Luteimonas aquatica sp. nov., isolated from fresh water from Southern Taiwan

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A yellow-pigmented bacterial strain, designated RIB1-20T, isolated from fresh water was investigated by means of a polyphasic taxonomic approach. The cells were Gram-negative, rod-shaped and non-spore-forming. Phylogenetic analyses with the 16S rRNA gene sequence showed that the strain formed a monophyletic branch towards the periphery of the evolutionary radiation occupied by the genus Luteimonas, its two closest neighbours being Luteimonas composti CC-YY255T (96.1 % sequence similarity) and Luteimonas mephitis B1953/27.1T (95.8 %). Strain RIB1-20T was clearly distinguished from both of those type strains using phylogenetic analysis, DNA–DNA hybridization, fatty acid composition data and a range of physiological and biochemical characteristics. It is evident from the genotypic and phenotypic data that strain RIB1-20T represents a novel species of the genus Luteimonas, for which the name Luteimonas aquatica sp. nov. is proposed. The type strain is RIB1-20T (=BCRC 17731T =LMG 24212T).

During the characterization of micro-organisms in water samples collected from a freshwater spring located in Kaohsiung County, Taiwan, a yellow-coloured strain, designated RIB1-20T, was isolated and maintained on R2A agar (BD Difco) plates after incubation at 25 °C for 3 days. Subcultivation was performed on R2A agar at 25 °C for between 48 and 72 h. On this medium, strain RIBI-20T was able to grow at 15–37 °C, but not at 10 or 40 °C. The organism was able to grow on R2A, nutrient agar (BD Difco) and tryptic soy agar (BD Difco).

Cells were observed with phase-contrast microscopy (DM 2000; Leica) in the lag, exponential and stationary phases of growth to ascertain their morphology. Motility was tested by means of the hanging-drop method. The Gram stain set S kit (BD Difco) and the Ryu non-staining KOH method (Powers, 1995) were used to test the Gram-staining reaction. Accumulation of poly-β-hydroxybutyrate granules was investigated using light microscopy after staining of the cells with Sudan black. Colony morphology was observed on R2A agar, using a stereoscopic microscope (SMZ 800; Nikon). Details of the morphology are given in the species description.

The pH range for growth was determined by measuring the OD600 of cultures grown on nutrient broth (BD Difco) adjusted to various pH values (pH 4–10, in increments of 1.0 pH unit), prior to sterilization, using appropriate biological buffers (Chung et al., 1995). To investigate NaCl tolerance, nutrient broth was prepared according to the formula of the BD Difco medium, while NaCl concentrations were varied (0, 0.5 and 1.0–10.0 %, w/v, in increments of 1.0 %). Growth under anaerobic conditions was determined after incubating strain RIB1-20T in an Oxoid AnaeroGen system. Strain RIB1-20T was examined for a broad range of phenotypic properties. Catalase, oxidase, DNase, arginine dihydrolase, urease and lipase activities and hydrolysis of starch, casein and Tweens 20, 40, 60 and 80 were determined using standard methods (Gerhardt et al., 1994; Lánya, 1987; MacFaddin, 2000). Phenotypic characteristics, biochemical data, carbon-source utilization results (Biolog GN2) and API ZYM and API 20NE (both from bioMérieux) profiles were investigated. For G+C content determinations, DNA was prepared and degraded enzymically into nucleosides as described by Mesbah et al. (1989). The nucleoside mixture obtained was then separated by using HPLC. The DNA G+C content of strain RIB1-20T was 70.3 mol%.

The 16S rRNA gene sequence was analysed as described previously Chen et al. (2001). Analysis of the sequence data was performed using BioEdit (Hall, 1999) and MEGA.
version 3.1 (Kumar et al., 2004), after the performance of multiple alignments of the data by CLUSTAL_X (Thompson et al., 1997). A distance matrix method (with distance options according to Kimura’s two-parameter model; Kimura, 1983), including clustering using neighbour-joining (Saitou & Nei, 1987) (Fig. 1 and Supplementary Fig. S1), available in IJSEM Online, and a discrete character-based maximum-parisimony method (Kluge & Farris, 1969) were used. In each case, bootstrap percentages based on 1000 replications were calculated. The 16S rRNA gene sequence of strain RIB1-20T was a continuous stretch of 1481 bp. Sequence-similarity calculations (over 1350 bp) indicated that strain RIB1-20T was most closely related to Luteimonas composti CC-YY255T (96.1 % similarity) and Luteimonas mephitis B1953/27.1T (95.8 %). Lower levels of sequence similarity (<94.0 %) were found with respect to representative members of the other genera shown in Fig. 1 and Supplementary Fig. S1.

DNA–DNA hybridization experiments were performed with L. composti CCUG 53595T and L. mephitis DSM 12574T using the method described by Ezaki et al. (1989). The results indicated low levels of relatedness between strain RIB1-20T and its closest phylogenetic neighbours, L. composti CCUG 53595T (48 ± 5 %) and L. mephitis DSM 12574T (23 ± 2 %). These values are clearly below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne et al., 1987).

Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (Microbial ID; Sasser, 1990). The fatty acid profile of strain RIB1-20T (Table 1) was similar to those given for L. composti and L. mephitis (Young et al., 2007; Finkmann et al., 2000), but there were some significant differences (Table 1).

Sensitivity to antibiotics was tested by spreading cells of strain RIB1-20T (density of 0.5 McFarland standard) on Mueller–Hinton agar (BD Difco) plates and adding antibiotic discs (Oxoid) containing the following: 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 μg), streptomycin (10 μg), sulfamethoxazole (23.75 μg) plus trimethoprim (1.25 μg) or tetracycline (30 μg). The effect of antibiotics on cell growth was assessed after 3 days and susceptibility was scored on the basis of the distance from the edge of the clear zone to the edge of the disc. If the distance was greater than 3 mm the strain was classified as susceptible, if the distance was between 1 and 3 mm the strain was classified as moderately susceptible and if the clear zone was less than 1 mm the strain was considered resistant.

The results of physiological characterization are given in the species description and in Table 2; L. composti CCUG 53595T and L. mephitis DSM 12574T were tested for comparison.

Strain RIB1-20T was capable of producing acid from various carbohydrates. However, carbon-substrate utilization tests involving organic acids as the substrates produced few positive results. In API 20NE tests, strain RIB1-20T showed positive reactions for β-glucosidase, protease, β-galactosidase, oxidase and catalase and for assimilation of glucose, mannose, N-acetylglucosamine, maltose and malate; negative results were obtained for nitrate and nitrite reduction, indole production, glucose acidification, arginine dihydrose and urease and for assimilation of arabinose, mannitol, glucuronate, caprate, adipate, citrate and phenyl acetate. With the API ZYM system, strain RIB1-20T showed positive reactions for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase and produced negative reactions for lipase (C14), trypsin, α-chymotrypsin, x-galactosidase, β-glucuronidase, x-mannosidase and x-fucosidase.

On the basis of these results, we propose that strain RIB1-20T represents a novel species of the genus Luteimonas, for which the name Luteimonas aquatica sp. nov. is proposed.

**Fig. 1.** Phylogenetic tree, based on 16S rRNA gene sequences, for strain RIB1-20T and related taxa. Distances and clustering with the neighbour-joining method were performed by using the software package MEGA, version 3.1. Accession numbers are shown in parentheses. Bootstrap percentages (based on 1000 replications) are shown at branching points. Bar, 0.01 substitutions per nucleotide position. An extended version of this tree is available as Supplementary Fig. S1.
Table 1. Fatty acid compositions of strain RIB1-20T and type strains of Luteimonas species

<table>
<thead>
<tr>
<th>Fatty acid Strain</th>
<th>Strain RIB1-20T</th>
<th>L. mephitis DSM 12574T</th>
<th>L. composti CCUG 53595T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated straight-chain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10:0</td>
<td>–</td>
<td>0.1</td>
<td>–</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.2</td>
<td>0.7</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>2.4</td>
<td>1.6</td>
<td>4.7</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Saturated branched</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C10:0</td>
<td>–</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>iso-C11:0</td>
<td>4.0</td>
<td>5.2</td>
<td>6.5</td>
</tr>
<tr>
<td>anteiso-C11:0</td>
<td>–</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>iso-C13:0</td>
<td>0.2</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>iso-C14:0</td>
<td>0.3</td>
<td>1.5</td>
<td>1.9</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>25.3</td>
<td>47.4</td>
<td>25.5</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>6.2</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>4.4</td>
<td>5.6</td>
<td>13.8</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>21.5</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>1.9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C18:0</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C15:1</td>
<td>–</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>iso-C17:1:1o9c</td>
<td>22.3</td>
<td>18.7</td>
<td>25.5</td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C11:0 3-OH</td>
<td>6.7</td>
<td>5.3</td>
<td>5.5</td>
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<tr>
<td>iso-C12:0 3-OH</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed features*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>0.9</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unknown</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ECL 11.799</td>
<td>1.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ECL 14.263</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cyclopropane acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>–</td>
<td>0.2</td>
<td>–</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contained C16:1o7c and/or iso-C15:0 2-OH. Summed feature 4 contained anteiso-C17:1 B and/or iso-C17:1 I.

Description of Luteimonas aquatica sp. nov.

Luteimonas aquatica (aqua’ti.ca. L. fem. adj. aquatica found in water, aquatic).

Cells are aerobic, Gram-negative, non-spore-forming, non-motile and rod-shaped. Poly-β-hydroxybutyrate granules

Table 2. Phenotypic characteristics that separate strain RIB1-20T from L. composti CCUG 53595T and L. mephitis DSM 12574T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain RIB1-20T</th>
<th>L. composti CCUG 53595T</th>
<th>L. mephitis DSM 12574T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Fresh water</td>
<td>Food waste</td>
<td>Ammonia biofilter</td>
</tr>
<tr>
<td>Reduction of nitrate</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Activity of (API ZYM):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>z-Chymotrypsin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>z-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Assimilation of (API 20NE):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Arabinose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Malate</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oxidation of (Biolog GN2):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Arabitol</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>z-D-Glucose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Formic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>z-Ketobutyric acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>z-Ketoglutaric acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>z-Ketovaleric acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Inosine</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
are not accumulated. After 24 h growth on R2A agar at 25 °C, the mean cell size is about 0.5 μm in width and 0.8–1.0 μm in length. Forms variable colonies that are round with umbolic elevation, smooth-edged and yellow-pigmented. Colonies are approximately 1.2–1.8 mm in diameter on R2A agar after 72 h incubation at 25 °C. Grows at 15, 25, 30 and 37 °C, 0–3 % NaCl and pH 7–8. Optimal growth occurs at 25 °C, 0 % NaCl and pH 7.0. Positive for the following: catalase (weak), cytochrome oxidase and hydrolysis of starch, casein and Tween 20 (weak), 40 (weak), 60 (weak) and 80 (weak). Negative for DNase and lipase (corn oil) activities. Major fatty acids (>20 %) are iso-C₁₅ : 0, iso-C₁₇ : 0 and C₁₇ : 1ω7c. The DNA G+C content of the type strain is 70.3 mol%. Additional phenotypic properties are shown in Table 2. The following compounds are utilized as sole carbon sources (in the Biolog GN2 test system): dextrin, glycogen, Tweens 40 and 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, D-fructose, L-fucose, gentiobiose, α-D-glucoside, D-psicose, D-sorbitol, sucrose, trehalose, turanose, pyruvic acid methyl ester, acetic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, β-hydroxybutyric acid, p-hydroxyphenylacetic acid, α-ketobutyric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, sebacic acid, bromosuccinamic acid, succinic acid, L-alanine, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycylic L-aspartic acid, glycolic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-proline, L-serine, L-threonine, urocanic acid, inosine, uridine and α-D-glucose 1-phosphate. Does not oxidize the remaining substrates of the Biolog GN2 test panel. Resistant to kanamycin, penicillin G and streptomycin, but sensitive to gentamicin, rifampicin, tetracycline, ampicillin, novobiocin, chloramphenicol, nalidixic acid and sulfamethoxazole plus trimethoprim.

The type strain, RIB1-20 T (=BCRC 17731 T = LMG 24212 T), was isolated from a freshwater sample from southern Taiwan.

Acknowledgements

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Table 2. cont.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain</th>
<th>L. composti</th>
<th>L. mephitis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RIB1-20 T</td>
<td>CCUG 53595 T</td>
<td>DSM 12574 T</td>
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<tr>
<td>Susceptibility to:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>S</td>
<td>R</td>
<td></td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>70.3</td>
<td>68.1</td>
<td>67.0</td>
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References


