A New Species of Actinomycete, *Amycolata alni*

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A new species of the genus *Amycolata*, *Amycolata alni*, is proposed for strains which were mostly isolated from root nodules and rhizospheres of alder trees and were formerly assigned to the species *Amycolata autotrophica*. Strains of this new species could be differentiated from representatives of *A. autotrophica* by deoxyribonucleic acid homology data (17 to 29% relatedness), as well as by their ability to grow on salicin and D-gluconate, their failure to produce acid from cellobiose and meso-inositol, their resistance to penicillin and carbenicillin (10 µg/ml), and other characteristics. Phenotypic features and low levels of deoxyribonucleic acid homology distinguish *A. alni* from two other species of the genus *Amycolata*, *Amycolata saturnea* and *Amycolata hydrocarbonoxydans*. The type strain of *A. alni* is strain 3LS (= VKM Ac-901).

Eleven phenotypically similar nocardioform actinomycetes having a type IV cell wall composition and lacking mycolic acids were isolated from nitrogen-fixing root nodules and rhizospheres of two alder species, *Alnus glutinosa* and *Alnus incana* (29; L. S. Sharaya, Diss. C. Sci. thesis, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, USSR, 1985). According to the identification keys and species descriptions that were available at that time (3, 14, 15), these isolates were assigned to the species *Nocardia autotrophica* (20; Y. B. Malishkaite, Diss. C. Sci. thesis, University of Moscow, Moscow, USSR, 1984). Recently, *N. autotrophica* was included as the type species in the genus *Amycolata*, which was proposed, along with the genus *Amycolatopsis*, to accommodate nocardioform actinomycetes having cell wall chemotype IV and lacking mycolic acids (19). Besides the type strain, strain ATCC 19727, the description of *Amycolata autotrophica* included 28 strains of various origins assigned to *N. autotrophica* by Gordon et al. (14). Many physiological characteristics exhibited by these 28 strains are variable or different from those of the type strain (19), thus indicating that the species *Amycolata autotrophica* may be a heterogeneous taxon.

Variations in deoxyribonucleic acid (DNA) guanine-plus-cytosine (G+C) ratios (60 to 72 mol%) and DNA homology values (37 to 67%) (24) among five strains from the group studied by Gordon et al. (14) have shown that strains of different genospecies may have been placed in *Amycolata autotrophica* primarily on the basis of physiological criteria. Recently, genetic heterogeneity among strains assigned to *Amycolata autotrophica*, including the strains from alder associations, was demonstrated by restriction fragment analysis of total genomic DNAs (10). These data showed that the alder isolates are distantly related to the type strain of *Amycolata autotrophica*.

Because of the obvious differences in genome structure among strains of the same species, as well as the above-mentioned physiological variations, we compared strains assigned to *Amycolata autotrophica* more extensively. On the basis of DNA-DNA hybridization data and chemotaxonomic and physiological characteristics, we propose a new species, *Amycolata alni*, for 14 of the strains tested, including all 11 isolates from root nodules and rhizospheres of alders.

(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. The organisms used in this study are listed in Table 1. All of the strains assigned by us to *Amycolata alni* were originally received as *Amycolata autotrophica* strains. The type strain of *Amycolata autotrophica* was included in our work as two separate cultures (cultures DSM 535^T and RIA 1008^T). Since these two cultures exhibited indistinguishable phenotypic characteristics and 100% DNA homology, as well as identical restriction banding patterns of their total genomic DNAs (10) and the same plasmid profiles (Dobritsa, unpublished data), we refer to only one of them below, strain DSM 535^T (T = type strain).

Morphology. Morphological characteristics were observed by phase-contrast microscopy of either living cultures or cultures prepared by the technique of Okami and Suzuki (25), which were fixed with a glacial acetic acid-ethanol-chloroform mixture (1:6:3) and then treated with tannin and finally stained with a 0.2% crystal violet solution. For microscopy, we used 3-, 7-, or 14-day-old cultures grown on either peptone-yeast extract agar (peptone, 5 g; yeast extract, 3 g; glucose, 5 g; K₂HPO₄, 0.2 g; distilled water, 1 liter; agar, 15 g) or water agar supplemented with 0.1% casein hydrolysate.

Physiology. Physiological characteristics were studied by using previously described procedures (13, 15, 16, 27). Utilization of amino acids was determined in glucose-asparagine medium (glucose, 10 g; L-asparagine, 0.5 g; K₂HPO₄, 0.5 g; distilled water, 1 liter; pH 6.8) with a corresponding amino acid substituted for asparagine. Susceptibility to certain concentrations of growth inhibitors and antibiotics was checked on peptone-yeast extract agar. For some antibiotics, minimal inhibitory concentrations were determined by the agar dilution method (12), using Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.).

Cultural characteristics were observed on the media used for physiological tests.

Cell chemistry. Cell wall peptidoglycan preparations were obtained by the method of Schleifer and Kandler (28) and were further purified of proteins by boiling with 2% sodium dodecyl sulfate. The amino acid composition of a peptidoglycan was determined by using a Biotronik model LC 6000 E amino acid analyzer.

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TABLE 1. List of strains used^a

Strain designation as received	Other strain designation(s)	Received from:
Strains assigned by us to <i>Amycolata alni</i>		
3LS ^T	VKM Ac-901 ^T	Sharaya
6LS	VKM Ac-914	Sharaya
10LS		Sharaya
11LS	VKM Ac-915	Sharaya
12LS		Sharaya
14LS		Sharaya
19LS		Sharaya
23LS		Sharaya
24LS	VKM Ac-916	Sharaya
25LS		Sharaya
26LS	VKM Ac-917	Sharaya
VKM Ac-759		VKM
VKM Ac-1066	NCIB 8939, IMRU 1337	VKM
VKM Ac-813 ^b		VKM
Reference and other strains		
<i>Amycolata autotrophica</i> DSM 535 ^T	ATCC 19727 ^T , VKM Ac-941 ^T	Schlegel
<i>Amycolata autotrophica</i> RIA 1008 ^T	ATCC 19727 ^T	RIA
<i>Amycolata autotrophica</i> VKM Ac-1065	DSM 43103, IMRU 1577	VKM
<i>Amycolata autotrophica</i> VKM Ac-1067	JCM 4010	VKM
<i>Amycolata autotrophica</i> VKM Ac-1068	DSM 43098, IMRU 1398	VKM
<i>Amycolata saturnea</i> VKM Ac-781 ^T	ATCC 15809 ^T	VKM
<i>Amycolata hydrocarbonoxydans</i> VKM Ac-799 ^T	ATCC 15104 ^T	VKM
<i>Micrococcus luteus</i> VKM B-1314 ^T	ATCC 15307 ^T	VKM

^a Strains with designations ending in LS are isolates from alder associations. ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; IMRU, Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, New Brunswick; JCM, Japan Collection of Microorganisms, Saitama, Japan; NCIB, National Collection of Industrial Bacteria, Aberdeen, United Kingdom; RIA, All-Union Research Institute for Antibiotics, Moscow, USSR; VKM, All-Union Collection of Microorganisms, Pushchino, USSR; Schlegel, H. G. Schlegel, Institut für Mikrobiologie, Universität Göttingen, Göttingen, Federal Republic of Germany; Sharaya, L. S. Sharaya, All-Union Collection of Microorganisms, Pushchino, USSR.

^b Strain VKM Ac-813 is a subculture from strain NCIB 8939 (= VKM Ac-1066), which was found to have lost a small plasmid (Dobritsa, unpublished data).

Cellular fatty acid methyl ethers were prepared by heating 40 to 50 mg of a lyophilized biomass in a mixture containing 200 µl of methanol and 50 µl of acetyl chloride at 85°C until the reagents were completely evaporated. The methyl ethers were extracted with *n*-hexane and were analyzed by using a Perkin-Elmer model 3500 gas-liquid chromatograph equipped with a flame ionization detector and a type SE-30 capillary column.

Menaquinones were extracted and purified by the method of Collins and Jones (6), and their compositions were determined by using a model MX-1310 mass spectrometer. Phospholipids were isolated, analyzed by thin-layer chromatography, and identified by using the methods of Minnikin et al. (23). Phosphatidylcholine was identified with the Dragendorff reagent (17).

DNA base composition and DNA-DNA hybridization. High-molecular-weight DNA was prepared as described previously (9) or by the technique of Bradley et al. (2), with some modifications. The G+C content of the DNA was determined from its thermal denaturation midpoint (21) in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) by using a Beckman model DU-8B spectrophotometer equipped with a thermoprogrammer. DNA base composition was calculated from the equation given by Owen and Pitcher (26), modified for another reference DNA. DNA from *Micrococcus luteus* VKM B-1314^T, which contains 72 mol% G+C (21), was used as the reference.

The extent of DNA homology was determined by the membrane filter method, as modified by Meyer and Schleifer (22) and Stackebrandt et al. (30). ³H-labeled DNA was obtained by in vitro nick translation, using a type N.5000 nick translation kit (Radiochemical Centre, Amersham, United Kingdom) and deoxy[³H]cytidine 5'-triphosphate.

The radioactivities of the DNA preparations ranged from 10⁴ to 10⁵ cpm/µg. A 0.1- to 0.2-µg portion of fragmented, denatured [³H]DNA was incubated with a filter (type BA-85; diameter, 5.5 mm; Schleicher & Schuell, Dassel, Federal Republic of Germany) loaded with 7 to 10 µg of unlabeled DNA in 200 µl of 3× SSC containing 35% formamide at 60°C for 24 h. The amount of filter-fixed DNA was estimated by the method of Burton (4) after the radioactivity was counted and the filter was washed with toluene.

RESULTS

Morphology. The morphological properties of some of the strains received by us as *Amycolata autotrophica* have been studied and described in detail previously (1, 14, 18, 19, 29). In particular, the strains isolated from alder associations were characterized by their ability to divide swollen hyphal fragments in transverse and longitudinal planes (1, 18, 29), which has never been reported previously for *Amycolata autotrophica*. Thus, the purpose of this study was to compare the morphological characteristics of the alder isolates with those of the type strain of *Amycolata autotrophica* grown under the same conditions. All of the strains examined were shown to have similar morphological properties which are characteristic of the group of nocardioform organisms. The cultures formed true mycelia with diameters of 0.5 to 1.5 µm (sometimes up to 2 µm), which tended to break down into rod-shaped and oval elements after 2 to 4 days. Chains of spores were produced on both the substrate and aerial hyphae, with diameters somewhat larger than those of the hyphae. When living cultures were observed by phase-contrast microscopy, local hyphal thickenings up to 2 to 2.5 µm in diameter and of variable lengths (from 2 to 8 µm and

more) were detected in all of the strains, including strain DSM 535^T, although these thickenings were observed more often in fresh isolates than in strains stored in culture collections for a long time. In stained preparations, transverse and longitudinal septa were observed within these structures.

Cultural properties. The cultural characteristics of *Amycolata autotrophica* and *Amycolata alni* strains were similar, corresponding to those given in the description of *Amycolata autotrophica* (19). All of the strains produced aerial mycelia which were white to cream colored. However, the color of the substrate hyphae produced by a strain, especially on organic media, was very specific, so that the strains of *Amycolata autotrophica* produced orangish yellow vegetative mycelia, while the strains of *Amycolata alni* formed orangish brown to brown substrate mycelia.

Chemotaxonomic properties. A study of the sugar and amino acid compositions of six strains isolated from alder associations, together with the previous data on strain 3LS^T (29), confirmed that all of the cultures had a type IV cell wall composition (*meso*-diaminopimelic acid, arabinose, and galactose). No mycolic acids were detected. The amino acid compositions of the peptidoglycans were similar in the cultures tested. The molar ratio of amino acids (ratio of alanine to glutamic acid to diaminopimelic acid) was about 2:1:1, corresponding to the Al γ peptidoglycan (28).

The principal menaquinone component in *Amycolata autotrophica* DSM 535^T and *Amycolata alni* 3LS^T and 6LS was a type MK-8(H₄) menaquinone. Both strains of *Amycolata autotrophica* (DSM 535^T and VKM Ac-1065) and strains of *Amycolata alni* (3LS^T, VKM Ac-759, and VKM Ac-1066) had type PIII phospholipid patterns (the diagnostic phospholipids are phosphatidylcholine and phosphatidylmethylethanolamine).

The fatty acid spectra of representative strains of *Amycolata autotrophica* and *Amycolata alni* contained 17 to 18 components in amounts of more than 0.2% (Table 2). The principal components were saturated branched-chain acids of the iso and anteiso types (14-methylpentadecanoic acid [iso-16:0 acid] and 14-methylhexadecanoic acid [anteiso-17:0 acid]). Straight-chain saturated fatty acids (15:0, 16:0, and 17:0 acids) and unsaturated fatty acids (hexadecenoic acid [16:1 acid] and 14-methyl-pentadecenoic acid [iso-16:1 acid]), as well as 10-methyl-branched fatty acids (10-methyl-16:0 acid and 10-methyl-17:0 acid), were detected in amounts up to 10%. Tuberculostearic acid (10-methyl-18:0 acid) was present in trace amounts (less than 0.2%).

DNA base composition and DNA-DNA homology. The DNA G+C contents ranged from 71 to 73.5 mol% in the four *Amycolata autotrophica* strains and from 72 to 74 mol% in the strains of *Amycolata alni* (Table 3). The DNAs from the strains of *Amycolata alni* shared only 17 to 29% homology with the DNAs from the strains of *Amycolata autotrophica* (Table 3). The levels of DNA homology among the strains of *Amycolata autotrophica* were 51 to 78%, and the levels of DNA homology among the strains of *Amycolata alni* were 67 to 94%. Strains of both species exhibited low levels of DNA relatedness (usually less than 18%) to the type strains of *Amycolata saturnea* and *Amycolata hydrocarbonoxydans* (data not shown).

Physiology. More than 150 characteristics of the strains assigned to *Amycolata alni* and *Amycolata autotrophica* and the type strains of the two other *Amycolata* species were studied. On the whole, the 14 *Amycolata alni* strains were characterized, compared with the other organisms, by wider spectra of hydrolytic enzymes and utilizable carbon and

TABLE 2. Cellular fatty acid compositions of *Amycolata autotrophica* and *Amycolata alni*^a

Fatty acid ^b	% In:	
	<i>Amycolata autotrophica</i>	<i>Amycolata alni</i>
ai-14:0	0.3–0.8 ^c	0.2–0.4
14:0	0.1–0.3	0.1–0.2
i-15:0 ^d	4.5–15.8	1.6–3.7
ai-15:0		0.8–9.5
15:1	0.7–0.8	0.3–1.6
15:0 ^d	0.6–1.6	1.4–2.6
i-16:1	5.0–6.8	5.8–8.6
i-16:0 ^d	18.6–36.5	36.2–39.6
16:1	5.0–6.8	5.8–8.6
16:0 ^d	3.3–5.5	5.8–8.1
X ^d	2.6–9.1	0.5–1.7
Me-17:0	2.5–6.1	2.7–5.8
i-17:0	2.8–8.5	4.4–14.4
ai-17:0	13.6–15.2	14.3–18.9
17:0 ^d	1.1–2.4	4.5–9.6
Me-18:0 ^d	1.8–3.4	3.5–6.9
ai-18:0 ^d	0.4–0.8	1.2–7.5
18:0 ^d	0.2–0.3	0.5–1.0
Me-19:0	tr	tr

^a The strains used were the strains listed in Table 3; in addition, *Amycolata alni* 26LS was included.

^b i, iso; ai, anteiso; X, unidentified constituent; Me, 10-methyl-branched. The first number indicates the number of carbon atoms, and the second number indicates the number of double bonds.

^c Range expressed as percentage of the total amount.

^d The relative amounts of these fatty acids are specific for each of the two species.

nitrogen sources, as well as lower susceptibility to growth inhibitors and antibiotics. A detailed characterization of those properties which were common to most strains of *Amycolata alni* is given below in the description of this species. A summary of differentiating characteristics for *Amycolata* spp. is presented in Table 4.

DISCUSSION

Besides the type strain of *Amycolata autotrophica* (19, 31), this study included 11 strains isolated from root nodules and rhizospheres of alders (Sharaya, Diss. C. Sci. thesis) and four strains (strains VKM Ac-1065, VKM Ac-1068, VKM

TABLE 3. DNA base compositions and levels of DNA-DNA homology for *Amycolata autotrophica* and *Amycolata alni* strains

Organism	G+C content (mol%)	% Homology with labeled DNA from:	
		<i>Amycolata autotrophica</i> DSM 535 ^T	<i>Amycolata alni</i> 3LS ^T
<i>Amycolata autotrophica</i> strains			
DSM 535 ^T	72	100	21
VKM Ac-1067	72	78	17
VKM Ac-1068	71	51	24
VKM Ac-1065	73.5	55	29
<i>Amycolata alni</i> strains			
3LS ^T	72	26	100
6LS	73	24	71
11LS	73	29	94
VKM Ac-759	74	24	67
VKM Ac-1066	73.5	23	88
VKM Ac-813	73	28	78

TABLE 4. Differentiating characteristics of *Amycolata* spp.

Characteristic	<i>Amycolata</i> <i>autotrophica</i> (4 strains)	<i>Amycolata</i> <i>saturnea</i> VKM Ac-781 ^T	<i>Amycolata</i> <i>hydrocarbonoxydans</i> VKM Ac-799 ^T	<i>Amycolata</i> <i>alni</i> sp. nov. (14 strains)
Decomposition of:				
Adenine	+	—	—	+
Hypoxanthine	—	+	—	+
Tyrosine	—	+	—	+
Xanthine	—	+	—	V
Decarboxylation of:				
Benzoate	V	+	—	+
Citrate	+	—	—	+
Production of:				
Amylase	—	—	+	+
Urease	+	+	—	+
Growth on 5% NaCl	+	—	—	+
Acid produced from:				
Adonitol	+	—	—	+
D-Cellobiose	+	+	+	—
meso-Erythritol	+	—	+	+
Galactose	+	—	+	+
meso-Inositol	+	+	+	—
D-Lactose	—	—	+	—
D-Mannitol	+	+	—	+
α-Methyl-D-glucoside	V ^b	+	—	V
D-Salicin	—	—	+	+
D-Sorbitol	V ^b	—	—	+
Growth on sole carbon sources				
D-Gluconate	—	+	+	+
α-Methyl-D-glucoside	—	+	+	+
meso-Inositol	+	+	+	—
Utilization as nitrogen sources				
L-Histidine	—	—	—	+
L-Proline	V	+	—	+
Resistance to carbenicillin (10 µg/ml)	—	—	—	+
Color of substrate hyphae	Yellow to orange	Orange	Orange	Brown to orange

^a +, Positive; —, negative; V, variable.^b The type strain is negative.

Ac-1066, and VKM Ac-813 [a derivative of strain VKM Ac-1066]) belonging to the group studied by Gordon et al. (14). We have no information about two other strains (strains VKM Ac-759 and VKM Ac-1067) except that they originated from the culture collections of A. Takamiya and A. Tubaki, respectively, who first described (31) the species *Streptomyces autotrophicus* (*Amycolata autotrophica*). Our study showed that morphologically and chemotaxonomically all of these strains constitute a homogeneous group. In all of them, including the type strain of *Amycolata autotrophica* (strain DSM 535), local hyphal thickenings with longitudinal and transverse septa, which were demonstrated previously in strain 3LS^T (1, 18, 29), were observed at different frequencies. Evidently, the description of *Amycolata autotrophica* (19) should be supplemented by characterization of these structures.

The chemotaxonomic properties of all of the strains were shown to be similar and correspond to those of the genus *Amycolata* (19), as follows: meso-diaminopimelic acid, arabinose, and galactose in the cell wall, lack of mycolic acids, peptidoglycan A1γ, type PIII phospholipid pattern, and major menaquinones of the MK-8(H₄) type. In accordance with the data of Collins et al. (5) reported for strains ATCC 19727^T (= DSM 535^T) and NCIB 8939 (= VKM Ac-1066), we failed to detect in the strains which we tested type MK-8(H₂) menaquinones, which are included in the description of *Amycolata autotrophica* (19). The fatty acid spectra of the strains which we studied were similar; however, differences in the relative amounts of some fatty acids (iso-15:0, 15:0,

iso-16:0, 16:0, 17:0, methyl-18:0, anteiso-18:0, and 18:0 acids) were found between representative strains of *Amycolata autotrophica* and *Amycolata alni*.

The DNA base compositions of the strains which we examined ranged between 71 and 74 mol% G+C. Thus, the upper limit of the DNA G+C content for the genus *Amycolata* should be raised to 74 mol% (rather than 71 mol% [19]). The somewhat higher value (72 versus 70 mol%) obtained for the type strain of *Amycolata autotrophica* by us compared with the data reported by Lechevalier et al. (19) was probably due to the method of DNA isolation which we used (9), which yielded more intact DNA preparations.

Ten of the strains tested, which we received as *Amycolata autotrophica*, are divided into two groups on the basis of DNA-DNA hybridization data, which agrees with the results of a numerical taxonomic study (L. I. Evtushenko, L. G. Tomashevskaya, and P. A. Shary, Mikrobiologiya, in press). This shows that strains formerly assigned to *Amycolata autotrophica* represent at least two distinct species of the genus *Amycolata*. In this study, one of these species, *Amycolata autotrophica*, was represented by four strains, including type strain DSM 535, which displayed DNA-DNA homology levels ranging from 51 to 78% and formed a single cluster (Evtushenko et al., in press). The strains of another group constitute a separate taxospecies (Evtushenko et al., in press) and exhibit high degrees of DNA relatedness (67 to 94%), corresponding to a single genospecies. The close genetic relatedness among some strains of the latter species was demonstrated by the identical or similar banding pat-

terns of their genomic DNA restriction fragments (10, 11) and by the same plasmid composition (8). The members of this species have low levels of DNA homology with all *Amycolata* species described previously, cluster at some distance by numerical taxonomic analysis (Evtushenko et al., in press), and display a number of phenotypic characteristics which differentiate them from *Amycolata autotrophica*, *Amycolata saturnea*, and *Amycolata hydrocarbo-noxydans*. Evidently, they represent a new species of the genus *Amycolata*, for which we propose the name *Amycolata alni*. Most strains of the new species (11 of 14 strains) were isolated from the rhizoplanes and rhizospheres of alders (Sharaya, Diss. C. Sci. thesis). Among them, strain 3LS (= VKM Ac-901) is the best-studied organism (1, 7, 8, 10, 11, 18, 20, 29; Malishkaite, Diss. C. Sci. thesis; Sharaya, Diss. C. Sci. thesis) and therefore is designated the type strain of *Amycolata alni*. Three other strains (strains VKM Ac-759, VKM Ac-1066, and VKM Ac-813 [a variant of strain VKM Ac-1066]) have been stored in culture collections for a long time, and strain VKM Ac-1066 (= NCIB 8939) is rather well studied (5, 14, 15, 24). Below is a description of the new species.

Amycolata alni sp. nov. *Amycolata alni* (al'ni. L.adj. *alni*, of *Alnus*, alder, referring to the isolation of the type strain and some other strains from alder associations). Substrate and aerial mycelia are formed; both fragment into rod-shaped and oval elements. The aerial mycelium (0.7 to 1.0 µm wide) is white to cream colored, and the vegetative mycelium is orange to brown. Chains of spores (up to 1.5 µm in diameter) are abundantly formed on the aerial hyphae. The vegetative hyphae are characterized by pronounced pleomorphisms, forming thickenings up to 2 µm in diameter, as well as cells of a polygonal shape and their conglomerates. Formation of swollen hyphal segments (up to 2.5 µm in diameter and up to 10 µm long) with transverse and longitudinal septa is a characteristic trait.

Nitrate reductase, phosphatase, and urease are produced by most strains. Adenine, esculin, gelatin, hypoxanthine, starch, Tween 40, Tween 60, Tween 80, tyrosine, and xanthine are hydrolyzed. Allantoin, casein, and cellulose are not hydrolyzed. Acetate, aconitate, benzoate, citrate, formate, fumarate, α-ketoglutarate, lactate, malate, maleate, propionate, pyruvate, sebacate, succinate, and *trans*-aconitate are utilized. Produces acid from and grows on adonitol, L-arabinose, *meso*-erythritol, D-fructose, galactose, D-glucose, glycerol, α-methyl-D-glucoside, D-maltose, D-manitol, L-ribose, D-salicin, D-sorbitol, DL-sucrose, trehalose, and D-xylose as sole carbon sources. Acid is produced from D-arabinose, but growth is variable. No acid is formed from D-cellobiose, dulcitol, *meso*-inositol, D-lactose, D-mannose, melibiose, L-rhamnose, and D-sorbose. Grows on D-glucuronate but not on D-glucuronate as a sole carbon source. Assimilates as nitrogen sources asparagine, glutamine, ornithine, arginine, leucine, proline, and histidine. Very weak growth on media containing other amino acids as sole nitrogen sources. Moderate growth on water agar.

Tolerates 7% NaCl, 0.001% phenol, and 0.01% thymol. Susceptible to the following antibiotics: monomycin (8 µg/ml), roseofungin (10 µg/ml), neomycin (4 µg/ml), fucidin (10 µg/ml), streptomycin (4 µg/ml), chloramphenicol (32 µg/ml), gentamicin (4 µg/ml), rifampin (0.125 µg/ml), kanamycin (16 µg/ml), and vancomycin (0.5 µg/ml). In contrast to *Amycolata autotrophica*, susceptible to nalidixic acid (64 µg/ml), novobiocin (0.25 µg/ml), and tetracycline (1 µg/ml) but resistant to ampicillin (1 to 16 µg/ml), benzylpenicillin (0.5 to 10 µg/ml), carbenicillin (1 to 10 µg/ml), ristomycin (0.05 µg/

ml), tobramycin (5 µg/ml), and vancomycin (0.25 µg/ml). No growth in lysozyme broth (50 µg/ml).

The major fatty acids are the following saturated branched-chain acids of the iso and anteiso types: iso-16:0 acid (up to 40%) and anteiso-17:0 acid (up to 20%). 10-methyl-17:0 and 10-methyl-18:0 acids, as well as tuberculostearic acid (traces), are also present. The principal menaquinone component is MK-8(H₄). The G+C content of the DNA is 72 to 74 mol% (as determined by the thermal denaturation method).

Isolated from root nodules and rhizospheres of alders (*Alnus glutinosa* [L.] Gaertn. and *Alnus incana* [L.] Moench.), as well as from other sources.

The type strain is strain 3LS (= VKM Ac-901).

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