Algoriphagus olei sp. nov., isolated from oil-contaminated soil

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A motile, Gram-negative, pinkish red-pigmented bacterium (strain CC-Hsuan-617T) was isolated from an oil-contaminated soil near an oil refinery located in Kaohsiung County, Taiwan. 16S rRNA gene sequence analysis showed that strain CC-Hsuan-617T clustered with Algoriphagus mannitoliivorans (97.5 % sequence similarity), Algoriphagus aquatilis (98.0 %) and Algoriphagus boritolerans (97.5 %), followed by Algoriphagus ornithinivorans (97.1 %) and Algoriphagus alkaliphilus (97.1 %). The fatty acid profile of the novel strain was slightly different from those reported for recognized Algoriphagus species. The quinone system contained menaquinone MK-7 as the predominant component. The major polar lipids were phosphatidylethanolamine, an unknown polar lipid, an unknown phospholipid and an unknown aminophospholipid. The main cell polyamine was sym-homospermidine; lesser amounts of spermine and spermidine were also found. The results of DNA–DNA hybridization, and physiological and biochemical tests allowed the genotypic and phenotypic differentiation of strain CC-Hsuan-617T from recognized Algoriphagus species. Strain CC-Hsuan-617T is thus considered to represent a novel species of the genus Algoriphagus, for which the name Algoriphagus olei sp. nov. is proposed. The type strain is CC-Hsuan-617T (=BCRC 17886T=CCUG 57471T).

During the characterization of micro-organisms from an oil-contaminated soil near the oil refinery located in Kaohsiung County, Taiwan, strain CC-Hsuan-617T was isolated and then subsequently routinely grown on nutrient agar (Hi-Media) after incubation at 30 °C for 48 h. Subcultivation was done on nutrient agar at 30 °C for 48 h up to 3 days. On this medium, strain CC-Hsuan-617T was able to grow at 25–40 °C, but not at 45 or 15 °C. The organism was able to grow on nutrient agar, tryptone soy (TS) agar (Hi-Media) and R2A agar (Oxoid). Gram-staining was performed as described by Gerhardt et al. (1994). Accumulation of poly-β-hydroxybutyrate granules was observed under light microscopy after staining cells with Sudan black. Phenotypic characteristics, biochemical tests (Gerhardt et al., 1994) and carbon source utilization (Biolog-GNII), and API ZYM enzyme profiles, API 20E and API 20NE (bioMérieux) were also investigated. Physiological tests were performed according to Kämpfer et al. (1991). Antibiotic susceptibility testing was carried out by using ABT STAPH 5 strips (bioMérieux) according to the manufacturer’s recommendations. Fluorescence was tested after plating on King’s B medium for 48 h. For analysis of DNA G+C content, a DNA sample was prepared and degraded enzymically into nucleosides as described by Mesbah et al. (1989). The nucleoside mixture obtained was then separated via HPLC. The DNA G+C content of strain CC-Hsuan-617T was 43 mol%. DNA–DNA hybridization experiments were performed with the type strain of Algoriphagus man nitoliivorans by using the method described by Ziemke et al. (1998), except that for nick translation, 2 μg DNA was labelled during 3 h of incubation at 15 °C.

Cell morphology was observed under a Zeiss light microscope at ×1000 magnification with cells that had been grown for 3 days at 30 °C on nutrient agar (Oxoid).
The results are given in the species description. The pH range for growth was determined by measuring the optical density (wavelength 595 nm, OD595) of the culture grown in nutrient broth (Difco), which was adjusted prior to sterilization to various pH (3–11 at intervals of 0.5 pH units) by using appropriate biological buffers (Chung et al., 1995). Growth at various temperatures (10–50 °C) was measured in nutrient broth. Growth under anaerobic conditions was determined after incubation in an Oxoid AnaeroGen system on nutrient agar. Growth was again recorded by measuring OD595 of the culture.

16S rRNA gene sequence analysis was performed as described previously (Young et al., 2005). Analysis of the sequence data was performed by using the software package MEGA version 2.1 (Kumar et al., 2001), after multiple alignments of the data via CLUSTAL_X (Thompson et al., 1997). A distance matrix method (distance options according to the Kimura two-parameter model), including clustering with the neighbour-joining method (Fig. 1), and a discrete character-based maximum-parsimony method were used. In each case, bootstrap values were calculated based on 1000 replications. The 16S rRNA gene sequence of strain CC-Hsuan-617T was a continuous stretch of 1375 bp. Strain CC-Hsuan-617T was related most closely to the type strains of *Algoriphagus aquatilis* (98.0 % 16S rRNA gene sequence similarity), *Algoriphagus mannitolivorans* (97.5 %), *Algoriphagus boritolerans* (97.5 %), *Algoriphagus ornithinivorans* (97.1 %) and *Algoriphagus alkaliphilus* (97.1 %); lower levels of 16S rRNA gene sequence similarity (<97.0 %) were found between strain CC-Hsuan-617T and the type strains of all other species shown in Fig. 1 in the family *Flexibacteraceae*.

Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (MIDI; Microbial ID). The fatty acid profile of strain CC-Hsuan-617T (given in the species description below) was similar to those for recognized *Algoriphagus* species, but showed some minor differences (Table 1). Conditions for growth of biomass used for chemotaxonomic analyses and the methods for extraction and analyses of polyamines, quinone system components and polar lipids were as described by Busse & Auling (1988), Tindall (1990a, b), Altenburger et al. (1996) and Stolz et al. (2007). The quinone system comprised menaquinone MK-7 (99.4 %) as the predominant component, with MK-6 as a trace component (0.6 %). This is in accordance with the emended description of the genus *Algoriphagus* provided by Nedashkovskaya et al. (2004).

The profile of major polar lipids comprised phosphatidylethanolamine, and an unknown polar lipid and an unknown aminolipid (exhibiting almost identical chromatographic behaviour; Fig. 2), an unknown phospholipid and an unknown aminophospholipid. Copa-Patiño et al. (2008) noted that the polar lipid profile of *Algoriphagus hitonicola* supports its affiliation to the genus but provided no supporting data; no details concerning polar lipid

![Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences available from GenBank/EMBL/DDBJ (accession numbers in parentheses) constructed after multiple alignments of the data by CLUSTAL_X (Thompson et al., 1997). Distances (options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were determined by using the software package MEGA version 2.1 (Kumar et al., 2001). Bootstrap percentages based on 1000 replications are given at branch points. Bar, 0.02 nt substitutions per nucleotide position.](image-url)
Table 1. Fatty acid composition (%) of strain CC-Hsuan-617T and *Algoriphagus* species

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*Summed features consist of one or more fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 contains iso-C15:0 2-OH, C16:1:07c and/or C16:1:07t; summed feature 4 contains iso-C17:1 I, anteiso-C17:1 B and/or C16:1:07t.

Composition are available for other recognized *Algoriphagus* species. However, the polar lipid of strain CC-Hsuan-617T is significantly different from other representatives of the Bacterioideae, such as members of the genera *Hymenobacter* (Buczolits et al., 2002, 2006), *Chryseobacterium* (Kämpfer et al., 2003) and *Arcicella* (Kämpfer et al., 2009). Following polar lipid analysis, two red pigment spots were detected that exhibited highly hydrophobic chromatographic behaviour and stained positively with α-naphthol, indicating the presence of a sugar moiety. The main cell polyamine was sym-homospermidine [26.6 μmol (g dry weight)⁻¹], with lesser amounts of spermine [9.1 μmol (g dry weight)⁻¹] and spermidine (4.3 μmol (g dry weight)⁻¹]. A trace amount of putrescine [0.2 μmol (g dry weight)⁻¹] was detected. As far as we are aware, no data have been reported regarding the polyamine patterns of recognized *Algoriphagus* species. A similar polyamine pattern with sym-homospermidine as the predominant component was reported for *Cyclobacterium marinum* (Hamana & Nakagawa, 2001), which is closely related to *Algoriphagus* species (Fig. 1), suggesting that this trait is characteristic of the clade including these genera.

Results of the physiological characterization are given in the species description and in Table 2. Strain CC-Hsuan-617T was non-fluorescent, unable to produce acid from various carbohydrates and showed few positive results for carbon substrate utilization tests with organic acids as substrate. It was positive for ONPG and indole production (API 20E), and positive for oxidase, aesculin hydrolysis, -galactosidase, -acetylglucosaminidase and assimilation of D-glucose, D-mannose, N-acetylglucosamine and maltose (API 20NE). These results were confirmed with the methods described by Kämpfer et al. (1991). Results with the API-ZYM system are given in the species description. Strain CC-Hsuan-617T showed a relatively low level of DNA–DNA relatedness (Wayne et al., 1987) to A. mannitolivorans KCTC 12050T (20.8%).

On the basis of the data presented, we consider that strain CC-Hsuan-617T represents a novel species of the genus.
Algoriphagus, for which the name Algoriphagus olei sp. nov. is proposed.

**Description of Algoriphagus olei sp. nov.**

*Algoriphagus olei* (o’le.i. L. gen. neut. n. olei of/from oil, as the type strain was isolated from oil-contaminated soil).

Cells are Gram-negative, aerobic, non-motile rods (0.4–0.8 μm in diameter and non-fluorescent. Optimal temperature for growth is 32 °C. Optimal pH for growth is 8.0; growth occurs at pH 7 and 9, but not at pH 6.0 or 10. Growth occurs in the presence of 0–3.0% (w/v) NaCl; optimal growth occurs in the presence of 0–1% (w/v) NaCl. No anaerobic growth is seen on plain nutrient agar or TS agar at 32 °C. Colonies on complex standard media at 32 °C are pinkish red, smooth, shiny and convex with a spreading edge, 1.0–2.0 mm in diameter and non-fluorescent. Optimal temperature for growth is 32 °C. Optimal pH for growth is 8.0; growth occurs at pH 7 and 9, but not at pH 6.0 or 10. Growth occurs in the presence of 0–3.0% (w/v) NaCl; optimal growth occurs in the presence of 0–1% (w/v) NaCl. No anaerobic growth is seen on plain nutrient agar or tripticine soy agar supplemented with nitrate. No accumulation of poly-β-hydroxybutyrate granules is observed. Shows aerobic metabolism. Oxidase-, catalase- and aesculin-positive. Nitrate is not reduced to nitrite. The fatty acid profile of the type strain is shown in Table 1. The quinone system comprises menaquinone MK-7 as the predominant component. The polar lipid profile consists of phosphatidylethanolamine, an unknown polar lipid, an unknown phospholipid and an unknown aminophospholipid. The main cell polyamine is sym-homospermidine; lesser amounts of spermine and spermidine are found. The red pigment consists of at least two highly hydrophobic components which contain a sugar moiety. Positive for utilization, H2S production, urease, acetoin production, lysine decarboxylase and ornithine decarboxylase, citrate utilization, H2S production, urease, acetoin production, gelatinase, oxidation of D-mannitol, inositol, D-sorbitol, L-rhamnose, amygdalin and L-arabinose, fermentation of glucose, and assimilation of L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In the API ZYM system, positive for alkaline phosphatase, esterase, succinic acid monomethyl ester, acetic acid, α-ketobutyric acid, α-ketovaleric acid, propionic acid, L-alaninamide, L-alanine, L-glutamic acid, glycolyl-L-aspartic acid, L-leucine, L-ornithine, L-threonine, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Negative for α-cyclodextrin, dextrin, glycojen, Tween 40, Tween 80, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, i-erythritol, L-fucose, myo-inositol, D-mannitol, D-psicose, L-rhamnose, D-sorbitol, xylitol, pyruvic acid methyl ester, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucaric acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinic acid, glucuronamide, D-alanine, L-alanyl glycine, L-asparginine, L-aspartic acid, glycolyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol and DL-α-glycerol phosphate. Positive reactions (API 20E and 20NE) for β-galactosidase (ONPG), oxidase, aesculin, indole production, and assimilation of D-glucose, D-mannose, N-acetylglucosamine and maltose, but negative reactions for nitrate reduction, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase, citrate utilization, H2S production, urease, acetoin production, gelatinase, oxidation of D-mannitol, inositol, D-sorbitol, L-rhamnose, amygdalin and L-arabinose, fermentation of glucose, and assimilation of L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In the API ZYM system, positive for alkaline phosphatase, esterase,
Table 2. Differential phenotypic characteristics between strain CC-Hsuan-617\textsuperscript{7} and recognized "Algoriphagus" species

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<tr>
<td>Gentamicin</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Tetracycline</td>
<td>-</td>
<td>ND</td>
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<td>DNA G+C content (mol%)</td>
<td>43</td>
<td>43</td>
<td>43,5</td>
<td>39–41</td>
<td>41</td>
<td>42,5</td>
<td>37–40</td>
<td>37</td>
<td>42</td>
<td>42</td>
<td>43</td>
<td>38</td>
<td>35</td>
<td>49</td>
<td>39–42</td>
<td>41</td>
<td>43,8</td>
</tr>
</tbody>
</table>
esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase, but negative for lipase, α-galactosidase, β-glucuronidase and β-fucosidase. Sensitive to cotrimoxazole, erythromycin, clindamycin, tetracycline, minocycline, vancomycin, teicoplanin, rifampicin, nor/quinolones 2G, levofloxacin, fusidic acid, nitrofurantoin and quinupristin-dalfopristin. Resistant to penicillin, gentamicin, Coagulase and oxacillin. Further physiological characteristics are given in Table 2.

The type strain, CC-Hsuan-617T (=BCRC 17886T=CCUG 57471T), was isolated from an oil-contaminated soil near an oil refinery located in Kaohsiung County, Taiwan.

Acknowledgements

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