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Actimicrobium antarcticum gen. nov., sp. nov., of the Family *Oxalobacteraceae*, Isolated from Antarctic Coastal Seawater

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Abstract A Gram-negative, non-motile, catalase- and oxidase- positive, strictly aerobic, and short rod-shaped bacterium that was designated strain KOPRI 25157^T was isolated from coastal seawater sample in Antarctica. The temperature and pH ranges for growth on R2A agar were 10–20°C, and 5.0–10.0, respectively. Phylogenetic analyses of the 16S rRNA gene sequence of strain KOPRI 25157^T showed it to belong to the family *Oxalobacteraceae* of the class *Betaproteobacteria*, and it formed a distinct clade from other recognized members of the family. DNA G + C content was 65.9 mol%. Major ubiquinone was Q-8. Predominant cellular fatty acids were $C_{16:1}$ $\omega 7c$/15 iso 2OH (56.4%) and $C_{16:1}$ (30.5%). Major polar lipids were phosphatidylglycerol, phosphatidylethanolamine, and unknown lipid. On the basis of these data, it is proposed that strain KOPRI 25157^T is the representative of a novel genus, for which the name *Actimicrobium* gen. nov. is proposed in the family *Oxalobacteraceae*. The type strain for *Actimicrobium antarcticum* sp. nov. is KOPRI 25157^T (=JCM 16673^T=KCTC 23040^T).

The GenBank accession number for the 16S rRNA gene sequence of strain KOPRI 25157^T is HQ699437.

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Introduction

The order *Burkholderiales* in the class *Betaproteobacteria* was circumscribed on the basis of phylogenetic analysis of 16S rRNA gene sequences and contains four families, *Alcaligenaceae*, *Burkholderiaceae*, *Comamonadaceae*, and *Oxalobacteraceae*. Among them, the family *Oxalobacteraceae* contains the genera *Collimonas, Duganella, Herbaspirillum, Herminiimonas, Janthinobacterium, Massilia, Naxibacter, Oxalobacter, Oxalicibacterium, Telluria,* and *Undibacterium*. These have been obtained from various environmental and biotic samples including soil [1, 16, 25], drinking water [6, 10], air [24], and blood [22]. The family is metabolically diverse and includes strict anaerobes, strict aerobes, and nitrogen-fixing organisms [8].

During the study of microbial diversity in the Antarctic, we isolated yellowish white colonies at low temperatures. 16S rRNA gene sequence analysis showed that the isolate belonged to the family *Oxalobacteraceae*. The morphological, physiological and chemical characteristics, and phylogenetic analyses, indicated that the strain should be classified as representing a novel member of the family *Oxalobacteraceae* [8]. In this report we propose the inclusion of the strain in a new genus and novel species name *Actimicrobium antarcticum* gen. nov., sp. nov.

Materials and Methods

Isolation of the Strain

Strain KOPRI 25157^T was isolated in November 2005 from Antarctic coastal sea water (62° 14′ S 58° 44′ W) where melted glaciers entered the area. Two hundred micro liters of sample diluted 10^{-1} in sterilized sea water was spread onto R2A (Difco) and incubated aerobically at 4°C for 2 weeks.

Amplification of 16S rDNA and Phylogenetic Analyses

The 16S rRNA gene was amplified from a single colony with universal primers, 27F and 1492R, as previously described [14, 15]. PCR products were purified using AccuPrep PCR Purification Kit (Bioneer, Korea) and directly sequenced using primers, 27F, 785F, 926R, and 1492R, as previously described [14]. The sequence was compared with the sequences of all type strains in EzTaxon database [3], aligned with those of type strains of each genus in *Oxalobacteraceae*. Phylogenetic trees were constructed by the neighbor-joining (NJ) [21], maximum parsimony (MP) [7], and maximum-likelihood (ML) [4] methods using the PAUP program [23]. Evolutionary distance matrix for the NJ methods was generated according to the Kimura's two-parameter model [12]. MP and ML trees were constructed by heuristic search option. The confidence level of the tree topology was evaluated by bootstrap analysis using 1,000 replication [5].

Phenotypic and Biochemical Analyses

To examine phenotypic characteristics, strain KOPRI 25157^T was grown on R2A agar (Difco) at 4, 10, 15, 20, 25, 30, 37, and 40°C. Growth at different pH (between pH 5.0 and 10.0 at internals of 1.0 pH unit) was determined on R2A agar at 20°C. Tolerance of strain KOPRI 25157^T to NaCl was determined by growing on R2A medium (Difco) with various concentrations of NaCl (between 0 and 2% (w/v) at intervals of 0.5% and between 2 and 10% (w/v) at intervals of 2.0%). Cell size and morphology were determined by scanning electron microscopy. Motility was tested on motility medium (marine broth, 0.4% agar). Catalase activity was determined by bubble formation in a 3% (v/v) H_2O_2 solution and oxidase activity was determined by oxidation of 1% *p*-aminodimethylaniline oxalate. Growth in anaerobic condition was examined in the anaerobic chamber with air of 90% nitrogen, 5% carbon dioxide, and 5% hydrogen at 16°C for up to 4 days on marine agar 2216 (Difco). Other biochemical tests were performed with the API 20E, API 20NE, API 50CHB, and API ZYM systems (bioMérieux) using cells grown on R2A agar at 15°C for 6 days.

Chemotaxonomic Analyses

The G+C content was determined as described [19] by analysis of deoxyribonucleosides using HPLC equipped with a Discovery C18 reversed-phase column (5 μm, 150 × 4.6 mm; Supelco). Fatty acid methyl esters (FAME) analysis was performed by gas chromatography according to the Microbial Identification system (MIDI; database TSBA40) using 1-day-old fresh cells grown at 20°C on R2A agar. Isoprenoid quinones were extracted from 2-day-old cells and analyzed using RP-TLC [13, 26]. Polar lipids of strain KOPRI 25157^T was extracted by an integrated approach [20] and determined using two-dimensional TLC on aluminum plate coated with silica gel as previously described [20]. The TLC plates were developed and detected by procedures as previously described [2].

Results and Discussions

An almost full sequence of 16S rRNA gene of strain KOPRI 25157^T was obtained (1,455 bp) and deposited in GenBank database under the accession number HQ699437. Preliminary sequence comparison with 16S rRNA gene sequence from EzTaxon [3] revealed that the isolate showed highest sequence similarity to *Herminiimonas glaciei* UMB49^T (96.8%, 1378/1424), followed by *H. saxobsidens* NS11^T (96.8%, 1406/1453), *H. fonticola* S-94^T (96.3%, 1371/1424), and *H. arsenicoxydans* ULPAs1^T (96.1%, 1396/1453). To clarify the phylogenetic position of strain KOPRI 25157^T, NJ, MP, and ML analysis were conducted including sequences of 43 members of family *Oxalobacteraceae*. As shown in the phylogenetic tree (Fig. 1), strain KOPRI 25157^T formed an independent phyletic lineage within the family *Oxalobacteraceae* by ML and MP analyses (Fig. 1). The relationship of the strain with other genera of the family was not supported by high bootstrap values. In NJ analysis, strain KOPRI 25157^T formed a sister group with a monophyletic lineage of the genus *Herminiimonas*. However, bootstrap support for the relationship was very low (<50%). These phylogenetic analyses indicated the uniqueness of the 16S rRNA gene sequence of the strains. Therefore, strain KOPRI 25157^T was considered to represent a new genus and species in the family *Oxalobacteraceae* of the order *Burkholderiales* (Fig. 1).

Morphological, physiological, and biochemical characteristics of KOPRI 25157^T are presented in the species description and Table 1. Strain KOPRI 25157^T was Gram-negative, non-motile, short rod-shaped (0.5–0.9 × 0.8–1.5 μm^2, Fig. 2), and strictly aerobic. Colonies were yellowish white, translucent, glistening, flat, smooth surfaced, and circular. The growth temperature range of the strain on R2A agar medium was 10–20°C with an optimum growth temperature 20°C and the

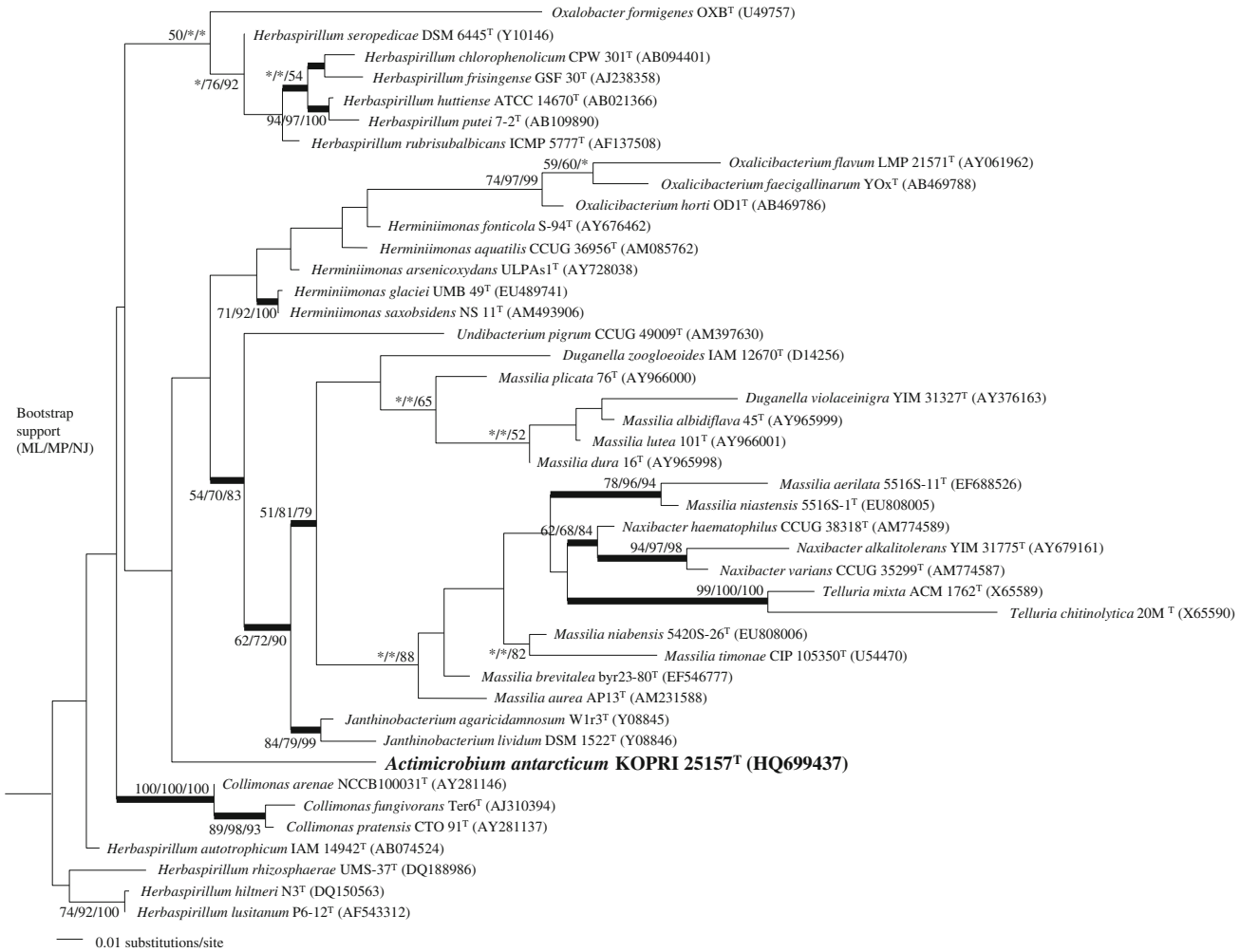

Fig. 1 Phylogenetic position of KOPRI 25157 in the *Oxalobacteraceae* based on 16S rRNA gene sequences. The tree was reconstructed by the heuristic search with the maximum likelihood criterion. Branches that were conserved in maximum likelihood, maximum parsimony, and neighbor-joining analyses were presented by *thick lines*. Percent bootstrap supports (>50%) were given at each node (ML/MP/NJ). *Acidovorax delafieldii* ATCC 17505^T and *Ideonella dechloratans* ATCC 51718^T were used as an outgroup (data not shown); * <50%

pH range for growth was 5.0–10.0, with optimum 6.0. The strain grew in 0–1.0% sea salt and NaCl concentration. The strain was positive for oxidase and catalase. Other physiological characteristics were given in the species description. Differential characteristics of strain KOPRI 25157^T was presented in Table 1, along with data for type species of related genera in *Oxalobacteraceae*.

The DNA G+C content of strain KOPRI 25157^T was 65.9 mol%, and ubiquinone 8 was the only isoprenoid quinone present, which is a characteristic trait of all members of the *Betaproteobacteria* [27]. The major polar lipid of KOPRI 25157^T is phosphatidylglycerol, phosphatidylethanolamine, and unknown lipid (Fig. S1). The fatty acid composition of strain KOPRI 25157^T showed large amounts of $C_{16:1}$ ω7c/15 iso 2OH and $C_{16:1}$ predominating (Table 1).

In conclusion, phylogenetic data show KOPRI 25157^T to form a distinct clade from other recognized members of the *Oxalobacteraceae* (Fig. 1). In addition, combinations of phenotypic and chemotaxonomic characteristics can be used to differentiate strain KOPRI 25157^T from other related genera in the family *Oxalobacteraceae* (Table 1). The results of the present polyphasic study therefore indicate that strain KOPRI 25157^T represents a novel species of a new genus, for which the name *Actimicrobium antarcticum* gen. nov., sp. nov. is proposed.

Description of gen. nov

Actimicrobium (Ac.ti.mi.cro'bi.um. Actimicrobium. L. n. acta, the sea-shore; N.L. neut. n. microbium, microbe; N.L. neut. n. Actimicrobium, a microbe (bacterium) isolated from sea-shore).

Table 1 Comparison of characteristics separating strain KOPRI 25157^T from closely related taxa in *Oxalobacteraceae*

Character	1	2	3	4	5	6	7	8
Cell diameter >1 μm	–	–	v	+	+	–	–	+
Motility	Non-motile	Monopolar	Monopolar	Monopolar	Monopolar	Polar (>1)	Monopolar	Non-motile
Growth on nutrient agar	+	v	ND	+	ND	+	–	+
Temp. (opt.)	10–20 (20)	28–30	10–35 (30)	4–30 (25)	28–37	4–55 (28–37)	20–45 (30–35)	4–30
pH (opt.)	5–10 (6)	(7.2)	6–9.5 (7.5–8)	7–8	ND	5.5–12 (7–9)	(7)	ND
NaCl	<1%	ND	ND	ND	<3%	0.03	<1.5%	ND
Catalase	+	v	+	+	+	+	+	+
Oxidase	+	v	+	+	V	v	w	+
Citrate utilization	–	+	+	–	–	–	+	ND
Urease	–	+	ND	–	V	+	v	ND
Gelatinase	–	+	–	v	+	+	+	ND
Reduction of nitrates to nitrites	–	–	v	v	V	–	–	ND
Major fatty acid (>7%)	$C_{16:0}$, $C_{16:1}$ ω7c/15 iso 2OH	$C_{16:1}$ ω7c/15 iso 2OH, $C_{10:0}$ 3-OH, $C_{18:1}$ ω7c	$C_{16:0}$, $C_{16:1}$ ω7c, $C_{17:0}$ cylo, $C_{18:1}$ ω7c	$C_{16:0}$, $C_{16:1}$ ω7c/15 iso 2OH	$C_{16:0}$, $C_{16:1}$ ω7c, $C_{18:1}$	$C_{16:0}$, $C_{16:1}$ ω7c	$C_{16:0}$, $C_{16:1}$ ω7c, $C_{17:0}$ cylo, $C_{18:1}$ ω7c	$C_{16:0}$, $C_{16:1}$ ω7c/15 iso 2OH, $C_{18:1}$ ω7c
DNA G + C content (mol%)	65.9	63–64	52–59	61–67	62–67	62–63	67–72	50.6

Strains and genera: *1* KOPRI 25157^T, *2 Duganella* [9, 16], *3 Herminiimonas* [6, 18], *4 Janthinobacterium* [8, 17], *5 Massilia* [22, 24], *6 Naxibacter* [10, 25], *7 Telluria* [1, 8], *8 Undibacterium pigrum* CCUG 49009^T [11]

Data were obtained in this study for the strain KOPRI 25157 ^{T} and from previous studies for the other genera

+ growth or positive reaction, *w* weak reaction, – no growth or negative reaction, *ND* no data

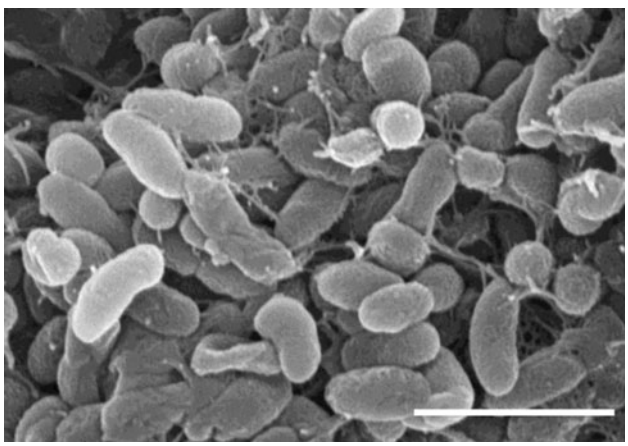

Fig. 2 Scanning electron micrograph of *Actimicrobium antarcticum* KOPRI25157^T. Cells were grown on R2A agar for 3 days at 20°C. *Bars* 2.0 μm

Cells are aerobic, gram negative, non-motile rods. DNA G+C content of the type species is 65.9 mol%. Major respiratory quinone is Q8. The major cellular fatty acids are $C_{16:1}$ ω7c/15 iso 2-OH (summed feature 3) and $C_{16:0}$. Oxidase- and catalase-positive. The predominant polar lipid constituents are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, unknown aminolipids, and unknown polar lipid. As determined by 16S rRNA gene sequence analysis, the genus *Actimicrobium* is a member of the family *Oxalobacteraceae* within the class *Betaproteobacteria*. The type species is *Actimicrobium antarcticum*.

Description of sp. nov

Actimicrobium antarcticum (antarc'ti.cum. L. neut. adj. antarcticum, southern, antarctic, from the Antarctic, referring to the place).

In addition to the description of the genus, the species is characterized as follows. Cells are short rods (0.5–0.9 × 0.8–1.5 μm^2). Positive for cytochrome oxidase in API 20NE. Positive in API 50CHB, the strain produce acid from D-galactose, D-glucose, D-fructose, D-manose,

D-maltose, and glycogen. Activities of alkaline phosphatase, esterase lipase (C8), leucine arylamidase, and valine arylamidase are positive (API ZYM). Beta-galactosidase, arginine dihydrolase, lysine dicarboxylase, ornithine decarboxylase, and tryptophane deaminase are negative in API 20E, and also tests for H_2S, indole, and acetoin production, citrate utilization, urease, and gelatinase are negative. Fermentation/oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose are negative. The quinone system is ubiquinone Q-8 and the fatty acids compositions are $C_{10:0}$ (0.2%), $C_{10:0}$ 3-OH (3.2%), $C_{12:0}$ (2.9%), $C_{16:1}$ $\omega 7c$/15 iso 2-OH (56.4%), $C_{16:0}$ (30.5%), $C_{18:1}$ $\omega 7c$ (3.7%), $C_{18:0}$ (0.7%), and 11 methyl $C_{18:1}$ $\omega 7c$ (2.4%). It was isolated from coastal sea water in Antarctica. The type strain is KOPRI 25157^T (=JCM 16673^T=KCTC 23040^T).

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