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Pacificamonas flava gen. nov., sp. nov., a Novel Member of the Family *Sphingomonadaceae* Isolated from the Southeastern Pacific

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Abstract Strain JLT2015^T was isolated from surface seawater of the Southeastern Pacific. The strain was Gram-negative, aerobic, motile by gliding, and rod shaped. The dominant fatty acids were $C_{18:1\omega7c}$, $C_{16:0}$, and $C_{16:1\omega7c}$. The major respiratory ubiquinone was Q-10, and the predominant polyamine pattern was spermidine. The components of the polar lipid profile were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and sphingoglycolipid. The DNA G+C content was 64.2 %. Phylogenetic analysis based on 16S rRNA gene sequence revealed strain JLT2015^T belonged to belong to the family *Sphingomonadaceae*, exhibiting 94.7 % 16S rRNA gene sequence similarity with *Novosphingobium pentaromativorans*. On the basis of the taxonomic data presented, together with phylogenetic and genetic characteristics, strain JLT2015^T is considered to represent a novel genus, for which the name *Pacificamonas flava* gen. nov., sp. nov. is proposed. The type strain of *Pacificamonas flava* is JLT2015^T (=LMG27364^T = CGMCC1.12401^T).

The GenBank/EMBL/DDBJ Accession number for the 16S rRNA gene sequence of strain JLT2015^T is JX845636.

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Introduction

The family *Sphingomonadaceae* was established by Kosako et al. [19]. The genus *Sphingomonas* was first described by Yabuuchi et al. in 1990 [38] and later divided into four genera, *Sphingomonas Sphingobium*, *Novosphingobium*, and *Sphingopyxis* by Takeuchi et al. [31]. At the time of writing, 14 validly published genera have been added to the family, such as the genera *Sphingomicrobium* [14] and *Parasphingopyxis* [36]. Species of this clade share several phenotypic traits, including the presence of yellow-colonies, glycosphingolipids, the predominant quinone profiles (Q-10), and the major polyamine patterns (spermidine) [22, 31].

The species in the family originate from a diversity of environments, such as seawater, freshwater, polychlorinated-dioxin-contaminated soil, tubeworm, and hot-spring conditions [3, 10, 12, 14, 36]. During the cruise of the China Global Ocean Sampling in 2011, strain JLT2015^T was isolated. Based on the polyphasic characterization that included phylogenetic, chemotaxonomic, and other phenotypic properties presented, strain JLT2015^T is considered to represent a novel genus within the family *Sphingomonadaceae*, for which the name *Pacificamonas flava* gen. nov., sp. nov. is proposed.

Materials and Methods

Bacterial Strain and Culture Conditions

Strain JLT2015^T was isolated from a surface seawater sample from the Southeastern Pacific (102°35′W, 3°41′S) in September 2011. The strain was isolated and maintained on marine agar 2216 (MA; BD) after incubation at 30 °C

for 48 h and was preserved in marine broth 2216 (MB; BD) as glycerol suspensions (15 %, v/v) at −80 °C.

Morphological, Physiological, and Biochemical Analysis

Cell morphology was observed by transmission electron microscopy (JEM-1230; JEOL USA) for cells grown on MA at 25 °C for 48 h. Gliding motility and aerobic/anaerobic culture test were determined by the semi-solid puncture method. The Gram-stain reaction was examined as described by Gerhardt et al. [9]. Hydrolysis of casein, DNA, starch, gelatin, and aesculin was determined according to Smibert and Krieg [27]. The intracellular accumulation of polyhydroxyalkanoates (PHAs) was tested by Nile blue A staining [23]. Oxidase, catalase activity, and other biochemical and physiological properties were performed with the API 20 E, API 20 NE, API ZYM strips (bioMerieux) according to the manufacturer's instruction, and results were recorded after 48 h of incubation at 25 °C. Growth on sole carbon sources and nitrogen sources was tested by using Microlog GN_2 plates (Biolog) according to the procedures of Garland [8]. Susceptibility to antibiotics was investigated on MA plates using antibiotic disks (Kirby–Bauer) method as described by Fraser and Jorgensen [7] and Andrews [1]. The antibiotic disks included the following components and concentrations: ampicillin (10 μg), carbenicillin (100 μg), chloromycetin (5 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), neomycin (30 μg), novobiocin (5 μg), penicillin (10 μg), polymyxin B (300 μg), rifampicin (5 μg), streptomycin (10 μg), tetracycline (30 μg), and vancomycin (30 μg).

Growth at various NaCl concentrations was investigated in MB medium with final NaCl concentrations of 0, 0.5, and 1–13 %, at intervals of 1 %. Growth at different temperatures (4, 10, 20, 25, 30, 35, 40, and 50 °C) and pH values (pH 4–10, in increments of 1 pH unit) was determined by adjusting the final pH of MB medium after autoclaving to 4, 5, 6, 7, 8, 9, and 10 with HCl or NaOH (2 % NaCl, 30 °C).

Chemotaxonomic Analysis

Cellular fatty acids were extracted according to the standard MIDI protocol, and analysis was carried out as described by Komagata and Suzuki [18]. Polar lipids were separated by two-dimensional TLC. The polar lipids were identified by two-dimensional thin-layer chromatography according to Collins et al. [4] and Kates [17], using Merck silica gel 60F254 plates (10 by 20 cm) and chloroform–methanol–water (65:25:4, vol/vol) in the first dimension and chloroform–methanol–acetic acid–water (80:12:15:4, vol/vol) in the second dimension. The general detection reagent, sulfuric acid–ethanol (1:2, by vol.), was also used to detect total polar lipids. Respiratory ubiquinones were extracted as described by Tindall [34, 35] and analyzed using reverse-phase HPLC. For the polyamines analysis, cells biomass were harvested on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2), extracted as described as previously [2] and analyzed by HPLC using the apparatus described by Stolz et al. [29].

Molecular Analysis

The genomic DNA was extracted according to the method of Marmur [21], and the yield and concentration were measured with spectrophotometer Nanodrop 2000 (Thermo Scientific, USA). The DNA G+C content of strain JLT2015^T was estimated via a massive parallel pyrosequencing technology (Roche 454 GS FLX). This Whole Genome Shotgun information has been deposited at DDBJ/EMBL/GenBank under the accession AMRV00000000 [33].

The 16S rRNA gene of strain JLT2015^T was amplified with universal bacterial primers 27F and 1492R [5]. Phylogenetic analysis based on 16S rRNA gene sequences was performed as described by Yoon et al. [39]. To determine approximate phylogenetic affiliation, the 16S rRNA gene sequence (1,383 bp) of strain JLT2015^T (GenBank accession number JX845636) was compared with those available from the GenBank database by using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi; NCBI). Phylogenetic analysis was performed by using BioEdit [13]. Phylogenetic trees were constructed by using the neighbor-joining method [25], maximum-parsimony, and maximum-likelihood method [6] within the MEGA 5 software [32].

Results and Discussion

Morphological, Physiological, and Biochemical Characteristics

Cells of strain JLT2015^T were Gram-negative, aerobic, motile by gliding, and rod shaped (Fig. 1). Colonies were orange, circular, opaque, and convex with entire margins on MA medium. Growth occurred at 20–30 °C (optimum 25 °C), at pH 5–12 (optimum 5–11) and in 0.5–7 % (w/v) NaCl (optimum 2 %). Strain JLT2015^T was positive for catalase and oxidase. It hydrolyzed aesculin, Tween 40, and Tween 80. Strain JLT2015^T did not reduce nitrate to nitrite, like members of neighboring genera *Sphingobium* and *Sphingopyxis* [31]. Intracellular polyhydroxyalkanoates (PHAs) were not detected in strain JLT2015^T, but PHAs accumulation was commonly found in the members

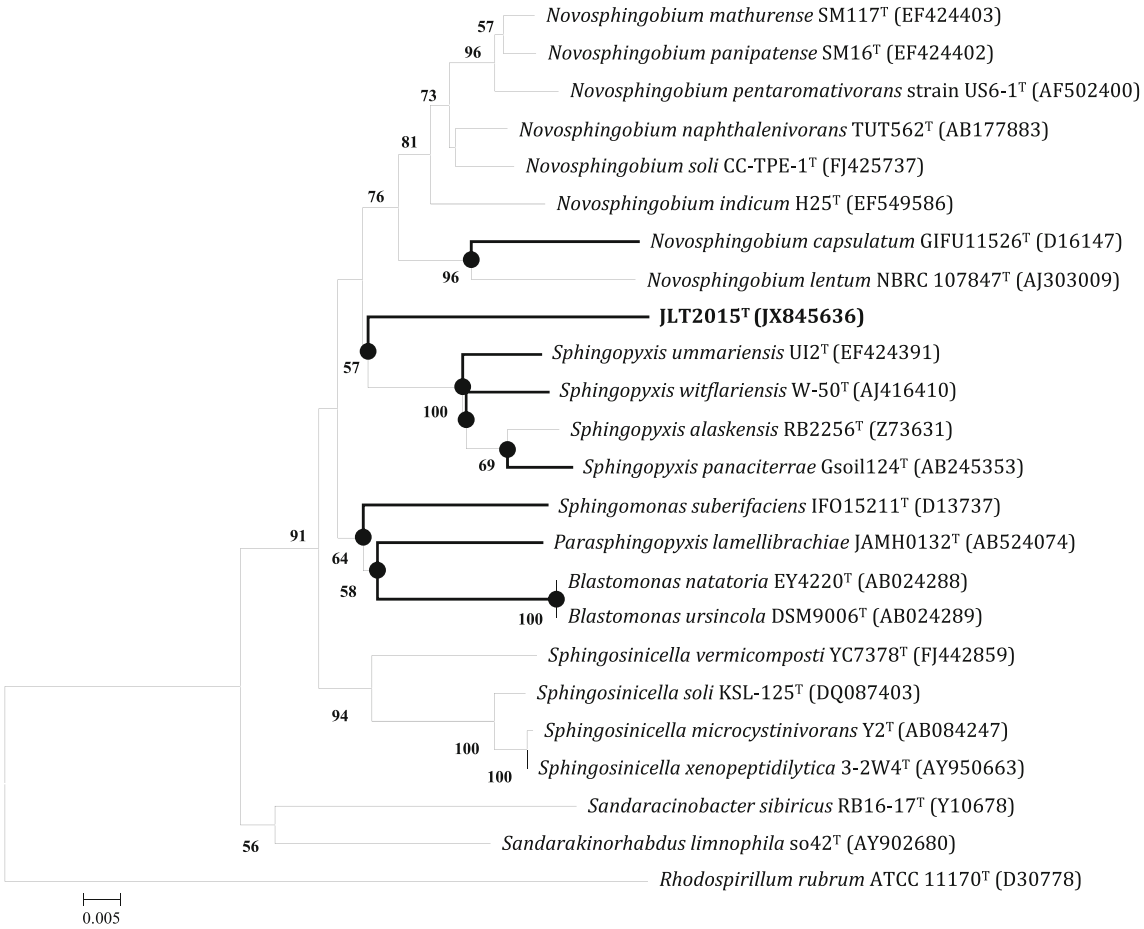

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain JLT2015^T and representatives of the family *Sphingomonadaceae*. *Rhodospirillum rubrum* ATCC 11170^T was used as outgroup. Bootstrap percentages analyses based on 1,000 replications and only values >50 % are shown. *Filled circles* indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Bar* 0.005 substitutions per nucleotide position

of the genera *Sphingopyxis* and *Sphingomona*s [11]. Strain JLT2015^T was found to be susceptible to rifampicin, neomycin, and penicillin. Detailed results from the phenotypic and biochemical tests are given in the species description and Table 1.

Chemotaxonomic Characteristics

The major cellular fatty acids of strain JLT2015^T were $C_{18:1}\omega7c$ (30.2 %), $C_{16:0}$ (17.3 %), and $C_{16:1}\omega7c$ (14.1 %) (Table 2). The major fatty acid of members of neighboring genera *Sphingopyxis, Sphingobium, Sphingomona*s, and *Novosphingobium* is $C_{18:0}$ [31]. The dominant respiratory ubiquinone was Q-10 and the polyamine pattern contained the major compound (g dry weight) spermidine (17.7 μg), trace amounts of spermine (0.5 μg), and putrescine (0.2 μg). The DNA G+C content of strain JLT2015^T was determined to be 64.2 %. The polar lipid profile consisted of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and sphingoglycolipid (SGL) (Fig. S2).

Phylogenetic Analysis

Phylogenetic tree constructed using the neighbor-joining algorithm based on 16S rRNA gene sequences revealed that strain JLT2015^T belonged to the family *Sphingomonadaceae* (Fig. 1). The relationships among strain JLT2015^T and the type strains of the family *Sphingomonadaceae* were also maintained in the tree constructed using the maximum-likelihood and maximum-parsimony methods (Fig. S3 and S4). Based on 16S rRNA gene sequence phylogenetic analysis, the closest taxa of the family *Sphingomonadaceae* were the genus *Novosphingobium* and *Sphingopyxis*. Strain JLT2015^T showed the highest sequence similarity to *Novosphingobium pentaromativorans* US6-1^T (94.7 %), followed by *Sphingopyxis alaskensis* RB2256^T (94.6 %), *Novosphingobium*

Table 1 Differential characteristics of strain JLT2015^T and type strains of closely related neighboring genera within the family *Sphingomonadaceae*

Characteristic	1	2	3	4	5	6	7	8	9
Colony color	O	Y(B)	PY(B)	Y(LY)	Y	Y	Y	Y	PY
Source	seawater	sediment	seawater	soil	soil	soil	dump	sludge	mineral water
Motility	+	–	+	–	–	+	+	+	+
Oxidase	+	–	+	+	–	+	+	+	+
Catalase	+	+	+	+	–	–	+	+	+
Hydrolysis of:									
Aesculin	+	+	+	+	–	–	+	–	+
Nitrate reduction[a]	–	–	–	–	+	+	+	–	–
Indole production	–	–	–	+		ND	–	–	–
PHAs accumulation	–	–	+	–	–	ND	+	ND	ND
Assimilation of									
L-Arabinose	–	–	–	–	+	+	–	–	–
Maltose	–	+	+	+	+	–	–	+	–
Malate	–	–	+	–	+	+	+	–	+
D-Mannitol	–	–	–	+	–	–	–	–	+
D-Mannose	–	–	–	+	–	–	–	–	–
Major non-polarfatty acid	18:1ω7c	18:1ω7c	17:1ω6c	18:1ω7c	18:1ω7c	18:1ω7c	18:1ω7c	17:1ω6c	18:1ω7
major 2-hydroxy fatty acids	14:0 2-OH	14:0 2-OH	15:0 2-OH	14:0 2-OH	14:0 2-OH	14:0 2-OH	14:0 2-OH	15:0 2-OH	14:0 2-OH

All species were Gram-negative, rod shaped; assimilation of D-glucose; sphingoglycolipid (SGL) and spermidine as the major polar lipid and polyamine are present. All strains were negative for hydrolysis of gelatin; assimilation of *N*-acetylglucosamine

+ Positive, – Negative, *ND* no data, *Y* yellow, *O* orange, *PY* pale yellow, *B* beige, *LY* light yellow

[a] Nitrate reduction of column 2, 4, and 7 was different from previously reported [26, 28, 30]

Strains: 1. JLT2015^T; 2. *Novosphingobium pentaromativorans* US6-1^T; 3. *Sphingopyxis alaskensis* RB2256^T; 4. *Novosphingobium naphthalenivorans* TUT562^T; 5. *Novosphingobium panipatense* SM16^T; 6. *Novosphingobium soli* CC-TPE-1^T; 7. *Sphingopyxis ummariensis* UI2^T; 8. *Sphingopyxis witflariensis* W-50^T; 9. *Sphingopyxis taejonensis* JSS54^T. Data in column 3 are from Vancanneyt et al. [37] and Godoy et al. [11]; Data in column 6 and 8 are from Kämpfer et al. [15, 16]; Data in column 9 are from Lee et al. [20] and Pal et al. [24]

*naphthalenivoran*s TUT562^T (94.5 %), and *Novosphingobium panipatense* SM16^T (94.1 %).

Strain JLT2015^T can be distinguished from its closest phylogenetic taxa within the family *Sphingomonadaceae* not only by phylogenetic analysis but also by a range of different phenotypic characteristics such as colony color, PHAs accumulation, and nitrate reduction (Table 1). Some fatty acids for example anteiso-$C_{15:0}$, $C_{18:1\omega9c}$, $C_{15:0\ 3\text{-}OH}$, and cyclo-$C_{19:0\omega8c}$ are only detected in the novel strain JLT2015^T (Table 2). On the basis of phylogenetic analysis and phenotypic characteristics, strain JLT2015^T is considered to represent a novel species of a new genus, for which the name *Pacificamonas flava* gen. nov., sp. nov. is proposed.

Description of *Pacificamonas* gen. nov.

(Gr. fem. adj. *pacifica*, peaceful, referring to the Pacific Ocean; Gr. fem. n. monas, a unit, monad; N.L. fem. n. *Pacificamonas*, a monad isolated from the Pacific Ocean.)

Cells are Gram-negative, aerobic, motile by gliding, and rod shaped. PHA granules are not produced. Catalase and oxidase are positive. The major cellular fatty acids of strain JLT2015^T are $C_{18:1\omega7c}$, $C_{16:0}$, and $C_{16:1\omega7c}$. The predominant respiratory ubiquinone is Q-10, and the polyamine is spermidine. Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and sphingoglycolipid as the major polar lipids are present. The type species is *Pacificamonas flava.*

Description of *Pacificamonas flava* sp. nov

(L. fem. adj. *flava*. golden yellow)

The type strain exhibits the following properties in addition to those given for the genus. Cells are 1.5–2.0-μm long and 0.3–0.5-μm wide. Colonies are convex, opaque, orange, and circular with entire margins (about 0.8–1.2 mm in diameter) on MA medium. Growth occurred at 20–30 °C (optimum 25 °C); at pH 5–12 (optimum 5–11) and in 0.5–7 % (w/v) NaCl (optimum

Table 2 Cellular fatty acid compositions of strain JLT2015^T and type strains of the most closely related neighboring genera

Fatty acid	1	2	3	4	5	6	7	8	9
Straight-chain									
$C_{10:0}$	–	–	–	1.7	–	–	–	–	–
$C_{12:0}$	3.9	2.3	–	–	–	–	–	–	–
$C_{14:0}$	3.2	2.3	tr	–	–	–	–	–	–
$C_{15:0}$	–	–	2.8	tr	–	tr	–	7.0	2.3
$C_{16:0}$	17.3	8.6	7.3	8.3	7.1	7.3	10.9	5.8	23.0
$C_{17:0}$	–	–	2.7	tr	tr	–	–	3.8	1.6
$C_{18:0}$	8.4	2.4	–	8.4	2.7	tr	4.4	–	–
Branched saturated acids									
anteiso-$C_{15:0}$	2.0	–	–	–	–	–	–	–	–
Unsaturated acids									
$C_{16:1\omega5c}$	2.1	2.2	1.1	–	4.5	1.9	3.1	1.2	1.6
$C_{16:1\omega7c}/C_{17:1\omega6c}$	14.1	9.8	33.2	17.1	12.5	17.3	24.0	54.4	21.5
$C_{17:1\omega8c}$	–	–	–	–	–	1.7	–	6.2	1.8
$C_{18:1\omega7c}$	30.2	55.1	26.5	55.9	59.3	49.9	44.7	9.0	28.3
$C_{18:1\omega9c}$	1.2	–	–	–	–	–	–	–	–
Branched unsaturated acids									
$C_{14:0\ 2\text{-OH}}$	9.7	6.0	1.3	9.0	6.6	8.2	6.1	1.0	4.4
$C_{15:0\ 2\text{-OH}}$	–	tr	4.6	–	–	2.4	–	7.0	2.3
$C_{15:0\ 3\text{-OH}}$	1.5	–	–	–	–	–	–	–	–
$C_{16:0\ 2\text{-OH}}$	–	2.4	1.4	–	–	1.0	2.5	tr	1.5
11-Methyl $C_{18:1\omega7c}$	–	5.0	1.7	–	3.4	7.6	2.0	2.8	–
cyclo-$C_{19:0\omega8c}$	4.2	–	–	–	–	–	–	–	–

Values are percentages of total fatty acids

tr Trace (<1 %), – not detected or not reported

Strains: 1. JLT2015^T; 2. *Novosphingobium pentaromativorans* US6-1^T; 3. *Sphingopyxis alaskensis* RB2256^T; 4. *Novosphingobium naphthalenivorans* TUT562^T; 5. *Novosphingobium panipatense* SM16^T; 6. *Novosphingobium soli* CC-TPE-1^T; 7. *Sphingopyxis ummariensis* UI2^T; 8. Sphingopyxis witflariensis W-50^T; 9. *Sphingopyxis taejonensis* JSS54^T. Data in column 3 are from Vancanneyt et al. [37]; Data in column 6 and 8 are from Kämpfer et al. [15, 16]; Data in column 9 are from Lee et al. [20]

2 %). Positive for hydrolysis of aesculin, catalase, oxidase, and Voges–Proskauer test. Negative for hydrolysis of casein, DNA, starch, gelatin, indole, and H_2S production. Nitrate is not reduced. Acid is not produced from glucose, mannitol, inositol, sorbitol, rhamnose, melibiose, amygdalin, and arabinose as examined in the API20E. According to API ZYM tests, estérase (C4), estérase lipase (C8), leucine, valine and cystine arylamidase, trypsine, alkaline and acid phosphatase, naphtol phosphohydrolase, and *N*-acetyl-β–glucosaminidase are positive. The following carbon substrates are utilized in assay with Biolog GN_2 microplates: dextrin, Tween 40, Tween 80, L-arabinose, i-erythritol, D-fructose, α-D-glucose, lactulose, methyl pyruvate, monomethyl- succinate, acetic acid, β-hydroxy butyric acid, and L-proline. Cells are sensitive to rifampicin, neomycin, and penicillin but resistant to vancomycin, carbenicillin, kanamycin, gentamicin, polymyxin B, ampicillin, tetracycline, chloromycetin, erythromycin, novobiocin, streptomycin, and lincomycin. The DNA G+C content of strain JLT2015^T was 64.2 %. The type strain is JLT2015^T (= LMG27364^T = CGMCC1.12401^T), which was isolated from Southeastern Pacific surface seawater.

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