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Acidiphilium aminolytica sp. nov.: An Acidophilic Chemoorganotrophic Bacterium Isolated from Acidic Mineral Environment

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Abstract. *Acidiphilium aminolytica* is proposed for a species of the genus *Acidiphilium*. *Acidiphilium aminolytica* can be phenotypically differentiated from all other species of the genus *Acidiphilium*. The seven strains of this species that have been studied are Gram-negative, aerobic, mesophilic, non-sporeforming, motile, and rod-shaped bacteria. They grow between pH 3.0 and 6.0, but not at pH 6.5. They yield positive results in tests for hippuric acid hydrolysis, catalase and urease production. Oxidase, esculin hydrolysis, and β-galactosidase tests are negative. They can use D-glucose, D-galactose, inositol, sorbitol, L-lysine, L-glutamate, L-arginine, β-alanine, DL-4-aminobutyrate, DL-5-aminovalerate, sperimine, or diaminobutane as a sole carbon source, but cannot use elemental sulfur and ferrous iron as an energy source. The DNA base composition is 58.7–59.2 G + C mol%. The major isoprenoid quinone is ubiquinone with ten isoprene units (Q-10). The major fatty acid is the $C_{18:1}$ fatty acid. Two ornithine amide lipids, the $C_{18:1}$ fatty acid esters of α-N-3-hydroxystearylornithyltaurine and α-N-3-hydroxystearylornithine, are detected as the polar aminolipid. DNA relatedness between this species and the other species of *Acidiphilium*, the genera *Acidomonas*, and *Acidobacterium* was 29 to 2%. These results indicate that this new species should be placed in the genus *Acidiphilium*. The type strain (strain 101) of *A. aminolytica* is JCM 8796.

Harrison [3, 4] characterized the acidophilic heterotrophic bacteria isolated from acidic mineral environments and proposed the name *Acidiphilium* for this new genus. Members of this genus were obligately acidophilic Gram-negative bacteria and failed to grow on elemental sulfur or ferrous iron. The genus *Acidiphilium* now consists of five species: *A. angustum* [16]. *A. cryptum* [3], *A. facilis* [16], *A. organovorum* [12], and *A. rubrum* [16]. We have also isolated a number of acidophilic heterotrophic bacteria from the acidic mineral environments and sewage [6]. We have previously described several strains of bacteria containing menaquinone as the major isoprenoid quinone and proposed *Acidobacterium capsulatum* gen. nov., sp. nov. for them [8, 9].

However, among our isolates we found strains that could not be identified because several characteristics were different from those of the recognized *Acidiphilium* and *Acidobacterium* species.

We selected seven strains from our isolates that have the same characteristics, i.e., hippuric acid hydrolysis, urease production, and assimilation of some amino acids. An extensive study was carried out to clarify the taxonomic position of these organisms.

Our results indicated that the strains form a single group both phenotypically and genetically, and they can be distinguished from any known species on the basis of the DNA base composition and several phenotypic characteristics. We concluded that these seven strains constitute a new species in the genus *Acidiphilium*, and the name *Acidiphilium aminolytica* sp. nov. is proposed.

Materials and Methods

Bacterial strains and culture conditions. The seven strains and eight reference strains that we studied are listed in Table 1. The seven strains were all isolated from acidic mineral environments [6]. The reference strains were used for comparison of the pheno-

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Table 1. Source of strains studied

Strain	Source
Isolate[a] 99	Acidic mine drainage
Isolate 101	Acidic mine drainage
Isolate 119	Acidic mine drainage
Isolate 1521	Mud
Isolate 1522	Mud
Isolate 154	Acidic mine drainage
Isolate 15H	Acidic mine drainage
Acidiphilium cryptum ATCC 33463^T	Thiobacillus ferrooxidans culture
Acidiphilium angustum ATCC 35903^T	Acidic mine drainage
Acidiphilium facilis ATCC 35904^T	Acidic mine drainage
Acidiphilium rubrum ATCC 35905^T	Acidic mine drainage
Acidiphilium organovorum ATCC 43141^T	Thiobacillus ferrooxidans culture
Acidomonas methanolica IMET 10945^T	Septic methanol-yeast process
Acidobacterium capsulatum JCM 7670^T	Acidic mine drainage[a]

[a] All isolated strains were obtained from Yanahara mine (Dowa Kogyo Co., Okayama, Japan). ^{T} indicates the type strain of each species. ATCC, American Type Culture Collection; IMET, Zentralinstitut für Mikrobiologie und experimentelle Therapie; JCM, Japan Collection of Microorganisms.

typic and chemotaxonomic characteristics. The cultures were maintained on basal salts–glucose–yeast extract (GYE) medium containing 0.6% gellan gum [6], the culture conditions have been previously described [8].

Morphological physiological and chemotaxonomical characteristics. Cell shape, cell size, Gram reaction, and colonial appearance were observed with the cells grown on GYE gellan gum plates for 1–3 days at 30°C. Motility was determined microscopically in overnight cultures grown at 25°C on GYE plates. The methods for physiological characterization of the strains have been previously described [7, 8]. API 50CH, 50AO, and 50AA galleries (bioMérieux, France) were used for utilization of carbon compounds as the sole carbon source. Hippuric acid hydrolysis was detected with ninhydrin [13], and urease activity was observed on Christensen medium [1] after 24 h incubation and by the method of Wichlacz and Unz [15]. DNA base composition, quinone systems, and cellular fatty acid composition were determined by the method previously described [8]. The ubiquinones purchased from Sigma Chemical Co. (St. Louis, Missouri, USA) were used as the standard Q-10 and Q-9 during HPLC analysis. The thin-layer chromatography of ornithine lipids was done with silica gel plates (Merck, Germany) and the solvent systems (v/v/v) chloroform–methanol–28% (w/v) ammonia water, 70:30:4 (A), and chloroform–methanol–acetone–acetic acid–water, 65:10:20:10:3 (B) [10].

DNA–DNA hybridization. DNA hybridization was carried out by the membrane filter methods previously described [11]. DNAs from strain 101 and the type strain of *A. cryptum* and *A. facilis* were radiolabeled by in vitro nick translation with a commercial kit (product code TRK.625 and N.5500A, Amersham, England). The reaction was carried out at 68°C, where the optimum reassociation occurs.

Results

Morphology. The seven selected strains were all strictly aerobic, Gram-negative, motile, non-spore-forming, rod-shaped bacilli (0.5–0.8 μm in width and 1.0–1.8 μm in length) and occurred singly, in pairs, or occasionally in short chains. Capsules were not observed around the cells. The colonies on the GYE gellan gum plates after 3 days were circular (4–5 mm in diameter), smooth, opaque, and had a white to pale light-brown color.

Phenotypic characteristics. The results are shown in Table 2. All strains grew in the pH range of 3.0–6.0, but not at pH 6.5. Growth of the bacteria occurred between 20°C and 37°C, but no growth was observed above 42°C. Three of the seven strains (1521, 1522, 15H) grew on a medium containing 3.5% sodium chloride (NaCl).

All seven strains gave positive results in tests for catalase, urease, and hippuric acid hydrolysis. Oxidase, β-galactosidase, and esculin hydrolysis were negative. Acetate and succinate did not inhibit their growth at 0.25 mM and 4 mM, respectively, but lactate inhibited two strains (119, 154) at 2 mM. All strains utilized the following substances as their sole carbon source: D-glucose, D-galactose, D-fructose, L-arabinose, D-xylose, D-ribose, inositol, mannitol, sorbitol, arabitol, gluconate, L-lysine, L-arginine, L-histidine, L-ornithine, L-glutamate, β-alanine, DL-4-aminobutyrate, DL-5-aminovalerate, diaminobutane, spermine, and creatine, but none of the strains utilized methanol, ethanol, cellobiose, maltose, lactose, or starch, nor did they utilize ferrous iron or elemental sulfur as the sole energy source.

Chemotaxonomic characteristics. The seven strains possessed similar G + C contents of DNA, isoprenoid quinone types, and fatty acid compositions (Tables 3 and 4). Their G + C contents of DNA ranged from 58.7 to 59.2 mol%. Ubiquinone with ten isoprene units (Q-10) was detected as the major isoprenoid quinone. A menaquinone spot was not detected on thin-layer chromatography. The major fatty acid was the $C_{18:1}$ fatty acid. The hydroxy fatty acids detected were 2-hydroxymyristic acid and 3-hydroxypalmitic acid. Two ninhydrin-positive and phos-

Table 2. Characteristics among the Isolates, *Acidiphilium*, *Acidomonas*, and *Acidobacterium* species

Characteristic	No. of strains positive	Results for type strain	*A. cryptum*	*A. angustum*	*A. facilis*	*A. rubrum*	*A. organovorum*	*A. methanolica*	*A. capsulatum*
Motility	7	+	+	+	+	+	+	–	+
Pigmentation	0	–	–	+[a]	–	+[b]	–	–	+[c]
Capsule	0	–	–	–	–	–	–	–	+
Catalase	7	+	+	+	+	+	+	+	+
Oxidase	0	–	–	W	W	W	–	+	–
Urease	7	+	–	+	+	+	–	–	–
β-Galactosidase	0	–	–	–	–	–	–	–	+
Hydrolysis of hippurate	6	+	–	–	–	–	+	–	–
Hydrolysis of esculin	0	–	–	–	–	–	–	–	+
Growth at pH 3.0	7	+	+	+	+	+	+	+	+
Growth at pH 6.5	0	–	–	–	–	–	–	–	–
Growth at 37°C	7	+	+	+	+	+	+	+	+
Growth at 42°C	0	–	+	–	–	–	–	–	–
Growth on 3.5% NaCl	3	–	+	–	+	–	+	+	–
Growth on Fe^{2+} or S^0	0	–	–	–	–	–	–	–	–
Growth inhibition by the following substances:									
0.25 mM acetate	0	–	+	+	–	+	+	–	+
2 mM lactate	2	–	+	+	–	+	+	–	–
4 mM succinate	0	–	+	+	–	+	+	–	+
Growth on a sole carbon source:									
D-Glucose	7	+	+	+	+	+	+	+	+
D-Galactose	7	+	+	+	+	+	+	–	+
L-Arabinose	7	+	+	+	+	+	+	–	+
D-Xylose	7	+	+	+	+	+	+	–	+
Mannitol	7	+	+	+	+	+	+	–	–
D-Arabitol	7	+	+	+	+	+	+	–	–
Sorbitol	7	+	+	–	–	–	+	–	–
Inositol	7	+	–	–	–	–	+	–	–
Glycerol	2	–	+	+	+	+	+	+	–
Cellobiose	0	–	–	–	–	–	–	–	+
β-Gentiobiose	0	–	–	–	–	–	–	–	+
Methanol	0	–	–	–	–	–	–	+	–
Ethanol	0	–	–	–	+	W	–	+	–
Spermine	7	+	–	–	–	–	–	–	–
L-Lysine	7	+	–	–	–	–	–	–	–
β-Alanine	7	+	–	–	–	–	–	–	–
Creatine	5	+	–	–	–	–	–	–	–
Diaminobutane	7	+	–	–	+	–	–	–	–
DL-5-Aminovalerate	7	+	–	–	+	–	–	–	–
DL-4-Aminobutyrate	7	+	–	+	+	–	–	–	–
L-Glutamate	7	+	+	+	+	+	+	–	–
L-Arginine	7	+	+	–	+	–	+	–	–
Tween 80	7	+	–	–	+	–	+	–	–

[a], pink; [b], violet; [c], orange; W, weakly positive or slight growth.

phorus-free spots were detected at R_f 0.41 and 0.30 (solvent system A) and R_f 0.27 and 0.45 (solvent system B), respectively. These spots were identified as $C_{18:1}$ fatty acid esters of α-N-3-hydroxystearyl-ornithyltaurine and α-N-3-hydroxystearylonithine by having the same R_f values as the ornithine lipids from *A. organovorum* [10].

DNA–DNA homology. Labeled DNA from the strain 101[T] was 100 to 76% related to unlabeled DNAs from six other strains in hybridization reactions carried out at 68°C, while the DNAs from *Acidiphilium*, *Acidomonas*, and *Acidobacterium* strains showed low relative binding ratios against the strain 101[T] (Table 5).

Discussion

The seven strains shared almost all of the same phenotypic characteristics as shown in Table 2. They had the same DNA base composition, cellular fatty acid composition, and isoprenoid quinone system. The strains showed high levels of similarity in DNA–DNA hybridization among the isolated strains. Therefore, they can be considered to form a single group from a chemotaxonomic as well as phenotypic point of view.

Three genera, *Acidiphilium*, *Acidomonas*, and *Acidobacterium*, have already been reported as acidophilic, mesophilic, and heterotrophic bacteria [5, 8, 14]. The isolates, *Acidiphilium* and *Acidobacterium* species, were isolated from the same acidic

Table 3. Isoprenoid quinone type and DNA base composition of the Isolates, *Acidiphilium, Acidomonas*, and *Acidobacterium* species[a]

	G + C content mol%	Isoprenoid quinone type (%)						
		Q-8	Q-9	Q-10	Q-11	MK-7	MK-8	MK-9
Isolates:								
99	59.1	2	7	91				
101	58.7	1	8	90	tr			
119	59.0	1	9	89	tr			
1521	58.8	3	7	90				
1522	59.0	3	8	88	tr			
154	59.2	2	8	90				
15H	58.9	1	7	92				
A. cryptum	67.5	4	6	90				
A. angustum	63.2	1	6	92	tr			
A. facilis	64.4	1	15	84				
A. rubrum	64.0	1	11	87	tr			
A. organovorum	67.5	3	9	88				
A. methanolica	64.7	tr	12	87	tr			
A. capsulatum	60.8					1	98	tr

[a] Abbreviations: MK, menaquinone; Q, ubiquinone; tr, trace amounts (less than 1%).

Table 4. Cellular fatty acid compositions of the Isolates, *Acidiphilium, Acidomonas*, and *Acidobacterium* species[a]

	Straight-chain fatty acids										Branched-chain fatty acids			Cyc.	2-Hydroxy acids		3-Hydroxy acids		
	12:0	14:1	14:0	15:0	16:1	16:0	17:1	17:0	18:1	18:0	i-15:0	i-17:1	i-17:0	19:0	14:0	16:0	14:0	16:0	18:0
Isolates:																			
99		tr			12				60	6				10	3			2	tr
101		tr	tr		10				64	5				7	4			1	tr
119			tr	tr	10				57	9				6	6			tr	tr
1521		tr	tr		8				63	5				6	4			tr	tr
1522		tr			8				65	7				11	3			tr	tr
154		tr	tr		13				65	8				9	2			1	tr
15H			tr		11				66	4				9	3			1	tr
A. cryptum	3	tr	tr	tr	1				57	8				16			1		tr
A. angustum			tr	3	4				44	7				21			3	1	tr
A. facilis		2	tr	tr	8				65	2				7	4		2	1	tr
A. rubrum			tr		5	6			48	6				18			3		tr
A. organovorum	2	tr			tr	1			52	9				21			1		
A. methanolica			tr			9			62					1	6	7	2	3	
A. capsulatum			tr	1	4	4	4	2	23	3	55	1	3	tr					

[a] Abbreviations: i,iso-type, branched-chain fatty acids; Cyc., cyclopropane acid; carbon atoms : double bonds; tr, trace amounts (less than 1%).

mineral environments. Table 6 shows a comparison of the selected strains with those acidophilic species with which they might be identified. The seven strains can be clearly distinguished from other species by a number of characteristics.

The seven strains shared almost the same phenotypic characteristics, cellular fatty acid composition, ubiquinone system, and polar aminolipid as the genus *Acidiphilium*, whereas these bacteria can be distinguished from *Acidobacterium* by chemotaxonomic and phenotypical characteristics [5, 8, 10].

However, the seven strains can be readily distinguished from five described species of *Acidiphilium* on the basis of some chemotaxonomic and phenotypic characteristics. Urease, DNA base composition, and 2-hydroxy fatty acid composition distinguish these strains from *A. cryptum* and *A. organovorum*, and pigment production, 2-hydroxy fatty acid composition, and assimilation of carbon sources distinguish them from *A. angustum* and *A. rubrum*. The seven strains have phenotypic and chemotaxonomic characteristics similar to those of *A. facilis*, but it is unlikely that these strains can hydrolyze hippuric acid and assimilate a number of amino acids and amines. These strains also have a different DNA base composition and low relative

Table 5. Relatedness of DNA among the Isolates and other related organisms

	Relative binding ratio (%)		
	101	*A. cryptum*	*A. facilis*
Isolates:			
99	100	4	13
101	100	5	11
119	94	5	15
1521	93	3	12
1522	90	3	12
154	76	3	11
15H	94	5	10
A. cryptum	8	100	7
A. angustum	9	18	8
A. facilis	29	5	100
A. rubrum	6	15	11
A. organovorum	8	24	13
A. methanolica	9	1	12
A. capsulatum	2	0	8

binding ratio against *A. facilis* on DNA–DNA homology.

Thiobacillus acidophilus, an organism that was isolated from a culture of *Thiobacillus ferrooxidans*, resembles our seven strains in growth on a wide variety of organic compounds [2]. However, *T. acidophilus* is acidophilic and a facultative autotroph. It obtains energy from elemental sulfur as well as organic compounds. Our strains are strictly heterotrophic and cannot utilize elemental sulfur or ferrous iron as an energy source.

From the acidophilic point of view, members of the genus *Acidomonas*, *Acidiphilium*, and *Acidobacterium*, may be considered to resemble these isolates, but members of *Acidomonas* are acidophilic and facultatively methylotrophic bacteria [14]. Our seven strains cannot utilize methanol as the sole carbon source and can be differentiated from *Acidomonas* spp. by several phenotypic and chemotaxonomic characteristics, as shown in Tables 2–4.

From these results, we conclude that the isolates in this study constitutes a new species belonging to the genus *Acidiphilium* and propose the name *Acidiphilium aminolytica* sp. nov. The characteristics for differentiating the species *A. aminolytica* from related species are shown in Table 6.

Acidiphilium aminolytica sp. nov. (a.mi.no.lyti.ca. M.L.n.aminum amine; Gr.adj.lytica dissolving; M.L.adj. aminolytica amine dissolving). Gram-negative, aerobic, non-sporeforming, motile, and rod-shaped (0.5–0.8 μm by 1.0–1.8 μm) bacteria. The colonies on GYE gellan gum plate are circular (4–5 mm), smooth, opaque, and colored white to pale light brown. They grow at pH 3.0–6.0, but not at pH 6.5. They give positive results in tests

Table 6. Characteristics that differentiate *Acidiphilium aminolytica* from related species

	A. aminolytica	*A. cryptum*	*A. angustum*	*A. facilis*	*A. rubrum*	*A. organovorum*	*A. methanolica*	*A. capsulatum*
Acidophilic	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	−	+
Pigmentation	−	−	+[a]	−	+[b]	−	−	+[c]
Capsule	−	−	−	−	−	−	−	+
Urease	+	−	+	+	+	−	−	−
Hydrolysis of hippurate	+	−	−	−	−	+	−	−
Hydrolysis of esculin	−	−	−	−	−	−	−	+
β-Galactosidase	−	−	−	−	−	−	−	+
Assimilation of:								
D-Galactose	+	+	+	+	+	+	−	+
D-Xylose	+	+	+	+	+	+	−	+
Cellobiose	−	−	−	−	−	−	−	+
β-Gentiobiose	−	−	−	−	−	−	−	+
Methanol	−	−	−	−	−	−	+	−
Sorbitol	+	+	−	−	−	+	−	−
Inositol	+	−	−	−	−	+	−	−
Spermine	+	−	−	−	−	−	−	−
L-Lysine	+	−	−	−	−	−	−	−
β-Alanine	+	−	−	−	−	−	−	−
Diaminobutane	+	−	−	+	−	−	−	−
DL-5-Aminovalerate	+	−	−	+	−	−	−	−
DL-4-Aminobutyrate	+	−	+	+	−	−	−	−
Mol% G + C content of DNA	58.7	67.5	63.2	64.4	64.0	67.5	64.7	60.8
Quinone system	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	Q-10	MK-8
Major fatty acid	$C_{18:1}$	$C_{18:1}$	$C_{18:1}$	$C_{18:1}$	$C_{18:1}$	$C_{18:1}$	$C_{18:1}$	iso-$C_{15:0}$
2-Hydroxy fatty acids	$C_{14:0}$	−	−	$C_{14:0}$	−	−	$C_{14:0}$, $C_{16:0}$	−
Ornithine amide lipids	+	+	+	+	+	+	+	−

[a], pink; [b], violet; [c], orange; Q, ubiquinone; MK, menaquinone.

for urease, hippuric acid hydrolysis, and catalase. Oxidase, β-galactosidase, and esculin hydrolysis are negative. Growth occurs at 25–37°C, but not at 42°C. Growth also occurs in the presence of 0.25 mM acetate and 4 mM succinate. Chemoorganotrophs: D-glucose, D-galactose, D-fructose, L-arabinose, D-xylose, D-ribose, inositol, mannitol, sorbitol, arabitol, gluconate, L-lysine, L-arginine, L-histidine, L-ornithine, L-glutamate, β-alanine, DL-4-aminobutyrate, DL-5-aminovalerate, diaminobutane, spermine, and creatine are utilized as the sole carbon source, but not methanol, ethanol, cellobiose, maltose, lactose, or starch. Elemental sulfur and ferrous iron are not used as an energy source. The DNA base composition is 58.7–59.2 mol%. The major isoprenoid quinone is ubiquinone with ten isoprene units. The major fatty acid is $C_{18:1}$ fatty acid, and 2-hydroxymyristic acid and 3-hydroxypalmitic acid are contained. Two ornithine amide lipids, $C_{18:1}$ fatty acid esters of α-N-3-hydroxystearylornithyltaurine and α-N-3-hydroxystearylornithine, are detected as a polar aminolipid.

Strain 101 is designated the type strain of *Acidiphilium aminolytica* and has been deposited with the Japan Collection of Microorganisms as JCM 8796.

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