Pseudoamycolata halophobica gen. nov., sp. nov.

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A new genus and species, *Pseudoamycolata halophobica*, is proposed for two closely related strains formerly assigned to *Amycolata* (*Nocardia*) *autotrophica*. The following characteristics are important for defining the genus *Pseudoamycolata*: a type IV cell wall composition (presence of *meso*-diaminopimelic acid, arabinose, and galactose); lack of mycolates; phospholipid type PII (presence of phosphatidylethanolamine); menaquinone MK-8(H₄) as the major menaquinone; iso- and anteiso-branched fatty acids; and a guanine-plus-cytosine content of 72 mol%. *P. halophobica* is readily differentiated from morphologically similar members of the genera *Amycolata* and *Amycolatopsis* by phospholipid composition and menaquinone composition, respectively. In addition, it can be distinguished from *Amycolata* and *Amycolatopsis* species by low levels of deoxyribonucleic acid-deoxyribonucleic acid homology (12 to 15 and 7 to 10%, respectively) and a number of physiological characteristics. The type strain of *P. halophobica* is strain VKM Ac-1069 (= DSM 43089).

Actinomycetes which contain *meso*-diaminopimelic acid, arabinose, and galactose in their cell envelopes (wall chemotype IV sensu Lechevalier and Lechevalier [12]) but lack mycolic acids were considered to form the *Micropolyspora* aggregate group (5) containing the genera *Actinopolyspora*, *Faenia* (*Micropolyspora*), *Pseudonocardia*, *Saccharomonospora*, and *Saccharopolyspora*, as well as several species of uncertain generic affiliation previously misclassified in the genera *Nocardia* and *Streptomyces*. More recently, the taxonomy of this group has changed substantially, and new genera and reclassifications have been proposed. In 1986, three new genera of mycolateless cell wall type IV actinomycetes were described. The genus *Kibdelosporangium* (15) was proposed to accommodate organisms that produce long chains of aerial spores and sporangiumlike structures, and the genera *Amycolata* and *Amycolatopsis* (13) were erected to accommodate mycolateless organisms that were formerly classified in the genus *Nocardia*. Certain chemotaxonomic characteristics, especially compositions of polar lipids, menaquinones, and cellular fatty acids, have been shown to be of significant value in defining and grouping genera of mycolateless wall chemotype IV actinomycetes (3, 13). In particular, the genera *Amycolata* and *Amycolatopsis* have been defined mainly on the basis of phospholipid composition (type PIII for the genus *Amycolata* and type PII for the genus *Amycolatopsis*) and menaquinone composition [MK-8(H₂,H₄) for the genus *Amycolata* and MK-9(H₂,H₄) for the genus *Amycolatopsis*] (13).

Amycolata autotrophica, the type species of the genus *Amycolata* (13), includes about 30 poorly investigated strains of various origins, which were assigned to *Nocardia* (*Amycolata*) *autotrophica* by Gordon et al. (6) primarily on the basis of physiological studies. Our recent taxonomic study (4) showed that some of these organisms should be transferred to a new species, *Amycolata alni*. Two other strains, strains VKM Ac-1069^T (T = type strain) and VKM Ac-1078, which were received by us as *Amycolata autotrophica*, were found to be clearly distinguished from *Amycolata* species on the basis of their phospholipid pattern (type PII sensu Lechevalier et al. [11]); these strains initially came from M. P. Lechevalier (The Waksman Institute) as *Nocardia* sp. strains of soil origin (strains SS1/1^T and N11,

respectively). Subsequently, they were assigned to *Nocardia autotrophica* by Gordon et al. (6).

In this paper we show that these organisms have chemotaxonomic characteristics that preclude their placement in any of the previously described genera of the order *Actinomycetales*. On the basis of chemotaxonomic, deoxyribonucleic acid (DNA), morphological, and physiological studies, we propose a new genus, *Pseudoamycolata*, and a new species, *Pseudoamycolata halophobica*, to accommodate these two mycolateless wall type IV strains.

(Some of the data have been presented elsewhere [V. N. Akimov, S. V. Dobritsa, L. I. Evtushenko, and P. A. Shary, Abstr. 2nd Conf. Taxon. Automatic Identification Bacteria, Prague, Czechoslovakia, 1987].)

MATERIALS AND METHODS

Bacterial strains. Strains VKM Ac-1069^T (= DSM 43089^T = IMRU 1300^T = M. P. Lechevalier SS1/1^T) and VKM Ac-1078 (= DSM 43092 = IMRU 1333 = M. P. Lechevalier N11) were studied. Type strains of *Amycolata* and *Amycolatopsis* species were used for comparison (see Tables 1 and 2).

Phenotypic characterization. Tests for more than 150 characteristics, including morphological, physiological, biochemical, and nutritional tests, were carried out. The methods which we used have been described previously (4).

Cell chemistry. Cell wall, phospholipid, and menaquinone compositions were determined as described previously (4). Cellular fatty acid methyl ethers were prepared and analyzed by gas-liquid chromatography as described previously (4). 2-Hydroxy fatty acids were identified on thin-layer chromatograms as described by Kroppenstedt (9).

DNA characterization. The methods used for preparation and purification of DNA and the conditions used to determine guanine-plus-cytosine (G+C) contents by the thermal denaturation method and DNA relatedness by the membrane filter method have been described previously (2, 4).

RESULTS

Morphological and cultural characteristics. A study of cultural and morphological characteristics of strains VKM Ac-1069^T and VKM Ac-1078 showed that both of these strains produced white to cream aerial mycelium and yel-

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TABLE 1. DNA base compositions and DNA-DNA homologies among strains of *Pseudoamycolata*, *Amycolatopsis*, and *Amycolata* species

Strain	G+C content (mol%)	% Homology with labeled DNA from:	
		<i>Pseudoamycolata halophobica</i> VKM Ac-1069 ^T	<i>Amycolatopsis orientalis</i> VKM Ac-866 ^T
<i>Pseudoamycolata halophobica</i> VKM Ac-1069 ^T	72	100	10
<i>Pseudoamycolata halophobica</i> VKM Ac-1078	72	61	7
<i>Amycolatopsis orientalis</i> VKM Ac-866 ^T	66	7	100
<i>Amycolatopsis mediterranei</i> VKM Ac-798 ^T	70	10	14
<i>Amycolatopsis rugosa</i> IMET 7650 ^T	67	10	14
<i>Amycolatopsis sulphurea</i> IMET 7649 ^T	68	7	15
<i>Amycolata autotrophica</i> DSM 535 ^T	72	12	5
<i>Amycolata alni</i> VKM Ac-901 ^T	72	12	ND ^a
<i>Amycolata saturnea</i> VKM Ac-781 ^T	ND	15	ND

^a ND, Not determined.

lowish orange substrate mycelium. Hyphae broke into rod-shaped and oval units. Short chains of spores were produced on the aerial hyphae. In cultures of strain VKM Ac-1078, swollen hyphal segments (up to 3 µm long) with transverse and longitudinal septa were often observed, which is characteristic of *Amycolata* species, *Amycolata autotrophica*, and *Amycolata alni* (4, 10). Strain VKM Ac-1069^T rarely displayed such structures.

Cell chemistry. A cell wall analysis showed the presence of *meso*-diaminopimelic acid, arabinose, and galactose, indicating a type IV cell wall composition. No mycolic acids were detected. The principal nitrogenous phospholipid was phosphatidylethanolamine, and phosphatidylmethylethanolamine was present in small amounts; no phosphatidylcholine- or glucosamine-containing phospholipids were detected (type II phospholipid pattern sensu Lechevalier et al. [11]). The principal menaquinone component found in both strains was menaquinone MK-8(H₄). As minor constituents, menaquinones MK-8(H₂) and MK-9(H₄,H₆) were detected in strain VKM Ac-1069^T, while menaquinone MK-8(H₆) was found in strain VKM Ac-1078. The fatty acids were predominantly saturated branched-chain acids of the iso and anteiso types, as follows (the numbers in parentheses are the percentages of the total fatty acids): iso-C_{16:0} acid (19 to 26%), anteiso-C_{17:0} acid (17 to 21%), iso-C_{15:0} acid (7 to 9%), and iso-C_{15:0} acid (5.5 to 9%). Straight-chain saturated (C_{15:0} and C_{16:0}) and unsaturated (C_{16:1}) fatty acids were detected in amounts ranging from 3 to 8%. 10-Methyl-branched fatty acids (10-methyl-C_{17:0} and 10-methyl-C_{18:0} acids) were also present as minor constituents (3 to 6.5%), including trace amounts (less than 0.2%) of tuberculostearic acid (10-methyl-C_{19:0} acid). 2-Hydroxy fatty acids were also detected by thin-layer chromatography.

DNA characterization. The G+C contents of the DNAs of both strains were 72 mol% (Table 1). The values for *Amycolata* species range from 68 to 74 mol% (4, 13) (Table 1), while those for *Amycolatopsis* species are in the range between 66 and 73 mol% (8, 13) (Table 1).

Values of 7 to 10% DNA-DNA homology were found for strains VKM Ac-1069^T and VKM Ac-1078 with the type strains of *Amycolatopsis* species. The levels of homology were slightly higher (12 to 15%) with the type strains of *Amycolata* species. The degree of binding between the DNAs from strains VKM Ac-1069^T and VKM Ac-1078 was 61% (Table 1).

Physiological characteristics. About 70% of the 150 physiological characteristics examined were similar for strains VKM Ac-1069^T and VKM Ac-1078. However, these strains varied in their patterns of utilization of carbon compounds

(glycerol, *meso*-inositol, maltose, sorbitol, sucrose, and benzoic acid), their hydrolytic (hydrolysis of casein) and nitrate reductase activities, and their susceptibilities to some concentrations of antibiotics and growth inhibitors (strain VKM Ac-1078 is more resistant to carbenicillin, erythromycin, gramicidin, lincomycin, monomycin, neomycin, novobiocin, oligomycin, rifampin, ristomycin, streptomycin, tetracycline, and tobramycin). A detailed characterization of the physiological properties is given below in the description of *Pseudoamycolata halophobica* sp. nov. The physiological characteristics that differentiate *Pseudoamycolata halophobica* and *Amycolatopsis* species are shown in Table 2.

DISCUSSION

Our results show that strains VKM Ac-1069^T and VKM Ac-1078 are characterized by cell wall chemotype IV and the absence of mycolates. Previously, the following eight genera of mycolateless cell wall type IV actinomycetes have been described: *Actinopolyspora*, *Amycolata*, *Amycolatopsis*, *Faenia*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharomonospora*, and *Saccharopolyspora*. However, strains VKM Ac-1069^T and VKM Ac-1078 cannot be unambiguously assigned to any of these taxa because these strains have a type PII phospholipid pattern and major menaquinones of the MK-8(H₄) type, a combination which is different from the combinations of characteristics which define related taxa (Table 3).

Upon initial observation, strains VKM Ac-1069^T and VKM Ac-1078 morphologically resemble members of the genus *Amycolata*, especially *Amycolata autotrophica* and *Amycolata alni* (4, 10). These strains also have cultural and physiological characteristics, DNA G+C contents, and menaquinone and fatty acid compositions that are consistent with their assignment to the genus *Amycolata*. However, strains VKM Ac-1069^T and VKM Ac-1078 have phospholipid type PII rather than phospholipid type PIII, which is characteristic of the genus *Amycolata* (13). The difference in phospholipid composition, which is a strong taxonomic criterion, leads us to conclude that these two strains should be excluded from the genus *Amycolata*.

Accordingly, these strains cannot belong to the genera *Actinopolyspora*, *Faenia*, *Pseudonocardia*, and *Saccharopolyspora*, members of which have type PIII phospholipid compositions and differ from our strains in a number of other attributes (1, 3, 13, 16) (Table 3).

Three genera of mycolateless cell wall type IV actinomycetes, *Kibdelosporangium*, *Saccharomonospora*, and *Amycolatopsis*, are characterized by type PII phospholipid

TABLE 2. Differentiating physiological characteristics of *Pseudoamycolata halophobica* and *Amycolatopsis* species

Organism	Decarboxy- lation of: position of xan- thine	Produce- tion of:	Growth on 5% NaCl	Acid produced from:																Utilization of the fol- lowing com- pounds as carbon sources:		Utilization of the following compounds as nitrogen sources:				Color of substrate mycelium on peptone- yeast ex- tract agar				
				Ben- zoate	Cit- rate	Nitrate reduc- tase	Urease	Adon- itol	L- Arab- inose	D- Arab- inose	D- Cello- biose	meso- Eryth- ritol	meso- Inosi- tol	D- Lac- tose	D- Malt- ose	Meli- biose	α- Methyl- D-glu- coside	L- Rham- nose	D- Sali- cin	Treh- lose	D- Xy- lose	D- Gluc- uro- nate	D- Gluc- onate	Argi- nine	L- Histi- dine		Orni- thine	Phenyl- alanine	Gly- cine	
<i>P. halophobica</i> (2 strains)	+	V	-	V	+	-	-	-	-	-	V	-	V	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	Orange	
<i>Amycolatopsis</i> <i>orientalis</i>	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Cream	
VKM Ac-866 ^T	-	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Cream	
<i>Amycolatopsis</i> <i>mediterranei</i>	-	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Cream	
VKM Ac-798 ^T	+	+	-	+	+	+	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	+	Pink	
<i>Amycolatopsis</i> <i>rugosa</i> IMET 7650 ^T	-	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Yellow
<i>Amycolatopsis</i> <i>sulphurea</i> IMET 7649 ^T	-	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Yellow
<i>Amycolatopsis</i> <i>azurea</i> DSM 43854 ^{Tb}	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Grey
<i>Amycolatopsis</i> <i>fastidiosa</i> DSM 43855 ^{Tb}	-	-	+	+	ND	+	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	ND	ND	ND	ND	ND	ND	Cream

^a +, Positive; -, negative; V, variable; ND, not determined.^b Data from reference 8 and this study.

TABLE 3. Chemotaxonomic and DNA characteristics of mycolateless wall type IV actinomycetes^a

Genus	Characteristic fatty acids ^b	Phospholipid type	Major menaquinone(s)	G+C content (mol%)
<i>Actinopolyspora</i>	I, A, Me	PIII	MK-9(H ₄)	64
<i>Faenia</i>	I, A, Me, diMe	PIII	MK-9(H ₄)	66-68
<i>Saccharopolyspora</i>	I, A, Me, diMe	PIII	MK-9(H ₄)	69
<i>Pseudonocardia</i>	I, A, Me, diMe	PIII	MK-8(H ₄)	71-72
<i>Amycolata</i>	I, A, Me	PIII	MK-8(H ₂ ,H ₄)	68-74
<i>Amycolatopsis</i>	I, A, Me	PII	MK-9(H ₂ ,H ₄)	66-73
<i>Saccharomonospora</i>	I, A, Me	PII	MK-9(H ₄)	67-69
<i>Kibdelosporangium</i>	I, A	PII	ND ^c	66
<i>Pseudoamycolata</i>	I, A, Me	PII	MK-8(H ₄)	72

^a Data from references 3, 4, 8, 13, 14, and 15 and this study. The DNA base compositions of *Pseudonocardia* (*Pseudonocardia thermophila* ATCC 19285^T and *Pseudonocardia compacta* DSM 43592^T), *Saccharopolyspora* (*Saccharopolyspora hirsuta* ATCC 27875^T), and *Saccharomonospora* (*Saccharomonospora viridis* ATCC 15386^T and *Saccharomonospora mesophilica* VKM Ac-1249^T) are our unpublished data.

^b I, Iso; A, anteiso; Me, 10-methyl branched; diMe, 10,15-dimethyl branched.

^c ND, Not determined.

patterns. Our strains can be readily differentiated from members of the genus *Kibdelosporangium* (14, 15), as its members produce long chains of aerial spores and sporangiumlike structures. *Saccharomonospora* species can be readily differentiated from strains VKM Ac-1069^T and VKM Ac-1078 by morphological and cultural traits, as well as by menaquinone composition (1, 3, 7, 13) (Table 3).

The strains which we studied correspond closely to the description given for the genus *Amycolatopsis* by Lechevalier et al. (13), with one distinctive difference. Our strains have predominantly menaquinones of the MK-8(H₄) type [versus MK-9(H₂,H₄) for *Amycolatopsis* (13)]. However, the taxonomic position of strains VKM Ac-1069^T and VKM Ac-1078 is not immediately apparent from these data, as other actinomycete genera are known to have menaquinones with variable isoprenoid chain lengths (16). The search for genetic relationships between our strains and the type strains of *Amycolatopsis* species (Table 1) yielded only insignificant degrees of DNA binding (7 to 10%), which seems sufficient to warrant separate generic status for strains VKM Ac-1069^T and VKM Ac-1078. It is interesting that homology values were slightly higher (12 to 15%) with the type strains of *Amycolata* species. In addition, strains VKM Ac-1069^T and VKM Ac-1078 have physiological characteristics which clearly differentiate them from all members of the genus *Amycolatopsis* (Table 2), as well as *Amycolata* species (4).

In view of the distant genetic relatedness to both *Amycolata* and *Amycolatopsis* species and their distinctive chemical and physiological characteristics, we believe that the strains which we studied merit a new genus. This conclusion is supported by the results of a numerical taxonomy study (L. I. Evtushenko, L. G. Tomashevskaya, and P. A. Shary, Mikrobiologiya, in press). Therefore, we propose that strains VKM Ac-1069 and VKM Ac-1078 be classified in a new genus, *Pseudoamycolata*, as *Pseudoamycolata halophobica* sp. nov. and that strain VKM Ac-1069 be designated as the type strain of the species. Certain differences in physiological characteristics between strains VKM Ac-1069^T and VKM Ac-1078 might be considered as corresponding to the taxonomic rank of species. However, the 61% level of DNA relatedness between these strains (Table 1) indicates that they belong to the same species. Below we give descriptions of *Pseudoamycolata* gen. nov. and *Pseudoamycolata halophobica* sp. nov.

***Pseudoamycolata* gen. nov.** *Pseudoamycolata* (Pseu. do.a.my.co.la'ta. Gr. adj. *pseudes*, false; M. L. fem. n. *Amycolata*, a genus of actinomycetes; M. L. fem. n. *Pseudoamycolata*, false *Amycolata*). Actinomycetes that

form vegetative and aerial hyphae which tend to break down into rod-shaped and oval fragments. Short chains of spores may be produced on the aerial hyphae. Gram positive, non-acid fast. Mesophilic, aerobic, catalase positive. Non-motile. Type IV cell wall composition (*meso*-diaminopimelic acid, arabinose, and galactose as diagnostic constituents). Mycolates absent. Type PII phospholipid pattern (phosphatidylinositol mannosides, diphosphatidylglycerol, and phosphatidylethanolamine, as well as small amounts of phosphatidylmethylethanolamine, are present). Saturated branched-chain fatty acids of the iso and anteiso types are predominant; straight-chain saturated, unsaturated, 2-hydroxy, and 10-methyl-branched fatty acids, including tuberculostearic acid (traces), are present as minor constituents. The major menaquinone is MK-8(H₄). The G+C content of the DNA is 72 mol% (as determined by the thermal denaturation method). The type species is *Pseudoamycolata halophobica*.

***Pseudoamycolata halophobica* sp. nov.** *Pseudoamycolata halophobica* (ha.lo.pho'bi.ca. Gr. n. *hals*, salt, the sea; Gr. n. *phobos*, fear, dread; L. fem. adj. *halophobica*, salt-fearing, referring to the inability to grow in the presence of 3% NaCl). Morphology, chemotaxonomy, and general characteristics are as given above for the genus. In addition, swollen hyphal segments (up to 3 µm long) with transverse and longitudinal septa may be observed in cultures. The aerial mycelium is white to cream colored, and the substrate mycelium ranges from yellowish orange to brown, depending on the medium (the color of the substrate mycelium on peptone-yeast extract agar is orange).

Acid is produced from fructose, D-galactose, D-glucose, L-rhamnose, and xylose. Acid production from glycerol, *meso*-inositol, maltose, sorbitol, and sucrose is variable. No acid is produced from adonitol, L- and D-arabinose, cellobiose, erythritol, melibiose, α-methyl-D-glucoside, raffinose, ribitol, ribose, salicin, and trehalose. Decarboxylates aconitate, formate, lactate, malate, malonate, propionate, pyruvate, sebacate, succinate, and *trans*-aconitate but not citrate, maleate, and salicylate. Decarboxylation of benzoate is variable. No growth on D-glucuronate and D-glucuronate as sole carbon sources. Hydrolyzes arbutin, esculin, xanthine, urea, Tween 40, Tween 60, and gelatin but not allantoin and starch; variable results are obtained for hydrolysis of casein. Alanine, arginine, hydroxyproline, ornithine, phenylalanine, proline, threonine, tryptophan, and valine but not glycine, histidine, leucine, lysine, and tyrosine are assimilated as sole nitrogen sources. Production of nitrate reductase is variable.

No growth in the presence of 3% NaCl, 0.001% sodium

azide, or 0.000001% crystal violet. Susceptible to roseofungin (10 µg/ml), polymyxin B (20 µg/ml), fucidin (1 µg/ml), metacycline (1 µg/ml), lincomycin (10 µg/ml), penicillin (10 µg/ml), tetracycline (10 µg/ml), and novobiocin (50 µg/ml).

The G+C content of the DNA is 72 mol%.

Isolated from soil.

The type strain is strain VKM Ac-1069 (= DSM 43089 = IMRU 1300 = M. P. Lechevalier SS1/1).

Description of the type strain. The description of the type strain is the same as that given above for the species, with the following differences: acid is produced from glycerol and meso-inositol but not from maltose, sorbitol, and sucrose; nitrate reductase negative; no decarboxylation of benzoate; and no hydrolysis of casein.

Additional comments. White et al. (17) have described an actinomycete strain, strain NRRL B-16216, which has the same chemotaxonomic and DNA characteristics as *Pseudoamycolata halophobica*. Strain NRRL B-16216 has a type IV cell wall composition, no mycolates, a type PII phospholipid pattern, major menaquinones of the MK-8(H₂,H₄) type, and a G+C content of 73 mol%. Its physiological characteristics most closely resemble those of *Amycolatopsis orientalis*. However, the difference in menaquinone composition and a low level of DNA homology with *Amycolatopsis orientalis* (11.5%) have not permitted the authors to assign strain NRRL B-16216 to the genus *Amycolatopsis*. In our opinion, this strain might belong to the genus *Pseudoamycolata*.

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