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Amino Acid Sequence Analysis of Ribosomal Protein AT-L30 from Members of the Family *Pseudonocardiaceae*

KOZO OCHI*

National Food Research Institute, 2-1-2 Kannondai, Tsukuba, Ibaraki 305, Japan

The phylogenetic relationships of the genera belonging to the family *Pseudonocardiaceae* were examined by a novel approach, amino acid sequencing of ribosomal AT-L30 proteins. The results of partial amino acid sequencing of AT-L30 preparations revealed that the members of the family *Pseudonocardiaceae* are divided into four clusters; the first cluster contains the genus *Actinopolyspora*, the second cluster contains the genus *Saccharopolyspora*, the third cluster contains the genus *Amycolatopsis*, and the fourth cluster contains the genera *Amycolata*, *Pseudonocardia*, *Saccharomonospora*, and *Kibdelosporangium*, indicating a close phylogenetic relationship between the genera *Amycolata* and *Pseudonocardia*. The genus *Actinokineospora* is closely related to the genus *Saccharothrix*, and these two genera formed a cluster separate from the clusters for the genera of the *Pseudonocardiaceae*. These results agree in almost all respects with previous 16S rRNA sequencing work by Embley et al. (T. M. Embley, J. Smida, and E. Stackebrandt, Syst. Appl. Microbiol. 11:44–52, 1988) and Warwick et al. (S. Warwick, T. Bowen, H. McVeigh, and T. M. Embley, Int. J. Syst. Bacteriol. 44:293–299, 1994), thus supporting the proposal of Warwick et al. that the genera *Amycolata* and *Pseudonocardia* should be combined in an emended genus, *Pseudonocardia*. However, a discrepancy was found between the present study and that of Warwick et al. In the present study, the *Nocardia-Rhodococcus* group and the *Saccharothrix-Actinokineospora* group were both recovered within the clade for the family *Pseudonocardiaceae*.

The wall chemotype IV actinomycetes are a group of actinomycetes which contain *meso*-diaminopimelic acid, arabinose, and galactose in their cell walls and can be divided into at least two groups based on the presence or absence of mycolic acids. Members of the genera *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Rhodococcus*, classified in a group, contain mycolic acids (reviewed by Goodfellow and Cross [11]). Another group, which includes the genera *Pseudonocardia* (which replaces *Amycolata*), *Actinopolyspora*, *Amycolatopsis*, *Saccharomonospora*, and *Saccharopolyspora*, is characterized by the absence of mycolic acids (reviewed by Lechevalier [22]), and the members of the group share a number of chemotaxonomic features with each other (3, 5, 6, 9, 10, 14, 20, 24). Therefore, on the basis of 16S rRNA sequence analysis, together with such chemotaxonomic similarity, Embley et al. (8) proposed an amended family, *Pseudonocardiaceae*, for the mycolateless wall chemotype IV actinomycetes. Subsequently, on the basis of the results of 16S rRNA sequence analysis, Bowen et al. (1, 2) proposed that the genera *Amycolata* and *Kibdelosporangium* should also be included in this family. The family *Pseudonocardiaceae* contains a number of economically important organisms, including the producers of erythromycin, rifamycin, and vancomycin (4). All current members of the family have been reported to have a chemotype IV cell wall (21, 23). The genus *Actinokineospora* was proposed by Hasegawa (13) for a new genus with a mycolateless chemotype IV cell wall. The genus *Saccharothrix*, originally thought to be closely related to the genus *Nocardiopsis* on the basis of morphology (21), has been shown to be related to members of the family *Pseudonocardiaceae* on the basis of chemotaxonomic and 16S rRNA sequence results (1). However, unlike members of the family *Pseudonocardiaceae*, *Saccharothrix* strains have chemotype III walls (21). For this reason, it was deemed premature to transfer the genus *Saccharothrix* to the family *Pseudonocardiaceae* (1). The phylogenetic position of these actinomycetes with mycolateless chemotype IV or III cell walls was investigated by using reverse transcriptase sequencing of 16S rRNA (1, 7, 8). The most recent study by Warwick et al. (38) demonstrated that the genera *Amycolata* and *Pseudonocardia* are always recovered as a mixed group in phylogenetic trees. Accordingly, they proposed that these genera should be reclassified in an emended genus *Pseudonocardia*. (In this paper, for convenience, we use the name *Amycolata* instead of the unified name *Pseudonocardia*.) On the other hand, the phylogenetic branching pattern did not unambiguously resolve whether the members of the genera *Saccharothrix* and *Actinokineospora* should be placed in the family *Pseudonocardiaceae* (38). Therefore, these two genera will remain outside the family *Pseudonocardiaceae* until additional data become available to decide the issue.

I have recently used ribosomal protein analysis to investigate the taxonomy of actinomycetes and developed a novel method for classification and identification (26, 28), based on the heterogeneity of ribosomal proteins. While there is striking variability in the electrophoretic mobilities of ribosomal AT-L30 proteins among genera of actinomycetes, these proteins exhibit electrophoretic mobilities that are specific for each genus (27, 32, 33). On the basis of this observation, Ochi and Yoshida (35) analyzed the AT-L30 proteins of members of the family *Pseudonocardiaceae* by using two-dimensional polyacrylamide gel electrophoresis (PAGE) and demonstrated that the genera *Actinopolyspora*, *Saccharopolyspora*, *Amycolatopsis*, *Pseudonocardia*, and *Saccharomonospora* can be distinguished by the electrophoretic properties of the AT-L30 proteins. Subsequently, Ochi et al. (30, 34) also found that amino acid sequence analysis of the AT-L30 proteins, together with electrophoretic analysis, can be a useful basis for classification at the genus level. The aim of this study was to review the family *Pseudonocardiaceae* by using our amino acid sequence analysis method.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. Almost all were type strains obtained from the Japan Collection of Microorganisms,

* Phone: 0298-38-8125. Fax: 0298-38-7996.

TABLE 1. Strains used in this study and REM values of ribosomal AT-L30 proteins

Species[a]	Strain designation	REM[b]
Actinopolyspora halophila	ATCC 27976^T	46.0[c]
Actinopolyspora mortivallis	JCM 7550^T (ATCC 49777^T)	42.5[c]
Saccharopolyspora hirsuta subsp. *hirsuta*	JCM 3170^T (ATCC 27875^T)	66.5[c]
Saccharopolyspora rectivirgula	JCM 3057^T (ATCC 33515^T)	67.0[c]
Amycolatopsis azurea	JCM 3275^T (DSM 43854^T)	73.0[c]
Amycolatopsis orientalis subsp. *orientalis*	JCM 4600^T (ATCC 19795^T)	71.0[c]
Amycolatopsis fastidiosa	JCM 3276^T (ATCC 31181^T)	68.0
Amycolata autotrophica (*Pseudonocardia autotrophica*)	JCM 4348^T (ATCC 19727^T)	81.0[c]
Amycolata saturnea (*Pseudonocardia saturnea*)	JCM 3187^T (ATCC 15809^T)	ND[d]
Amycolata hydrocarboxydans (*Pseudonocardia hydrocarboxydans*)	JCM 3392^T (ATCC 15104^T)	88.5[c]
Pseudonocardia thermophila	JCM 3095^T (ATCC 19285^T)	86.0[c]
Saccharomonospora glauca	JCM 7444^T (DSM 43769^T)	98.0[c]
Saccharomonospora caesia	JCM 3098^T (DSM 43044^T)	96.0
Saccharomonospora viridis	JCM 3036^T (ATCC 15386^T)	100[c]
Actinokineospora riparia	JCM 7471^T (ATCC 49499^T)	28.0
Saccharothrix australiensis	JCM 3370^T (ATCC 31497^T)	70.0
Saccharothrix aerocolonigenes	JCM 4614^T (ATCC 23870^T)	65.0
Saccharothrix mutabilis subsp. *mutabilis*	JCM 3380^T (ATCC 31520^T)	71.0
Kibdelosporangium aridum	ATCC 39323^T	41.5[c]
Rhodococcus rhodochrous	JCM 3202^T (ATCC 13808^T)	47.5
Mycobacterium diernhoferi	JCM 6371^T (ATCC 19340^T)	ND
Corynebacterium diphtheriae	JCM 1310 (ATCC 11913)	ND
Nocardia asteroides	JCM 3384^T (ATCC 19247^T)	59.0
Streptomyces parvulus	ATCC 12434^T	17.0[c]

[a] The names in parentheses are emended names.
[b] The electrophoretic mobilities (in the first dimension) of the AT-L30 proteins are expressed as REMs compared with the electrophoretic mobility of the *Saccharomonospora viridis* AT-L30 protein. The mobility of protein AT-L30 from *Saccharomonospora viridis* JCM 3036^T was defined as unity (100).
[c] Data from references 27, 32, 34, and 35.
[d] ND, not determined.

Wako-shi, Saitama, Japan, and the American Type Culture Collection, Rockville, Md. *Actinopolyspora mortivallis* JCM 7550^T (T = type strain) is a strain which was proposed recently as a new species (40), and *Actinokineospora riparia* JCM 7471^T is a strain which was proposed as a new species representing a new genus (13).

Preparation of total ribosomal proteins. The halophilic bacteria, *Actinopolyspora halophila* and *Actinopolyspora mortivallis*, were grown in a medium containing 20% (wt/vol) NaCl as previously described (35). Other strains (Table 1) were grown in soluble starch-polypeptone-yeast extract medium at 30°C as previously described (33), except for *Pseudonocardia thermophila*, which was grown at 55°C. Ribosomal proteins were prepared from 70S ribosomes by extraction with acetic acid, as previously described (26), by the method of Hardy et al. (12). The total ribosomal protein samples obtained in this way contained 20 to 30 mg of protein per ml.

Two-dimensional PAGE. Two-dimensional PAGE by the method of Kaltschmidt and Wittmann (17) has been described in detail previously (26). After each slab gel was stained with Coomassie blue, the AT-L30 protein spot was identified. The electrophoretic mobilities (in the first dimension) of the AT-L30 proteins were expressed as relative electrophoretic mobilities (REMs) compared with the electrophoretic mobility of the *Saccharomonospora viridis* AT-L30 protein, which exhibited the greatest mobility of all of the actinomycete AT-L30 proteins examined (27). The amounts of the total ribosomal proteins applied to the PAGE gels for the purpose of REM determination were 400 to 500 μg. However, 5 to 10 mg of the total ribosomal proteins were used when preparing the AT-L30 protein sample for amino acid sequence analysis.

Determination of amino acid sequence. After two-dimensional PAGE, the proteins were extracted from the gels and used for amino acid sequence analysis as previously described (31). Determination of a maximum of 26 N-terminal amino acids was possible when about 150 pmol (corresponding to about 1.5 μg) of AT-L30 preparation was analyzed.

RESULTS

Two-dimensional PAGE analysis of ribosomal proteins. The ribosomal proteins from more than 20 species belonging to the genera of the family *Pseudonocardiaceae* and related genera (Table 1) were studied. Table 1 shows that species belonging to the same genus exhibited similar electrophoretic properties of AT-L30 proteins as expressed by REMs. In contrast, each genus displayed REMs that were distinct from those of other genera. The only exception was *Pseudonocardia thermophila* (the type species of the genus *Pseudonocardia*), with an REM of 86.0, which overlapped with that of the genus *Amycolata* (REMs, 81.0 to 88.5). I attempted to analyze another *Pseudonocardia* species, *Pseudonocardia compacta* (15), but it was too difficult to prepare ribosomal proteins from this species, perhaps because of its extremely slow growth. Therefore, this organism was not included in the present study. *Actinokineospora riparia* had a low REM value (28.0) that was clearly distinct from those of other genera, supporting the taxonomic status of this organism in a new genus. Although these results provided additional evidence that actinomycete taxa of the mycolateless wall chemotype IV should be classified as distinct genera, I further analyzed these organisms by sequencing the N-terminal amino acids of the AT-L30 proteins.

Amino acid sequences of AT-L30 proteins. The AT-L30 proteins from members of the family *Pseudonocardiaceae* were subjected to amino acid sequence analysis, and the results are presented in Fig. 1. The members of the mycolic acid-containing genera *Nocardia*, *Rhodococcus*, *Corynebacterium*, and *Mycobacterium* were also analyzed for reference. It is immediately evident from Fig. 1 that species belonging to the same genus displayed sequences with a high level of homology. There were 16 variable positions within the 20 N-terminal amino acids among the members of the family *Pseudonocardiaceae*. To express quantitatively the levels of similarity of the amino acid sequences (SAS values), I determined the frequency of appearance of a particular amino acid in 20 N-terminal amino acids of each AT-L30 protein. Smaller numbers of amino acids were compared in several combinations. Nondetermined positions were not weighted for the calculation of SAS values. The SAS values determined in this way for every combination of strains tested are shown in Table 2. High SAS values (84 to 94%) were detected within the genera *Actinopolyspora*, *Saccharopolyspora*,

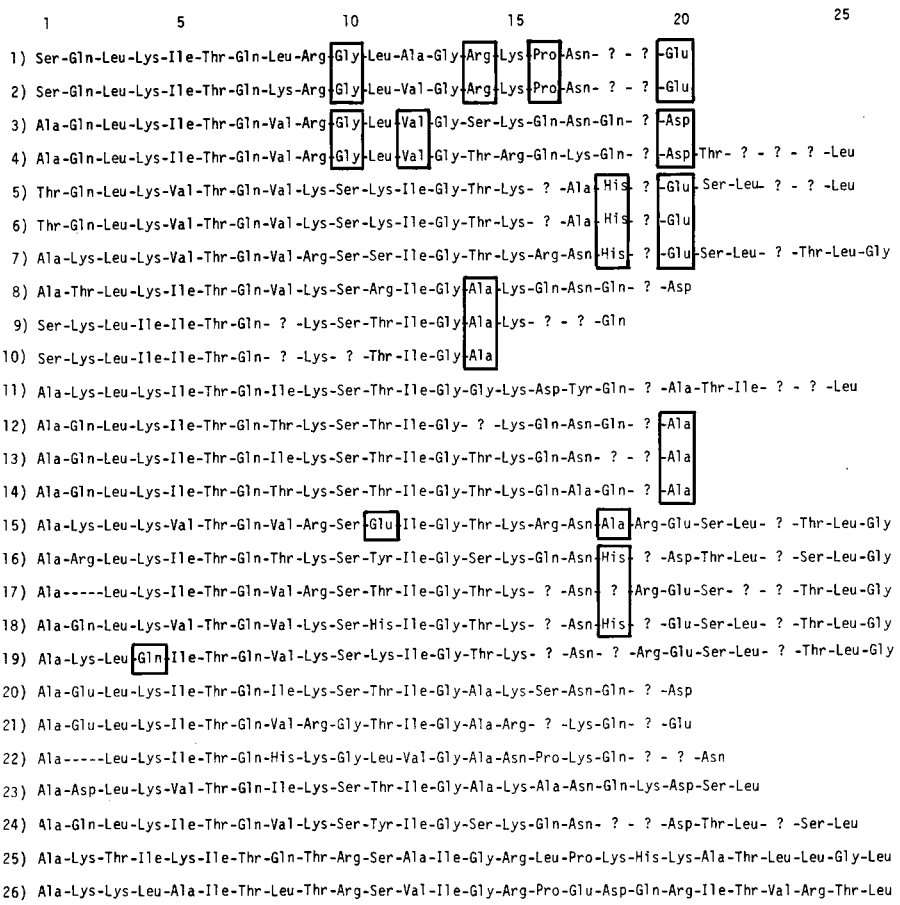

FIG. 1. Primary structures of N termini of AT-L30 proteins from various species of the genera belonging to or closely related to the family *Pseudonocardiaceae*. The data for *Escherichia coli* and *Bacillus stearothermophilus* are from references 36 and 18, respectively. Question marks indicate amino acids that were not determined. The dotted lines indicate deletions. The amino acids which characterize each genus are boxed. Lines: 1, *Actinopolyspora halophila*; 2, *Actinopolyspora mortivallis*; 3, *Saccharopolyspora hirsuta* subsp. *hirsuta*; 4, *Saccharopolyspora rectivirgula*; 5, *Amycolatopsis azurea*; 6, *Amycolatopsis orientalis* subsp. *orientalis*; 7, *Amycolatopsis fastidiosa*; 8, *Amycolata autotrophica*; 9, *Amycolata saturnea*; 10, *Amycolata hydrocarboxydans*; 11, *Pseudonocardia thermophila*; 12, *Saccharomonospora glauca*; 13, *Saccharomonospora caesia*; 14, *Saccharomonospora viridis*; 15, *Actinokineospora riparia*; 16, *Saccharothrix australiensis*; 17, *Saccharothrix aerocolonigenes*; 18, *Saccharothrix mutabilis* subsp. *mutabilis*; 19, *Kibdelosporangium aridum*; 20, *Rhodococcus rhodochrous*; 21, *Mycobacterium diernhoferi*; 22, *Corynebacterium diphtheriae*; 23, *Nocardia asteroides*; 24, *Streptomyces parvulus*; 25, *Escherichia coli* K-12; 26, *Bacillus stearothermophilus* NCA 1503.

and *Saccharomonospora*. In contrast, low SAS values (65 to 76%) were detected within the genus *Saccharothrix*.

On the basis of SAS values presented in Table 2, I constructed a dendrogram (Fig. 2). The genera *Nocardia*, *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* were included in the dendrogram for reference. As Fig. 2 shows, 15 species of the genera in the *Pseudonocardiaceae* formed separate lines from the mycolic acid-containing genera *Mycobacterium* and *Corynebacterium* and were divided into four clusters; the first contained the genus *Actinopolyspora*, the second contained the genus *Saccharopolyspora*, the third contained the genus *Amycolatopsis*, and the fourth contained the genera *Amycolata*, *Pseudonocardia*, *Saccharomonospora*, and *Kibdelosporangium*. Surprisingly, *Nocardia asteroides* and *Rhodococcus rhodochrous* were recovered within the clade that includes the family *Pseudonocardiaceae*, exhibiting a close relationship with the genera *Pseudonocardia* and *Amycolata*. Similarly, the members of the wall chemotype III genus *Saccharothrix* and the wall chemotype IV genus *Actinokineospora* were also recovered within the clade for the family *Pseudonocardiaceae*, but these two genera were more clearly distinguished from the clusters for the genera of the family *Pseudonocardiaceae* (Fig. 2).

Characteristics of amino acid sequences. In addition to simple comparison on the homology level of amino acid sequences of AT-L30 proteins, we have recently found that it is possible to pinpoint amino acids located at specific positions which characterize each genus (29, 31). Amino acids which characterize each genus are indicated by boxes in Fig. 1. The genus *Actinokineospora* is characterized by glutamic acid at position 11 and alanine at position 18.

DISCUSSION

In a previous study, Ochi and Yoshida (35) created a sound basis for PAGE data for the family *Pseudonocardiaceae*. The observed electrophoretic heterogeneity of AT-L30 proteins within the family reflected the results of investigations of 16S rRNA analysis by Bowen et al. (1) and Embley et al. (7, 8). In the present study, I confirmed our previous conclusion by using another approach: amino acid sequence analysis of AT-L30 proteins. Although previously the genera *Amycolatopsis* and *Saccharopolyspora* were not distinguished from each other with respect to REM values (35), the present study demonstrated clearly that these genera are separable at the genus level (Fig. 2). In the dendrogram (Fig. 2), the genera *Actinopolyspora* and *Saccharopolyspora* exhibited deep branching from other mem-

TABLE 2. SAS values for the amino acid sequences of AT-L30 proteins[a]

Species	SAS value (%)																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. *Actinopolyspora halophila*	—																						
2. *Actinopolyspora mortivallis*	89	—																					
3. *Saccharopolyspora hirsuta*	67	72	—																				
4. *Saccharopolyspora rectivirgula*	56	61	84	—																			
5. *Amycolatopsis azurea*	47	47	44	44	—																		
6. *Amycolatopsis orientalis*	47	47	44	44	100	—																	
7. *Amycolatopsis fastidiosa*	50	50	53	47	72	72	—																
8. *Amycolata autotrophica*	44	44	68	58	56	56	58	—															
9. *Amycolata saturnea*	50	50	47	40	53	53	53	73	—														
10. *Amycolata hydrocarboxydans*	55	55	45	45	55	55	55	73	100	—													
11. *Pseudonocardia thermophila*	39	39	47	42	50	50	53	63	80	73	—												
12. *Saccharomonospora glauca*	53	53	67	56	59	59	56	78	79	70	78	—											
13. *Saccharomonospora caesia*	50	50	61	56	65	65	61	72	71	64	78	94	—										
14. *Saccharomonospora viridis*	44	44	58	58	67	67	53	68	73	64	74	94	89	—									
15. *Actinokineospora riparia*	50	50	53	47	67	67	89	58	53	55	53	56	61	53	—								
16. *Saccharothrix australiensis*	44	44	63	47	56	56	58	74	60	64	58	78	72	68	53	—							
17. *Saccharothrix aerocolonigenes*	59	59	65	59	65	65	82	71	64	55	65	75	76	71	83	65	—						
18. *Saccharothrix mutabilis*	53	53	56	50	83	83	78	67	53	55	56	71	76	67	78	67	76	—					
19. *Kibdelosporangium aridum*	47	47	53	47	71	71	76	71	71	73	65	69	71	65	78	65	78	76	—				
20. *Rhodococcus rhodochrous*	44	44	58	47	50	50	53	79	80	73	74	78	78	68	53	68	71	61	65	—			
21. *Mycobacterium diernhoferi*	53	53	61	72	44	44	56	61	60	64	56	59	53	56	56	44	71	50	53	67	—		
22. *Corynebacterium diphtheriae*	53	59	61	67	35	35	33	56	53	64	50	53	47	50	33	44	47	41	44	56	65	—	
23. *Nocardia asteroides*	39	39	53	42	56	56	58	74	73	64	68	72	72	63	55	63	61	67	56	84	56	50	—
24. *Streptomyces parvulus*	50	50	79	63	61	61	58	84	67	64	63	83	78	74	58	84	71	72	71	74	56	50	68

[a] The SAS values for each pair of organisms for 20 N-terminal amino acids (but a smaller number of amino acids in several combinations) of the AT-L30 proteins were determined on the basis of the data shown in Fig. 1. Undetermined positions were not weighted for the calculation of SAS values.

bers of the family *Pseudonocardiaceae*. On the other hand, the genera *Amycolata*, *Pseudonocardia*, and *Kibdelosporangium* formed coherent lines. These results are entirely in agreement with the previous work on rRNA sequencing analysis by Warwick et al. (38) and Bowen et al. (1). In the work of Warwick et al. (38), the strains examined were classified into three groups on the basis of 16S rRNA sequence data: the first contained the *Actinoplanetes* group, comprising the genera *Micromonospora*, *Ampullariella*, and *Dactyrosporangium*; the second contained the mycolic acid-containing wall chemotype IV taxa such as *Nocardia*, *Rhodococcus*, *Mycobacterium*, and *Corynebacterium*; and the third contained the genera *Saccharothrix*, *Actinokineospora*, and members of the family *Pseudonocardiaceae*. However, the branching order for these three groups was unstable and varied with the choice of outgroup taxa and the method of analysis (38). In this respect, there is a significant discrepancy between my AT-L30 work and that of Warwick et al. (38), namely, that in my study both the *Nocardia-Rhodococcus* cluster and the *Saccharothrix-Actinokineospora* cluster were recovered as a sister group within a clade of the family *Pseudonocardiaceae* (Fig. 2), while in the study of Warwick et al. (38) these genera were recovered in clades outside the family *Pseudonocardiaceae*. No explanation can be offered at this time, but the work of Warwick et al. (38) explains the classification of mycolic acid-containing and nonmycolic acid-containing groups better. On the basis of my AT-L30 work (Fig. 2), it is possible that the members of the family *Pseudonocardiaceae* evolved from a common ancestor, with a loss of mycolic acid, in the process of development to the *Nocardia-Rhodococcus* group. In the light of this notion, the *Saccharothrix-Actinokineospora* group, another taxon without mycolic acid, may be taken as a member of the family *Pseudonocardiaceae*. These issues remain to be clarified. Another discrepancy found between our AT-L30 analysis and 16S rRNA analysis was that in the former method the genus *Saccharomonospora* was closely related to the genera *Pseudonocardia* and *Amycolata* (with respect to both REM value and amino acid sequence homology), while in the latter method it exhibited a close relationship to the genus *Amycolatopsis.*

The genus *Saccharothrix* was proposed by Labeda et al. (21) for a single isolate containing a chemotype III wall. *Actinokineospora riparia*, a new genus proposed by Hasegawa (13), has chemotype IV walls. Despite their difference in wall chemotypes, these two taxa were closely related (Fig. 2). The close phylogenetic relatedness of the genera *Actinokineospora* and *Saccharothrix* was also reported by Warwick et al. (38) on the basis of 16S rRNA sequence analysis. Recent investigations by Takeuchi et al. (37) showed that the cell wall of the genus *Actinokineospora* (and also of the genera *Amycolatopsis*, *Kibdelosporangium*, *Pseudonocardia*, and *Saccharomonospora*) was chemotype III, displaying *meso*-diaminopimelic acid in the peptidoglycan and galactose among the whole-cell sugars, while the content of arabinose was very low. However, since the location and amount of arabinose may vary significantly among members of the family *Pseudonocardiaceae* (4, 31), it is deemed that the distinction between wall chemotype IV and wall chemotype III among members of the family may not be as taxonomically significant as was once thought (1, 4, 38). The taxonomic status of the genus *Actinokineospora* as a distinct genus is, however, emphasized by the marked difference in REM values between *Actinokineospora riparia* (REM, 28.0) and the genus *Saccharothrix* (REMs, 65.0 to 71.0) (Table 1). In the study by Warwick et al. (38), the phylogenetic branching pattern did not unambiguously resolve whether the members of the genera *Actinokineospora* and *Saccharothrix* should be placed in the family *Pseudonocardiaceae*. My results of AT-L30 protein analyses also did not resolve this issue (Fig. 2). High SAS values detected within genera indicate that species within those genera are closely related to each other. Low SAS values between genera indicate their differences from each other.

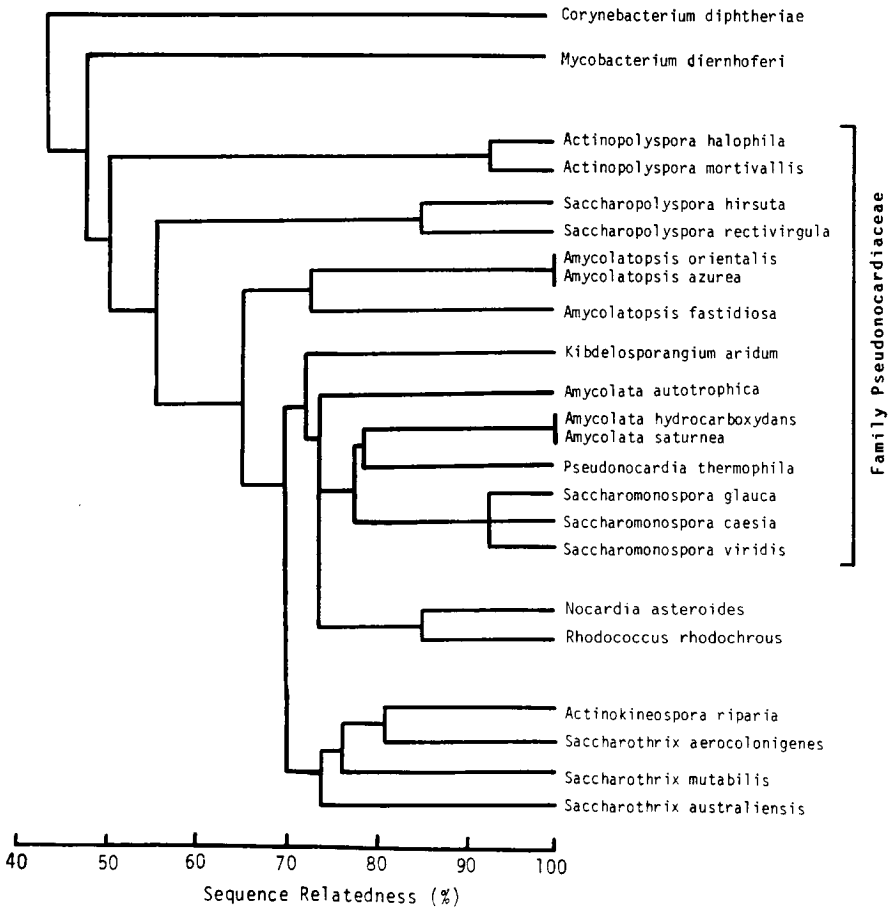

FIG. 2. Clustering of AT-L30 protein SAS values. The dendrogram was drawn from the data in Fig. 1.

Therefore, the low SAS values observed within the genus *Saccharothrix* (Fig. 2) signify that this genus contains phylogenetically distant species.

It is noteworthy that in agreement with the results of Warwick et al. (38), my study showed that the relationship between *Amycolatopsis orientalis* (the type species of the genus) and *Amycolatopsis azurea* was fully supported, while *Amycolatopsis fastidiosa* was separated by long branches from these two species (Fig. 2). Since these *Amycolatopsis* species share many phenotypic and chemical characteristics (16, 24), it is unlikely that *Amycolatopsis fastidiosa* is misclassified. Conceivably, the genus *Amycolatopsis*, as well as the genus *Saccharothrix* (see above), is a taxon which contains phylogenetically distant species.

It is interesting that *Amycolata hydrocarboxydans* and *Amycolata saturnea* are related more closely to *Pseudonocardia thermophila* than to *Amycolata autotrophica* (Fig. 2). Moreover, as presented in Table 1, these four organisms exhibit similar electrophoretic properties of AT-L30 proteins (REMs, 81.0 to 88.5). These results, together with other morphological and chemotaxonomic evidence (6, 19, 22, 24), fully support the proposal of Warwick et al. (38) that the genera *Pseudonocardia* and *Amycolata* be combined as a single genus.

The amino acid located at position 11 in Fig. 1 (position 12 in the numbering for *Escherichia coli*) has been shown to be an important signature for classification at the genus level, as demonstrated for the genera *Escherichia*, *Bacillus*, *Staphylococcus*, *Thermoactinomyces*, and *Streptomyces* (29, 31). However, variation in the amino acid at position 11 was found within a single genus of the family *Pseudonocardiaceae* (Fig. 1). Nevertheless, the presence of different amino acids at position 11 in the genera *Saccharothrix* and *Actinokineospora* (Fig. 1) can be taken in support of *Actinokineospora* as a distinct genus, since this position has never been occupied by glutamic acid in other actinomycete species examined so far.

Polyphasic taxonomy is the goal of modern procaryotic taxonomy, as stated by Wayne et al. (39) and Murray et al. (25). In these reports, polyphasism has been perceived as the integration of phylogenetic rRNA (or rDNA) sequence data with the distribution of chemotaxonomic and other phenotypic/epigenetic markers. Thus, it is widely believed that sequence analysis of rRNA molecules is the best means presently available for the construction of phylogenies. Several hundred variable positions are routinely compared in 16S rRNA sequencing analyses. In contrast, less than 20 variable positions were used in the AT-L30 sequencing analyses in this study. This rather small number of variable positions may be insufficient to accurately reflect phylogenetic relationships and may define the taxonomic limit of this technique. However, the ribosomal protein analysis, as demonstrated in the present and previous papers, can be helpful in reviewing microbial phylogeny on the basis of molecular data other than rRNA sequencing.

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