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# *Algoriphagus shivajiensis* sp. nov., isolated from Cochin back water, India☆

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ABSTRACT

Novel orange-pigmented, Gram-negative, rod-shaped, non-motile bacteria, designated strains NIO-S3$^T$ and NIO-S4, were isolated from a water sample collected from Cochin back waters, Thanneermukkom and Arookutty, Kerala, India. Both strains were positive for oxidase and catalase activities, and hydrolyzed gelatin and Tween 40. The predominant fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3OH, $C_{16:1}$ $\omega$7c/$C_{16:1}$ $\omega$6c (summed feature 3) and iso-$C_{17:1}$ $\omega$9c/$C_{16:0}$ 10-methyl (summed feature 9), whereas MK-7 was the major respiratory quinone, and phosphatidylethanolamine, two unidentified phospholipids and one unidentified lipid were the only polar lipids. The DNA G+C content of the two strains was 43.7 and 43.6 mol%, respectively. The 16S rRNA gene sequence analysis indicated that they were members of the genus *Algoriphagus* and closely related to *Algoriphagus olei* CC-Hsuan-617$^T$, *Algoriphagus aquatilis* A8-7$^T$, *Algoriphagus aquaeductus* LMG 24398$^T$ and *Algoriphagus mannitolivorans* DSM 15301$^T$, with pairwise sequence similarities of 96.8, 96.6, 96.2 and 96.2%, respectively. DNA–DNA hybridization between strains NIO-S3$^T$ and NIO-S4 showed a relatedness of 89%. Based on data from the current polyphasic study, the strains are proposed as a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus shivajiensis* sp. nov. is proposed. The type strain of *A. shivajiensis* is NIO-S3$^T$ (=JCM 17885$^T$ = MTCC 11066$^T$).

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The genus *Algoriphagus* belongs to the family "*Cyclobacteriaceae*" of the phylum *Bacteroidetes*, and comprises Gram-negative, non-motile, rod-shaped, pink- to orange-pigmented, organoheterotrophic, strictly aerobic bacteria with an absence of flexirubin and a DNA G+C content ranging between 35 and 44 mol% [6,18,20]. The type species of the genus is *Algoriphagus ratkowskyi*. Species of the genus *Algoriphagus* have been isolated from different habitats, including sea ice, seawater, algal mats, marine sediments, soil, fresh water, corals, a marine solar saltern, oil-contaminated soil, microbial mats in Antarctic lakes, an athalassohaline lagoon and non-saline alkaline groundwater [9,18,22,28,30,34–36]. At the time of writing, the genus *Algoriphagus* accommodates 19 recognized species (Euzeby, http://www.bacterio.cict.fr/a/algoriphagus.html). In addition, the descriptions of the novel species *Algoriphagus aquaeductus*, *Algoriphagus faecimaris*, *Algoriphagus jejuensis* and *Algoriphagus namhaensis* were recently proposed [14,15,21,23]. The present study used a polyphasic taxonomic approach for the characterization and classification of strains NIO-S3$^T$ and NIO-S4, which were isolated from brackish water. From the results of phylogenetic and phenotypic analyses, the strains were proposed as the representatives of a novel species of the genus *Algoriphagus*.

Strains NIO-S3$^T$ and NIO-S4 were isolated from water samples collected from Cochin back waters, Thanneermukkom (9°52′47.09″N 76°19′27.82″E) and Arookutty (9°41′17.13″N 76°23′34.71″E) villages, respectively, Kerala State, India, on 26th July 2010. The samples that yielded the strains had a pH of 7.0. For isolation of bacteria, 1 mL of each water sample was serially diluted in 1% saline water and 100 μL were then plated on half strength ZoBell marine agar (MA) medium [37] and incubated at room temperature for 15 days. Out of the different morphotypes obtained, two pale-orange colonies were further selected and characterized. Sub-cultivation of the isolates was carried out on half strength MA at 30 °C. Stock cultures of the isolates in marine broth with 10% glycerol were preserved at −80 °C.

Strains NIO-S3$^T$ and NIO-S4 were characterized simultaneously with *Algoriphagus olei* CC-Hsuan-617$^T$, *Algoriphagus mannitolivorans* DSM 15301$^T$, *A. aquaeductus* LMG 24398$^T$ and *Algoriphagus aquatilis* A8-7$^T$. Colony morphology was examined following growth on MA at 30 °C for 48 h. Cell morphology and motility were observed by using phase contrast microscopy. Motility was

☆ The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains NIO-S3$^T$ and NIO-S4 are FR872716 and JN205302, respectively.

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**Table 1**
Phenotypic features that distinguish strains NIO-S3$^T$ and NIO-S4 from the closely related species of the genus *Algoriphagus*.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Colony color | Pale orange | Pale orange | Orange | Pinkish red | Pink | Orange |
| Cell size (width × length) in μm | 0.5–0.6 × 1.0–1.5 | 0.5–0.6 × 1.0–1.5 | 0.4–0.6 × 1.3–2.0 | 0.4–0.5 × 1.0–2.5[a] | 0.5–2.5 × 1.5–15.0 | 0.2–0.4 × 1.6–4.0 |
| Salinity optimum (%) | 1–2 | 1–2 | 1 | 0–1 | 0 | 0.5–1 |
| Metabolism | Strictly aerobic | Strictly aerobic | Strictly aerobic | Strictly aerobic | Aerobic to microaero-philic | Strictly aerobic |
| Temperature optimum (°C) | 30 | 30 | 30–37 | 30–35[a] | 25–30 | 30 |
| pH growth range (optimum) | 7–11 (7–8) | 7–11 (7–8) | 6–11 (7) | 7–9 (8) | 7–9 (8) | 5.5–10.5 (7.5) |
| Biochemical tests | | | | | | |
| Citrate utilization | + | + | − | − | − | w |
| Indole | − | − | − | + | − | − |
| Hydrolysis of | | | | | | |
| Aesculin | − | − | + | + | − | + |
| ONPG | − | − | + | + | + | + |
| Urea | − | − | w | − | − | − |
| Utilization of | | | | | | |
| Cellobiose | + | + | w | − | + | w |
| Dulcitol | − | − | − | w | − | + |
| Fructose | w | − | − | − | w | + |
| Galactose | + | + | − | + | + | − |
| Inulin | − | − | − | w | − | − |
| Malonate | + | + | − | − | − | w |
| Mannitol | − | − | + | − | − | − |
| Melibiose | + | + | w | − | − | + |
| Melezitose | w | − | − | − | − | + |
| Methyl α-D-glucoside | w | w | w | − | w | + |
| Methyl α-D-mannoside | w | − | − | − | − | − |
| Raffinose | w | w | + | − | w | + |
| Salicin | − | − | + | − | + | − |
| Trehalose | + | + | + | −[a] | + | + |
| Xylitol | w | − | + | − | − | − |
| Xylose | w | − | + | w | − | − |
| Antibiotic susceptibility (μg per disc unless indicated) | | | | | | |
| Ampicillin (10) | R | M | S | R | S | M |
| Bacitracin (10) | M | M | S | S | S | S |
| Cephalexin (30) | R | M | S | R | M | S |
| Cephalothin (30) | R | R | S | S | S | S |
| Ciprofloxacin (5) | R | R | S | S | S | S |
| Gentamycin (10) | M | M | S | R[a] | M | S |
| Methicillin (5) | M | M | S | M | S | R |
| Neomycin (30) | R | R | R | S | S | S |
| Nitrofurantoin (300) | R | R | M | S | R | M |
| Penicillin G (10 units) | S | M | S | R | S | M |
| Tetracycline (30) | S | S | R | S[a] | S | S |
| Tobramycin (10) | R | M | M | R | M | R |
| Vancomycin (30) | R | R | R | S | R | R |

Taxa: 1, *Algoriphagus shivajiensis* strain NIO-S3$^T$; 2, *Algoriphagus shivajiensis* strain NIO-S4; 3, *Algoriphagus mannitolivorans* DSM 15301$^T$; 4, *Algoriphagus olei* CC-Hsuan-617$^T$; 5, *Algoriphagus aquaeductus* LMG 24398$^T$; 6, *Algoriphagus aquatilis* A8-7$^T$.
All the data from the present study. All strains were rod-shaped, positive for catalase and oxidase activities, and utilized glucose, lactose, mannose and sucrose. All strains were negative for lysine and ornithine decarboxylase, phenylalanine deaminase activities, $H_2S$ production, methyl red and Voges Proskauer reactions, whereas agar and cellulose were not hydrolyzed, and adonitol, arabitol, D-arabinose, erythritol, glycerol, inositol, rhamnose, sodium gluconate, sorbitol and sorbose were not utilized. All strains were sensitive to (μg per disc) amoxicillin (20), azithromycin (15), chloramphenicol (30), erythromycin (15), nalidixic acid (30), norfloxacin (10), novobiocin (30), rifampicin (5), streptomycin (10) and trimethoprim (5), and resistant to kanamycin (30). +, positive; −, negative; w, weak; M, moderately sensitive; R, resistant; S, sensitive.

[a] The data of strain *Algoriphagus olei* CC-Hsuan-617$^T$ (cell size, optimum temperature, utilization of trehalose, susceptibility to gentamycin and tetracycline) differed from Young et al. [36].

also assessed on motility–indole–lysine HiVeg$^{TM}$ medium (cat. no. MV847; HIMEDIA) with 2 g $L^{-1}$ agar (by inoculating the active culture suspension using a sterile needle and checking for spreading of the growth on the medium), as well as under a phase contrast microscope. Motility was checked using the method described by Bernardet et al. [5]. Growth at 4, 10, 18, 30, 37 and 40 °C was assessed on MA, and salt tolerance [0,1, 2, 3, 4, 5, 6, 8 and 10% (w/v) NaCl] was ascertained using nutrient agar (NA) containing ($L^{-1}$) peptone (5 g), beef extract (3 g) and agar (20 g). Growth of strains NIO-S3$^T$ and NIO-S4 at pH 5, 6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11 and 12 was assessed on trypticase soy agar (TSA) buffered with citric acid/NaOH (for pH 5 and 6), $NaHPO_4/Na_2HPO_4$ (for pH 7 and 8), glycine/NaOH (for pH 9 and 10) or Tris–HCl or NaOH (for pH 11 and 12). Biochemical and enzymatic characterizations, carbon substrate utilization, acid production and antibiotic susceptibility of the strains were performed using previously described methods [2].

Pigments were extracted and analyzed as described by Anil Kumar et al. [3]. For fatty acid analysis, strains NIO-S3$^T$, NIO-S4, *A. olei* CC-Hsuan-617$^T$, *A. mannitolivorans* DSM 15301$^T$, *A. aquaeductus* LMG 24398$^T$ and *A. aquatilis* A8-7$^T$ were grown on TSA at 30 °C for 2–3 days. Cellular fatty acid methyl esters (FAMEs) were obtained from cells by saponification, methylation and extraction following the MIDI protocol. They were then separated by GC (6890), and identified and qualified with the Sherlock Microbial Identification System (MIDI-6890 with database TSBA6). Polar lipids were extracted and analyzed according to the method described by Komagata and Suzuki [12]. Menaquinones and polar lipids were

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