Algoriphagus faecimaris sp. nov., isolated from coastal sediment

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A Gram-negative, non-motile, non-sporulating bacterial strain, designated LYX05^T, was isolated from coastal sediment of Qingdao, China, on the coast of the Yellow Sea. Strain LYX05^T was aerobic and heterotrophic. The strain grew optimally at 37 °C and pH 7.5 and in the presence of 2% (w/v) NaCl. Colonies were 1–2 mm in diameter, circular, reddish orange and shiny with entire edges on marine agar medium. Cells were rods (0.3–0.5 µm wide and 0.8–1.6 µm long). The dominant fatty acids were iso-C_{15:0} (40.82%) and C_{16:0} (10.45%). The DNA G + C content was 42.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain LYX05^T was phylogenetically related to the members of the genus *Algoriphagus* and the closest relative was *Algoriphagus hitonicola* 7-UAH^T (95.8% 16S rRNA gene sequence similarity). On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain LYX05^T was considered to represent a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus faecimaris* sp. nov. is proposed. The type strain is LYX05^T (=JCM 16561^T =DSM 23095^T =LMG 25474^T).

The genus *Algoriphagus* was proposed by Bowman *et al.* (2003) for heterotrophic, Gram-negative, non-motile, strictly aerobic, pink-pigmented, rod-like, cold-adapted strains, at which time the genus was represented by a single species, *Algoriphagus ratkowskyi*. Phylogenetically, the genus is distantly related to the genus *Cyclobacterium* within the phylum *Bacteroidetes*. On the basis of phylogenetic, phenotypic and genotypic evidence, the members of the genera *Chimaericella* and *Hongiella*, *Chimaericella alkaliphila* (Tiago *et al.*, 2006), *C. boritolerans* (Ahmed *et al.*, 2007), *Hongiella marincola* (Yoon *et al.*, 2004), *H. mannitolivorans*, *H. ornithinivorans* and *H. halophilus* (Yi & Chun, 2004), were reclassified into the genus *Algoriphagus* (Nedashkovskaya *et al.*, 2004, 2007). Currently, the genus *Algoriphagus* contains 19 species. The majority of these were isolated from marine environments or saline lakes (Copa-Patiño *et al.*, 2008; Park *et al.*, 2010; Van Trappen *et al.*, 2004); *Algoriphagus terrigena* was discovered in a soil sample (Yoon *et al.*, 2006), *Algoriphagus aquatilis* was isolated from freshwater (Liu *et al.*, 2009) and *Algoriphagus olei* was recovered from an oil-contaminated soil near an oil refinery (Young *et al.*, 2009). In

this study, we report the taxonomic characterization of an *Algoriphagus*-like bacterial strain, LYX05^T, which was isolated from coastal sediment of Jiaozhou Bay, near the estuary of the river Lichun at Qingdao, China, on the coast of the Yellow Sea (36° 09' N 120° 21' E).

Strain LYX05^T was isolated using the standard dilution-plating technique on marine agar 2216 (MA; Difco) after incubation at 28 °C for 2 days. The morphological, physiological and biochemical characteristics of strain LYX05^T were investigated together with two reference strains, the environmentally similar *Algoriphagus marincola* DSM 16067^T and *A. ratkowskyi* LMG 21435^T, which were obtained from the DSMZ and the BCCM, respectively. The isolate and the reference strains were routinely cultivated on MA at 37, 28 and 16 °C, respectively. Cell morphology and the presence of flagella were determined using transmission electron microscopy (JEM-1200EX; JEOL) with cells grown on MA at 37 °C for 16 h. The Gram reaction, catalase and oxidase activities, absorption maxima of crude extracts and hydrolysis of casein, gelatin, starch and Tweens 20, 40 and 80 were determined as described by Tindall *et al.* (2007). The presence of flexirubin-like pigments was tested according to Reichenbach (1989). Growth in the absence of NaCl was examined in trypticase soy broth (Difco) prepared without NaCl. Growth with 1–10% (w/v) NaCl (in increments of 1%) was investigated in marine broth 2216 (MB; Difco).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LYX05^T is GU072591.

Two supplementary figures and a supplementary table are available with the online version of this paper.

Growth at pH 3.5–10 (in increments of 0.5 pH units) was determined in MB with the pH adjusted using the appropriate biological buffers. Growth was measured by monitoring OD₆₀₀ for 3 days. Growth at 0, 4, 16, 28, 37, 42 and 45 °C was measured on MA. Growth under anaerobic conditions was determined by incubation on MA in an enclosed cylinder with 10 g gallic acid and 100 ml 10 % (w/v) NaOH to absorb oxygen at 28 °C for 5 days.

Carbon-source assimilation was determined using GN MicroPlates (Biolog). Acid production from different carbohydrates was determined using API 50 CH test strips and 50 CHB/E medium (bioMérieux) according to the manufacturer's instructions except that sterile 1.5–2 % (w/v) NaCl was used to prepare the inocula. Additional biochemical assays and enzyme activities were detected with the API 20 E, API 20 NE and API ZYM systems (bioMérieux), according to the manufacturer's instructions except that sterile 1.5 % (w/v) NaCl was used to prepare the inocula. Susceptibility to antibiotics was determined on MA using filter-paper discs containing various antibiotics.

The DNA G + C content was determined by the method of Mesbah & Whitman (1989). For isoprenoid quinone analysis, cell mass of strain LYX05^T and *A. marincola* DSM 16067^T was obtained from cultures prepared in MB at 28 °C. Analysis of fatty acid methyl esters of strain LYX05^T, *A. marincola* DSM 16067^T and *A. ratkowskyi* LMG 21435^T was carried out according to the standard protocol of the Sherlock Microbial Identification System (Microbial ID) (Xie & Yokota, 2003) with cells cultured in MB at 28 °C for 4 days.

The phylogenetic position of strain LYX05^T was determined by 16S rRNA gene sequence analysis. Genomic DNA was prepared according to the protocol of Niemann *et al.* (1997). The 16S rRNA gene sequence was amplified using oligonucleotide primers complementary to highly conserved regions of bacterial 16S rRNA genes. The forward primer was 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse primer was 1492R (5'-GGTTACCTTGT-TACGACTT-3'). Sequences were initially analysed using BLAST (<http://www.ncbi.nlm.nih.gov>; <http://www.eztaxon.org>; Chun *et al.*, 2007) and, from these search results, selected sequences were collected manually. After multiple alignment using CLUSTAL X (Thompson *et al.*, 1997), phylogenetic trees were reconstructed with the neighbour-joining and maximum-parsimony methods using MEGA version 4 (Tamura *et al.*, 2007), according to the default settings.

The morphological, physiological and biochemical characteristics of strain LYX05^T are shown in Table 1, the species description and Supplementary Fig. S1 (available in IJSEM Online). The phenotypic properties of strain LYX05^T were in agreement with those given for the genus *Algoriphagus* (Bowman *et al.*, 2003), such as the absence of motility, the marine habitat and the production of non-diffusible pink and red pigments. However, cell extracts of strain LYX05^T showed an absorption peak maximum at 482 nm, no acid production from amygdalin, salicin or

melezitose and utilization of D-fructose, lactose, maltose and D-mannose. These and other characters differentiated strain LYX05^T from its closest phylogenetic neighbours (Table 1).

An almost-complete 16S rRNA gene sequence of strain LYX05^T, comprising 1397 nt, was determined. In the neighbour-joining phylogenetic tree, strain LYX05^T fell within the cluster comprising the genus *Algoriphagus* and clustered closely with *A. marincola* SW-2^T (Fig. 1). A similar topology was found with the maximum-parsimony algorithm (Supplementary Fig. S2). Strain LYX05^T exhibited 92.7–95.8 % 16S rRNA gene sequence similarity with the type strains of the 19 species of the genus *Algoriphagus*. The isolate was most closely related to *A. hitonicola* 7-UAH^T (95.8 % 16S rRNA gene sequence similarity) and *A. marincola* SW-2^T (95.1 %).

The predominant isoprenoid quinone detected in strain LYX05^T was menaquinone-7 (MK-7) and the dominant fatty acid was iso-C_{15:0} (40.82 %; Supplementary Table S1), as described for the genus *Algoriphagus* (Nedashkovskaya *et al.*, 2004, 2007). The DNA G + C content of strain LYX05^T was 42.5 mol%, which is similar to values described for *A. marincola* (43.0 mol%) and *A. hitonicola* (43.4 mol%; Table 1).

On the basis of phylogenetic, phenotypic and chemotaxonomic evidence, strain LYX05^T represents a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus faecimaris* sp. nov. is proposed.

Description of *Algoriphagus faecimaris* sp. nov.

Algoriphagus faecimaris (fa.e.ci.ma'ris. L. n. *faex*, *faecis* sediment; L. n. *mare*, -is the sea; N.L. gen. n. *faecimaris* of a sediment of the sea).

Cells are Gram-negative, non-sporulating, non-flagellated, strictly aerobic rods, 0.3–0.5 µm wide and 0.8–1.6 µm long. Colonies are 1–2 mm in diameter, circular, reddish orange and shiny with entire edges after 2 days on MA medium at 37 °C. Grows at 0–42 °C (optimum 37 °C), at pH 6–12.5 (optimum pH 7.5) and with 0–8 % (w/v) NaCl (optimum 2 % NaCl). Nitrate is reduced to nitrite. Positive for oxidase and hydrolysis of aesculin and weakly positive for catalase, but negative for arginine dihydrolase and urease. Indole and hydrogen sulfide are not produced. Flexirubin-type pigments are not formed. Degrades gelatin, starch and Tween 20, degrades Tweens 40 and 80 weakly and does not degrade agar or casein. Acid is produced from D-galactose, D-glucose, D-fructose, aesculin, maltose, methyl α-D-glucopyranoside, cellobiose, melibiose, sucrose, turanose and potassium 5-ketogluconate, but not from amygdalin, glycerol, erythritol, salicin, melezitose, methyl α-D-mannopyranoside, N-acetylglucosamine, inulin or potassium 2-ketogluconate. With API ZYM, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase and acid phosphatase, weakly positive for cystine

Table 1. Phenotypic characteristics of strain LYX05^T and its closest phylogenetic neighbours in the genus *Algoriphagus*

Strains: 1, *Algoriphagus faecimaris* sp. nov. LYX05^T; 2, *A. marincola* DSM 16067^T; 3, *A. ratkovskyi* LMG 21435^T; 4, *A. hitonicola* 7-UAH^T; 5, *A. ornithinivorans* JC2052^T; 6, *A. vanfongensis* KMM 6241^T. Data in parentheses in columns 2 and 3 and all data in columns 4–6 were taken from Yoon *et al.* (2004), Bowman *et al.* (2003), Copa-Patiño *et al.* (2008), Yi & Chun (2004) and Nedashkovskaya *et al.* (2007); other data were from this study. All strains are positive for aerobic metabolism, catalase, alkaline phosphatase and acid production from D-glucose. All strains are negative for gliding motility, production of flexirubin-type pigments, H₂S and indole production, urease and acid production from glycerol. +, Positive; w, weakly positive; v, variable; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6
Pigment absorption maximum (nm)	482	486	ND	481	480	ND
Nitrate reduction	+	–	–	+	–	–
NaCl concentration for growth (%)	0–8	1–9	0–6	1.5–5	0–10	0–8
Growth temperature (°C)	0–42	16–45 (10–45)	0–28 (–2 to 25)	20–40	10–40	12–35
Hydrolysis of:						
Casein	–	+	+	ND	–	–
DNA	+	+	–	+	+	–
Gelatin	+	–	–	ND	+	+
Starch	+	+	– (v)	+	+	–
Tween 40	w	+	+	ND	+	–
Tween 80	w	+	–	–	+	–
Acid production from:						
Amygdalin	–	+	+	+	ND	ND
D-Fructose	+	+	w	+	–	–
D-Galactose	+	+	w	ND	–	–
D-Lyxose	–	–	+	ND	ND	ND
D-Mannitol	–	–	+	ND	–	ND
D-Mannose	w	+	+	+	–	+
Melezitose	–	+	+	ND	ND	ND
Salicin	–	+	+	+	ND	ND
D-Xylose	–	+	+	–	–	+
N-Acetylglucosamine	–	+	+	+	–	+
Utilization of:						
L-Arabinose	–	+	+	ND	–	+
D-Galactose	–	+	+	ND	–	ND
myo-Inositol	+	–	+	ND	–	–
D-Mannitol	–	–	+	–	–	–
D-Sorbitol	–	–	+	ND	–	–
N-Acetyl-D-glucosamine	+	–	+	ND	+	+
Susceptibility to:						
Ampicillin	–	+	–	ND	+	–
Doxycycline	–	–	– (+)	ND	+	+
Gentamicin	–	–	–	ND	–	+
Kanamycin	–	–	–	–	+	–
Neomycin	–	–	–	–	–	+
Tetracycline	–	+	+	ND	+	+
DNA G + C content (mol%)	42.5	(43)	(35)	43.4	38	43.8

arylamidase and β -glucosidase and negative for lipase (C14), trypsin, α -chymotrypsin, α - and β -galactosidase, α -glucosidase, β -glucuronidase, β -mannosidase and α -fucosidase. Sensitive to (μ g per disc, unless indicated otherwise) ciprofloxacin (5), vancomycin (30), cefobid (75), cephalothin V (30), piperacillin (100) and chloromycetin (30); resistant to norfloxacin (10), polymyxin B (300 U), compound sulfamethoxazole (75), furazolidone (300), clindamycin (2), rocephin (30), amikacin (30), gentamicin (10), kanamycin (30), neomycin (30), tetracycline (30), doxycycline (30),

minocycline (30), erythromycin (15), oxacillin sodium (1), ampicillin (10), cephalothin IV (30), cephalothin VI (30), cefuroxime (30), ceftazidime (30) and midcamycin (30). The dominant fatty acids are iso-C_{15:0} and C_{16:0}. The DNA G + C content of the type strain is 42.5 mol%.

The type strain is LYX05^T (=JCM 16561^T =DSM 23095^T =LMG 25474^T), which was isolated from coastal sediment of Jiaozhou Bay, near the estuary of the river Lichun at Qingdao, on the Yellow Sea coast of China.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain LYX05^T and members of the genus *Algoriphagus*. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. The sequence of *Flexibacter flexilis* ATCC 23079^T was used as an outgroup. Bar, 0.005 nucleotide substitutions per position.

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