC 15332 ^T)	A-19 ^e
<i>Streptomyces fradiae</i>	JCM 4579 ^T (= ATCC 19760 ^T)	G-68
<i>Streptomyces fragilis</i>	JCM 4187 ^T (= ATCC 23908 ^T)	G ^c
<i>Streptomyces fulvissimus</i>	JCM 4129 ^T (= ATCC 27431 ^T)	A-10
<i>Streptomyces glaucescens</i>	JCM 4377 ^T (= ATCC 23622 ^T)	A-28
<i>Streptomyces graminofaciens</i>	JCM 4762 ^T (= ATCC 12705 ^T)	A-26
<i>Streptomyces griseoflavus</i>	JCM 4479 ^T (= ATCC 25456 ^T)	A-37
<i>Streptomyces griseoincarnatus</i>	JCM 4381 ^T (= ATCC 23917 ^T)	A-13 ^d
<i>Streptomyces griseoluteus</i>	JCM 4765 ^T (= ATCC 12768 ^T)	C-43
<i>Streptomyces griseoruber</i>	JCM 4642 ^T (= ATCC 23919 ^T)	A-21
<i>Streptomyces griseoviridis</i>	JCM 4643 ^T (= ATCC 23920 ^T)	A-17
<i>Streptomyces griseus</i>	JCM 4644 ^T (= ATCC 23921 ^T)	A-1B ^d
<i>Streptomyces halstedii</i>	JCM 4584 ^T (= ATCC 19770 ^T)	A-1C
<i>Streptomyces hygroscopicus</i>	IFO 13472 ^T (= ATCC 27438 ^T)	A-32 ^d
<i>Streptomyces intermedius</i>	JCM 4483 ^T (= ATCC 25461 ^T)	A-1A ^d
<i>Streptomyces kanamyceticus</i>	JCM 4775 ^T (= ATCC 12853 ^T)	B-42 ^d
<i>Streptomyces lateritius</i>	JCM 4389 ^T (= ATCC 19913 ^T)	H ^c
<i>Streptomyces lavendulae</i> subsp. <i>lavendulae</i>	IFO 12789 ^T (= ATCC 19777 ^T)	F-61
<i>Streptomyces longisporoflavus</i>	JCM 4396 ^T (= ATCC 23932 ^T)	A-39
<i>Streptomyces luridus</i>	JCM 4591 ^T (= ATCC 19782 ^T)	F-62
<i>Streptomyces lydicus</i>	JCM 4492 ^T (= ATCC 25470 ^T)	A-29
<i>Streptomyces massasporeus</i>	JCM 4593 ^T (= ATCC 19785 ^T)	D ^c
<i>Streptomyces microflavus</i>	JCM 4496 ^T (= ATCC 25474 ^T)	A-23
<i>Streptomyces misakiensis</i>	JCM 4653 ^T (= ATCC 23938 ^T)	F-66
<i>Streptomyces nigrescens</i>	JCM 4401 ^T (= ATCC 23941 ^T)	A-29 ^d
<i>Streptomyces noboritoensis</i>	JCM 4557 ^T (= ATCC 25477 ^T)	A-33
<i>Streptomyces nodosus</i>	JCM 4656 ^T (= ATCC 14899 ^T)	A-35 ^e
<i>Streptomyces nogalater</i>	JCM 4799 ^T (= ATCC 27451 ^T)	A-34
<i>Streptomyces novaecaesareae</i>	JCM 4800 ^T (= ATCC 27452 ^T)	J ^c

Continued

TABLE 1—Continued

Species or subspecies	Strain	Cluster of Williams et al. ^a
<i>Streptomyces ochraceiscloreticus</i>	JCM 4801 ^T (= ATCC 15814 ^T)	A-41
<i>Streptomyces olivaceoviridis</i>	JCM 4499 ^T (= ATCC 25478 ^T)	A-20
<i>Streptomyces pactum</i>	JCM 4809 ^T (= ATCC 27456 ^T)	C-44
<i>Streptomyces parvulus</i>	ATCC 12434 ^T	A-12 ^d
<i>Streptomyces phaeochromogenes</i>	JCM 4659 ^T (= ATCC 23945 ^T)	A-40
<i>Streptomyces poonensis</i>	JCM 4815 ^T (= ATCC 15723 ^T)	A-22
<i>Streptomyces prasinopilosus</i>	JCM 4404 ^T (= ATCC 19799 ^T)	A-37 ^d
<i>Streptomyces prasinosporeus</i>	JCM 4816 ^T (= ATCC 17918 ^T)	A-38
<i>Streptomyces prunicolor</i>	JCM 4508 ^T (= ATCC 25487 ^T)	A-11
<i>Streptomyces psammoticus</i>	JCM 4434 ^T (= ATCC 25488 ^T)	F-67
<i>Streptomyces purpureus</i>	JCM 3172 ^T (= ATCC 27787 ^T)	F-65
<i>Streptomyces ramulosus</i>	JCM 4604 ^T (= ATCC 19802 ^T)	C ^c
<i>Streptomyces rimosus</i> subsp. <i>rimosus</i>	JCM 4667 ^T (= ATCC 23955 ^T)	B-42
<i>Streptomyces rochei</i>	JCM 4074 ^T (= ATCC 23956 ^T)	A-12
<i>Streptomyces sulphureus</i>	JCM 4835 ^T (= ATCC 27468 ^T)	C ^c
<i>Streptomyces tendae</i>	JCM 4610 ^T (= ATCC 19812 ^T)	A-12 ^d
<i>Streptomyces thermonitrificans</i>	JCM 4841 ^T (= ATCC 23385 ^T)	A-36 ^d
<i>Streptomyces thermoviolaceus</i> subsp. <i>thermoviolaceus</i>	JCM 4843 ^T (= ATCC 19283 ^T)	C-45 ^d
<i>Streptomyces thermovulgaris</i>	JCM 4520 ^T (= ATCC 25501 ^T)	A-36
<i>Streptomyces tubercidicus</i>	JCM 4558 ^T (= ATCC 25502 ^T)	C-47
<i>Streptomyces varsoviensis</i>	JCM 4523 ^T (= ATCC 25505 ^T)	C-46
<i>Streptomyces venezuelae</i>	IFO 13096 ^T (= ATCC 25508 ^T)	A-6 ^c
<i>Streptomyces violaceus</i>	JCM 4533 ^T (= ATCC 25515 ^T)	A-6
<i>Streptomyces violaceusniger</i>	JCM 4850 ^T (= ATCC 27477 ^T)	A-32
<i>Streptomyces viridochromogenes</i>	JCM 4856 ^T (= ATCC 14920 ^T)	A-27
<i>Streptomyces xanthochromogenes</i>	JCM 4612 ^T (= ATCC 19818 ^T)	F-63
<i>Streptomyces xanthocidicus</i>	JCM 4862 ^T (= ATCC 27480 ^T)	F-66 ^d
<i>Streptomyces yerevanensis</i>	JCM 3065 ^T (= DSM 43167 ^T)	D ^c

^a See reference 34.^b T = type strain.^c Single-member cluster.^d Subjective synonym in each cluster.^e Allied species in each cluster.

structure of streptomycetes by using the AT-L30 analysis method and the numerical phenetic data of Williams et al. (34).

MATERIALS AND METHODS

Bacterial strains. The *Streptomyces* species used in this study (Table 1) are species which were listed in *Bergey's Manual of Systematic Bacteriology* by Williams et al. (33). All of the strains were type strains that were obtained from the Japan Collection of Microorganisms, Saitama, Japan, the Institute of Fermentation, Osaka, Japan, and the American Type Culture Collection, Rockville, Md. Most of the strains were grown in soluble starch-Polypeptone-yeast extract medium at 30°C (26); the thermophilic actinomycetes *Streptomyces thermonitrificans* and *Streptomyces thermoviolaceus* were grown at 37 and 50°C, respectively.

Preparation of total ribosomal proteins and two-dimensional PAGE. The total ribosomal protein preparation and two-dimensional PAGE procedures were performed by using the method of Kaltschmidt and Wittmann (7), as described in detail previously (17, 24). The electrophoretic mobilities (in the first dimension) of the AT-L30 proteins were expressed as relative electrophoretic mobilities compared with the electrophoretic mobility of *Saccharomonospora viridis* AT-L30 protein, which exhibited the greatest mobility of all of the actinomycete AT-L30 proteins examined (26).

Determination of amino acid sequences. The amino acid sequences of AT-L30 protein samples were determined by using a model 470A protein sequencer (Applied Biosystems, Foster City, Calif.) as described previously (24, 25). Determination of a maximum of 26 N-terminal amino acids was possible when 100 to 150 pmol of an AT-L30 preparation was used. The 26 N-terminal amino acids

firmly that members of the genera *Kitasatospora* and *Streptomyces* should be classified in the genus *Streptomyces* (24), as suggested previously on the basis of 16S rRNA analysis data (32, 35). The aim of this study was to survey the taxonomic

represent nearly one-half of the whole AT-L30 protein, assuming that the L30 protein of *E. coli* and the AT-L30 proteins of streptomycetes are the same size.

RESULTS

Two-dimensional PAGE analysis of ribosomal proteins. The ribosomal proteins of 66 species of the genus *Streptomyces*, as listed by Williams et al. (33) on the basis of numerical phenetic classification data (34), were analyzed by two-dimensional PAGE. The only two species not included were *Streptomyces cyaneus* and *Streptomyces gelaticus*, whose ribosomal proteins were difficult to prepare. The electrophoretic mobilities of the proteins identified as AT-L30 proteins in the first dimension of gel electrophoresis were similar for all of the species examined; these proteins exhibited relative electrophoretic mobilities of about 20. These results are consistent with previous observations (18, 19) in that, although there is electrophoretic heterogeneity of AT-L30 proteins among actinomycete genera, each genus has a relative electrophoretic mobility typical of that genus.

N-terminal amino acid sequence analysis of AT-L30 proteins. On the basis of the amino acid sequence homology data (Fig. 1), the strains were classified into five groups (groups I to IV and a nongrouped category). The members of groups I to IV exhibited the same sequence within each group, whereas the strains in the nongrouped category exhibited sequences typical of each species. The same sequences were found in *Streptomyces bambergiensis* and *Streptomyces griseoflavus* and in *Streptomyces thermovulgaris* and *Streptomyces cellulosa*. For convenience, these pairs of species were classified in the nongrouped category. Altogether, 19 different sequences were found in the genus *Streptomyces*. Group I was characterized by Arg at position 2; group II strains contained Arg and Val at positions 2 and 5; group III strains contained Gln and Val at positions 2 and 8; and group IV strains contained Gln at position 2. It is especially noteworthy that all of the members of groups I to IV had Tyr at position 11, a marker position for classification at the genus level (20, 24). In contrast, several species classified in the nongrouped category (*Streptomyces tubercidicus*, *Streptomyces rimosus*, *Streptomyces sulphureus*, and *Streptomyces albus*) had Phe, Ile, or Val at this position. Ser at position 14, together with Tyr at position 11, has also been considered a signature amino acid characteristic of the genus *Streptomyces* (24). Interestingly, in *Streptomyces sulphureus* and *Streptomyces albus*, Ser at position 14 was also replaced by Thr, indicating that the taxonomic position of these species within the genus was isolated. The amino acids which characterized the species at various loci are indicated in Fig. 1. The N-terminal sequence (Met-Arg-Ile) found in *Streptomyces ramulosus* and *Streptomyces ochraceiscleroticus* was especially characteristic.

To express quantitatively the levels of similarity of the amino acid sequences (SAS values), the frequency of appearance of the same amino acid in 26 N-terminal amino acids of the AT-L30 proteins was determined. A difference in the amino acid at position 11 was weighted twofold. On the basis of SAS values determined in this way for a combination of all strains, a dendrogram was drawn (Fig. 2). This dendrogram shows that six clusters were identified. The first cluster contains the members of groups I and II along with several other species; the second cluster contains the members of groups III and IV, as well as several other species; the third cluster contains *Streptomyces ramulosus* and *Streptomyces ochraceiscleroticus*; the fourth cluster contains only *Streptomyces rimosus*; the fifth cluster contains *Streptomyces aurantiacus* and *Streptomyces tuber-*

cidicus; and the sixth cluster contains *Streptomyces albus* and *Streptomyces sulphureus*.

Comparison of the amino acid sequences within one cluster defined by numerical taxonomy. Fifteen species obtained from the culture collection of my laboratory which have been considered subjective synonyms of a cluster representative of Williams et al. (33) were analyzed, and the results are shown in Fig. 3. These results, including the results obtained for three species studied previously (24), are summarized in Table 2, which shows the results of a comparison of the amino acid sequences of the strains tested and their cluster representatives. It is evident that in general, but not always, strains placed in the same cluster by Williams et al. (34) had identical or very similar amino acid sequences, indicating that there was good agreement between the AT-L30 analysis data and the numerical phenetic data. For example, *Streptomyces violaceusniger* and *Streptomyces hygroscopicus*, both of which were classified in cluster A-32 by numerical taxonomy methods (34), exhibited complete amino acid sequence similarity. Close phylogenetic relationships between *Streptomyces aminophilus* and *Streptomyces albus* (both classified in cluster A-16), between *Streptomyces cacaoi* and *Streptomyces albus* (both classified in cluster A-16), and between *Streptomyces intermedius* and *Streptomyces albidoflavus* (both classified in A-1A) were especially evident, with characteristic amino acids at specific positions (Table 2). However, discrepancies were also found. For example, *Streptomyces kanamyceticus*, *Streptomyces nodosus*, *Streptomyces thermoviolaceus*, and *Streptomyces venezuelae* exhibited differences at three loci compared with their cluster representatives; these differences correspond to SAS values as low as 88%. Apparently, these four species are classified in the wrong clusters.

DISCUSSION

This work was a continuation of previous work (24) involving the *Streptomyces* species listed by Williams et al. (33). Two main points can be focused on. First, the data from the ribosomal protein AT-L30 analysis provide further evidence that the genus *Streptomyces* is well circumscribed. This was demonstrated not only by the constant relative electrophoretic mobility value (about 20) of the AT-L30 proteins of the *Streptomyces* species examined but also by the phylogenetic coherence based on N-terminal amino acid sequence homology data. Second, considerable agreement between the results of the AT-L30 analyses and the results of the numerical phenetic study of Williams et al. (34) was found. There also were numerous disagreements in details. Despite the significance of streptomycete taxonomy for industrial microbiology, the results of only a few phylogenetic analyses have been published previously, and these analyses dealt with limited numbers of species (30, 32, 35). Almost all of these studies were performed by using phenetic or chemotaxonomic methods. Therefore, it is difficult at the present time to construct a comprehensive picture in which results of rRNA analyses are included. It is also difficult to discuss my data in relation to previously published DNA homology data (12–14) since the latter data involve few species of the numerous *Streptomyces* species used in this work. Seki and coworkers (28a) are now attempting to survey the streptomycetes by analyzing numerous strains with 16S rRNA sequencing methods. Discrepancies between my AT-L30 data and the numerical phenetic data of Williams et al. (34) and Kämpfer et al. (8) are also evident. Each of the groups classified on the basis of AT-L30 analysis data (groups I to IV) contained species belonging to widely separated clusters in the studies of Williams et al. (34) (Fig. 1) and Kämpfer et al. (8),

Group I		1	5	10	15	20	25	
<i>S. aburaviensis</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. amakusaensis</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							B
<i>S. anulatus</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. atroolivaceus</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. aureofaciens</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? -Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. badius</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							C
<i>S. californicus</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. finlayi</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							I
<i>S. fradiae</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							G
<i>S. graminofaciens</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. griseoruber</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? -Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. halstedii</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. lavendulae</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							F
<i>S. microflavus</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. noboritoensis</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. psammoticus</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							F
<i>S. purpureus</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							F
<i>S. violaceusniger</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. xanthochromogenes</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							F
<i>S. yerevanensis</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							D
Group II		1	5	10	15	20	25	
<i>S. bikiniensis</i>	Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							F
<i>S. exfoliatus</i>	Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. lateritius</i>	Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Asp-Thr-Leu- ? -Ser-Leu-Gly							H
<i>S. luridus</i>	Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							F
<i>S. pactum</i>	Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							C
<i>S. phaeochromogenes</i>	Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. prunicolor</i>	Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. varsoviensis</i>	Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							C
Group III		1	5	10	15	20	25	
<i>S. chromofuscus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. filipinensis</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. flaveolus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. fragilis</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							G
<i>S. glaucescens</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. griseoluteus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							C
<i>S. griseoviridis</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. massaporeus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							D
<i>S. nogalater</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. olivaceoviridis</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. poonensis</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. prasinosporus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr- ? - ? - ? -Leu-Gly							A
<i>S. rochei</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly							A
<i>S. violaceus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly							A
<i>S. viridochromogenes</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Asp-Thr-Leu- ? -Ser-Leu-Gly							A

FIG. 1. Primary structures of N termini of AT-L30 proteins from *Streptomyces* species listed in Bergey's *Manual of Systematic Bacteriology*. Question marks indicate amino acids that were not determined. The amino acids that characterize the species are enclosed in boxes. The letters on the right indicate the cluster groups of Williams et al. (34).

Group IV		1	5	10	15	20	25	
<i>S. albobiflavus</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly						E
<i>S. antibioticus</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly						A
<i>S. aurantiogriseus</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly						A
<i>S. canus</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly						A
<i>S. fulvissimus</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly						A
<i>S. novaecaesareae</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Asp-Thr-Leu- ? -Ser-Leu-Gly						J
Non-grouped		1	5	10	15	20	25	
<i>S. bambergensis</i>		Ala-Gln-Leu-Arg-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly						A
<i>S. griseoflavus</i>		Ala-Gln-Leu-Arg-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly						A
<i>S. thermovulgaris</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Thr-Leu-Gly						A
<i>S. cellulosa</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Thr-Leu-Gly						A
<i>S. diastaticus</i>		Pro-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Asp-Thr-Leu- ? -Thr-Leu-Gly						A
<i>S. longisporoflavus</i>		Ala-Gln-Leu-Lys-Val-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Asp-Thr-Leu- ? -Ser-Leu-Gly						A
<i>S. misakiensis</i>		Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Glu-Thr-Leu-Arg-Ser-Leu-Gly						F
<i>S. chattanoogensis</i>		Ala-Arg-Leu-Gln-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? -Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly						A
<i>S. lydicus</i>		Ala-His-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly						A
<i>S. albidoflavus</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Lys-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? - ? -Leu-Gly						A
<i>S. ramulosus</i>		Met-Arg-Ile-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Thr-Leu-Gly						C
<i>S. ochraceiscleroticus</i>		Met-Arg-Ile-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly						A
<i>S. aurantiacus</i>		Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Phe-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly						C
<i>S. tubercidicus</i>		Ala-His-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Phe-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly						C
<i>S. rimosus</i>		Ala-Ser-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Ile-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly						B
<i>S. sulphureus</i>		Ala-Arg-Leu-Lys-Ile-Thr-Gln-Asn-Lys-Ser-Val-Ile-Gly-Thr-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu- ? -Thr-Leu-Gly						C
<i>S. albus</i>		Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Arg-Ser-Val-Ile-Gly-Thr-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Thr-Leu-Gly						A

FIG. 1—Continued.

although the following tendencies are evident: group III is composed mainly of organisms that were classified in cluster group A by Williams et al. (34), and groups I and II contain members of cluster group F of Williams et al. (Fig. 1). In addition, no relationship between the four groups and a classical streptomycete character (aerial spore mass color) was found. On the other hand, good agreement was found between the AT-L30 results and the results of 16S rRNA sequencing (28a). For example, group I as defined by AT-L30 data corresponded to specified clusters defined by the 16S rRNA data of Seki, while group III corresponded to another cluster of Seki. The agreement between the AT-L30 results and the 16S rRNA results was also evident from the close relationships between *Streptomyces lydicus* and *Streptomyces chattanoogensis*, between *Streptomyces albidoflavus* and *Streptomyces diastaticus* (both common in freshwater habitats), between *Streptomyces aureofaciens* and *Streptomyces psammotus*, and among *Streptomyces californicus*, *Streptomyces microflavus*, and *Streptomyces finlayi* (Fig. 2) (28a).

The phylogenetic data presented in Fig. 2 are considered taxonomically significant even though a relatively small number of characteristics are involved. In numerical phenetic taxonomy studies (33, 34), a close relationship among *Streptomyces anulatus* (cluster A-1B), *Streptomyces halstedii* (cluster A-1C), and *Streptomyces aburaviensis* (cluster A-2) was found. These species were all classified as members of group I in the AT-L30 study. Similarly, *Streptomyces californicus* (cluster A-9)

and *Streptomyces fulvissimus* (cluster A-10), *Streptomyces filipinensis* (cluster A-30) and *Streptomyces antibioticus* (cluster A-31), and *Streptomyces lavendulae* (cluster F-61) and *Streptomyces xanthochromogenes* (cluster F-63) were found to be related to one another by numerical taxonomy (34). *Streptomyces rimosus*, *Streptomyces aurantiacus*, *Streptomyces tubercidicus*, *Streptomyces sulphureus*, and *Streptomyces ramulosus*, all of which were placed in rather isolated phylogenetic positions within the genus *Streptomyces* (Fig. 2), have been identified by numerical taxonomy as members of minor cluster group B or C, which is differentiated from major cluster group A. These results are all in good agreement with the results of AT-L30 analyses. Also, *Streptomyces albus*, which was classified in major cluster group A by numerical taxonomy by Williams et al. (34), was placed in a phylogenetic position that was far removed from the majority of streptomycetes (Fig. 2). Furthermore, in numerical taxonomy studies (34) no close relationship between *Streptomyces albus* (cluster A-16) and *Streptomyces sulphureus* (cluster C) was found. However, a close relationship between these two species was detected on the basis of the results of AT-L30 analyses (Fig. 2) and 16S rRNA sequence analyses (28a). Also, the rather distant taxonomic position of *Streptomyces albus* within the genus has been pointed out by Stackebrandt and coworkers (30, 35). Interestingly, unlike the results of Williams et al. (34), the results of another numerical analysis of phenetic traits, the analysis of Kämpfer et al. (8), indicated that *Streptomyces albus* occupied a rather isolated

FIG. 2. Clustering based on AT-L30 protein SAS values. The dendrogram was drawn by using the data in Fig. 1. The members of groups I to IV are shown in Fig. 1. The letters on the right indicate the cluster groups of Williams et al. (34).

taxonomic position within the genus *Streptomyces*, which is consistent with our phylogenetic data. *Streptomyces chattanoogaensis* (cluster A-35) and *Streptomyces thermovulgaris* (cluster A-36) were found to be closely related by numerical taxonomy (34) but not by AT-L30 analysis (Fig. 2). In summary, the AT-L30 sequence analysis data agreed more with the rRNA analysis data of Seki (28a) and of Stackebrandt and coworkers

(30, 35) than with the numerical phenetic analysis data. This is not surprising because AT-L30 analyses and rRNA analyses are both based on molecular analysis of ribosomal components, while numerical taxonomy is based mainly on phenetic data analysis.

Members of the genus *Chainia* form sclerotia embedded in colonies on agar media or in shake cultures. Members of the genus *Microellobosporia* form club-shaped sporangia that contain short rows of nonmotile spores and are borne on both the aerial and substrate mycelia. Members of the genus *Kitasatoa* have also been reported to form club-shaped sporangia on both substrate and aerial hyphae, but produce spores that are motile when they are placed in water. Species of these three genera have been reclassified in the genus *Streptomyces* (4-6). *Streptomyces flaveus* and *Streptomyces yerevanensis* were previously classified in the genus *Microellobosporia*. *Streptomyces ochraceiscleroticus* and *Streptomyces poonensis* were classified in the genus *Chainia*, and *Streptomyces purpureus* was classified in the genus *Kitasatoa*. As shown in this study, all of these species exhibited characteristics typical of the genus *Streptomyces*, in terms of their relative electrophoretic mobility values and their levels of amino acid sequence homology with other representative streptomycetes. The presence of Tyr at position 11 emphasized the fact that these organisms should be included in the genus *Streptomyces*.

In a previous study (24), it was shown that all of the *Streptomyces* species examined had Tyr at position 11. In this study, however, I found a few exceptions to this, including *Streptomyces albus*, the type species of the genus. Nevertheless, inclusion of these species in the genus *Streptomyces* is supported by their relative electrophoretic mobility values (about 20), which are typical of the genus, and their relatively high SAS values (80 to 86%) (Fig. 2), which are similar to the SAS values of the majority of streptomycetes.

It is clear that ribosomal protein AT-L30 analysis is valuable for defining the genus *Streptomyces*. My finding that there are at least 19 different N-terminal amino acid sequences of AT-

	1	5	10	15	20	25
<i>S. aminophilus</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Asn-Arg-Ser-Val-Ile-Gly-Thr-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Thr- ? -Gly					
<i>S. bluensis</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly					
<i>S. cacaoi</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Asn-Arg-Ser-Val-Ile-Gly-Thr-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Thr-Leu-Gly					
<i>S. cyanoalbus</i>	Ala-Gln-Leu-Arg-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly					
<i>S. flaveus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys					
<i>S. griseoincarnatus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly					
<i>S. intermedius</i>	Pro-Gln-Leu-Lys-Ile-Thr-Gln-Lys-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? -Arg-Asp-Thr-Leu- ? -Ser-Leu-Gly					
<i>S. kanamyceticus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly					
<i>S. nigrescens</i>	Ala-His-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly					
<i>S. nodosus</i>	Ala-Gln-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly					
<i>S. prasinopilosus</i>	Ala-Gln-Leu-Arg-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Ser-Leu-Gly					
<i>S. tendae</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp					
<i>S. thermotritificans</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Thr-Leu-Gly					
<i>S. thermoviolaceus</i>	Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His-Arg-Asp-Thr-Leu-Arg-Thr-Leu-Gly					
<i>S. xanthocidicus</i>	Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn-His- ? -Asp-Thr-Leu-Arg-Ser-Leu-Gly					

FIG. 3. Primary structures of N termini of AT-L30 proteins from *Streptomyces* species which were listed as subjective synonyms of cluster representatives by Williams et al. in *Bergey's Manual of Systematic Bacteriology*. The data for cluster representatives are shown in Fig. 1.

TABLE 2. Results of a comparison of amino acid sequences of AT-L30 proteins from strains classified in a single cluster by Williams et al.^a

Species examined ^b	Species in same cluster (cluster) ^c	Results of a comparison of amino acid sequences
<i>Streptomyces intermedius</i>	<i>Streptomyces albidoflavus</i> (A-1A)	Different at one locus, but characterized at position 8
<i>Streptomyces griseus</i>	<i>Streptomyces anulatus</i> (A-1B)	Agreement
<i>Streptomyces venezuelae</i>	<i>Streptomyces violaceus</i> (A-6)	Different at three loci, in agreement with group II
<i>Streptomyces parvulus</i>	<i>Streptomyces rochei</i> (A-12)	Agreement
<i>Streptomyces tendae</i>	<i>Streptomyces rochei</i> (A-12)	Agreement
<i>Streptomyces griseoincarnatus</i>	<i>Streptomyces cellulosa</i> (A-13)	Different at one locus, in agreement with group III
<i>Streptomyces aminophilus</i>	<i>Streptomyces albus</i> (A-16)	Different at one locus, but characterized at positions 9, 11, and 14
<i>Streptomyces cacaoi</i>	<i>Streptomyces albus</i> (A-16)	Different at one locus, but characterized at positions 9, 11, and 14
<i>Streptomyces flaveus</i>	<i>Streptomyces diastaticus</i> (A-19)	Different at one locus, in agreement with group IV
<i>Streptomyces nigrescens</i>	<i>Streptomyces lydicus</i> (A-29)	Agreement
<i>Streptomyces hygroscopicus</i>	<i>Streptomyces violaceusniger</i> (A-32)	Agreement
<i>Streptomyces nodosus</i>	<i>Streptomyces chattanoogensis</i> (A-35)	Different at three loci, in agreement with group IV
<i>Streptomyces thermotritrificans</i>	<i>Streptomyces thermovulgaris</i> (A-36)	Agreement
<i>Streptomyces cyanoalbus</i>	<i>Streptomyces griseoflavus</i> (A-37)	Agreement
<i>Streptomyces prasinopilosus</i>	<i>Streptomyces griseoflavus</i> (A-37)	Agreement
<i>Streptomyces bluensis</i>	<i>Streptomyces longisporoflavus</i> (A-39)	Different at one locus, in agreement with group III
<i>Streptomyces kanamyceticus</i>	<i>Streptomyces rimosus</i> (B-42)	Different at three loci, in agreement with group III
<i>Streptomyces thermoviolaceus</i>	<i>Streptomyces aurantiacus</i> (C-45)	Different at three loci, in agreement with group III
<i>Streptomyces xanthocidicus</i>	<i>Streptomyces misakiensis</i> (F-66)	Different at one locus, in agreement with group I

^a See reference 34.^b Sequence data for *Streptomyces griseus*, *Streptomyces hygroscopicus*, and *Streptomyces parvulus* were obtained from reference 24.^c Clusters of Williams et al. (34).

L30 proteins within the genus *Streptomyces* is important. The agreement found between numerical phenetic data and AT-L30 amino acid sequence data is encouraging (Table 2), but several discrepancies which I found may be taxonomically significant and should be clarified by other approaches, such as DNA relatedness analysis. In a previous study it was demonstrated that *Thermoactinomyces vulgaris* and *Thermoactinomyces thalophilus*, whose classification at the species level has been confusing, could be clearly distinguished by a difference in their AT-L30 amino acid sequences (20). As stressed previously (22), sequence analyses of L30 proteins may be useful for demonstrating that some *Pseudomonas fluorescens* strains or *Pseudomonas putida* strains represent separate species. It has been pointed out that *Streptomyces griseoflavus* JCM 4142 is a misidentified strain (24). This strain should be assigned to *Streptomyces ochraceiscleroticus* (or a related species) on the basis of its complete AT-L30 sequence and the characteristic N-terminal sequence Met-Arg-Ile (Fig. 1). Thus, ribosomal protein AT-L30 analysis is valuable not only for taxonomy at the genus level but also for identifying unknown isolates to the species level.

Polyphasic taxonomy is the goal of modern procaryotic taxonomy (16, 31), and it is widely believed that rRNA molecules and sequence analysis of rRNA molecules are the best means presently available for constructing phylogenies. The classification of Williams et al. (33, 34), which was based on numerical phenetic analysis data, is considered a useful basis for streptomycete taxonomy. However, results of phylogenetic analyses in which 16S rRNA sequencing and AT-L30 amino acid sequencing are used may need to be included in streptomycete classification schemes, as demonstrated in this study.

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