Pseudomonas alcaliphila sp. nov., a novel facultatively psychrophilic alkaliphile isolated from seawater

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Facultatively psychrophilic alkaliphilic strains were isolated from seawater obtained off the coast of Rumoi, Hokkaido, Japan. They were Gram-negative, aerobic straight rods with polar flagella. The isolates were catalase- and oxidase-positive and able to grow at 4 °C, but not at 40 °C. They produced acid from D-glucose under aerobic conditions. The isolates reduced nitrate to nitrite and hydrolysed casein and gelatin, but not starch or DNA. NaCl was required for growth at pH 10 but was not required at neutral pH. The major isoprenoid quinone was ubiquinone-9 (Q-9) and the DNA G+C content was 62·3–63·2 mol%. The whole-cell fatty acids mainly consisted of $C_{16:0}$, $C_{16:1(9c)}$ and $C_{18:1(9c)}$, with 3-OH $C_{10:0}$ and 3-OH $C_{12:0}$ as the hydroxyl fatty acids. A larger amount of *trans*-unsaturated fatty acid, $C_{16:1(9t)}$ was observed when the cells were grown at pH 7 compared to when cells were grown at pH 10. Phylogenetic analysis based on 16S rRNA gene sequencing indicated that the bacteria are members of the genus *Pseudomonas*. Analysis of DNA–DNA relatedness data with several close phylogenetic neighbours revealed a low level of hybridization (less than 61%). On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, it is concluded that these isolates represent a separate new species. Accordingly, the name *Pseudomonas alcaliphila* is proposed. The type strain is AL15-21^T (= JCM 10630^T = IAM 14884^T).

Keywords: *Pseudomonas alcaliphila*, alkaliphilic, 16S rRNA, DNA–DNA hybridization, *cis*/*trans* unsaturated fatty acids

INTRODUCTION

There have been many reports concerning micro-organisms that are able to grow under extreme environmental conditions such as alkaline and acidic conditions, high and low temperatures and very high pressure under the deep sea (Horikoshi & Grant, 1991). Such organisms are very important in the fields of biotechnology and for basic research to clarify the physiology for adaptation to extreme environments and to study the genome and biodiversity. A large number of alkaliphiles have been isolated from a variety of environments for industrial applications and for basic research of their physiology and genetics for adaptation to an alkaline environment. Most of these isolates belong in the genus *Bacillus* and there are only a few reports on Gram-negative, alkaliphilic micro-organisms. To date, more than ten novel species of alkaliphilic *Bacillus* species have been identified (Nielsen *et al*., 1995; Fritze, 1996; Yumoto *et al*., 1998). Although there are several reports on alkaliphilic *Pseudomonas* strains, these strains have not been identified at the species level (Jones *et al*., 1998; Kim *et al*., 1996; Na *et al*., 1996). Alkaliphilic strains belonging to genera other than *Bacillus* and *Pseudomonas* have also been reported (Horikoshi, 1991; Ikeda *et al*., 1994). In several alkaliphilic *Bacillus* strains, the mechanisms of alkaliphily have been studied (Guffanti *et al*., 1986; Krulwich *et al*., 1997, 1998). However, it is not known whether this information is applicable in other taxa. Therefore, any new alkaliphilic micro-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of AL15-21^T is AB030583.

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organism not belonging to the genus *Bacillus* might be a good candidate to study the variation in the mechanisms involved in the adaptation of micro-organisms to an alkaline environment.

In this study, facultatively alkaliphilic micro-organisms were isolated from seawater obtained off the coast of Rumoi, Hokkaido, Japan. These isolates were examined based on their phenotypic and chemotaxonomic characteristics, and their phylogenetic position and DNA–DNA relatedness in relation to other strains. It was found that the strains should be classified as a new species belonging to the genus *Pseudomonas*.

METHODS

Bacterial strains and cultivation. Three strains of alkaliphilic micro-organisms were independently isolated at the same time from the same seawater sample obtained off the coast of Rumoi, Hokkaido, Japan, using PYA (peptone/yeast extract/alkaline) agar medium consisting of 8 g peptone (Kyokuto), 3 g yeast extract (Merck), 15 g agar, 1 g K_2HPO_4, 3·5 mg EDTA, 3 mg $ZnSO_4 . 7H_2O$, 10 mg $FeSO_4 . 7H_2O$, 2 mg $MnSO_4 . 4H_2O$, 1 mg $CuSO_4 . 5H_2O$, 2 mg $Co(NO_3)_2 . 6H_2O$, and 1 mg H_3BO_3 in 1 l $NaHCO_3/Na_2CO_3$ buffer (100 mM in deionized water; pH 10) at 27 °C. Growth at pH 7 was tested in PYA broth containing 100 mM NaH_2PO_4/Na_2HPO_4 buffer. Cells for chemotaxonomic analysis were harvested in late exponential phase after cultivation with reciprocal shaking (140 strokes min^{-1}) at 27 °C. In addition to these isolates, *Pseudomonas oleovorans* MCIMB 6576^T, *Pseudomonas pseudoalcaligenes* JCM 5968^T, *Pseudomonas nitroreducens* JCM 2782^T and *Pseudomonas mendocina* JCM 5966^T were used as reference strains for DNA–DNA relatedness. The micro-organisms were cultivated using nutrient broth containing 5 g peptone (Kyokuto), 3 g meat extract (Kyokuto) and 5 g NaCl in 1 l deionized water, pH 7·0 at 25, 26, 30 and 37 °C.

Physiological and biochemical characteristics. For the phenotypic characterization, PYA medium was used as the basal medium. The culture was incubated at 27 °C for 2 weeks and the experiment was performed more than twice unless otherwise stated. Pullulanase and gas production from nitrate were performed according to the methods described by Morgan *et al.* (1979) and Watahiki *et al.* (1983), respectively. Determination of substrate utilization as the sole carbon and energy source was performed in US medium containing 0·2 % substrate, 2 g NH_4Cl, 2 g Na_2HPO_4, 1 g KH_2PO_4, 0·1 g $MgSO_4 . 7H_2O$, 0·05 g $CaCl_2 . 2H_2O$ and 1 ml trace minerals in 1 l $NaHCO_3/Na_2CO_3$ buffer (100 mM; pH 10). The trace minerals included (per 100 ml) 1·8 g EDTA . 2Na, 5·0 g $ZnSO_4 . 7H_2O$, 5·0 g $FeSO_4 . 7H_2O$, 1·5 g $MnSO_4 . 4H_2O$, 0·4 g $CuSO_4 . 5H_2O$, 0·25 g $Co(NO_3)_2 . 6H_2O$ and 0·1 g H_3BO_3. Other physiological and biochemical characteristics were examined according to the methods of Yumoto *et al.* (1998) and as described in Cowan & Steel's Manual (Barrow & Feltham, 1993).

Electron microscopy. For observation of negatively stained cells under TEM, cells were grown on PYA agar and suspended in physiological saline solution. A small drop of suspension was placed on a carbon-coated copper grid and the cells were negatively stained with 1 % phosphotungstic acid for observation under TEM (model H-800; Hitachi). For SEM, cells were grown on PYA agar and were immersed in a 2 % glutaraldehyde solution in 0·1 M cacodylate buffer (pH 7·0) for 2 h. After washing three times with 0·1 M cacodylate buffer, they were fixed in 1 % OsO_4 for 2 h and dehydrated in a graduated ethanol series (50–100 %) and substituted with amyl acetate. The preparations were dried to a critical point in CO_2, fixed on a specimen mount and sputter-coated with platinum and palladium. The specimens were observed under SEM (model S-4000; Hitachi) at 3·0 kV.

Analysis of isoprenoid quinones. Isoprenoid quinones were extracted by treating 0·5 g freeze-dried cells with 150 ml chloroform/methanol (2 : 1, v/v) for 2 h in a reciprocal shaker (120 strokes min^{-1}) at room temperature. The extracted solution was concentrated to dryness and dissolved in acetone. The resulting solution was concentrated, separated by TLC using benzene as the solvent and the isoprenoid quinones were recovered from the TLC plate using acetone. The obtained isoprenoid quinones were analysed using HPLC equipped with a 3·9 mm × 150 mm Novapak C_{18} column (Waters); methanol/2-propanol (1 : 1, v/v) was used as the solvent at room temperature. The HPLC system consisted of a solvent delivery pump (model L-7100; Hitachi) and a spectrophotometric detector (model L-7400; Hitachi) set at 275 nm.

Analysis of whole-cell fatty acids. Whole-cell fatty acids were extracted from 100 mg freeze-dried cells, esterified by acid methanolysis and analysed by GLC equipped with a flame-ionization detector (model GC 353; GL Sciences) and a 0·25 mm (i.d.) × 100 m, 0·2 µm film SP-2560 column (Supelco) at an oven temperature of 140 °C (initially for 15 min) increased to 240 °C at 4 °C min^{-1}. Fatty acids were identified by comparing them with fatty acid methyl ester standards purchased from Supelco and GL Sciences, and using GC/MS (model INCOS 50; Finnigan mat) connected to a GLC (model 3400; Varian).

DNA base composition and DNA–DNA hybridization. DNA was prepared from bacterial cells according to the method of Marmur (1961). The G+C content of the DNA was determined according to the method of Tamaoka & Komagata (1984). The levels of DNA relatedness were determined fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microplates.

Amplification of 16S rRNA and sequencing. The 16S rRNA gene was amplified by PCR. The sequences of primers used for amplification were 5′-AGAGTTTGATCCTGGCTCA-G-3′ and 5′-AAGGAGGTGATCCAA/GCCGCA-3′, corresponding to positions 8–27 and 1521–1540, respectively, in the 16S rRNA sequence of *Escherichia coli* (Brosius *et al.*, 1978). The 1·5 kb PCR product was directly sequenced by the dideoxynucleotide chain-termination method using a DNA sequencer (model 373A; Applied Biosystems). Multiple alignments of the sequence were performed, nucleotide substitution rates (K_{nuc}) were calculated and a neighbour-joining phylogenetic tree (Kimura, 1980; Saitou & Nei, 1987) was constructed using the CLUSTAL W program (Thompson *et al.*, 1994). The similarity values of the sequences were calculated using the GENETYX computer program (Software Development).

RESULTS

Morphology

Colonies of strains AL15-21^T, AL15-22 and AL15-2 on PYA agar were circular and colourless; cells were Gram-negative, polarly flagellated straight rods, 0·3–

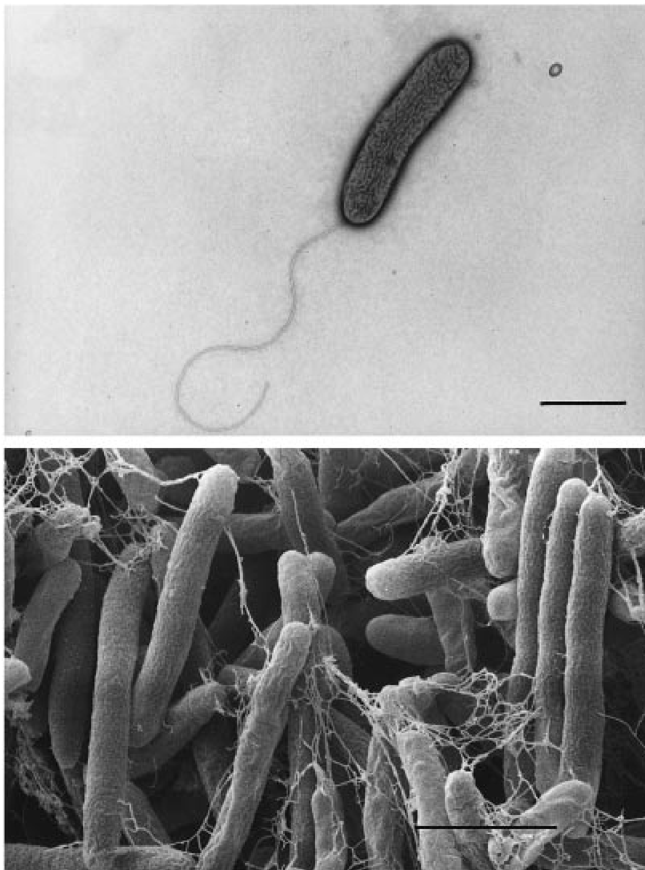

Fig. 1. (top) TEM of a negatively stained cell of *P. alcaliphila* AL15-21^T, showing polar flagellation; bar, 2 µm. (bottom) SEM of cells of platinum/palladium-coated *P. alcaliphila* AL15-21^T, showing rough surfaces of the cells; bar, 2 µm.

0·5 × 1·5–3·0 µm in size (Fig. 1, top). Spore formation was absent and Gram staining was negative. By SEM, a rough surface was observed that was not seen in the reference strains (Fig. 1, bottom).

Phenotypic characteristics

Physiological and biochemical characteristics of the three isolated strains are listed in Table 1.

Chemotaxonomic characteristics

The isoprenoid quinones isolated from three isolates using TLC were analysed by HPLC. Analysis revealed that ubiquinone-9 (Q-9) was the predominant isoprenoid quinone in these strains. GLC analysis revealed that the methyl ester derivatives of fatty acids of these strains consisted of $C_{16:0}$, $C_{16:1(9c)}$ and $C_{18:1(9c)}$, with 3-OH $C_{10:0}$ and 3-OH $C_{12:0}$ as the hydroxyl fatty acids. Among the constituents, $C_{18:1(9c)}$ was the major component, comprising 42·7–47·5% of the total fatty acid. The content of *trans*-unsaturated fatty acid, $C_{16:1(9t)}$, was increased when the cells were grown at pH 7 compared to when the cells were grown at pH 10. Results of the analysis of the three isolates are shown in Table 2.

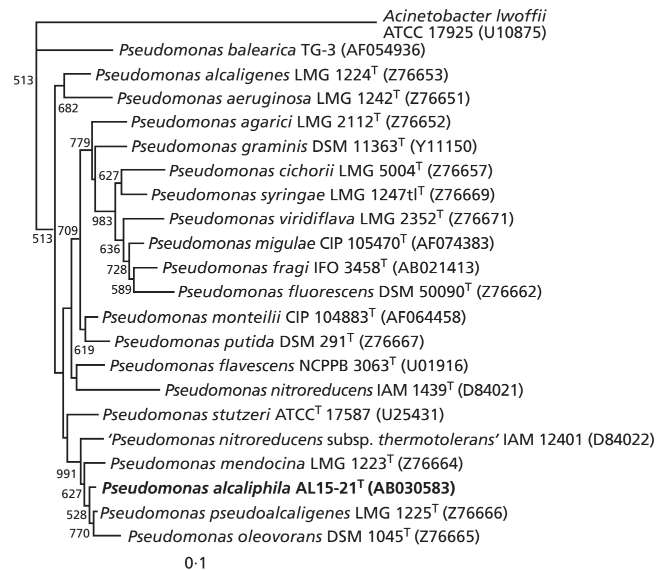

Fig. 2. Phylogenetic tree of *Pseudomonas alcaliphila* AL15-21^T and other *Pseudomonas* strains derived from 16S rRNA gene sequence data, using the neighbour-joining method for calculation. *Acinetobacterl woffii* was used as the outgroup for the phylogenetic tree. Numbers indicate bootstrap values greater than 500. Bar, 0·1 K_{nuc} unit.

16S rRNA gene sequence analysis

The sequence of 1489 bases of the 16S rRNA gene of strain AL15-21^T had 94·6–99·5% similarity to the 16S rRNA gene sequence of 27 strains of *Pseudomonas* species. A phylogenetic tree (Fig. 2) constructed by the neighbour-joining method showed that strain AL15-21^T belongs to the *P. mendocina* sublineage (Moore *et al.*, 1996) with 99·5, 98·6, 98·6 and 98·5% sequence similarities to *P. pseudoalcaligenes*, *P. oleovorans*, *P. mendocina* and '*P. nitroreducens* subsp. *thermotolerans*', respectively.

DNA base composition and DNA–DNA hybridization

The DNA G+C contents of strains AL15-21^T, AL15-22 and AL15-2 were 63·2, 63·8 and 62·3 mol%, respectively; these values fall within the definition range of the genus *Pseudomonas*. According to the results of 16S rRNA gene sequence analysis, strain AL15-21^T was closely related to *P. oleovorans*, *P. pseudoalcaligenes* and *P. mendocina*. The level of DNA relatedness between strain AL15-21^T, AL15-22 and AL15-2, the three closely related strains given above, and *P. nitroreducens* was estimated (Table 3). DNA relatedness results indicated that the three isolates were tightly related (more than 94% similarity), but

Table 1 Phenotypic characteristics of the three strains of *Pseudomonas alcaliphila*

All tests were performed at pH 10 and 27 °C except for growth at pH 7 and growth at various temperatures. +, Positive; −, negative; w, weakly positive.

Character	AL15-21^T/AL15-22/AL15-2
Flagellum	Polar/polar/polar
Form	Rods/rods/rods
Pigment	White/white/white
Gram stain	−/−/−
Catalase	+/+/+
Oxidase	+/+/+
Reduction of NO_3 to NO_2	+/+/+
Fermentation of glucose	−/−/−
Hydrolysis of:	
Casein, gelatin	+/+/+
Starch, pullulan, DNA, aesculin	−/−/−
Tween 20, 40, 60, 80	+/+/+
Growth at pH 7	+/+/+
Growth at (°C):	
4, 10, 20, 25, 30	+/+/+
40, 50	−/−/−
Growth in presence of NaCl (%):	
0	−/−/−
3, 5	+/+/+
7	+/−/+
9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20	−/−/−
Acid produced from:	
D-Glucose, D-xylose, mannitol, glycerol, fructose	+/+/+
L-Arabinose, sucrose, sorbitol, raffinose, rhamnose, *myo*-inositol, lactose, trehalose, cellobiose, maltose	−/−/−
Melibiose	w/−/−
D-Galactose	−/−/w
Utilization of:	
D-Glucose, mannitol, glycerol, fructose, DL-malic acid, succinic acid, L-proline, glutamate, L-arginine	+/+/+
L-Arabinose, D-xylose, D-mannose, melibiose, sucrose, sorbitol, raffinose, rhamnose, *myo*-inositol, D-galactose, lactose, trehalose, cellobiose, maltose, L-valine	−/−/−
Glycine	w/−/w

these isolates were obviously different (23·2–60·6% similarity) from *P. oleovorans*, *P. pseudoalcaligenes*, *P. mendocina* and *P. nitroreducens*.

DISCUSSION

Although the phenotypic characteristics of the three alkaliphilic isolates show a little variation, they can be considered as relatively homogeneous. Furthermore, results of DNA–DNA relatedness between AL15-21^T and the other isolates show more than 94% similarity (Table 3), indeed suggesting that all three isolates belong to the same species.

Phenotypic characteristics and G+C contents classified them as *Pseudomonas* species (Palleroni, 1984). Based on the results of 3-hydroxy fatty acid composition and quinone systems, these isolates belong in rRNA group I of Palleroni (1984) (Yamada *et al.*, 1982; Oyaizu & Komagata, 1983). Phylogenetic analysis based on 16S rRNA gene sequence showed that strain AL15-21^T belongs to the *P. mendocina* sublineage with more than 98% sequence similarity with the 16S rRNA gene of strains from the same sublineage. DNA–DNA hybridization values of less than 61% between strain AL15-21^T and strains belonging to the *P. mendocina* sublineage showed that the new group differed from the other members in this sublineage. Furthermore, the novel *Pseudomonas* strains can be differentiated phenotypically from most closely related species, e.g. from *Pseudomonas pseudoalcaligenes* by the inability to produce acid from D-glucose, hydrolysis of gelatin and Tween 80, growth at 4 °C, and the ability to grow at 42 °C (Table 4).

It is known that alkaliphilic *Bacillus* strains possess an

Table 2 Effect of culture pH on fatty acid composition in *Pseudomonas alcaliphila*

ND, Not detected.

Fatty acid	Strain AL15-21ᵀ		Strain AL15-22		Strain AL15-2	
	pH 7	pH 10	pH 7	pH 10	pH 7	pH 10
$C_{12:0}$	7·8	9·3	6·6	4·0	7·4	5·0
$C_{13:0}$	ND	0·5	0·1	0·2	0·2	0·3
$C_{14:0}$	0·5	0·3	0·3	ND	0·4	0·4
$C_{14:1(7c)}$	0·7	ND	0·1	ND	0·3	ND
$C_{15:0}$	0·8	2·2	0·7	1·6	0·8	2·3
3-OH $C_{10:0}$	0·5	0·5	0·2	0·6	0·2	0·6
$C_{16:0}$	17·5	17·4	20·0	17·0	19·9	18·1
$C_{16:1(9t)}$	3·3	0·5	3·8	0·2	3·0	0·3
$C_{16:1(9c)}$	15·7	16·6	16·9	17·5	19·3	18·4
$C_{17:0}$	1·0	2·7	0·8	2·4	0·7	3·5
3-OH $C_{12:0}$	1·8	3·3	1·5	4·6	1·7	4·7
$C_{18:0}$	1·3	0·9	0·9	0·6	1·0	0·8
$C_{18:1(7c)}$	1·0	0·3	0·9	ND	0·6	ND
$C_{18:1(9c)}$	45·5	42·7	45·3	47·5	43·2	42·4
$C_{18:1(11c)}$	0·5	0·9	0·4	0·5	0·5	0·5
Others	2·2	2·4	1·6	3·7	1·5	3·0

Table 3 DNA–DNA relatedness among strains examined in this study

Reassociation values are means of 2–4 determinations; statistical analysis was done by Student's *t*-test at $P = 0{\cdot}05$. ND, Not determined.

Species	Strain	Reassociation (%) with biotinylated DNA from:	
		P. alcaliphila AL15-2ᵀ	*P. pseudoalcaligenes* JCM 5968ᵀ
P. alcaliphila	AL15-21ᵀ	100	59·2±4·4
P. alcaliphila	AL15-22	94·3±2·3	60·6±3·8
P. alcaliphila	AL15-2	96·8±0·9	57·7±1·3
P. pseudoalcaligenes	JCM 5968ᵀ	56·7±3·6	100
P. mendocina	JCM 5966ᵀ	51·1±1·7	57·9±0·5
P. nitroreducens	JCM 2782ᵀ	23·2±2·1	24·2±1·4
P. oleovorans	MCIMB 6576ᵀ	51·2±3·6	ND

Na^+/H^+ antiporter for cytoplasmic pH regulation (Krulwich *et al.*, 1997). In addition to Na^+ transport systems, these strains possess also Na^+-dependent solute transport and flagellar rotation systems. However, an apparent Na^+ requirement for growth was not always observed in these micro-organisms because of strain differences in the affinity to Na^+ (Garcia *et al.*, 1983; Guffanti *et al.*, 1980; Krulwich *et al.*, 1982). On the other hand, the marine bacterium *Vibrio alginolyticus* possesses an Na^+ translocating respiratory component which works under alkali conditions, but not under neutral conditions (Tokuda & Unemoto, 1981, 1982), in addition to the Na^+-dependent solute transport (Tokuda *et al.*, 1982) and flagellar rotation systems (Atsumi *et al.*, 1992). In *P. alcaliphila*, an obvious Na^+ requirement was recognized when the cells were grown at pH 10 but not when grown at pH 7. This observation suggests that a different ion-transport system or respiratory system might operate when growing at a different pH.

Recently, *trans*-unsaturated fatty acids were detected in bacterial lipids as well as the commonly found *cis*-unsaturated fatty acid isomers (Keweloh & Heipieper, 1996). These membrane lipid constituents had greater concentration in bacterial cells exposed to various environmental stresses, e.g. heat, harmful compounds and high salinity (Heipieper *et al.*, 1996; Loffeld & Keweloh, 1996; Okuyama *et al.*, 1996). The increase in the amount of *trans*-unsaturated fatty acid is accompanied by an appropriate decrease in the corresponding *cis* isomer. *cis*/*trans* isomerization of unsaturated fatty acids occurs by direct isomerization of the double bond without a shift of its position. The

Table 4 Differential characteristics of *Pseudomonas alcaliphila* and related species

Symbols: +, 90% or more of strains are positive; −, 90% or more of strains are negative; ±, variable or slight reaction; D, reactions differ among strains; ND, no data. All tests for *P. alcaliphila* were performed at pH 7. Characteristics of other *Pseudomonas* species were cited from Palleroni (1984), Hildebrand *et al.* (1994), Bennasar *et al.* (1996), and Kiska & Gilligan (1999). Strains: 1, *Pseudomonas alcaliphila*; 2, *Pseudomonas pseudoalcaligenes*; 3, *Pseudomonas oleovorans*; 4, *Pseudomonas mendocina*; 5, *Pseudomonas flavescens*; 6, *Pseudomonas balearica*; 7, *Pseudomonas stutzeri*; 8, *Pseudomonas alcaligenes*; 9, *Pseudomonas aeruginosa*; 10, *Pseudomonas putida*.

Characteristic	1	2	3	4	5	6	7	8	9	10
Acid production from D-glucose	+	−	−*	+	ND	ND	+	−	+	+
Nitrate reduction	+	+	ND	+	ND	+	+	D	+	−
Gas from nitrate	−	−	−*	+	−	+	+	−	+	−
Arginine dihydrolase	+	D	ND	+	−	ND	−	D	+	+
Aesculin hydrolysis	−	−	−*	−	ND	ND	−	−	−	−
Gelatin hydrolysis	+	−	−	−	+	−	−	−	D	−
Starch hydrolysis	−	−	+	−	−	+	+	−	−	−
Tween 80 hydrolysis	+	−	−*	+	−	ND	+	D	±	D
Growth at 4 °C	+	−	−*	−	ND	ND	−	−	−	D
Growth at 42 °C	−	+	+*	+	−	+	D	−	+	−
DNA G+C content (mol%)	62·3–63·2	62–64	ND	62·9–64·3	63	64·1–64·4	60·6–66·3	64–68	67·2	60·7–62·5

* Tested in this study using type strain because data is not available from the literature.

conversion of *cis*-unsaturated fatty acids into *trans*-unsaturated fatty acids reduces the membrane fluidity (Diefenback *et al.*, 1992). In the present study, it was observed that the content of *trans*-unsaturated fatty acid, $C_{16:1(9t)}$, increased when the cells were grown at pH 7 compared with when cells were grown at pH 10. This is the first report of *cis*/*trans* isomerization occurring in response to the ambient pH. However, an estimate of membrane fluidity depending on the culture pH was not tried.

On the basis of the above results, the name *Pseudomonas alcaliphila* sp. nov. is proposed for this new group of organisms.

Description of *Pseudomonas alcaliphila* sp. nov.

Pseudomonas alcaliphila (al.ca.li′phi.la. N.L. adj. *alcaliphila* alkali-loving).

Cells are Gram-negative straight rods (0·3–0·5 × 1·5–3·0 µm), motile by means of a single polar flagellum. Colonies are circular and colourless. Catalase- and oxidase-positive. No growth is observed without NaCl in the medium at pH 10, whereas growth is seen at pH 7 in absence of NaCl. Growth occurs in media supplemented with 3–5% NaCl, but not in media with salinity higher than 9% at pH 10. Growth occurs at 4–30 °C, but no growth is observed at 40 °C or higher. Nitrate is reduced to nitrite. Acid is produced from D-glucose, D-xylose, mannitol, glycerol and fructose when grown at pH 10. No acid is produced from L-arabinose, sucrose, sorbitol, raffinose, rhamnose, *myo*-inositol, lactose, trehalose, cellobiose or maltose. Hydrolyses casein, gelatin, and Tween 20, 40, 60 and 80, but not starch, DNA, pullulan or aesculin. Utilizes D-glucose, mannitol, glycerol, fructose, DL-malic acid, succinic acid, L-proline, glutamate and L-arginine, but not L-arabinose, D-xylose, D-mannose, melibiose, sucrose, sorbitol, raffinose, rhamnose, *myo*-inositol, D-galactose, lactose, trehalose, cellobiose, maltose or L-valine. The major isoprenoid quinone is Q-9. The whole-cell fatty acids contain mainly $C_{16:0}$, $C_{16:1(9c)}$ and $C_{18:0}$, with 3-OH $C_{10:0}$ and 3-OH $C_{12:0}$ as the hydroxyl fatty acids. The DNA G+C content is 62·3–63·2 mol% (determined by HPLC). The type strain, AL15-21^T, has been deposited at The Institute of Physical and Chemical Research (RIKEN), Wako, Japan, and the IAM Culture Collection, University of Tokyo, Tokyo, Japan, as JCM 10630^T and IAM 14884^T, respectively.

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