The family *Pseudoalteromonadaceae*, belonging to the order *Alteromonadales* of the class *Gammaproteobacteria*, is a morphologically, metabolically and ecologically diverse group [1]. Members of this family are motile by means of one or several flagella, rod-shaped, strictly aerobic or facultatively anaerobic chemoorganotrophs [1–5]. In most species, the major isoprenoid quinone is ubiquinone Q-8. The major fatty acids are C_{16:0}, C_{16:1ω7c} and C_{18:1ω7c}. This family contains three genera, *Pseudoalteromonas*, *Algicola* and *Psychrosphaera*, members of which have been isolated from various habitats, such as coastal, open and deep-sea waters, sediments, marine invertebrates, fish and algae from marine environments.

During the characterization of micro-organisms from a brackish water sample collected from the Tsengwen River (GPS location: 23° 2’ 5” N 120° 9’ 40” E), Tainan County, Taiwan, a bacterial strain, designated LSN-49^T, was isolated on R2A agar (BD Difco) and subjected to detailed taxonomic analyses. Sub-cultivation was performed on R2A agar at 25°C for 48–72 h. The isolate was preserved at −80°C in R2A broth with 20% (v/v) glycerol or by lyophilization.

Genomic DNA was isolated using a bacterial genomic kit (DP02-150, GenMark Technology) and the 16S rRNA gene sequence was analyzed as described previously by Chen *et al.* [6]. Primers 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1541R (5’-AAGGAGGTATCTCAG-3’) were used for amplification of bacterial 16S rRNA genes by PCR [7, 8]. The PCR product was purified, and direct sequencing was performed by using sequencing primers 27F, 1541R, 520F and 800R [7, 8] with an ABI Prism 3730 DNA sequencer (Applied Biosystems). The sequenced length of the 16S rRNA gene was 1465 bp for LSN-49^T and this was compared with those available from the EzBioCloud [9], the
Ribosomal Database Project [10] and the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Analysis of the sequence data was performed using the software package BioEdit [11] and MEGA 7 [12], after multiple alignments of the data by CLUSTAL X version 2.0 [13]. Distances (corrected according to Kimura’s two-parameter model; [14]) were calculated and clustering was performed with the neighbour-joining method [15]. The maximum-likelihood [16], maximum-parsimony [17] and minimum-evolution [18] algorithms contained in the PHYLIP software package [19]. In each case bootstrap values were calculated based on 1000 replications.

The results of phylogenetic analyses based on 16S rRNA gene sequences indicated that LSN-49\(^T\) formed a distinct phylogenetic lineage with respect to the closely related genera *Pseudoalteromonas*, *Algicola* and *Psychrosphaera* in the family *Pseudoalteromonadaceae* in the neighbour-joining tree (Fig. 1). The overall topologies of the phylogenetic trees

![Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *Salsuginimonas clara* LSN-49\(^T\) and type strains of species in the class Gammaproteobacteria. Numbers at nodes are bootstrap percentages of over 70% based on the neighbour-joining (above nodes) and maximum-parsimony (below nodes) tree-making algorithms. Filled circles indicate branches of the tree that were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms. Open circles indicate the corresponding nodes were also recovered in the tree reconstructed with the maximum-parsimony algorithm. *Burkholderia cepacia* ATCC 25416\(^T\) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.](image-url)

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *Salsuginimonas clara* LSN-49\(^T\) and type strains of species in the family *Pseudoalteromonadaceae* in the class Gammaproteobacteria. Numbers at nodes are bootstrap percentages of over 70% based on the neighbour-joining (above nodes) and maximum-parsimony (below nodes) tree-making algorithms. Filled circles indicate branches of the tree that were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms. Open circles indicate the corresponding nodes were also recovered in the tree reconstructed with the maximum-parsimony algorithm. *Burkholderia cepacia* ATCC 25416\(^T\) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.
obtained with the maximum-likelihood and maximum-parsimony methods were similar. These findings were confirmed by analysis based on the minimum-evolution algorithm (see Fig. S1, available in the online Supplementary Material). The results of sequence similarity calculations (over 1400 bp) indicated that LSN-49T was closely related to the species of the genera *Pseudoalteromonas* (89.3–92.1 % 16S rRNA gene sequence similarity), *Algicola* (90.3–90.4 %) and *Psychrosphaera* (89.8–90.1 %), and showed the highest similarity with *Pseudoalteromonas fenneropenaei* rzy34T (92.1 %), *Pseudoalteromonas xiamenensis* Y2T (91.7 %) and *Pseudoalteromonas spongiae* UST010723-006T (91.0 %). Sequence similarities of less than 91 % were observed with the type strains of all other species listed in Fig. 1.

In this study, *Pseudoalteromonas fenneropenaei* KCTC 42730T (=rzy34T), *Pseudoalteromonas xiamenensis* JCM 18779T (=Y2T) and *Pseudoalteromonas spongiae* JCM 12884T (=UST010723-006T) were obtained from the culture collections. The three type strains were used as reference strains and evaluated together under identical experimental conditions to those for LSN-49T.

The morphology of bacterial cells was observed by phase-contrast microscope (DM 2000; Leica) and transmission electron microscopy (H-7500; Hitachi) (Fig. S2) using cells grown in R2A broth at 25°C for lag, exponential and stationary phases of growth. Cellular motility was tested by the hanging drop method [20]. The Gram Stain Set S (BD Difco) kit and the Ryu non-staining KOH method [21] were used for testing the Gram reaction. Colony morphology was observed on R2A agar using a stereo microscopic (SMZ 800; Nikon). Poly-β-hydroxybutyrate granule accumulation was examined under light microscopy after staining of the cells with Sudan black [22] and visualized by UV illumination after directly staining growing bacteria on plates containing Nile red [23].

The pH range for growth was determined by measuring the OD600 of R2A broth. The pH was adjusted prior to sterilization to pH 4–9 (at intervals of 1.0 pH unit) using appropriate biological buffers [24]: citrate/Na2HPO4 buffer, pH range 4.0–5.0; phosphate buffer, pH range 6.0–7.0; and Tris buffer, pH range 8.0–9.0. The temperature range for growth was determined on marine agar at 4–50°C (4, 10, 15, 20, 25, 30, 35, 37, 40, 45 and 50°C). To investigate the tolerance to NaCl, R2A broth was prepared according to the formula of the BD Difco medium with NaCl concentration adjusted to 0, 0.5 and 1.0–6.0 %, w/v (at intervals of 1.0 %). Growth under anaerobic conditions was determined after incubating LSN-49T on R2A agar in the Oxoid AnaeroGen system. Growth was tested on R2A, nutrient, Luria–Bertani, tryptophane and marine agars (all from Difco) under aerobic conditions at 25°C.

LSN-49T and the three reference strains were examined for a broad range of phenotypic properties. Activities of catalase, oxidase, DNase, urease, lipase (corn oil), hydrolysis of starch, casein, gelatin and Tween 20, 40, 60 and 80 were determined using standard methods [25]. Chitin hydrolysis activity was determined using chitinase-detection agar plates (CDA plates). Chitin hydrolysis was visualized by the formation of clear zone around the colonies on CDA plates. CDA plates were prepared as described previously by Wen et al. [26]. Hydrolysis of carboxymethyl cellulose (CM-cellulose) was tested according to the method described by Bowman [27]. Utilization of carbon sources was investigated as described by Chang et al. [28]. Substrates were added at a concentration of 0.1 % (w/v), and incubation was prolonged for 12 days at 25°C under aerobic conditions. Additional biochemical tests were performed using API ZYM, API 20NE and API 50CH kits (all from bioMérieux) according to the manufacturers’ instructions.

Sensitivity to antibiotics of LSN-49T was tested by the disc diffusion method after spreading cell suspensions (0.5 McFarland standard) on R2A agar. The discs (Oxoid) contained the following antibiotics: ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), rifampicin (5 µg), penicillin G (10 U), streptomycin (10 µg), sulfamethoxazole (23.75 µg) plus trimethoprim (1.25 µg) and tetracycline (30 µg). The effect of antibiotics on cell growth was assessed after 2 days at 25°C. A strain was considered to be susceptible to an antibiotic when the diameter of the inhibition zone was more than 12 mm, moderately susceptible at 10–12 mm and resistant at less than 10 mm as described by Nokhal and Schlegel [29]. LSN-49T was sensitive to penicillin G, ampicillin, chloramphenicol, gentamicin, rifampicin, kanamycin, tetracycline, novobiocin, streptomycin, nalidixic acid and sulfamethoxazole plus trimethoprim. Detailed results of physiological, biochemical and morphological characterization of LSN-49T are provided in the genus and species descriptions and Table 1.

The fatty acid profiles of LSN-49T, *Pseudoalteromonas fenneropenaei* KCTC 42730T, *Pseudoalteromonas xiamenensis* JCM 18779T and *Pseudoalteromonas spongiae* JCM 12884T were analyzed on cells grown on marine agar at 25°C for 2 days. Fatty acid methyl esters were prepared and separated according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0), analyzed by GC (5890 Series II Hewlett-Packard) and identified by using the RTSBA6.00 database of the microbial identification system [30]. The detailed fatty acid composition of LSN-49T was C9:0 (2.2 %), C10:0 (2.1 %), C12:0 (1.2 %), C14:0 (5.2 %), C16:0 (10.2 %), C17:0 (2.1 %), C10:0 3-OH (1.5 %), C11:0 3-OH (3.9 %), C12:0 3-OH (5.3 %), iso-C12:0 3-OH (1.7 %), iso-C16:0 (2.3 %), C15:0 10c (6.6 %), C16:1ω7c (6.6 %), C17:1ω8c (44.8 %), C18:1ω7c (8.5 %), summed feature 1 (iso-C15:1ω7c and C13:0 3-OH; 1.1 %) and summed feature 3 (C16:1ω7c and C16:1ω6c; 21.5 %) (Table S1). The major cellular fatty acids of LSN-49T (>10 %) were summed feature 3 (comprising C16:1ω7c and C16:1ω6c) and C17:1ω8c and C16:0.

The polar lipids of LSN-49T, *Pseudoalteromonas fenneropenaei* KCTC 42730T, *Pseudoalteromonas xiamenensis* JCM
Table 1. Characteristics that differentiate LSN-49<sup>T</sup> from other phylogenetically closely related species in the family Pseudoalteromonadaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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</thead>
<tbody>
<tr>
<td>Source</td>
<td>Brackish water</td>
<td>Sediment</td>
<td>Seawater</td>
<td>Sponge</td>
<td>Tunicate</td>
<td>Seawater</td>
<td>Kelp</td>
<td>Neritic seawater</td>
<td>Brackish water</td>
<td>Seawater</td>
<td>Abalone</td>
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<tr>
<td>Colony pigmentation</td>
<td>Shiny, Translucent</td>
<td>None</td>
<td>Dark red</td>
<td>Pale orange</td>
<td>Dark green</td>
<td>None</td>
<td>Red</td>
<td>Light yellow</td>
<td>None</td>
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<tr>
<td>Motility</td>
<td>+</td>
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<td>Relation to O&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>Growth at 0% NaCl</td>
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<td>Maximum NaCl concentration (% w/v)</td>
<td>3</td>
<td>6</td>
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<td>Nitrate reduction</td>
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<td>Catalase</td>
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<td>Casein</td>
<td>+</td>
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<td>Gelatin</td>
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<td>+</td>
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<td>Starch</td>
<td>+</td>
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<td>Distinctive major fatty acids (&gt;10%)</td>
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<td>Major polar lipids</td>
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<td>Major ubiquinone</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
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<td>Q-8</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>51.0</td>
<td>45.3†</td>
<td>45.1†</td>
<td>40.6†</td>
<td>42.2</td>
<td>43.3</td>
<td>46</td>
<td>42.0</td>
<td>38.7</td>
<td>49.5</td>
<td>42.5</td>
</tr>
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</table>

*SF3, Summed feature 3 comprises C<sub>16:1ω7c</sub> and/or C<sub>16:1ω6c</sub>. PE, phosphatidylethanolamine; PG, phosphatidylglycerol.
†Data for DNA G+C content for *Pseudoalteromonas fenneropenaei* KCTC 42730<sup>T</sup>, *Pseudoalteromonas xiamenensis* JCM 18779<sup>T</sup> and *Pseudoalteromonas spongiae* JCM 12884<sup>T</sup> were obtained from Ying *et al.* [33], Zhao *et al.* [34] and Lau *et al.* [45], respectively.
18779T and Pseudoalteromonas spongiae JCM 12884T were extracted from cells grown on MA at 25 °C for 2 days, and analyzed by two-dimensional TLC according to the method of Embley and Wait [31]. Molybdocphosphoric acid, ninhydrin, Zinazade reagent, Dragendorff reagent and α-naphthol reagent were used for the detection of the total polar lipids, amino lipids, phospholipids, choline-containing lipids and glycolipids, respectively. LSN-49T exhibited a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine, (PC), two uncharacterized aminophospholipids (APL1 and APL2), one uncharacterized glycolipid (GLI), four uncharacterized phospholipids (PL1–PL4) and four uncharacterized lipids (L1–L4) (Fig. S3).

LSN-49T exhibited a similar overall polar lipid profile to those of its closest relatives, Pseudoalteromonas fenneropenaeae KCTC 42730T, Pseudoalteromonas xiamenensis JCM 18779T and Pseudoalteromonas spongiae JCM 12884T, and they all had PE, PG, PC, APL1, PL1 and PL4. LSN-49T differed from those closest relatives in the presence and proportions of some uncharacterized polar lipids (Fig. S3). There were an uncharacterized aminophospholipid (APL2), an uncharacterized phospholipid (PL3) and an uncharacterized lipid (L3) present only in LSN-49T but absent in tints three closest relatives. In addition, an uncharacterized glycolipid (GL1) was detected in strain LSN-49T and Pseudoalteromonas spongiae JCM 12884T but not in the other two closest relatives. Apparently, Pseudoalteromonas xiamenensis JCM 18779T and Pseudoalteromonas spongiae JCM 12884T contained higher proportions of an uncharacterized phospholipid (PL4) than LSN-49T and Pseudoalteromonas fenneropenaeae KCTC 42730T.

The isoprenoid quinones of LSN-49T, Pseudoalteromonas fenneropenaeae KCTC 42730T, Pseudoalteromonas xiamenensis JCM 18779T and Pseudoalteromonas spongiae JCM 12884T were extracted from cells grown on MA at 25 °C for 2 days, and purified according to the method of Collins and analyzed by HPLC with a Spherisorb ODS column using methanol/1-chlorobutane (100 : 10, v/v) as the mobile phase by HPLC according to the protocol of Mesbah et al. [35], was 51.0±1.0 mol%.

Polyamines were extracted from strain LSN-49T, and analysis was carried out as described by Busse and Auling [36] and Busse et al. [37]. Cells were homogenized in 0.2 M perchloric acid (HClO4) and centrifuged. Polyamines in the resultant supernatant were treated with dansyl chloride solution (7.5 µg ml⁻¹ in aceton), and then were analyzed by HPLC on a D-7000 high-speed liquid chromatograph (Hitachi) with a UV–VIS detector L-7420 (Hitachi). The polyamine pattern of LSN-49T contained putrescine (PUT, 85.7 %) and spermidine (SPD, 14.3 %).

Several morphological, physiological and biochemical properties, such as colony pigmentation, growth at 0 % NaCl and growth at lower NaCl tolerance (3 %), distinguished LSN-49T from the type strains of species of the phylogenetically related genera in the family Pseudoalteromonadaceae, such as Pseudoalteromonas fenneropenaeae KCTC 42730T, Pseudoalteromonas xiamenensis JCM 18779T, Pseudoalteromonas spongiae JCM 12884T, Pseudoalteromonas tunicata D2T, Pseudoalteromonas haloplanktis ATCC 14393T, Algicola bacteriolitica IAM 14595T, Algicola sagamiensis B-10-31T, Psychrosphaera saromensis SA4-48T, Psychrosphaera aesteruarii PSC101T and Psychrosphaera halotiis KDW4T (Table 1). Furthermore, inability to grow at lower temperature (<20 °C) and ability to reduce nitrate to nitrite of LSN-49T separate it from these phylogenetically closely related strains except for Pseudoalteromonas fenneropenaeae KCTC 42730T. More importantly, LSN-49T could be also differentiated from these phylogenetically closely related strains on the basis of chemotaxonomic characteristics, such as the higher DNA G+C content (Table 1).

Additionally, the fatty acid profile of LSN-49T is compared with those of members of the most phylogenetically closely related genera in Table S1. Obviously, summed feature 3 (C₁₀₁₉₇c and/or C₁₆₀₆c) and C₁₆₀ were commonly found to be the major components in the type strains of phylogenetically related genera of the family Pseudoalteromonadaceae. However, LSN-49T, Pseudoalteromonas fenneropenaeae KCTC 42730T, Psychrosphaera aesteruarii PSC101T and Psychrosphaera halotiis KDW4T contained relatively higher amounts of C₁₇₁₈c (>10 %), and LSN-49T, Pseudoalteromonas haloplanktis JCM 20767T and Psychrosphaera aesteruarii PSC101T had relatively lower amounts of C₁₈₁₇₆c (<10 %). In addition, small amounts of C₁₆₀ (2.2 %) were found in LSN-49T only, and not in other type strains. Also, LSN-49T contained relatively higher amounts of C₁₇₁₈c and relatively smaller amounts of C₁₆₀ when compared with other type strains.

Phosphatidylethanolamine and phosphatidylglycerol were the major polar lipids detected in LSN-49T, along with small amounts of phosphatidylcholine, uncharacterized aminophospholipid, glycolipid, phospholipid and polar lipid. Phosphatidylethanolamine and phosphatidylglycerol have also been reported to be the major constituents of polar lipids in Psychrosphaera aesteruarii PSC101T and Psychrosphaera halotiis KDW4T [38].

It is now generally accepted that 16S rRNA gene sequence similarities between two bacteria of less than 95 % are an indication of affiliation to different genera [39]. LSN-49T most probably represents a novel species of a novel genus, since the 16S rRNA gene sequence similarity to its closest relatives with validly published names, Pseudoalteromonas fenneropenaeae nursery and Pseudoalteromonas xiamenensis Y2T, is 92.1 and 91.7 %, respectively. Moreover, LSN-49T...
can be readily distinguished from these closest phylogenetic neighbours by chemotaxonomic, physiological and biochemical characteristics. The low levels of 16S rRNA gene sequence similarity between LSN-49\(^T\) and all other members of the family \textit{Pseudoalteromonadaceae} together with differential phenotypic properties (Table 1), indicate that LSN-49\(^T\) represents a novel species of a novel genus within the family \textit{Pseudoalteromonadaceae}, for which the name \textit{Salsuginimonas clara} gen. nov., sp. nov. is proposed.

**DESCRIPTION OF \textit{SALSUGINIMONAS} GEN. NOV.**

\textit{Salsuginimonas} (Sal.su.gi.ni.mo’nas. L. n. salsugo -inis, salted water, brackish water; L. fem. n. monas, a unit, monad; N.L. fem. n. \textit{Salsuginimonas}, a monad from brackish water).

Cells are Gram-staining-negative, aerobic, motile by means of a monopolar flagellum, non-spore-forming and rod-shaped. Poly-\(\beta\)-hydroxybutyrate accumulation is observed. Oxidase and catalase are positive. The predominant quinone is Q-8. Major cellular fatty acids (\(>10\%\)) are summed feature 3 (comprising C\(_{16:1}\)ω7c and/or C\(_{16:1}\)ω6c), C\(_{17:1}\)ω8c and C\(_{16:0}\). Predominant polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The polyamine profile is composed of the major compound putrescine and moderate amounts of spermidine.

The type species is \textit{Salsuginimonas clara}. The DNA G+C content of the type strain of the type species is 51.0 mol%.

**DESCRIPTION OF \textit{SALSUGINIMONAS CLARA} SP. NOV.**

\textit{Salsuginimonas clara} (cla’ra. L. fem. adj. clara clear, bright, shining or brilliant, referring to the colony characteristics).

Displays the following properties in addition to those given in the genus description. Cells grow well on R2A, Luria-Bertani and marine agars and weakly on nutrient agar but not on trypticase soy agar. Cells are approximately 0.3–0.6 \(\mu\)m in diameter and 1.5–3.0 \(\mu\)m in length after 48 h of incubation on R2A agar at 25 \(^\circ\)C. Colonies are shiny, translucent, convex, round and smooth with entire edges. The colony size is approximately 0.9–3.0 \(\mathrm{mm}\) in diameter on R2A agar after 48 h of incubation at 25 \(^\circ\)C. Growth occurs at 20–40 \(^\circ\)C (optimum, 25–30 \(^\circ\)C), at pH 6–10 (optimum, pH 7–8) and with 0–3 \% (w/v) NaCl [optimum, 0–1 \% (w/v)].

Positive for hydrolysis of starch, casein, chitin, DNA and leucine arylamidase, valine arylamidase, \(\alpha\)-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, \(\alpha\)-glucosidase and N-acetyl-\(\beta\)-glucosaminidase activities are present and C14 lipase, cystine arylamidase, trypsin, \(\alpha\)-galactosidase, \(\beta\)-galactosidase, \(\beta\)-glucuronidase, \(\alpha\)-mannosidase and \(\alpha\)-fucosidase activities are absent. In the API 50CH tests, acid is produced from glycerol, d-ribose, d-glucose, d-mannitol, methyl \(\alpha\)-glucoside, ascorbic acid, maltose, glycogen, d-tagatose, 2-keto-enolase and 5-keto-gluconate but not from erythritol, d-arabinose, l-arabinose, d-xylene, L-xylene, d-adonitol, methyl \(\beta\)-xyloside, d-galactose, d-fructose, d-mannose, L-sorbitose, L-rhamnose, dulcitol, inositol, d-sorbitol, methyl \(\alpha\)-mannoside, N-acytelyglucosamine, amygdalin, arbutin, salicin, cellobiose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, xylitol, gentiobiose, turanose, d-lyxose, d-fucose, L-fucose, d-arabitol, L-arabitol, gluconate. N-acetyl-d-galactosamine, dextrin, glycogen, Tween 40 and 80 are utilized as sole carbon sources but D-glucose, d-fructose, d-galactose, lactose, trehalose, sucrose, raffinose, d-rhamnose, d-sorbitol, xylitol, acetic acid, succinic acid and glycerol are not utilized.

The type strain is LSN-49\(^T\) (=BCRC 81005\(^T\)=LMG 29726\(^T\)=KCTC 52439\(^T\)) isolated from a sample of brackish water collected from the Tsengwen River, Tainan County, Taiwan.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**References**


