

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

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The novel orange pigmented, Gram-negative, rod-shaped, non-motile bacteria, designated strains NIO-S3<sup>T</sup> 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<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3OH, C<sub>16:1</sub> ω7c/C<sub>16:1</sub> ω6c (summed feature 3) and iso-C<sub>17:1</sub> ω9c/C<sub>16:0</sub> 10-methyl (summed feature 9), MK-7 was the major respiratory quinone and phosphatidylethanolamine, two unidentified phospholipids and one unidentified lipid were the only polar lipids. The G + C content of DNA of strains NIO-S3<sup>T</sup> and NIO-S4 was 43.7 and 43.6 mol% respectively. The 16S rRNA gene sequence analysis indicated that strains NIO-S3<sup>T</sup> and NIO-S4 were members of the genus *Algoriphagus* and closely related to *Algoriphagus olei* CC-Hsuan-617<sup>T</sup>, *Algoriphagus aquatilis* A8-7<sup>T</sup>, *Algoriphagus aquaeductus* LMG 24398<sup>T</sup> and *Algoriphagus mannitolivorans* DSM 15301<sup>T</sup>, with pair-wise sequence similarities of 96.8, 96.6, 96.2 and 96.2% respectively. DNA-DNA hybridization between strains NIO-S3<sup>T</sup> and NIO-S4 showed a relatedness of 89%. Based on data from the current polyphasic study, strains NIO-S3<sup>T</sup> and NIO-S4 are proposed as a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus shivajiensis* sp. nov. is proposed. The type strain of *Algoriphagus shivajiensis* is NIO-S3<sup>T</sup> (= JCM 17885<sup>T</sup> = MTCC 11066<sup>T</sup>).

**Key words:** *Algoriphagus shivajiensis*; 16S rRNA gene based phylogeny; *Bacteroidetes*.

The genus *Algoriphagus* comprises Gram-negative, non-motile, rod-shaped, pink to orange pigmented, absence of flexirubin, organoheterotrophic, strictly aerobic bacteria with DNA G + C content ranging between 35-44 mol% [6, 18, 20] belongs to the family “*Cyclobacteriaceae*” of the phylum *Bacteroidetes*. The type species of the genus was *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, marine solar saltern, oil-contaminated soil, microbial mats in Antarctic lakes, athalassohaline lagoon and nonsaline alkaline groundwater [9, 18, 28, 30, 34, 36]. At the time of writing, the genus *Algoriphagus* accommodates 19 recognized species (Euzéby, <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]. In the present study we focused on the characterization and classification of strains NIO-S3<sup>T</sup> and NIO-S4, which were isolated from brackish water by using a polyphasic taxonomic approach. From the results of phylogenetic and phenotypic analyses, strains were proposed as the representatives of a novel species of the genus *Algoriphagus*.

Strains NIO-S3<sup>T</sup> and NIO-S4 were isolated from a 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 26<sup>th</sup> July 2010. The samples that yielded strains NIO-S3<sup>T</sup> and NIO-S4 had a pH of 7.0. For isolation of bacteria, 1 ml of the water sample was serially diluted in 1% saline water and 100 µl was 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 selected and characterized in the present study. 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<sup>T</sup> and NIO-S4 were characterized simultaneously with *Algoriphagus olei* CC-Hsuan-617<sup>T</sup>, *Algoriphagus mannitolivorans* DSM 15301<sup>T</sup>, *Algoriphagus aquaeductus* LMG 24398<sup>T</sup> and *Algoriphagus aquatilis* A8-7<sup>T</sup>. 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 also assessed on Motility-Indole-Lysine HiVeg<sup>TM</sup> medium (cat. no. MV847; HIMEDIA) with agar 2 g l<sup>-1</sup> (by inoculating the active culture suspension using a sterile needle and checking for spreading of the growth in the medium) and also under 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<sup>-1</sup>) peptone (5 g), beef extract (3 g) and agar (20 g). Growth of strains NIO-S3<sup>T</sup> 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<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> (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 strains NIO-S3<sup>T</sup> and NIO-S4 were performed by previously described methods [2].

Pigment were extracted and analysed as described by Anil Kumar *et al.* [3]. For fatty acid analysis, strains NIO-S3<sup>T</sup>, NIO-S4, *Algoriphagus olei* CC-Hsuan-617<sup>T</sup>, *Algoriphagus mannitolivorans* DSM 15301<sup>T</sup>, *Algoriphagus aquaeductus* LMG 24398<sup>T</sup> and *Algoriphagus aquatilis* A8-7<sup>T</sup> were grown on TSA at 30°C for two and three days. Cellular fatty acid methyl esters (FAMES) were obtained from cells by saponification, methylation and extraction following the protocol of MIDI. Cellular FAMES were separated by GC (6890) and identified and qualified with 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 determined on freeze-dried cells of strains NIO-S3<sup>T</sup> and NIO-S4. Menaquinones were extracted as described by Collins *et al.* [8] and were analyzed by HPLC [10]. The DNA of strains NIO-S3<sup>T</sup> and NIO-S4 were isolated according

to the procedure of Marmur [17] and the G+C content was determined from melting point ( $T_m$ ) curves [26] obtained by using a Lambda 2 UV-Vis spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package (Perkin Elmer). *Escherichia coli* DH5- $\alpha$  DNA was used as a standard in determining the DNA G + C content.

For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (Mo Bio Laboratories Inc.) and sequenced as described previously [13]. The resultant sequences of the 16S rRNA gene of strains NIO-S3<sup>T</sup> and NIO-S4 (1429 and 1461 nt respectively) were subjected to BLAST sequence similarity searches [1] and the EzTaxon server [7] was used to identify the nearest taxa. All the 16S rRNA gene sequences of members of the genus *Algoriphagus* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL\_X program [27] and the alignment was then corrected manually. A phylogenetic tree was constructed using the maximum-likelihood method using the PhyML program [11] and its topology was evaluated based on 100 resamplings. DNA-DNA hybridization between the two isolates was performed by the membrane filter method [29] as described previously [24, 25]. REP-PCR was performed according to the protocol described by Versalovic et al. [31]. RAPD-PCR was carried out with two random primers [RAPD1 - 5'-CTTGAGTGGGA-3' and RAPD2 - 5'-GAGATGACGA-3'] with 10 pm concentration. Initial denaturation at 95°C for 15 sec, and subsequent denaturation at 95°C for 5 sec. Primer annealing was performed at 35-40°C for 10 sec, and primer extension was performed at 72°C for 30 sec. The final extension step was 5 min at 72°C. Total 35 cycles were performed. Nearly equal concentration of the template DNA was taken for all the strain studied (113-120 ng/ $\mu$ l).

The phenotypic characteristics of the two isolates are listed in the species description and in Tables 1, 2 and Supplementary Table S1. The absorption spectrum of the ethanol extract of strains NIO-S3<sup>T</sup> and NIO-S4 showed a broad peak with a maximum around 476 nm, typical for carotenoid pigments [4]. Alkalinization of the extract did not cause a bathochromic shift of the peak. Therefore, strains NIO-S3<sup>T</sup> and NIO-S4 contained carotenoids pigments but no flexirubin pigments, as often observed for marine members of the phylum *Bacteroidetes* [3, 33]. The cellular fatty acid composition of strains NIO-S3<sup>T</sup> and NIO-S4 was dominated by of iso-C<sub>15:0</sub>, anteiso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3OH, C<sub>16:1</sub>  $\omega$ 7c/C<sub>16:1</sub>  $\omega$ 6c (summed feature 3) and iso-C<sub>17:1</sub>  $\omega$ 9c/C<sub>16:0</sub> 10-methyl (summed feature 9) (Table 2). Saturated fatty acids constituted 72.5 and 71.6% of the total fatty acids respectively. Two isolates shared nearly identical fatty acid profiles, their overall fatty acid composition was similar to those of the four reference strains and however, the two isolates could be clearly distinguished from the reference strains by high abundance of summed feature 9 and absence of iso-C<sub>15:1</sub> G and iso-C<sub>17:1</sub>  $\omega$ 9c. Compared with *Algoriphagus olei* CC-Hsuan-617<sup>T</sup>, *Algoriphagus mannitolivorans* DSM 15301<sup>T</sup>, *Algoriphagus*

*aquaeductus* LMG 24398<sup>T</sup> and *Algoriphagus aquatilis* A8-7<sup>T</sup> the composition of fatty acids differed considerably in strain NIO-S3<sup>T</sup> (Table 2). Two isolates contains three common major fatty acids in *Algoriphagus* strains are iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3-OH, and summed feature 3 comprising iso-C<sub>15:0</sub> 2-OH and/or C<sub>16:1</sub> ω7c (Nedashkovskaya and Ludwig, 2011) present in all type strains but slightly differs in *Algoriphagus jejuensis*, *Algoriphagus aquatilis*, *Algoriphagus faecimaris* and *Algoriphagus boritolers* (Lee et al., 2012; Liu et al., 2009; Nedashkovskaya et al., 2007b; Li et al., 2011). Two isolates differed with the type species of the genus *Algoriphagus ratkowskyi* with respect to the major fatty acids, anteiso-C<sub>15:0</sub>, iso-C<sub>16:1</sub> ω5c, summed feature 9 (iso-C<sub>17:1</sub> ω9c and/or C<sub>16:0</sub> 10-methyl). MK-7 was the only (100%) menaquinone present and the polar lipids consisted of phosphatidylethanolamine, two unidentified phospholipids and one unidentified lipid (Supplementary Fig. S1). Both strains NIO-S3<sup>T</sup> and NIO-S4 have similar composition and pattern (data not shown). The polar lipid pattern obtained for NIO-S3<sup>T</sup> agrees to those of the species already described in the genus *Algoriphagus* [15, 21, 23, 36]. The G + C content of DNA of strains NIO-S3<sup>T</sup> and NIO-S4 were 43.7 and 43.6 mol% respectively. These values are in accordance to the DNA G+C content range reported for *Algoriphagus* species [18].

The 16S rRNA gene analysis placed strains NIO-S3<sup>T</sup> and NIO-S4 within the genus *Algoriphagus*. The results indicated that the 16S rRNA gene sequence similarity of strains NIO-S3<sup>T</sup> and NIO-S4 shared 99.9% sequence similarity and 96.8, 96.6, 96.2 and 96.2% with *Algoriphagus olei* CC-Hsuan-617<sup>T</sup>, *Algoriphagus aquatilis* A8-7<sup>T</sup>, *Algoriphagus aquaeductus* LMG 24398<sup>T</sup> and *Algoriphagus manitolivorans* DSM 15301<sup>T</sup> respectively. The pair-wise sequence similarity with the other species of the genus *Algoriphagus* was between 92.3-96.2%. Phylogenetic tree obtained using the maximum-likelihood (Fig. 1) method revealed clustering of strains NIO-S3<sup>T</sup> and NIO-S4 with *Algoriphagus manitolivorans* DSM 15301<sup>T</sup> with a phylogenetic distance of 4.4% (95.6% similarity) and with strains *Algoriphagus olei* CC-Hsuan-617<sup>T</sup>, *Algoriphagus aquatilis* A8-7<sup>T</sup> and *Algoriphagus aquaeductus* LMG 24398<sup>T</sup> with a phylogenetic distances of 4.4, 4.9 and 5.6% (95.4, 95.1 and 94.4% similarity) respectively. The type species of the genus *Algoriphagus*, *Algoriphagus ratkowskyi* clustered distantly with phylogenetic distance of 11.7% (88.3% similarity). Nearly similar topology was observed for the maximum-parsimony and neighbour-joining trees (Supplementary Figs. 4 and 5). DNA-DNA hybridization between strains NIO-S3<sup>T</sup> and NIO-S4 showed a relatedness of 89%. This value was upper 70%, confirming that the novel strains are members of the same species [32]. And a number of differences were observed between the two strains NIO-S3<sup>T</sup> and NIO-S4 and also with *Algoriphagus olei* CC-Hsuan-617<sup>T</sup> and *Algoriphagus manitolivorans* DSM 15301<sup>T</sup> in Rep-PCR genomic fingerprint patterns (Supplementary Fig. S2). In case of RAPD-PCR with primer RAPD1 the banding pattern of two strains NIO-S3<sup>T</sup> and NIO-S4 is almost similar but different with the primer RAPD2. But the banding

pattern is different with the type strains *Algoriphagus olei* CC-Hsuan-617<sup>T</sup> and *Algoriphagus mannitolivorans* DSM 15301<sup>T</sup> with two primers used (Supplementary Fig. S3).

The main features of the two isolates are in line with the original and emended descriptions of the genus *Algoriphagus* but that they could be distinguished from published species with respect to a number of phenotypic characteristics like nitrate reduction; colour of colonies; temperature, salinity and pH growth range and optima; hydrolysis of complex substrates; acid production from and utilization a number of substrates; antibiotic susceptibility; fatty acid composition; polar lipids and DNA G+C content (Tables 1, 2 and Supplementary Table S1). Thus, the cumulative differences that strains NIO-S3<sup>T</sup> and NIO-S4 exhibits with the closely related type strains unambiguously supports the creation of a new species of the genus *Algoriphagus* for which the name *Algoriphagus shivajiensis* sp. nov. is proposed.

### **Description of *Algoriphagus shivajiensis* sp. nov.**

*Algoriphagus shivajiensis* (Shi.va.ji.en'sis. N. L. masc. adj. *shivajiensis* named after Dr. Shivaji, an eminent Indian microbiologist who has made a significant contribution to our knowledge of heterotrophic bacteria from diverse habitats).

Cells are Gram-negative strictly aerobic, rod shaped, non-motile, 0.5-0.6 µm wide and 1.0-1.5 µm long. Colonies on marine agar are circular, 1-3 mm in diameter, smooth, shiny, pale orange, translucent and raised with entire margins. Grows at 25 to 37 °C with an optimum temperature of 30°C and at 0-3% (w/v) NaCl, with optimum growth at 1-2%. Growth at pH 7 to 11 with an optimum pH of 7-8. Oxidase and catalase activities are present but lysine decarboxylase, ornithine decarboxylase, β-galactosidase and phenylalanine deaminase activities are absent. The methyl red and Voges-Proskauer reactions are negative. Gelatin and Tween 40 are hydrolysed but aesculin, casein, cellulose, DNA, starch, Tween 20, Tween 80 and urea are not hydrolysed. Nitrate is not reduced. H<sub>2</sub>S and indole are not produced. In the Hi25<sup>TM</sup> *Enterobacteriaceae* identification kit, acid is produced from cellobiose, galactose, glucose, lactose, malonate, mannose, melibiose, sucrose and trehalose after one week of incubation at optimum temperature and pH. In the HiCarbohydrate<sup>TM</sup> kit, assimilates cellobiose, citrate, fructose, galactose, glucose, L-arabinose, lactose, malonate, mannose, melibiose, melezitose, methyl α-D-glucoside, methyl α-D-mannoside, raffinose, sucrose, trehalose, xylitol and xylose but not adonitol, arabitol, D-arabinose, dulcitol, erythritol, glycerol, inositol, inulin, maltose, mannitol, myo-inositol, *N*-acetyl-D-glucosamine, rhamnose, salicin, sodium gluconate, sorbitol and sorbose. Susceptible to (µg per disc unless indicated) amoxicillin (20), azithromycin (15), chloramphenicol (30), erythromycin (15), nalidixic acid (30), norfloxacin (10), novobiocin (30), penicillin G (10 units/disc), rifampicin (5), streptomycin (10), tetracycline (30), trimethoprim (5); moderately resistant to bacitracin (10),

gentamycin (10), methicillin (5), and resistant to ampicillin (10), cephalexin (30), cephalothin (30), ciprofloxacin (5), kanamycin (30), neomycin (30), nitrofurantoin (300), tobramycin (10) and vancomycin (30). MK-7 was the only menaquinone present and the major polar lipids consisted of phosphatidylethanolamine, two unidentified phospholipids and one unidentified lipid. The DNA G + C content is 43.6-43.7 mol%. The type strain NIO-S3<sup>T</sup> (= JCM 17885<sup>T</sup> = MTCC 11066<sup>T</sup>) and NIO-S4 were isolated from a 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. Strain NIO-S4 is another reference strain.

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## Legends to Figures

**Fig. 1.** Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains NIO-S3<sup>T</sup> and NIO-S4 and representatives of the genus *Algoriphagus*. Numbers at the nodes are bootstrap values >50%. *Parapedobacter soli* DCY14<sup>T</sup> was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

**Supplementary Fig. S1.** Two-dimension thin-layer chromatogram of the total polar lipids of strain NIO-S3<sup>T</sup> after spraying of the plate with molybdato-phosphoric acid. Abbreviations: PE, phosphatidylethanolamine; L, unidentified lipid; PL1-2, unidentified phospholipids. The spots identified as phospho-, amino- or glyco-lipids by spraying molybdenum blue, ninhydrin and  $\alpha$ -naphthol reagents respectively.

**Supplementary Fig. S2.** Characteristic Rep-PCR genomic fingerprint patterns of strains NIO-S3<sup>T</sup> and NIO-S4, *Algoriphagus olei* CC-Hsuan-617<sup>T</sup> and *Algoriphagus manitolivorans* DSM 15301<sup>T</sup>. Lane 1 corresponds to 100 bp DNA Marker. Lane 2, 3, 4 & 5 corresponds to fingerprint patterns of strains *Algoriphagus manitolivorans* DSM 15301<sup>T</sup>, NIO-S3<sup>T</sup> and NIO-S4, *Algoriphagus olei* CC-Hsuan-617<sup>T</sup> respectively.

**Supplementary Fig. S3.** Characteristic RAPD-PCR genomic fingerprint patterns (done with two primers primer 1 (a) and primer 2 (b)) of strains NIO-S3<sup>T</sup> and NIO-S4, *Algoriphagus olei* CC-Hsuan-617<sup>T</sup> and *Algoriphagus manitolivorans* DSM 15301<sup>T</sup>. Lane 1 corresponds to 100 bp DNA Marker. Lane 2, 3, 4 & 5 corresponds to fingerprint patterns of strains NIO-S4, *Algoriphagus manitolivorans* DSM 15301<sup>T</sup>, *Algoriphagus olei* CC-Hsuan-617<sup>T</sup> and NIO-S3<sup>T</sup> respectively.

**Supplementary Fig. S4.** Maximum-parsimony phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains NIO-S3<sup>T</sup> and NIO-S4 and representatives of the genus *Algoriphagus*. Numbers at the nodes are bootstrap values >50%. *Parapedobacter soli* DCY14<sup>T</sup> was used as an outgroup.

**Supplementary Fig. S5.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains NIO-S3<sup>T</sup> and NIO-S4 and representatives of the genus *Algoriphagus*. Numbers at the nodes are bootstrap values >50%. *Parapedobacter soli* DCY14<sup>T</sup> was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

**Table 1.** Phenotypic features that distinguish strains NIO-S3<sup>T</sup> and NIO-S4 from the closely related species of the genus *Algoriphagus*.

Taxa: 1, *Algoriphagus shivajiensis* strain NIO-S3<sup>T</sup>; 2, *Algoriphagus shivajiensis* strain NIO-S4; 3, *Algoriphagus mannitolivorans* DSM 15301<sup>T</sup>; 4, *Algoriphagus olei* CC-Hsuan-617<sup>T</sup>; 5, *Algoriphagus aquaeductus* LMG 24398<sup>T</sup>; 6, *Algoriphagus aquatilis* A8-7<sup>T</sup>.

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<sub>2</sub>S production, methyl red and Voges Proskauer's reactions, agar and cellulose were not hydrolysed and not utilized adonitol, arabinol, D-arabinose, erythritol, glycerol, inositol, rhamnose, sodium gluconate, sorbitol and sorbose. 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.

	1	2	3	4	5	6
Colony colour	Pale orange	Pale orange	Orange	Pinkish red	Pink	Orange
Cell size (width x 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	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 microaerophilic	Strictly aerobic
Temperature optimum (°C)	30	30	30-37	*30-35	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)
<b>Biochemical:</b>						
Citrate utilization	+	+	-	-	-	w
Indole	-	-	-	+	-	-
<b>Hydrolysis of:</b>						
Aesculin	-	-	+	+	-	+
ONPG	-	-	+	+	+	+
Urea	-	-	w	-	-	-
<b>Utilization of:</b>						
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	+

	1	2	3	4	5	6
Salicin	-	-	+	-	+	-
Trehalose	+	+	+	*-	+	+
Xylitol	w	-	+	-	-	-
Xylose	w	-	+	w	-	-
<b>Anitibiotic susceptibility (<math>\mu</math>g per disc unless indicated):</b>						
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	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
Pencilin G (10 units)	S	M	S	R	S	M
Tetracycline (30)	S	S	R	*S	S	S
Tobramycin (10)	R	M	M	R	M	R
Vancomycin (30)	R	R	R	S	R	R

\*, The data of strain *Algoriphagus olei* CC-Hsuan-617<sup>1</sup> (cell size, optimum temperature, utilization of trehalose, suspetibility to gentamycin and tetracycline) differed from the Young et al. [36].

**Table 2.** Fatty acid profiles of strains NIO-S3<sup>T</sup> and NIO-S4 and closely related species of the genus *Algoriphagus*.

Taxa: 1, *Algoriphagus shivajiensis* strain NIO-S3<sup>T</sup>; 2, *Algoriphagus shivajiensis* strain NIO-S4; 3, *Algoriphagus mannitolivorans* DSM 15301<sup>T</sup>; 4, *Algoriphagus olei* CC-Hsuan-617<sup>T</sup>; 5, *Algoriphagus aquaeductus* LMG 24398<sup>T</sup>; 6, *Algoriphagus aquatilis* A8-7<sup>T</sup>.

Data from the present study. All four strains were grown on TSA plates at 30 °C for two days. Results are presented as a percentage of the total fatty acids. Fatty acids amounting to 5% or more of the total fatty acids are in bold. ND, not detected. Values of less than 1% for all strains are not shown. tr, traces (<1%)

Fatty acid composition	1	2	3	4	5	6
iso-C <sub>11:0</sub>	tr	1.4	1.8	1.0	tr	ND
anteiso-C <sub>11:0</sub>	1.3	2.1	1.2	1.3	tr	ND
iso-C <sub>14:0</sub>	tr	tr	tr	1.3	tr	1.1
C <sub>15:0</sub>	ND	ND	tr	1.5	ND	1.6
iso-C <sub>15:0</sub>	<b>36.7</b>	<b>32.8</b>	<b>42.0</b>	<b>29.1</b>	<b>30.5</b>	<b>39.2</b>
anteiso-C <sub>15:0</sub>	<b>6.6</b>	4.0	2.1	4.9	4.8	<b>6.2</b>
iso-C <sub>15:0</sub> 3OH	2.2	3.3	4.3	4.1	4.1	4.9
iso-C <sub>15:1</sub> G	ND	ND	<b>5.8</b>	1.9	tr	2.3
C <sub>15:1</sub> ω6c	3.6	1.4	1.7	2.4	tr	2.9
C <sub>16:0</sub>	1.5	2.4	tr	ND	tr	tr
iso-C <sub>16:0</sub>	2.8	1.7	1.1	4.1	2.9	2.2
iso-C <sub>16:0</sub> 3OH	1.5	1.0	tr	3.4	1.9	2.5
iso-C <sub>16:1</sub> H	tr	tr	tr	2.9	2.4	ND
C <sub>16:1</sub> ω5c	1.7	1.3	1.1	1.9	4.9	3.9
iso-C <sub>17:0</sub>	1.2	1.3	tr	tr	tr	tr
iso-C <sub>17:0</sub> 3OH	<b>9.9</b>	<b>9.4</b>	<b>7.9</b>	<b>8.3</b>	<b>7.9</b>	<b>6.8</b>
C <sub>17:1</sub> ω6c	2.7	1.2	1.5	2.9	1.7	tr
C <sub>17:1</sub> ω8c	1.0	ND	tr	tr	tr	tr
iso-C <sub>17:1</sub> ω9c	ND	ND	<b>10.9</b>	<b>5.6</b>	<b>8.1</b>	<b>8.3</b>
C <sub>19:0</sub> 10-methyl	1.1	2.6	ND	ND	tr	ND
C <sub>20:2</sub> ω6,9c	tr	2.1	tr	ND	1.1	tr
Summed feature 3	<b>11.5</b>	<b>8.6</b>	<b>7.1</b>	<b>14.2</b>	<b>24.1</b>	<b>14.9</b>
Summed feature 4	3.0	4.6	3.9	4.9	1.7	3.7
Summed feature 9	<b>5.8</b>	<b>8.5</b>	tr	1.2	1.6	tr

#Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c; Summed feature 4 contains iso-C<sub>17:1</sub> I and/or anteiso-C<sub>17:1</sub> B; Summed feature 9 contains iso-C<sub>17:1</sub> ω9c and/or C<sub>16:0</sub> 10-methyl.



**Supplementary Table S1.** Differentiating phenotypic properties of type strains of *Algoriphagus*

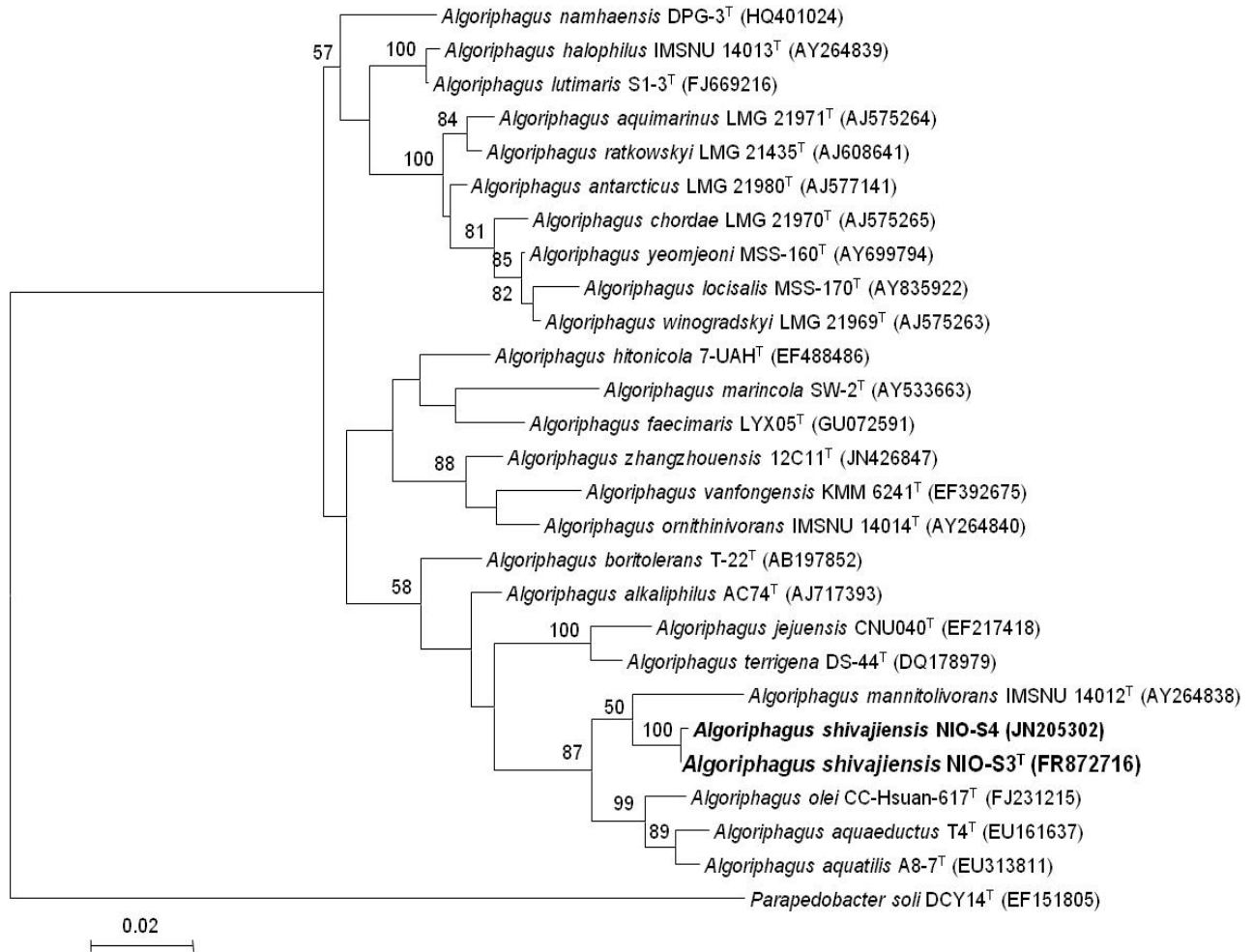
Taxa: 1, *Algoriphagus shivajiensis* sp. nov.; 2, *Algoriphagus mannitolivorans*; 3, *Algoriphagus olei*; 4, *Algoriphagus aquaeductus*; 5, *Algoriphagus aquatilis*; [Data for taxa 1 to 5 are from the present study except polar lipids and DNA G+C content of reference taxa] 6, *Algoriphagus alkaliphilus*; 7, *Algoriphagus antarcticus*; 8, *Algoriphagus aquimarinus*; 9, *Algoriphagus boritolerans*; 10, *Algoriphagus chordae*; 11, *Algoriphagus faecimaris*; 12, *Algoriphagus halophilus*; 13, *Algoriphagus hitonicola*; 14, *Algoriphagus jejuensis*; 15, *Algoriphagus locisalis*; 16, *Algoriphagus lutimaris*; 17, *Algoriphagus marincola*; 18, *Algoriphagus namhaensis*; 19, *Algoriphagus ornithinivorans*; 20, *Algoriphagus ratkowskyi*; 21, *Algoriphagus terrigena*; 22, *Algoriphagus vanfongensis*; 23, *Algoriphagus winogradskyi*; 24, *Algoriphagus yeomjeoni*; 25, *Algoriphagus zhangzhouensis*. Data for taxa 6 to 25 were taken from, Bowman et al. [6]; Copa-Patiño et al. [9]; Lee et al. [14]; Li et al. [15]; Nedashkovskaya et al. [20]; Nedashkovskaya et al. [18]; Oh et al. [21]; Park et al. [22]; Van Trappen et al. [30]; Yoon et al. [34]; Yoon et al. [35].

+, Positive; -, negative; w, weakly positive; v, variable; nd, no data available. All strains were positive for oxidase activity except *Algoriphagus hitonicola*. All strains were positive for catalase activity except *Algoriphagus faecimaris* (weakly positive). All strains were negative for H<sub>2</sub>S production except *Algoriphagus aquatilis*.

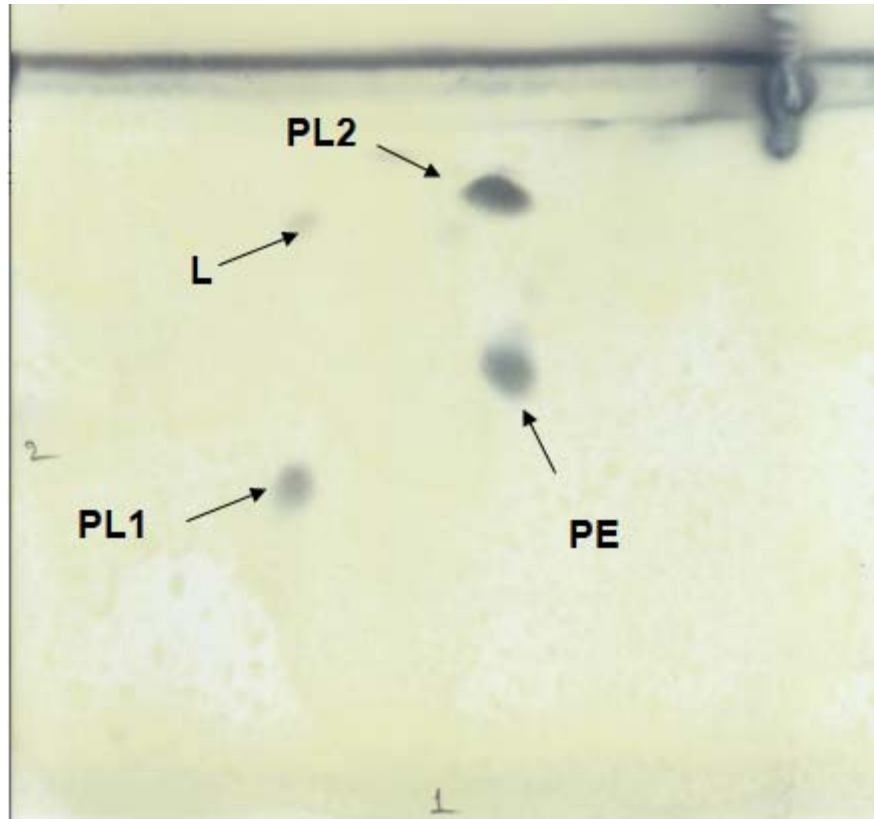
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
Nitrate reduction	-	+	-	+	-	+	-	-	-	-	+	-	+	-	-	-	v	-	-	v	+	-	+	-	-	
Salinity range for growth (%)	0-3	0-7	0-3	0-4	0-3	0-3	0-5	0-10	0-3	1-10	0-8	0-8	1.5-5	0-5	1-9	0-8	1-9	0.5-6	0-10	0-6	1-7	0-8	0-6	1-9	0-10	
Temperature range for growth (°C)	25-37	10-42	10-37	15-42	20-37	11-39	5-25	4-34	17-37	4-32	0-42	10-41	20-40	4-30	4-35	4-40	10-45	10-40	10-40	-2-25	10-36	12-35	4-39	4-35	4-40	
β-galactosidase	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+	
<i>Hydrolysis of:</i>																										
Agar	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	nd
Casein	-	-	-	+	w	-	-	+	nd	-	-	-	nd	-	-	+	+	+	-	+	+	-	-	+	-	
Gelatin	+	+	-	+	-	+	-	+	-	-	+	+	nd	+	-	+	-	+	+	v	-	+	+	v	-	
Starch	-	+	-	+	+	+	-	-	+	-	+	+	+	-	-	-	+	+	+	v	-	-	+	-	-	
DNA	-	+	-	+	-	+	-	+	nd	-	+	-	+	nd	nd	+	+	+	v	nd	-	-	-	-	nd	
Tween 20	-	-	-	+	+	nd	nd	+	nd	+	+	+	nd	+	+	nd	+	+	-	+	+	-	+	+	w	
Tween 40	+	-	-	-	+	nd	nd	+	nd	+	w	+	nd	+	+	nd	+	+	+	+	+	-	+	+	+	
Tween 80	-	-	-	-	+	nd	nd	+	nd	-	w	+	-	+	+	nd	+	+	+	-	+	-	-	+	+	
<i>Acid production from:</i>																										
L-arabinose	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	nd	
Cellobiose	+	+	-	-	w	+	-	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	nd	
D-fructose	-	-	-	-	w	+	-	nd	-	nd	+	nd	+	w	+	-	v	w	-	+	+	-	nd	+	nd	
D-galactose	+	-	-	-	-	+	-	-	+	+	+	-	nd	+	+	+	+	-	-	+	+	-	+	+	nd	
D-glucose	+	-	-	+	w	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	nd	
D-lactose	+	-	-	+	w	+	-	+	+	+	-	-	+	w	+	+	+	-	-	-	+	-	+	+	nd	
Maltose	-	-	-	+	w	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	nd	
D-mannose	+	-	-	+	w	+	-	+	+	+	w	nd	+	-	+	+	+	-	-	+	+	+	+	-	nd	
Melibiose	+	-	-	+	w	+	-	+	-	+	+	-	+	+	+	-	+	-	-	+	+	-	+	+	nd	
Raffinose	-	-	-	+	-	-	-	-	+	+	nd	-	+	+	w	+	+	-	-	+	+	-	+	v	nd	
L-rhamnose	-	-	-	+	-	-	-	+	+	+	nd	-	-	w	+	+	-	-	-	+	-	+	+	+	nd	
Sucrose	+	+	-	+	w	+	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	nd	
Trehalose	+	-	-	+	w	+	-	nd	-	nd	nd	nd	+	+	v	+	+	-	-	+	+	-	nd	+	nd	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
DL-xylose	-	-	-	-	-	-	-	+	-	+	-	+	-	+	+	-	+	w	-	+	+	+	+	+	+	nd
<i>N</i> -acetyl-D-glucosamine	-	-	-	+	-	-	-	+	+	-	-	+	+	+	nd	nd	-	nd	-	+	nd	+	+	-	+	nd
<i>Utilization of:</i>																										
L-arabinose	w	+	-	-	-	+	-	+	-	+	-	+	-	+	+	-	+	nd	-	+	+	+	+	-	-	
D-galactose	+	-	+	+	-	+	nd	nd	+	+	-	+	nd	w	-	-	+	nd	-	+	+	nd	+	+	+	
D-glucose	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-lactose	+	+	+	+	+	+	nd	+	+	+	-	+	+	w	+	+	+	nd	+	+	+	+	+	+	+	+
Maltose	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-mannose	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+
D-xylose	+	+	+	-	-	-	nd	+	-	+	-	+	-	+	-	+	+	w	+	+	+	+	+	+	+	+
D-mannitol	-	+	-	-	-	-	-	+	+	-	-	-	-	+	-	nd	-	nd	-	+	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	+	nd	-	-	-	-	-	-	+	nd	nd	-	nd	-	+	nd	-	-	nd	nd	
myo-Inositol	-	-	-	+	-	-	nd	-	-	-	+	-	-	nd	nd	nd	-	nd	-	+	-	-	-	-	-	nd
Glycerol	-	-	-	+	-	-	nd	-	nd	-	-	-	-	nd	nd	nd	-	nd	-	-	nd	-	-	-	-	nd
<i>N</i> -acetyl-D-glucosamine	-	+	+	+	+	-	-	+	-	+	+	+	+	+	nd	nd	nd	-	nd	+	+	nd	+	+	+	-
<i>Susceptibility to:</i>																										
Gentamicin	+	-	+	-	-	nd	nd	-	+	-	-	-	nd	-	-	+	-	+	-	-	-	+	-	-	-	-
Tetracycline	+	+	-	+	+	nd	nd	+	+	-	-	+	nd	+	-	-	+	-	+	+	-	+	v	-	-	-
DNA G+C content (mol%)	43.7	42	43	41.8	43	43.5	39-41	41	42.5	37-40	42.5	37	43.4	48.5	42	41.4	43	44.8	38	35	49	43.8	39-42	41	38.4	

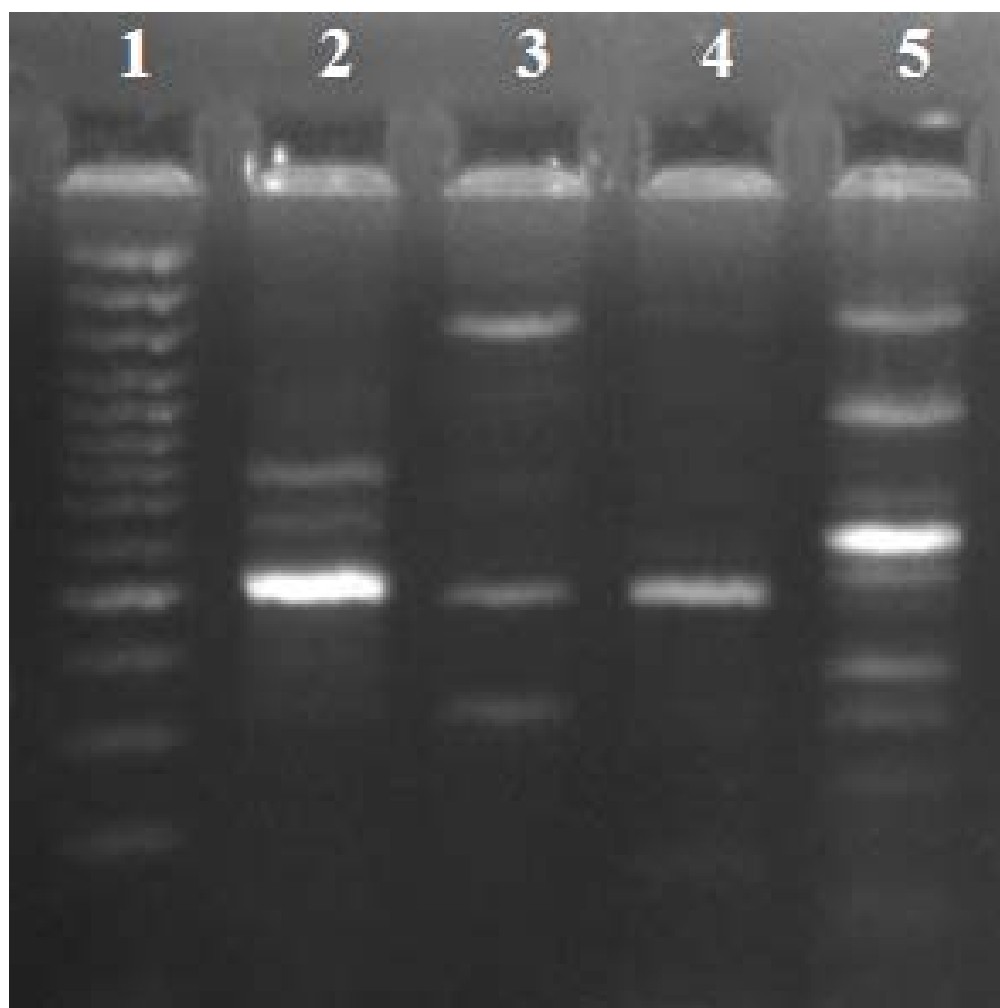




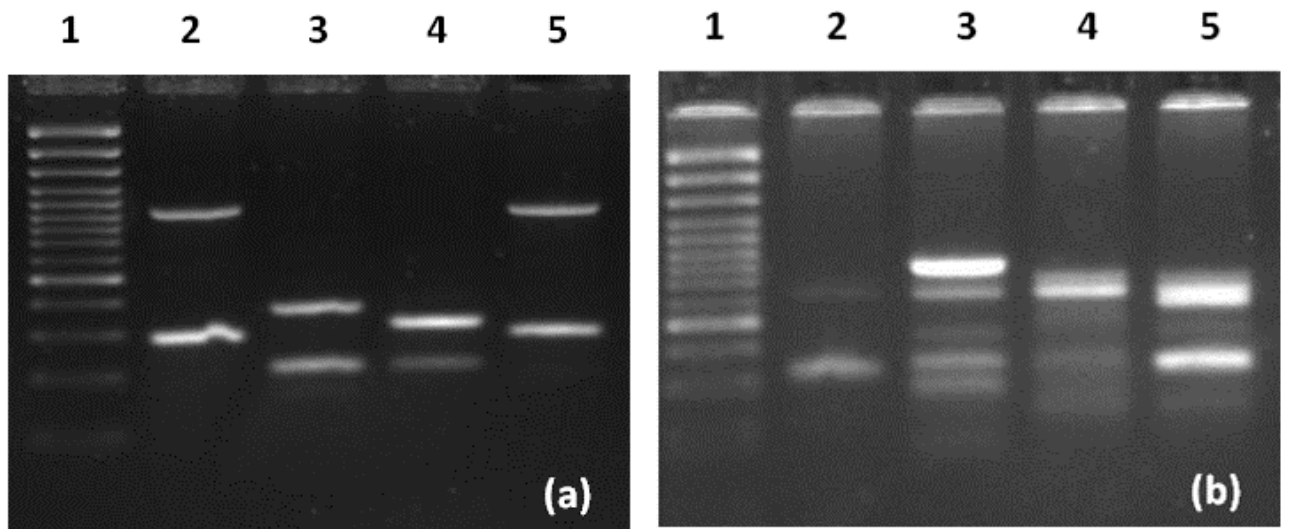
**Fig.1**



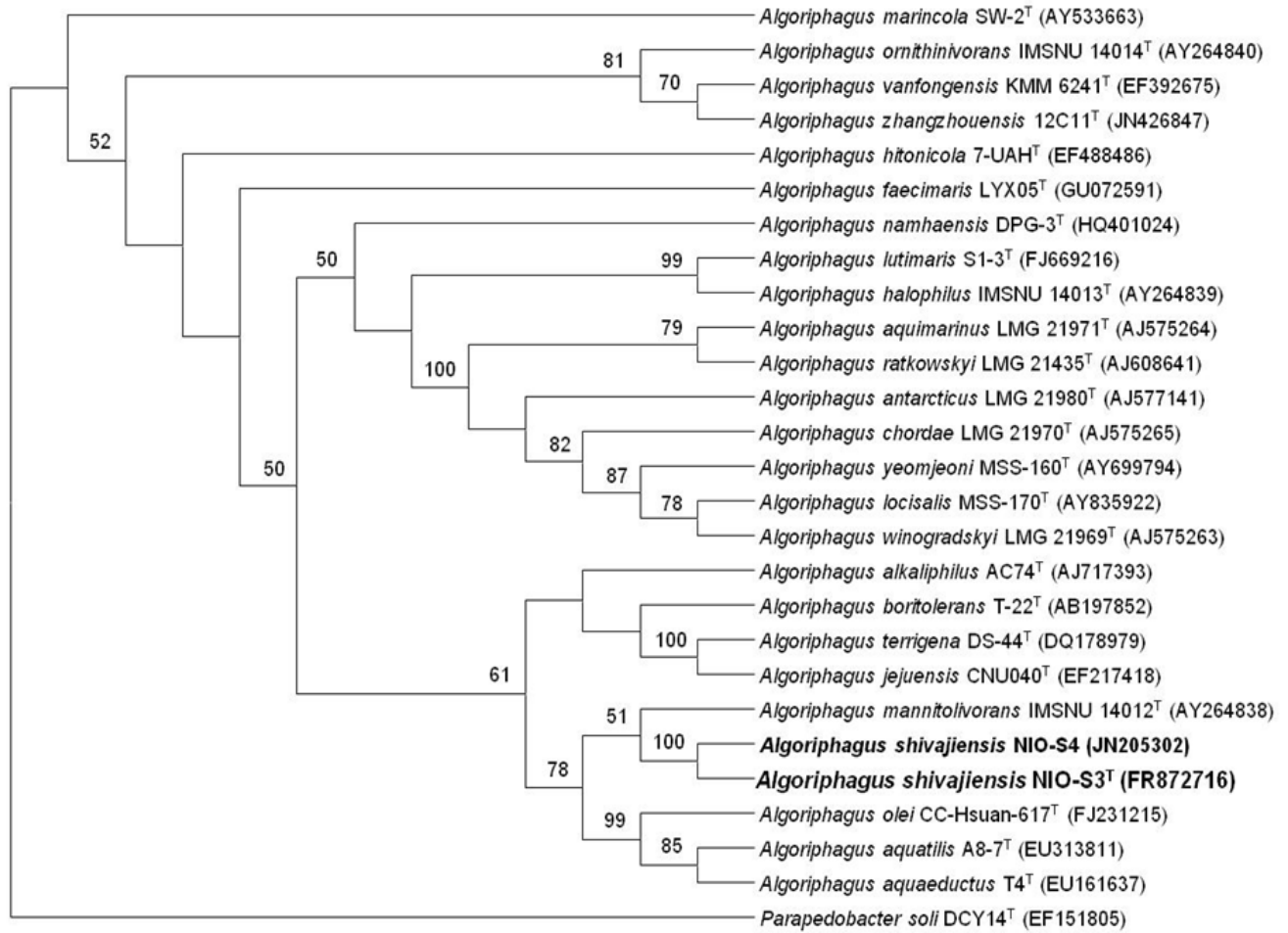
**Supplementary Fig. S1**



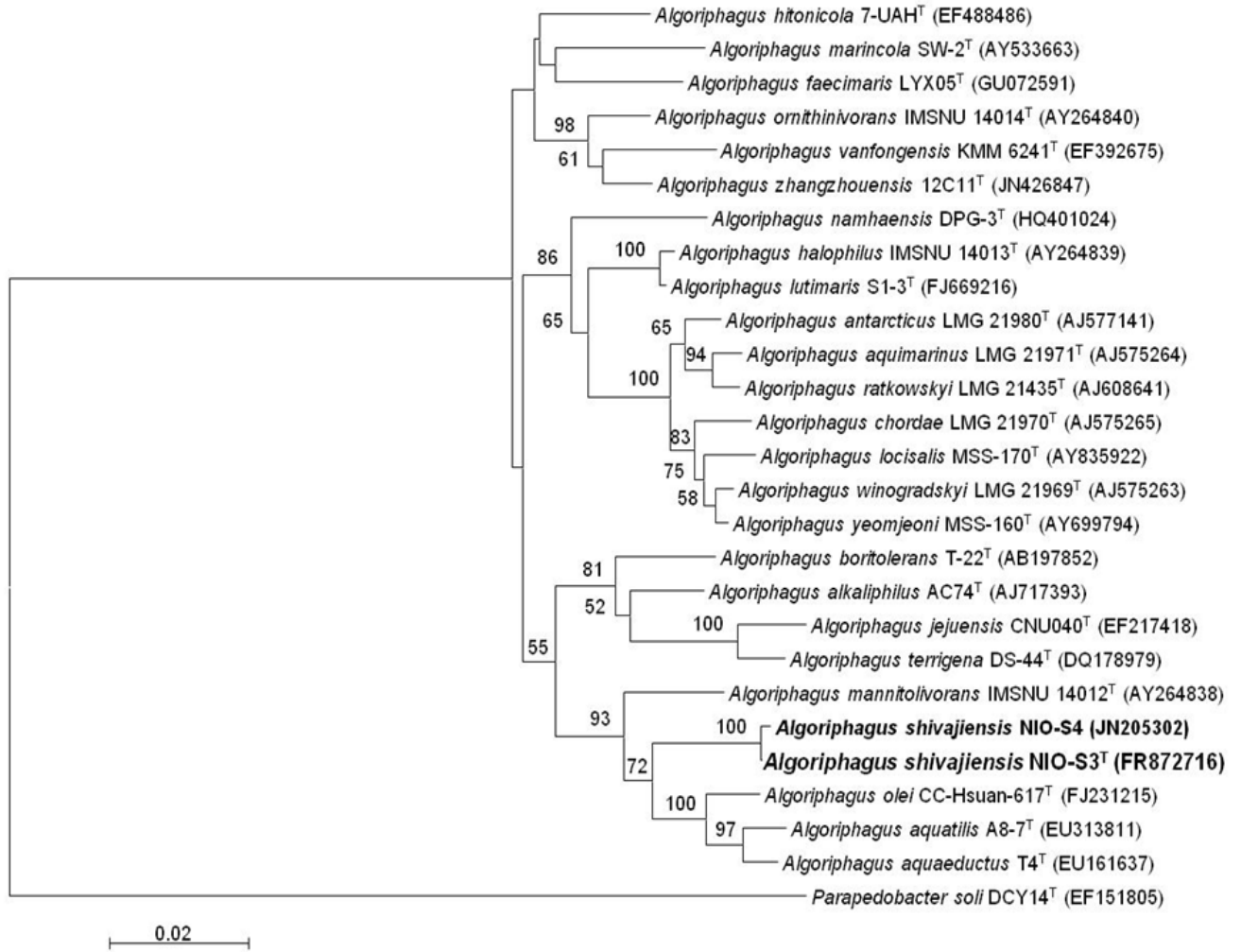
**Supplementary Fig. S2**



Supplementary Fig. S3



Supplementary Fig. S4



**Supplementary Fig. S5**