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Strain LMG 24366T was isolated from a marine electroactive biofilm grown on a stainless steel cathode (Genova, Italy) and was investigated by using a polyphasic taxonomic approach. This study demonstrated that strain LMG 24366T represents a novel species within the genus *Leisingera*, which shared 98.9 % 16S rRNA gene similarity with its nearest phylogenetic neighbour, *Leisingera methylhalidivorans*. Strain LMG 24366T grew on betaine (1 mM) as a sole carbon source, whereas no growth was observed on L-methionine (10 mM). The phenotypic and genotypic analyses showed that strain LMG 24366T could be differentiated from established *Leisingera* species and that it represented a novel species, for which the name *Leisingera aquimarina* sp. nov. is proposed. The type strain is LMG 24366T (=CCUG 55860T) and has a DNA G+C content of 61.4 mol%.

The genus *Leisingera* was created by Schaefer *et al.* (2002) to accommodate *Leisingera methylhalidivorans*. The only species of this genus was isolated from a tide pool off the coast of California and was able to grow on methyl bromide as a sole energy and carbon source (Schaefer *et al.*, 2002). Although oceans act as a sink for methyl bromide (Yvon & Butler, 1996), it was the first species shown to be able to grow on methyl bromide. Growth of *L. methylhalidivorans* has also been observed on methyl chloride, methyl iodide and methionine. These data indicate a role for *L. methylhalidivorans* in the degradation of methyl halides and thus in the release of bromide and chlorine atoms in the stratosphere, which contributes to the catalytic destruction of ozone (Butler, 1999).

The present study formed part of an analysis of the microbial diversity of a marine electroactive biofilm grown on a stainless steel cathode [EA BIOFILMS-508866 (NEST)], exposed to natural seawater at the ISMAR-CNR Marine Station, located in the port of Genoa, Italy (Faimali *et al.*, 2008; Vandecandelaere *et al.*, 2008). The biofilm was removed from the stainless steel cathode by sonication (Branson 3200) (90 s) in a sterile plastic tube containing 30 ml 0.85 % NaCl solution. Diluted cell suspensions (10⁻² to 10⁻⁶) were inoculated on marine agar 2216 (MA; Difco) and incubated aerobically at 20 °C for several days. Pure cultures were obtained and isolates were stored at −20 or −80 °C using MicroBank vials.

Whole-cell fatty acid methyl ester analysis, performed as described by Mergaert *et al.* (2001), indicated that strain LMG 24366T belonged to the *Alphaproteobacteria*. The predominant fatty acids were C₁₀ : 0 3-OH (2.0 %), C₁₂ : 0 3-OH (2.1 %), C₁₆ : 0 (3.5 %), C₁₆ : 0 2-OH (4.2 %), C₁₄ : 1 iso E (11.6 %), C₁₈ : 1ω7c (71.6 %) and an unknown fatty acid of equivalent chain-length 11.799 (2.7 %). The remaining fatty acids constituted minor fractions only (<1.0 %). The predominant fatty acids observed were very similar to those of *Phaeobacter daeponensis*, *Phaeobacter gallaeciensis*,

[The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LMG 24366T is AM900415.]
**Phaeobacter inhibens** and **L. methylhalidivorans** (Yoon et al., 2007), indicating that strain LMG 24366\(^T\) was closely related to **Phaeobacter** species and to **L. methylhalidivorans**.

DNA was extracted according to Pitcher et al. (1989) and an almost-complete 16S rRNA gene sequence was obtained (1396 bp) using the universal primers pA (5′-AGAGTTTGATCTTGCTGAG-3′) and pH (5′-AAGGAGGTGATCCAGCAGC-3′) (Edwards et al., 1989), as described by Margaert et al. (2001). The **Fasta** program was used to find the most similar sequences. These almost-complete 16S rRNA gene sequences (1248–1465 bp) were aligned using **Clustal X** (Thompson et al., 1997) and a neighbour-joining dendrogram was constructed (Saitou & Nei, 1987) using **BioNumerics** 4.61 software (Applied Maths) (Fig. 1).

The numerical analysis indicated that the nearest phylogenetic neighbour of strain LMG 24366\(^T\) was **L. methylhalidivorans** LMG 23656\(^T\) with 98.9% 16S rRNA gene sequence similarity. Members of the genus **Phaeobacter** (P. daeonensis LMG 24139\(^T\), P. inhibens LMG 22475\(^T\) and P. galleaciensis LMG 23163\(^T\)) (Martens et al., 2006; Yoon et al., 2007) were also closely related to strain LMG 24366\(^T\), with 16S rRNA gene sequence similarities of 96.9–97.2%.

Strains LMG 24366\(^T\), **L. methylhalidivorans** LMG 23656\(^T\), P. galleaciensis LMG 23163\(^T\) and P. inhibens LMG 22475\(^T\) were investigated by using repetitive-PCR fingerprinting using the BOX-A1R-primer 5′-CTACGGCAAGGCAGCGCTGACG-3′ (Rademaker et al., 2000; Versalovic et al., 1994). Numerical analysis, using **BioNumerics** 4.61 software showed that strain LMG 24366\(^T\) could be distinguished from its nearest phylogenetic neighbours (Fig. 2).

The DNA G+C content of strain LMG 24366\(^T\) was determined. DNA was enzymically degraded into nucleosides as described by Mesbah et al. (1989). The nucleoside mixture obtained was separated using a Water Breeze HPLC system and Xbridge Shield RP18 column thermostabilized at 37 °C. The solvent used was 0.02 M NH\(_4\)H\(_2\)PO\(_4\) (pH 4.0) with 1.5 % acetonitrile. Non-methylated lambda phage (Sigma) and **Escherichia coli** LMG 2093 DNA were used as calibration reference and control, respectively. The DNA G+C content of LMG 24366\(^T\) was 61.4 mol%, which correlates with that of the genus **Leisingera** as described by Schaefer et al. (2002).

DNA–DNA hybridization experiments were carried out with photobiotin-labelled probes in microplate wells, as described by Ezaki et al. (1989), using a HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements. The hybridization temperature used was 45 °C and reciprocal reactions were performed for each pair of strains. The mean DNA–DNA hybridization value between strain LMG 24366\(^T\) and its nearest phylogenetic neighbour, **L. methylhalidivorans** LMG 23656\(^T\), was 56±2 %; the mean DNA–DNA hybridization value between LMG 24366\(^T\) and **P. galleaciensis** LMG 23163\(^T\) was very low (2±0 %). These data indicated that LMG 24366\(^T\) represents a novel species within the genus **Leisingera** (Wayne et al., 1987).

The following morphological, physiological and biochemical characteristics were evaluated for strain LMG 24366\(^T\). Colony morphology was determined after 4 days incubation at 20 °C on MA. Cells were tested for their Gram reaction and the presence of catalase and oxidase activity.

Growth was examined on nutrient agar (NA), tryptcase soy agar (TSA), R2A and peptone/yeast extract/glucose (PYG) agar (Tan & Rüger, 1999). Growth on **L.-methionine** (10 mM) and betaine (1 mM) was tested as described by Martens et al. (2006). The optimal pH and the optimal growth temperature were determined using R2A supplemented with 1–20 % NaCl, incubated for 2 weeks at 20 °C, and MA incubated at 4–45 °C for 2 weeks, respectively. The effect of pH on growth was analysed using marine broth 2216 (Difco) with a pH that ranged from 5.0 to 10.0 (at intervals of 0.5 pH units), with incubation at 20 °C for 7 days.

Degradation of casein and chitin (Reichenbach & Dworkin, 1981), DNA [using DNA agar (Difco) containing 0.01 % toluidine blue (Merck)], starch and l-tyrosine (Barrow & Feltham, 1993) was tested and the reactions were read after 5 days incubation at 20 °C. Strain LMG 24366\(^T\) was inoculated on Sierra’s medium to determine its lipolytic activity and incubated for 10 days at 20 °C (Sierra, 1957).

The susceptibility to the following antibiotics (Oxoid) was examined on MA plates using the disc diffusion method: cefoxitin (30 μg), gentamicin (30 μg), erythromycin (15 μg), tetracycline (30 μg), streptomycin (25 μg), vancomycin (30 μg), trimethoprim (1.25 μg) and clindamycin (2 μg). Results were read after 5 days incubation at 20 °C.

Biochemical characteristics from commercial microtest galleries (API ZYM and API 20NE; bioMérieux) were studied according to the manufacturer’s instructions. API ZYM was read after 4 h incubation at 20 °C, whereas API 20NE was read after 48 h incubation at 20 °C.

The cell morphology of strain LMG 24366\(^T\) was determined by using transmission electron microscopy. Cells were negatively stained with 2 % uranyl acetate. Ultrathin sections were prepared and analysed as described by Mast et al. (2005) (Fig. 3).

The results of the phenotypic tests are given in Table 1. Strain LMG 24366\(^T\) could be differentiated from **L. methylhalidivorans** and **Phaeobacter** species. On the basis of the phylogenetic, genomic and phenotypic data, LMG 24366\(^T\) represents a novel species within the genus **Leisingera**, for which the name **Leisingera aquimarina** sp. nov. is proposed.

**Description of Leisingera aquimarina** sp. nov.


Cells are ovoid (1 × 1.4 μm), Gram-negative and motile by means of a single polar flagellum. Poly-β-hydroxybutyrate inclusion bodies are present. Colonies are dark beige–pink.
round and 1–2 mm in diameter after 3 days incubation on MA. Growth occurs after 2 days incubation at 20 °C on MA, but not on R2A, NA, TSA or PYG. Temperature range for growth is 4–37 °C; no growth occurs at 40 °C or higher. NaCl range for growth is 1–7 %; pH range for growth is 5.5–9.0; optimal pH for growth is 6.5–8. Growth occurs on betaine (1 mM) as a sole carbon source, but not on L-methionine (10 mM). Catalase- and oxidase-positive. Degradation of gelatin is weakly positive; does not degrade tyrosine, DNA, starch, casein, chitin, aesculin or Tween 80. Positive for leucine arylamidase activity; weak alkaline phosphatase, esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase activities. No activity is detected for esterase (C4), valine arylamidase, acid phosphatase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, arginine dihydrolase, urease or α-fucosidase. Nitrate is not reduced to nitrite or nitrogen. Indole is not produced and glucose is not fermented. Does not assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylgallosamin, maltose, potassium gluconate, capric acid, adipic acid, malate, citrate or phyllic acid. Susceptible to cefoxitin (30 μg), erythromycin (15 μg), tetracycline (30 μg) and

Fig. 1. Neighbour-joining dendrogram depicting the 16S rRNA gene sequences of strain LMG 24366T (*Leisingera aquimarina* sp. nov.) and related species. Bar, 1 % 16S rRNA gene sequence diversity. Bootstrap percentages (1000 replicates) above 50 % are shown.
The type strain, LMG 24366T (Leisingera aquimarina sp. nov.) was isolated from a marine electroactive biofilm grown on a stainless steel cathode (Genoa, Italy).

**Emended description of the genus Leisingera Schaefer et al. 2002**

The description is as given by Schaefer et al. (2002) and Martens et al. (2006) with the following additions. Do not degrade tyrosine, casein or DNA. Does not hydrolyse aesculin or gelatin. No indole production or fermentation of glucose. Do not grow on NA, R2A or PYG. Positive for leucine arylamidase activity. No activity is detected for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase or urease. Does not assimilate D-mannose, D-mannitol, maltose, potassium gluconate, capric acid, adipic acid or phenylacetic acid. Susceptible to cefoxitin (30 μg), erythromycin (15 μg), streptomycin (25 μg) and tetracycline (30 μg). Intermediately susceptible to gentamicin (10 μg). Resistant to vancomycin (30 μg), trimethoprim (1.25 μg) and clindamycin (2 μg).

**Emended description of Phaeobacter inhibens Martens et al. 2006**

The description is as given by Martens et al. (2006) with the following additions. Grows weakly on TSA; does not grow on NA, R2A or PYG. Positive for leucine arylamidase activity; weak acid phosphatase and N-acetyl-β-glucosaminidase activities. No activity is detected for α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase or urease. Susceptible to cefoxitin (30 μg), erythromycin (15 μg), streptomycin (25 μg) and tetracycline (30 μg). Intermediately susceptible to gentamicin (10 μg), resistant to vancomycin (30 μg), trimethoprim (1.25 μg) and clindamycin (2 μg).
Emended description of *Phaeobacter daeponensis* Yoon et al. 2007

The description is as given by Yoon et al. (2007) with the following additions. Grows weakly on TSA and NA; does not grow on PYG or R2A. Does not hydrolyse DNA. No fermentation of glucose. No activity is detected for urease. Susceptible to cefoxitin (30 μg) and vancomycin (30 μg). Intermediately susceptible to erythromycin (15 μg). Resistant to trimethoprim (1.25 μg) and clindamycin (2 μg).

Acknowledgements

This work is funded by the European Commission [EA BIOFILMS-508866 (NEST)]. The Fund for Scientific Research – Flanders (FWO – Vlaanderen) is acknowledged for financial support of P. V.

References


