The Phylogenetic Position of *Pseudoamycolata halophobica* (Akimov et al. 1989) and a Proposal To Reclassify it as *Pseudonocardia halophobica*

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The genus *Pseudoamycolata* was proposed for actinomycetes which closely resembled *Amycolata* species in most phenotypic features but lacked a key chemotaxonomic marker, phosphatidylcholine. We sequenced the 16S ribosomal DNA of *Pseudoamycolata halophobica* and found that this organism is very closely related to the genus *Pseudonocardia*, which now contains all of the strains formerly classified as members of the genus *Amycolata*. In light of these data, we believe that the absence of a single chemical characteristic is not sufficient to justify forming a new genus, and we propose that *Pseudoamycolata halophobica* (Akimov et al. 1989) should be reclassified as *Pseudonocardia halophobica*. The type strain is strain DSM 43089.

The family *Pseudonocardaceae* (2, 8) contains actinomycetes which lack mycolic acids and have chemotype IV cell walls (sensu Lechevalier and Lechevalier [16]). This taxon currently comprises the genera *Actinopolyspora*, *Amycolatopsis*, *Saccharomonospora*, *Saccharopolyspora*, *Kibdelosporangium*, and *Pseudonocardia* (2, 8, 21). There are relatively few stable characteristics which can be used to distinguish among the different genera (6), but in at least some cases differentiation can be achieved by using phospholipid and menaquinone compositions (7, 17). For example, *Amycolatopsis* species contain phosphatidylethanolamine as a characteristic phospholipid (type PII phospholipid pattern [15]) and contain partially hydrogenated menaquinones with nine isoprene units as their major menaquinones [MK-9(H₂,H₄)] (17). In contrast, members of the genus *Amycolata*, which otherwise look and behave like *Amycolatopsis* strains, contain phosphatidylcholine as well as phosphatidylethanolamine (phospholipid type PIII) and major amounts of hydrogenated menaquinones with eight isoprene units [MK-8(H₄)] (17). In 1989, a new genus, *Pseudoamycolata*, was proposed (1) for two strains which had been formerly classified as *Amycolata autotrophica* strains. The single species of this new genus strongly resembled *Amycolata* species in overall morphology and phenotype and contained MK-8(H₄) as its major menaquinone. However, this organism lacked phosphatidylcholine, and absence of this single characteristic was judged to be sufficient to distinguish the new genus *Pseudoamycolata*.

Comparative analysis of 16S rRNA sequences is currently the most powerful method for resolving higher taxonomic relationships among actinomycetes (2, 8, 9, 22). This approach has recently (21) been used to reexamine the phylogeny of members of the family *Pseudonocardaceae*. The data obtained led to the proposal that the genera *Amycolata* and *Pseudonocardia* should be combined in a redefined genus *Pseudonocardia*; this proposal is strongly supported by overall phenotype (6). In this investigation we used 16S rRNA analysis to determine the phylogenetic relationship between *Pseudoamycolata halophobica* and the genus *Pseudonocardia*.

MATERIALS AND METHODS

Bacterial strains. The type strain of *Pseudoamycolata halophobica*, strain DSM 43089, was grown at 25°C in Bennett's broth containing 0.1% (wt/vol) yeast extract, 0.1% (wt/vol) beef extract, 0.2% (wt/vol) N-Z amine type A, and 1.0% (wt/vol) glucose. Cells were harvested during the exponential growth phase, centrifuged to pellet the wet cell mass, and washed twice with TE buffer (pH 7.2).

Extraction of DNA, PCR amplification, and sequencing of rRNA genes. Cells were disrupted with a French pressure cell at 12,000 lb/in², and extraction of the resulting lysate, once with TE-equilibrated phenol and once with chloroform-isoamyl alcohol (24:1, vol/vol), was followed by ethanol precipitation. An aliquot of this DNA preparation was redissolved in sterile distilled water and reprecipitated by using polyethylene glycol 8000 (14). The resulting pellet was resuspended in distilled water, and 1-μl (10- to 100-ng) portions were added to PCR mixtures by using a hot start (12) and primers designed to amplify almost the entire 16S rRNA gene (5). The amplified products were precipitated and directly sequenced (5).

Phylogenetic analysis. The 16S rRNA gene sequence (1,438 bases) of *Pseudoamycolata halophobica* was aligned with sequences of members of the family *Pseudonocardaceae* (2, 8, 9, 21), and *Tsukamurella paurometabolum* (4) was used as an outgroup. Regions of rRNA sequence which could not be unambiguously aligned were removed, and 916 sequence positions were used to calculate a distance matrix by using the algorithm of Jukes and Cantor (13) in PHYLIP, version 3.5c (10). In order to assess the stability of the phylogenetic trees, the sequence data were resampled 100 times by using the bootstrapping procedure (SEQBOOT [10]). Tree diagrams were constructed by using the neighbor-joining method in (19) PHYLIP. For parsimony analysis we used the PAUP program (20).

Nucleotide sequence accession number. The sequence of *Pseudoamycolata halophobica* has been deposited in the EMBL data base under accession number Z14111.

RESULTS AND DISCUSSION

The sequencing method which we used generated 1,438 bases of 16S ribosomal DNA sequence of *Pseudoamycolata halophobica*. This sequence exhibited between 95.4 and 96.6% similarity (for 1,346 to 1,438 bases) to sequences obtained for

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FIG. 1. Distance tree produced by using neighbor joining, showing the phylogenetic position of *Pseudoamycolata halophobica*. An alignment of 916 bases was used in the analysis. The numbers at the nodes indicate the levels of bootstrap support (based on data for 100 replicates) for the groups to the right of the nodes. Scale bar (for horizontal distances) indicates 0.01 substitution per nucleotide position.

members of the genus *Pseudonocardia*. All of the phylogenetic trees placed *Pseudoamycolata halophobica* close to or within the radiation defined by species of the genus *Pseudonocardia* and at some distance from members of the genus *Amycolatopsis*. Distance matrix analysis (Fig. 1) and bootstrapping analysis consistently placed *Pseudoamycolata halophobica* together with *Pseudonocardia* species (95% of trees). Although the precise position of *Pseudoamycolata halophobica* was not resolved, this taxon most commonly appeared as a branch on the lineage leading to *Pseudonocardia compacta*, "*Streptomyces nitrificans*," and *Pseudonocardia autotrophica* (39% of trees).

Over the past few years the concept of polyphasic taxonomy has been adopted as a guiding principle for good bacterial classification (18). This concept advocates that the most stable and useful taxonomy is one that utilizes genotypic and phenotypic criteria and does not put undue weight on any one type of evidence. On the basis of such criteria, it is proposed in the accompanying paper that the genera *Amycolata* and *Pseudonocardia* should be combined in a redefined genus, *Pseudonocardia* (21). In contrast, the genus *Pseudoamycolata* was created for strains which differed from species of the genus *Amycolata* (which are now classified as members of the genus *Pseudonocardia*) by a single chemical characteristic: they lacked phosphatidylcholine. We did not detect phosphatidylcholine in the type strain of *Pseudoamycolata halophobica* (unpublished data). Phosphatidylcholine is produced in bacteria by sequential methylation of phosphatidylethanolamine (11), and it is conceivable (but not proven in this case) that relatively minor mutations could prevent this transformation. In any case, the absence of a single characteristic should not be used to determine relationships. This was demonstrated when the mycolate *Corynebacterium* species, *Corynebacterium amycolatum* (Collins et al. 1988), was described (3). This new species had chemotaxonomic characteristics consistent with inclusion in the genus *Corynebacterium*, yet it did not contain mycolic acids (3). Apart from the absence of phosphatidylcho-

line, *Pseudoamycolata halophobica* resembles species which are now classified as members of the genus *Pseudonocardia* (21) in all other characteristics. This organism has the same morphology as *Pseudonocardia* species and possesses cultural, physiological, fatty acid, menaquinone composition, and moles percent G+C of the DNA, characteristics which are consistent with membership in the genus *Pseudonocardia* (1, 6). We have now shown that it is closely related to members of the genus *Pseudonocardia* on the basis of 16S rRNA sequences.

A major goal of taxonomy is to produce stable classifications, and name changes should not be undertaken lightly. Balanced against this is the desire to reflect natural relationships. In the case described above, we believe that the weight of genotypic and phenotypic evidence argues strongly for reclassification of *Pseudoamycolata halophobica*. To this end, we propose that *Pseudodoamycolata halophobica* (Akimov, Evtushenko, and Dobritsa 1989) should be reclassified as *Pseudonocardia halophobica*. The inclusion of *Pseudonocardia halophobica* in the genus *Pseudonocardia* (Warwick, Bowen, McVeigh, and Embley 1994) means that the genus description should be emended to include strains which do not contain phosphatidylcholine. The species description remains the same as the description published previously (1).

ACKNOWLEDGMENTS

The phylogenetic analyses were carried out by using the SERC SEQNET facility at Daresbury, United Kingdom.

This project was funded by Glaxo Group Research.

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