Archives of Microbiology (2019) 201:705–712
https://doi.org/10.1007/s00203-019-01640-0

ORIGINAL PAPER

# Characterization of *Labrenzia polysiphoniae* sp. nov. isolated from red alga *Polysiphonia* sp.

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Received: 24 August 2018 / Revised: 21 January 2019 / Accepted: 20 February 2019 / Published online: 27 February 2019


## Abstract

A group of five Gram-negative aerobic halophilic bacteria was isolated from the red alga *Polysiphonia* sp. specimen collected from the Sea of Japan seashore and subjected to a taxonomic study. On the basis of 16S rRNA gene sequence analysis, the novel isolates were affiliated to the genus *Labrenzia* sharing the highest gene sequence similarities of 98.1–98.4% with the type strain of *Labrenzia suaedae* KACC 13772$^T$. The DNA–DNA hybridization values of 83–91% obtained between five novel strains, and 26 and 36% between two of the five novel strains and the closest neighbor *Labrenzia suaedae* KACC 13772$^T$ confirmed their assignment to the same separate species. Novel isolates were characterized by Q-10 as the major ubiquinone, by the predominance of $C_{18:1}\omega7c$ followed by 11-methyl $C_{18:1}\omega7c$ and $C_{14:0}$ 3-OH in their fatty acid profiles. Polar lipids consisted of phosphatidylcholine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, an unknown aminophospholipid, and an unknown phospholipid. Some of novel strains were found to inhibit growth of Gram-negative and Gram-positive test microorganisms. On the basis of phylogenetic analysis, DNA–DNA hybridization and phenotypic traits, a novel species with the name *Labrenzia polysiphoniae* sp. nov. (type strain KMM 9699$^T$ = rh46$^T$ = KACC 19711$^T$), is proposed.

**Keywords** *Labrenzia polysiphoniae* sp. nov. · Marine · Red alga · *Polysiphonia* sp.

## Introduction

The genus *Labrenzia* was proposed by (Biebl et al. 2007) for *Labrenzia alexandrii* as the type species of the genus and three species of the genus *Stappia* (Uchino et al. 1998) reclassified as *Labrenzia aggregata, Labrenzia marina*, and *Labrenzia alba*, and emended by Bibi et al. (2014). Two species, *Labrenzia suaedae* (Bibi et al. 2014) and *Labrenzia salina* (Camacho et al. 2016), were subsequently described. At the time of writing the genus *Labrenzia* comprises six recognized species, which have been isolated from sea water, marine sediments, dinoflagellates, oysters, and halophytes (Kim et al. 2006; Biebl et al. 2007; Bibi et al. 2014; Camacho et al. 2016). In the present study, the taxonomic position of five novel strains isolated from the red alga *Polysiphonia* sp. specimen collected from the Sea of Japan seashore was defined. On the basis of the phenotypic characteristics, DNA–DNA relatedness and phylogenetic analysis the novel species of the genus *Labrenzia, Labrenzia polysiphoniae* sp. nov. is described to accommodate five red alga-associated bacteria.

Communicated by Erko Stackebrandt.

The DDBJ/GenBank/EMBL accession number of the 16S rRNA gene sequence of strain KMM 9699T is LC381921.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00203-019-01640-0) contains supplementary material, which is available to authorized users.

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## Materials and methods

### Bacterial strains

A group of six bacteria designated rh32, rh44, rh45, rh46$^T$, rh51, and rh52 was isolated from red alga *Polysiphonia* sp. (*Polysiphonia* Greville, 1823, family *Rhodomelaceae*) specimen, which was collected from the Peter the Great Bay, the Sea of Japan, Russia, in September 2015 as described previously (Romanenko et al. 2017). The strains were grown aerobically on marine 2216 agar (MA) or in marine broth (MB) (all BD Difco), and stored at − 80 °C in the liquid MB supplemented with 30% (v/v) glycerol. The strains rh32, rh44, rh45, rh46$^T$, rh51, and rh52 have been deposited in the Collection of Marine Microorganisms (KMM), G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia, under numbers KMM 9685 (rh32), KMM 9697 (rh44), KMM 9698 (rh45), KMM 9699$^T$ (rh46$^T$), KMM 9704 (rh51), and KMM 9705 (rh52), respectively. The type strain KMM 9699$^T$ (= rh46$^T$) has been deposited in the Korean Agricultural Culture Collection (KACC), National Institute of Agricultural Science and Technology (NIAST), Suwon, Korea, as KACC 19711$^T$. The type strain *Labrenzia suaedae* KACC 13772$^T$ was kindly provided by the Korean Agricultural Culture Collection (KACC) and used in the phenotypic and genotypic analyses as a reference strain.

### Phenotypic characterization

Gram-staining, oxidase and catalase reactions, and motility (the hanging drop method) were determined as described by Gerhardt et al. (1994). The morphology of cells negatively stained with a 1% phosphotungstic acid was examined by electronic transmission microscopy [Libra 200 (Carl Zeiss), provided by the Far Eastern Centre of electronic microscopy, Zhirmunsky Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences] using cells grown in MB on carbon-coated 200-mesh copper grids. The following physiological tests, including hydrolysis of starch, casein, gelatin, Tween 80, DNA, L-tyrosine, chitin, xanthine, hypoxanthine, nitrate reduction (sulfanilic acid/α-naphthylamine test), and formation of $H_2S$ from thiosulfate, were studied using artificial sea water (ASW) as described in the previous papers (Romanenko et al. 2011, 2013). Growth at various concentrations of NaCl [0, 0.5, at 1.0–12.0% (in increments of 1.0%), w/v] was tested by adding appropriate concentrations of NaCl on ASW-based medium containing (per litre) 10.0 g Bacto peptone, 2.0 g yeast extract, 0.028 g $FeSO_4$, and 15.0 g agar. Growth at temperatures 5, 7, 15, 25, 28, 30, 35, 36, 37, 38, 39, 40, 41, and 42 °C was determined on MA for 4 days. Growth at pH 5.0–10.5 (at intervals of 0.5 pH units) was determined in MB adjusted with appropriate buffers as described by Hameed et al. (2014). Biochemical tests were carried out using API 20NE, API ID32 GN, API ZYM test kits (bioMérieux, France) as described by the manufacturer except the cultures were suspended in ASW. Antibiotic susceptibility of strains studied was examined using commercial paper discs (Research Centre of Pharmacotherapy, St. Petersburg, Russia) impregnated with the following antibiotics (mg per disc, unless otherwise indicated): ampicillin (10), benzylpenicillin (10 U), vancomycin (30), gentamicin (10), kanamycin (30), carbenicillin (100), chloramphenicol (30), neomycin (30), oxacillin (10), oleandomycin (15), lincomycin (15), ofloxacin (5), rifampicin (5), polymyxin (300 U), streptomycin (30), cephazolin (30), cephalexin (30), erythromycin (15), nalidixic acid (30), tetracycline (30), and doxocycline (10). For polar lipid and fatty acid analyses, six novel strains and *Labrenzia suaedae* KACC 13772$^T$ were grown on MA 2216 at 28 °C. Lipids were extracted using the extraction method of Folch et al. (1957). Two-dimensional thin-layer chromatography of polar lipids was carried out on Silica gel 60 $F_{254}$ (10 × 10 cm, Merck, Germany) using chloroform–methanol–water (65:25:4, v/v) for the first direction, and chloroform–methanol–acetic acid–water (80:12:15:4, v/v) for the second one (Collins and Shah 1984) and spraying with specific reagents (Collins et al. 1980). Respiratory lipoquinones were analyzed by reversed-phase high-performance thin-layer chromatography as described by Mitchell and Fallon (1990). Fatty acid methyl esters (FAMEs) were prepared according to the procedure of the Microbial Identification System (MIDI) (Sasser 1990). The analysis of FAMEs was performed using the GC-17A chromatograph (Shimadzu, Kyoto, Japan) equipped with capillary columns (30 m × 0.25 mm I.D.), one coated with Supecowax-10 and the other with SPB-5. Identification of FAMEs was accomplished by equivalent chain length values and comparing the retention times of the samples to those of standards (Standard bacterial acid methyl ester mix 47080-U, Supelco). In addition, FAMEs were analyzed using a GLC–MS Shimadzu GC–MS model QP5050 (column MDM-5S, the temperature program from 140 to 250 °C, at a rate of 2 °C/min). Production of bacteriochlorophyll *a* (Bchl *a*) was spectrophotometrically tested in methanolic extracts of cells grown on MA and MB in the dark as described by Lafay et al. (1995). DNA base composition was determined according to the methods of Marmur and Doty (1962) and Owen et al. (1969). DNA–DNA relatedness was measured by the hybridization method of De Ley et al. (1970) using a Cary 100 Bio UV–VIS spectrophotometer (Varian, Australia).

### 16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA of five new isolates was extracted using a commercial genomic DNA extraction kit (Fermentas; EU) following the manufacturer's instructions. The 16S rRNA genes were PCR-amplified (Devereux and Wilkinson 2004) with the universal bacterial primers 8F (5′-AGA GTTTGATCMTGGCTCAG-3′) and 1522R (5′-AAGGAG GTGATCCAGCCGCA-3′) (Edwards et al. 1989). The purified PCR fragments were sequenced using the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) and by the Big Dye v. 3.1 sequencing kit (Applied Biosystems).

The 16S rRNA gene sequences of five new isolates were compared with 16S rRNA gene sequences retrieved from the EMBL/GenBank/DDBJ databases using the FASTA program (Pearson and Lipman 1988). 16S rRNA gene sequences similarities for strains were calculated using Eztaxon service (Kim et al. 2012). Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis (MEGA, version 6.0) (Tamura et al. 2013). Phylogenetic trees were constructed by the neighbor-joining and the maximum-likelihood methods and the distances were calculated according to the Kimura two-parameter model. The robustness of phylogenetic trees was estimated by the bootstrap analysis of 1000 replicates.

### Antimicrobial assay

To examine antimicrobial compound production, strains KMM 9697, KMM 9699$^T$, and KMM 9704 were cultivated in 50 ml of MB for 3 days at 25 °C followed by extraction twice with an equal volume of ethyl acetate, washing with $H_2O$, drying over $Na_2SO_4$, and concentrating under reduced pressure. Subsequently, obtained extracts were examined for the presence of antimicrobial activity against *Staphylococcus aureus* CIP 65.8$^T$ by an agar diffusion method as described previously (Romanenko et al. 2007).

## Results and discussion

### Phylogenetic analysis

Comparative 16S rRNA gene sequence analysis showed that six novel strains belonged to the genus *Labrenzia*, while five of them, KMM 9697, KMM 9698, KMM 9699$^T$, KMM 9704, and KMM 9705, shared the highest gene sequence similarities of 98.1–98.4% with the type strain of *Labrenzia suaedae* KACC 13772$^T$, and strain KMM 9685 was close to *Labrenzia alba* CECT 5094$^T$ (99.9% sequence similarity). In the phylogenetic trees generated by the different algorithms strains, KMM 9697, KMM 9698, KMM 9699$^T$, KMM 9704, and KMM 9705 positioned as a distinct cluster adjacent to *Labrenzia suaedae* KACC 13772$^T$ within the genus *Labrenzia* (Fig. 1, Supplementary Fig. S1). The 16S rRNA gene sequence similarities obtained between these strains and the remaining *Labrenzia* species were lower the threshold similarity value of 97% proposed by Stackebrandt and Goebel (1994) and re-evaluated to 98.7–98.6% by Stackebrandt and Ebers (2006) and Kim et al. (2014), indicating that these novel strains could be assigned to the genus *Labrenzia* as an individual species.

### DNA–DNA hybridization

The DNA–DNA reassociation means between five novel strains were 83–91%. The DNA–DNA hybridization values between two of the novel strains, KMM 9699$^T$ and KMM 9704, and the closest neighbor *Labrenzia suaedae* KACC 13772$^T$ were measured to be 26% and 36%, respectively (Table 1). The DNA relatedness values confirmed an assignment of the novel isolates to the same separate species of the genus *Labrenzia* according to the value of 70% proposed by Wayne et al. (1987) for the bacterial species delineation.

### Phenotypic characterization

Cultural, physiological, and metabolic properties of novel strains are listed in Table 2 and in the species description. Novel isolates were aerobic, Gram-negative, ovoid- or rod-shaped bacteria motile by means of 2–5 subpolar and/or lateral flagella (Fig. 2, Supplementary Fig. S2). Cells with a single flagellum were rarely observed. Some bacteria are capable of producing extracellular material (Supplementary Fig. S2). In addition, cells could be observed as weakly motile or non-motile due to their property to lose flagella easily. Novel isolates were characterized by the inability to assimilate most organic substrates as gave negative results for the assimilation of carbon sources in API 32GN and API 20NE tests. The novel strains differed from each other in the reaction of tyrosine hydrolysis, β-galactosidase and trypsin API ZYM tests and antibiotic resistance profiles. Strain KMM 9685 differed from other novel isolates in many traits, as shown in Table 2, and was included to the present study for the comparative purpose.

Fatty acid profiles were similar with a large proportion of $C_{18:1}\omega7c$ followed by 11-Methyl $C_{18:1}\omega7c$, and $C_{14:0}$ 3-OH found in the novel isolates KMM 9697, KMM 9698, KMM 9699$^T$, KMM 9704, and KMM 9705. Unlike strain *Labrenzia suaedae* KACC 13772$^T$ the novel strains contained a small amount of $C_{18:2}$ and $C_{20:1}$, twice more 11-Methyl $C_{18:1}\omega7c$ and did not contain $C_{18:0}$ 3-OH (Table 3). The major isoprenoid quinone of novel strains was ubiquinone Q-10. The polar lipid composition of strains tested was found to be similar in major components

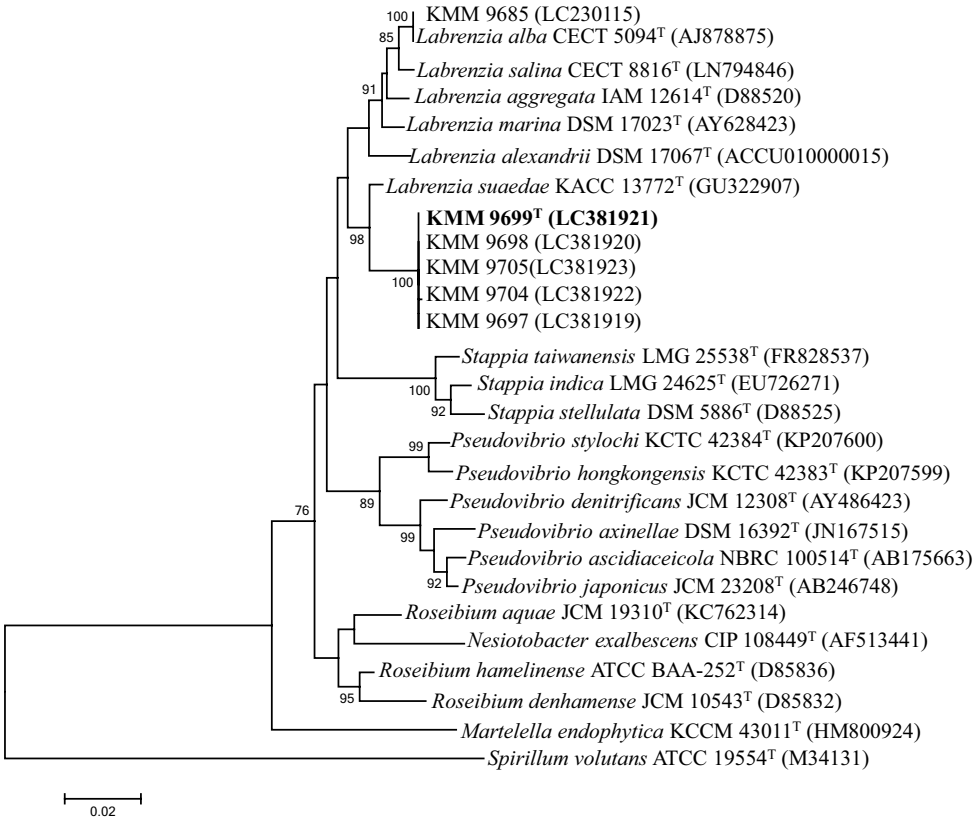


**Fig. 1** Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences available from the GenBank/EMBL/DDBJ databases (accession numbers are given in parentheses) showing phylogenetic position of the novel *Labrenzia* isolates and members of the genus *Labrenzia* and related taxa. Bootstrap values based on 1000 replications are given as percentages at the branching points. Numbers indicate percentages greater than 70%. Bar, 0.02 substitutions per nucleotide position

**Table 1** DNA–DNA relatedness among novel *Labrenzia* strains and *Labrenzia suaedae* KACC 13772$^T$

| Strain | DNA–DNA hybridization, % | |
|---|---|---|
| | KMM 9699$^T$ | KMM 9704 |
| *Labrenzia suaedae* KACC 13772$^T$ | 26 | 36 |
| KMM 9697 | 88 | 91 |
| KMM 9698 | 88 | n.d. |
| KMM 9704 | 83 | n.d. |
| KMM 9705 | 87 | n.d. |

*n.d.* not determined

and included phosphatidylcholine, PC, phosphatidylethanolamine, PE, phosphatidylmonomethylethanolamine, PMME, phosphatidylglycerol, PG, an unknown aminolipid, AL, and an unknown phospholipid, PL. *Labrenzia suaedae* KACC 13772$^T$ contained additionally an unknown lipid (Supplementary Fig. S3). Five novel strains and *L. suaedae* KACC 13772$^T$ did not contained sulphoquinovosyldiacylglyceride, (SQDG), which has been found in all other members of the genus *Labrenzia* (Biebl et al. 2007). This finding is congruent with the original description of Bibi et al. (2014) who reported lack of SQDG in *Labrenzia suaedae* KACC 13772$^T$ and emended description of the genus in terms of the variability of glycolipid SQDG in *Labrenzia* species (Bibi et al. 2014). Unlike all strains tested strain KMM 9685 contained SQDG that supported chemotaxonomically its assignment to the member of *L. alba* lineage.

Chemotaxonomic characteristics, including ubiquinone Q-10, the predominance of $C_{18:1\omega7c}$, and the presence of PC, PMME, PE, PG, Al, PL, and the DNA G + C content values of 56.5–58.0 mol% obtained for five novel strains supported their assignment to the genus *Labrenzia* (Biebl et al. 2007; Bibi et al. 2014; Camacho et al. 2016).

Some of novel strains were found to inhibit growth of one or two test cultures including *S. aureus, E. faecium* (weakly), *S. epidermidis* (weakly), and *Xanthomonas* sp. pv. *badrii* as described in a previous paper (Romanenko et al. 2017). Subsequently, antimicrobial activity against *S. aureus* was revealed in ethyl acetate extracts of liquor cultures as shown by the example of three strains, KMM 9697, KMM 9699$^T$, KMM 9704, pointing to the presence of low-molecular-weight metabolites, which could be responsible for their activity (Supplementary Fig. S4). It should be noted that the activity of some isolates was not consistently stable and changed with respect to test cultures. Further studies should be done to determine the structure of the antibacterial compounds and search for appropriate genes in the genome sequence of the respective strains.

**Table 2** Phenotypic characteristics of novel *Labrenzia* strains, *Labrenzia suaedae* KACC 13772$^T$, and *Labrenzia alba* CECT 5084$^T$

| Characteristic | KMM 9699$^T$ | KACC 13772$^T$ | KMM 9697 | KMM 9698 | KMM 9704 | KMM 9705 | KMM 9685 | CECT 5084$^{T\,b}$ |
|---|---|---|---|---|---|---|---|---|
| DNA G + C content (mol %) | 57.3 | 58.5[a] | 58.0 | 56.5 | 57.7 | 57.6 | n.d. | n.d. |
| Flagella number | 2–3 | 1 | 2–4 | 1–3 | 1–5 | 2–5 | n.d. | 1 |
| Growth in/at: NaCl (%) | 1–8 | 0–9 | 1–8 | 1–8 | 1–8 | 1–8 | 1–8 | 1–8 |
| 39 °C | + | + | (+) | (+) | + | (+) | − | − |
| 41 °C | − | + | − | − | − | − | − | − |
| Hydrolysis of | | | | | | | | |
| DNA | + | − | + | + | + | + | + | + |
| Tyrosine | S | + | S | − | − | − | (+) | − |
| Tween 80 | − | − | − | − | − | − | + | − |
| $H_2S$ production | − | + | − | − | − | − | − | − |
| API 20NE | | | | | | | | |
| Nitrate reduction | − | + | − | − | − | − | + | + |
| Gelatin hydrolysis | − | + | − | − | − | − | − | − |
| Assimilation of | | | | | | | | |
| Glucose | − | + | − | − | − | − | − | (+) |
| Arabinose | − | + | − | − | − | − | − | + |
| Mannitol | − | + | − | − | − | − | − | (+) |
| *N*-acetylglucosamine | − | + | − | − | − | − | − | − |
| Malate | − | + | − | − | − | − | − | + |
| Citrate | − | + | − | − | − | − | − | + |
| API ZYM | | | | | | | | |
| Esterase lipase C 8 | + | (+) | + | + | + | + | − | n.d. |
| Leucine arylamidase | + | (+) | + | + | + | + | + | n.d. |
| Trypsin | + | + | + | + | − | + | − | n.d. |
| Naphtol phosphohydrolase | (+) | + | + | + | + | (+) | + | n.d. |
| β-Galactosidase | + | + | − | − | − | (+) | − | n.d. |
| *N*-acetyl-β-glucosaminidase | − | + | − | − | − | − | + | n.d. |
| Sensitivity to antibiotics | | | | | | | | |
| Ampicillin | − | − | − | − | − | − | + | n.d. |
| Vancomycin | − | − | − | − | − | + | + | n.d. |
| Kanamycin | − | + | + | + | − | + | + | n.d. |
| Carbenicillin | − | − | − | − | − | + | + | n.d. |
| Nalidixic acid | + | − | + | + | + | + | + | n.d. |
| Cephazolin | − | − | (+) | − | − | + | − | n.d. |
| Cephalexin | − | + | (+) | (+) | + | + | + | n.d. |
| Tetracycline | (+) | (+) | + | + | + | + | + | n.d. |
| Doxocycline | − | − | + | + | + | + | + | n.d. |

Data were obtained from the present study unless otherwise indicated. +, Positive; −, negative; (+), weak reaction; S, slow reaction, n.d., not determined. All strains are positive for oxidase and catalase reactions and PNPG test, and negative for aesculin, casein and starch hydrolysis. All strains (excepting *L. alba* CECT 5084$^T$ for which no data available) are positive for alkaline phosphatase, esterase C4, acid phosphatase, and negative for lipase C 14, valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase, assimilation of mannose, gluconate, maltose, caprate, adipate, phenylacetate; are susceptible to gentamicin, rifampicin, ofloxacin, streptomycin, neomycin and erythromycin, and resistant to benzylpenicillin, lincomycin, oxacillin, oleandomycin and polymyxin

[a]Data from Bibi et al. (2014)

[b]Data from Pujalte et al. (2005)

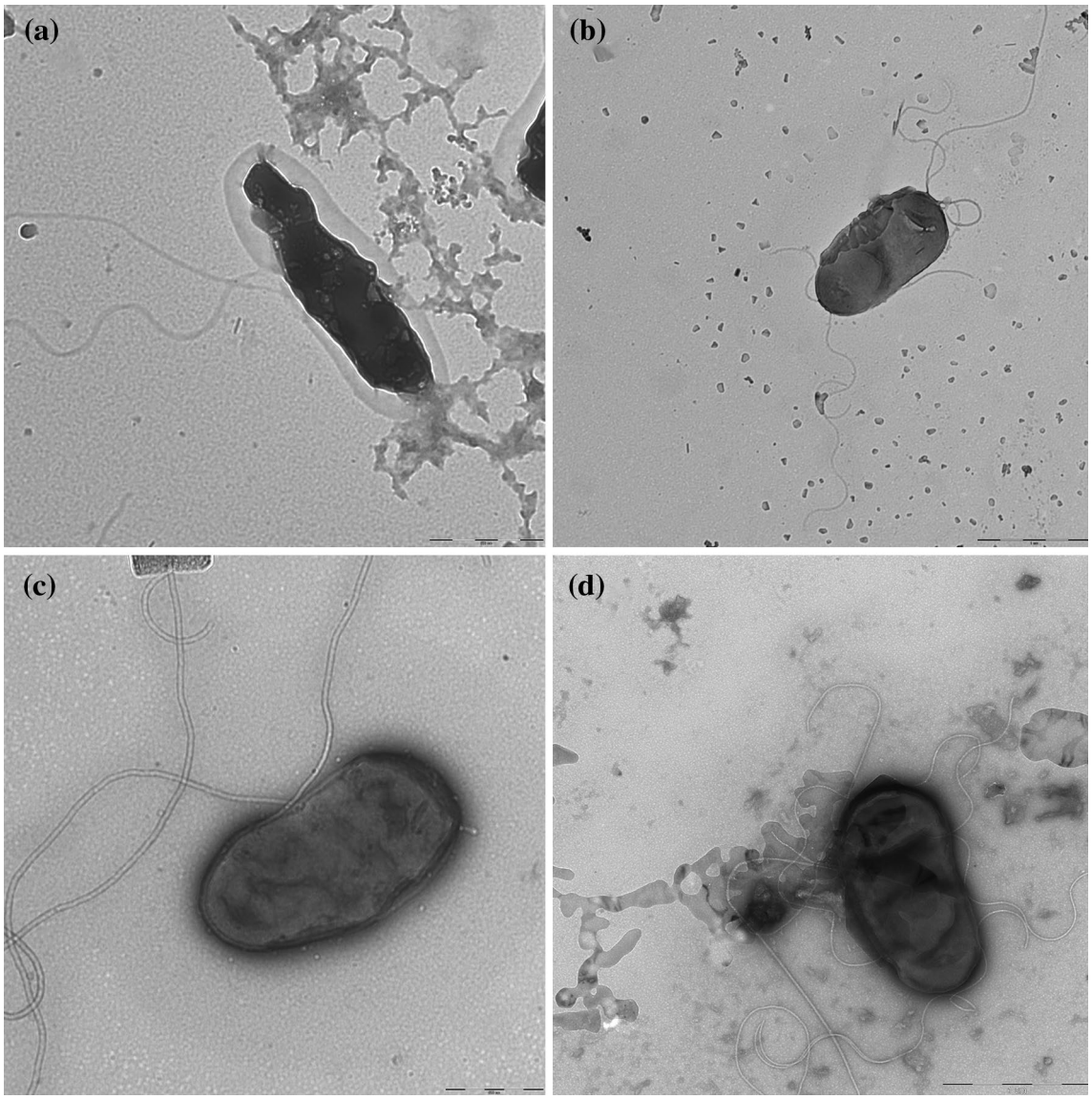


**Fig. 2** Transmission electron micrographs of strains: **a** KMM 9699$^T$; **b, c** KMM 9697; **d** KMM 9704 grown in MB for 24–36 h. Bars **a, c** 500 nm; **b, d** 1 μm

**Table 3** Fatty acid composition (%) of novel *Labrenzia* strains and *Labrenzia suaedae* KACC 13772$^T$

| Fatty acid | KMM 9699$^T$ | KACC 13772$^T$ | KMM 9697 | KMM 9698 | KMM 9704 | KMM 9705 | KMM 9685 |
|---|---|---|---|---|---|---|---|
| $C_{14:0}$ 3-OH | 16.34 | 15.20 | 13.41 | 18.54 | 14.47 | 15.48 | 10.02 |
| $C_{16:0}$ | 1.61 | 2.24 | 0.98 | 0.66 | 1.60 | 0.69 | 0.22 |
| $C_{18:2}$ | 1.42 | – | 1.24 | 1.17 | – | 1.49 | – |
| $C_{18:1}\omega 9c$ | 1.90 | – | 0.96 | 0.64 | 0.83 | 0.73 | – |
| $C_{18:1}\omega 7c$ | 52.37 | 68.16 | 52.59 | 55.88 | 59.52 | 55.76 | 61.50 |
| $C_{18:0}$ | 8.26 | 2.91 | 9.11 | 6.82 | 8.68 | 7.47 | 7.86 |
| 11-Methyl $C_{18:1}\omega 7c$ | 15.02 | 7.37 | 18.46 | 12.07 | 10.30 | 14.41 | 13.65 |
| $C_{18:0}$ 3-OH | – | 4.12 | – | 1.10 | – | – | – |
| $C_{20:1}$ | 3.08 | – | 3.26 | 3.13 | 4.59 | 3.98 | 6.75 |

Data were obtained from the present study. –, Not detected. Fatty acids representing < 1% in all strains tested are not shown

It is evident from the results obtained that novel isolates can be assigned to the genus *Labrenzia* on the basis of their physiological, biochemical, and chemotaxonomic characteristics. Five novel isolates could be distinguished from the most *Labrenzia* members which are monotrichous, in the number and flagella arrangement forming 2–5 subpolar and/or lateral flagella, in not being able to assimilate most compounds which are included to the API 20 NE and API 32GN strips, as well as in enzymes activities in API ZYM, in the absence of SQDG in polar lipids and antibiotic sensitivity profiles. These strains differed from *L. suaedae* KACC 13772$^T$ in the requirement of sodium ions for growth, in the inability to grow in 9% NaCl and at 41 °C, to reduce nitrate to nitrite, to produce $H_2S$ from thiosulfate, to hydrolyze gelatin, to assimilate glucose, arabinose, mannitol, *N*-acetylglucosamine, malate and citrate, and to produce *N*-acetyl-β-glucosaminidase. Differential phenotypic characteristics are listed in Table 1 and Supplementary Table 1. Based on the results obtained, it is proposed to assign five novel strains KMM 9697, KMM 9698, KMM 9699$^T$, KMM 9704, and KMM 9705 to the genus *Labrenzia* as representing a novel species, *Labrenzia polysiphoniae* sp. nov. with the type strain KMM 9699$^T$.

## Description of Labrenzia polysiphoniae sp. nov

***Labrenzia polysiphoniae*** **(po.ly.si.pho'ni.ae. N.L. fem. gen. n.** ***polysiphoniae*** **of** ***Polysiphonia*****, pertaining to the isolation source of red alga** ***Polysiphonia*** **sp.)**

Gram-negative, aerobic, oxidase-positive, catalase-positive, ovoid- or rod-shaped bacteria motile by means of 2–5 subpolar and/or lateral flagella cells, 0.6–0.8 μm in diameter and 1.5–2.0 μm in length. Bacteria are capable of producing extracellular material. Cells could be observed as weakly motile or non-motile due to their property to lose flagella easily. Cells occurred as a single or formed star-shaped aggregates. On Marine Agar 2216 hemi-transparent, lightly cream pigmented, shiny and smooth colonies of 2–3 mm diameter with the regular edges are produced. Grows on tryptic soya agar supplemented with sea water. Bchl *a* is not produced. Required NaCl for growth; growth occurred between 1 and 8% (w/v) NaCl with an optimum of 3–4% NaCl. The temperature range for growth was 7–39 °C with an optimum of 28–30 °C. The pH range for growth was 5.5–10.0 (optimal pH 6.5–7.5). Some strains could grow at pH 5.0–10.5. Negative for hydrolysis of casein, gelatin, chitin, starch, Tween 80, xanthine, hypoxanthine and production of $H_2S$ in conventional tests. Positive for DNA hydrolysis; hydrolysis of L-tyrosine is strain-dependent (the type strain reaction is slow). Negative for nitrate reduction in routine and API 20NE tests. According to the API 20NE, positive for PNPG test; and negative for aesculin and gelatin hydrolysis, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, D-gluconate, L-malate, caprate, adipate, citrate, and phenylacetate. According to the ID32 GN, strains could not assimilate any of substrates included to the ID32 GN gallery. Positive API ZYM test results were obtained for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase; and negative for lipase (C14), cystine arylamidase, valine arylamidase, α-chymotrypsin, α-galactosidase, β-glucosidase, α-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase; β-galactosidase and trypsin tests are variable (reactions are positive for the type strain). The major isoprenoid quinone is ubiquinone Q-10. Polar lipids consisted of phosphatidylcholine, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, an unknown aminophospholipid, and an unknown phospholipid. Fatty acid $C_{18:1}\omega7c$ was predominant followed by 11-Methyl $C_{18:1}\omega7c$, and $C_{14:0}$ 3-OH. Strains were susceptible to gentamicin, rifampicin, ofloxacin, streptomycin, nalidixic acid, neomycin, erythromycin, tetracycline, and resistant to ampicillin, benzylpenicillin, lincomycin, oxacillin, oleandomycin, and polymyxin. Susceptibility to vancomycin, kanamycin, carbenicillin, doxocycline, cephazolin, and cephalexin was strain-dependent (type strain was resistant to above antibiotics). The DNA G+C content is 56.5–58.0 mol%, the DNA G+C content of the type strain KMM 9699$^T$ is 57.3 mol%. The type strain of the species is strain KMM 9699$^T$ (=rh46$^T$=KACC 19711$^T$) isolated from red alga *Polysiphonia* sp. specimen, collected from the Sea of Japan seashore, Russia.

**Acknowledgements** We would like to thank Dr. Soon-Wo Kwon, Korean Agricultural Culture Collection, KACC, Microbial Genetics Division, National Institute of Agricultural Biotechnology, RDA, Republic of Korea, for providing the type strain *Labrenzia suaedae* KACC 13772$^T$ for comparative analyses. This study was supported by a programme of "Bioresource collections".

## Compliance with ethical standards

**Conflict of interest** All the authors have declared no conflict of interest.

## References

Bibi F, Jeong JH, Chung EJ, Jeon CO, Chung YR (2014) *Labrenzia suaedae* sp. nov., a marine bacterium isolated from a halophyte, and emended description of the genus *Labrenzia*. Int J Syst Evol Microbiol 64:1116–1122

Biebl H, Pukall R, Lünsdorf H, Schulz S, Allgaier M, Tindall BJ, Wagner-Döbler I (2007) Description of *Labrenzia alexandrii* gen. nov., sp.nov., a novel alphaproteobacterium containing bacteriochlorophyll *a*, and a proposal for reclassification of *Stappia*

*aggregata* as *Labrenzia aggregata* comb.nov., of *Stappia marina* as *Labrenzia marina* comb. nov. and of *Stappia alba* as *Labrenzia alba* comb. nov., and emended descriptions of the genera *Pannonibacter, Stappia* and *Roseibium*, and of the species *Roseibium denhamense* and *Roseibium hamelinense*. Int J Syst Evol Microbiol 57:1095–1107

Camacho M, Redondo-Gomez S, Rodriguez-Llorente I, Rohde M, Sproer C, Schumann P, Klenk HP, Montero-Calasanz MD (2016) *Labrenzia salina* sp. nov., isolated from the rhizosphere of the halophyte *Arthrocnemum macrostachyum*. Int J Syst Evol Microbiol 66:5173–5180

Collins MD, Shah HN (1984) Fatty acid, menaquinone and polar lipid composition of *Rothia dentosacariosa*. Arch Microbiol 137:247–249

Collins MD, Goodfellow M, Minnikin DE (1980) Fatty acid, isoprenoid quinone and polar lipid composition in the classification of *Curtobacterium* and related taxa. J Gen Microbiol 118:29–37

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Devereux R, Wilkinson SS (2004) Amplification of ribosomal RNA sequences. Molecular microbial ecology manual, 2nd edn, vol 3.01, Kluwer Academic Publishers, Dordrecht, pp 509–522

Edwards U, Rogall T, Blöcker H, Emde M, Böttger EC (1989) Isolation and direct complete nucleotide determination of entire genes: characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843–7853

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) (1994) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC

Hameed A, Shahina M, Lin SY, Lai WA, Hsu YH, Liu YC, Young CC (2014) *Aquibacter zeaxanthinifaciens* gen. nov., sp. nov., a zeaxanthin-producing bacterium of the family *Flavobacteriaceae* isolated from surface seawater, and emended descriptions of the genera *Aestuariibaculum* and *Gaetbulibacter*. Int J Syst Evol Microbiol 64:138–145

Kim BC, Park JR, Bae JW, Rhee SK, Kim KH, Oh JW, Park YH (2006) *Stappia marina* sp. nov., a marine bacterium isolated from the Yellow Sea. Int J Syst Evol Microbiol 56:75–79

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351

Lafay B, Ruimy R, Rausch de Traubenberg C, Breittmayer V, Gauthier MJ, Christen R (1995) *Roseobacter algicola* sp. nov., a new marine bacterium isolated from the phycosphere of the toxin-producing dinoflagellate *Prorocentrum lima*. Int J Syst Bacteriol 45:290–296

Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J Mol Biol 5:109–118

Mitchell K, Fallon RJ (1990) The determination of ubiquinone profiles by reversed-phase high performance thin-layer chromatography as an aid to the speciation of *Legionellaceae*. J Gen Microbiol 136:2035–2041

Owen J, Hill LR, Lapage SP (1969) Determination of DNA base composition from meltingprofiles in dilute buffers. Biopolymers 7:503–516

Pearson W, Lipman DJ (1988) Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 85:2444–2448

Pujalte MJ, Macián MC, Arahal DR, Garay E (2005) *Stappia alba* sp. nov., isolated from Mediterranean oysters. Syst Appl Microbiol 28:672–678

Romanenko LA, Uchino M, Frolova GM, Tanaka N, Kalinovskaya NI, Latyshev N, Mikhailov VV (2007) *Sphingomonas molluscorum* sp. nov., a novel marine isolate with antimicrobial activity. Int J Syst Evol Microbiol 57:358–363

Romanenko LA, Tanaka N, Svetashev VI, Kalinovskaya NI (2011) *Pacificibacter maritimus* gen. nov., sp. nov., isolated from shallow marine sediment. Int J Syst Evol Microbiol 61:1375–1381

Romanenko LA, Tanaka N, Svetashev VI, Kurilenko VV, Mikhailov VV (2013) *Luteimonas vadosa* sp. nov., isolated from seashore sediment. Int J Syst Evol Microbiol 63:261–1266

Romanenko LA, Kurilenko VV, Chernysheva NYU, Kalinovskaya NI, Dmitrenok PS, Popov RS, Mikhailov VV (2017) Diversity and antimicrobial activity of hydrobionts associated microorganisms from the Sea of Japan with the occurrence of tropodithietic acid producing bacteria. Microbiol Res J Int 20(6):1–14

Sasser M (1990) Microbial identification by gas chromatographic analysis of fatty acid methyl esters (GC-FAME).Technical Note 101. MIDI, Newark

Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microb Today 45:153–155

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Uchino Y, Hirata A, Yokota A, Sugiyama J (1998) Reclassification of marine *Agrobacterium* species: proposals of *Stappia stellulata* gen. nov., comb. nov., *Stappia aggregata* sp. nov., nom. rev., *Ruegeria atlantica* gen. nov., comb. nov., *Ruegeria gelatinovora* comb. nov., *Ruegeria algicola* comb. nov., and *Ahrensia kieliense* gen. nov., sp. nov., nom. rev. J Gen Appl Microbiol 44:201–210

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464