

***Shivajiella indica* gen. nov., sp. nov., a marine bacterium of the family “*Cyclobacteriaceae*” with nitrate reducing activity**

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The novel orange pigmented, Gram-negative-staining, rod-shaped, non-motile, strictly aerobic, designated strains NIO-S1^T and NIO-S2, were isolated from water sample of a pond adjacent to coast and algal mat from fish pond of Kakinada, India respectively. Both strains were positive for oxidase, catalase and β -galactosidase activities. The predominant fatty acids were iso-C_{15:0} (39.6 %), anteiso-C_{15:0} (9.9 %), iso-C_{17:0} 3OH (10.9 %) and C_{16:1 ω7c}/C_{16:1 ω6c} (summed feature 3) (5.7 %); contained MK-7 as the major respiratory quinone and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and three unidentified lipids as the polar lipids. Phylogenetic analysis indicated that strain NIO-S1^T was a member of the family “*Cyclobacteriaceae*” of the class “*Sphingobacteriia*” and clustered with the genera *Fontibacter*, *Cecembia* and *Aquiflexum* with a phylogenetic distance of 6.8, 9.0 and 12.2 % (93.2, 91.0 and 87.8 % similarity) respectively. DNA-DNA hybridization between strain NIO-S1^T and NIO-S2 showed a relatedness of 93% and rep-PCR banding patterns were similar. Based on data from the current polyphasic study, it is proposed that the new isolate be placed in a new genus and species for which the name as *Shivajiella indica* gen. nov., sp. nov. The type strain of *Shivajiella indica* is NIO-S1^T (= KCTC 19812^T = MTCC 11065^T).

Key words: *Shivajiella indica*, 16S rRNA gene sequence based phylogeny, chemotaxonomic analysis

The family “*Cyclobacteriaceae*”, a member of the class “*Sphingobacteriia*”, within the phylum *Bacteroidetes* was established by Nedashkovskaya and Ludwig [15]. The members of the family are gram negative, rod or ring/circle or horseshoe shaped, aerobic, chemo-organotrophic, pigmented, containing predominantly branched and saturated fatty acids (iso-C_{15 : 0} and anteiso-C_{15 : 0}). The members of the family were widely distributed in diverse habitats like hot spring water, soda lake water, marine surface water, sediment of an oilfield, sea urchin, alkaline ground water, coral, marine sediments, soil enriched with boron, microbial mats from Antarctic lakes, fresh water lake, brown alga, green alga, tidal flat sediment, salty water lagoon, marine solar saltern, sea ice and soil [11, 2, 3, 4, 8, 29, 17, 16, 24, 13, 18, 9, 30, 7, 31]. The family “*Cyclobacteriaceae*” is dynamic and a number of the new genera and species are added in the recent years. At the time of writing the family “*Cyclobacteriaceae*” comprises 8 validly described genera *Algoriphagus*, *Aquiflexum*, *Belliella*, *Cyclobacterium*, *Echinicola*, *Fontibacter*, *Indibacter* and *Nitritalea*. The species of the genera *Chimaericella* and *Hongiella* were reclassified and transferred to the genus *Algoriphagus* [16]. In the present study, we focused on the characterization and classification of the strains NIO-S1^T and NIO-S2, by using a polyphasic approach [25]. From the results of phylogenetic and phenotypic analyses, the strains were assigned to a new genus that belongs to the family “*Cyclobacteriaceae*”.

Strains NIO-S1^T and NIO-S2 were isolated from a sea water sample in a pond adjacent to the coast and from algal mat from a fish pond respectively at Kakinada (GPS Positioning 16°55'51.70"N 82°14'48.48"E and 16°57'48.81"N 82°16'03.03"E), Bay of Bengal, India on 09th September 2009. The samples that yielded strains NIO-S1^T and NIO-S2 had a pH of 7.5 and 7.8 respectively. For isolation of bacteria, 1 ml of the water sample serially diluted in 1 % saline water and from each dilution 100 µl was plated on Marine Agar 2216 (MA; HIMEDIA), and incubated at room temperature for 15 days. Two orange-pigmented isolates were purified by subsequent streaking on marine agar.

Strains NIO-S1^T and NIO-S2 were characterized simultaneously with *Fontibacter flavus* CC-GZM-130^T, *Cecembia lonarensis* LW9^T and *Aquiflexum balticum* DSM 16537^T were kindly provided by DSMZ, Dr. Shivaji and Dr. Chiu-Chung Young respectively. Colony morphology was examined following growth of the strain 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 HiVegTM medium (cat. no. MV847; HIMEDIA) with agar 2 gl⁻¹ (by inoculating the active culture suspension using sterile inoculation needle and checking for spreading of the growth in the medium around the inoculated line). Gram reaction was determined by using the HIMEDIA Gram Staining Kit according to manufacturer's protocol. Growth at 4, 10, 18, 25, 30, 37 and 40°C were ascertained using Zobell marine agar medium and salt tolerance [0, 1, 2, 3, 3.5, 4, 5, 6, 8 and 10% (w/v) NaCl] was ascertained using nutrient agar

(NA) medium containing (l^{-1}) peptone (5 g), beef extract (3 g) and agar (20 g). Growth of strains NIO-S1^T and NIO-S2 at pH 5, 6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11 and 12 was assessed on MA buffered with citric acid/NaOH (for pH 5 and 6), NaHPO₄/Na₂HPO₄ (for pH 7 and 8), glycine/NaOH (for pH 9 and 10) or Tris-HCl or NaOH (for pH 11 and 12). Biochemical characteristics were also assessed using the Hi25TM *Enterobacteriaceae* identification kit (cat. no. KB003) and the HiCarbohydrateTM kit parts A, B and C (cat. no. KB009) (HIMEDIA) according to the manufacturer's protocol. Please see the supplementary Table 2 for explanation regarding different test studied using Hi25TM *Enterobacteriaceae* and HiCarbohydrateTM kits. Biochemical and enzymatic characterization of the strains NIO-S1^T and NIO-S2 were also performed using Vitek 2 GN (bioMérieux), according to the manufacturer's protocol, except that sterile 2.0 % (w/v) NaCl was used to prepare the inoculum. The sensitivity to 22 different antibiotics using the disc diffusion method with commercially available discs (HIMEDIA). Pigment characteristics were determined by previously described methods [4] except that the pigments were extracted in to acetone.

Chemotaxonomic characteristics like fatty acids, polar lipids and quinones were determined by previously described methods [21]. For fatty acid analysis all five strains were grown on TSA plates with 2 % NaCl at 30 °C for two days. DNA G + C content and DNA-DNA hybridization was performed by the membrane filter method as described previously [21]. rep-PCR was performed using primers REP1R-I and REP2-I according to the protocol described by Versalovic et al. [26]. For 16S rRNA gene sequencing, DNA was prepared using a microbial DNA isolation kit (Mo Bio Laboratories Inc.) and sequenced as described previously Lane, [12]. The resultant sequences of the 16S rRNA gene (1385 and 1398 nt) were subjected to BLAST sequence similarity search [1] to identify the nearest taxa. To reveal the exact phylogenetic position of strains NIO-S1^T and NIO-S2 in the domain *Bacteria*, the 16S rRNA gene sequences were aligned against an ARB dataset using the ARB program package [14]. 16S rRNA gene sequences of the closely related members of the family "*Cyclobacteriaceae*" were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_X program [23] and the alignment was then corrected manually. Phylogenetic trees were constructed using tree-making algorithm, the maximum likelihood method using the PhyML program [10] and neighbor joining method using the MEGA4 package [22] and the resultant tree topologies were evaluated based on 100 resamplings.

Cells of strains NIO-S1^T and NIO-S2 were Gram-negative-staining, non-motile rods, 0.5-0.8 µm wide and 2.0-5.0 µm long (Supplementary Fig. S1). Colonies were circular, 2-3 mm in diameter, smooth, orange, translucent and raised with entire margins on marine agar. Strains were positive for oxidase and catalase activities. Strains NIO-S1^T and NIO-S2 reduced nitrate. Growth was observed at

25-37°C (optimum, 30°C), with 0-3% NaCl (optimum, 1-2%) and at pH 7-11 (optimum, 7.5). Other characteristics are presented in Table 1. Vitek GN card results of strains NIO-S1^T and NIO-S2 were presented in species description and Supplementary Table 1.

The absorption spectrum of the acetone extract of strains NIO-S1^T and NIO-S2 showed a broad peak with a maximum around 475 and 478 nm respectively, typical for carotenoid pigments [4, 6]. The cellular fatty acid composition of strain NIO-S1^T and NIO-S2 showed a spectrum of 17 fatty acids with a pronounced dominance of (>5 %) of iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{17:0} 3OH and C_{16:1 ω7c}/C_{16:1 ω6c} (summed feature 3) (Table 2). Saturated fatty acids constituted 79.6 % of the total fatty acids. Compared with *Fontibacter flavus* CC-GZM-130^T, *Cecembia lonarensis* LW9^T and *Aquiflexum balticum* BA160^T composition of fatty acids differed considerably in strains NIO-S1^T and NIO-S2 (Table 2). The menaquinone present was MK-7 which was also present in type strains. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and three unidentified lipids (Supplementary Fig. S2). Type strains *Fontibacter flavus* CC-GZM-130^T, *Cecembia lonarensis* LW9^T and *Aquiflexum balticum* BA160^T contains only phosphatidylethanolamine as the major phospholipid and diphosphatidylglycerol and phosphatidylglycerol were not present [11, 4]. But the presence of diphosphatidylglycerol and phosphatidylglycerol was also reported from other members of the phylum *Bacteroidetes*, including *Arcicella aurantiaca* [20], *Muricauda lutaonensis* [5] and *Pontibacter populi* [28] apart from phosphatidylethanolamine. The presence of phosphatidylglycerol was also reported from *Runella limosa* [19] member of *Cytophagaceae*.

The DNA G + C content of strains NIO-S1^T and NIO-S2 were 41.2 and 41.3 mol%. DNA hybridization between strains NIO-S1^T and NIO-S2 showed a relatedness of 93%. This value was upper 70%, confirming that the novel strains are members of the same species [27]. rep-PCR fingerprint pattern between strains NIO-S1^T and NIO-S2 was almost identical (Supplementary Fig. S3).

The phylogenetic relationships of strains NIO-S1^T and NIO-S2 were ascertained based on the 16S rRNA gene sequence similarity with other strains using BLAST sequence similarity search (NCBI-BLAST). The 16S rRNA gene sequence analysis placed strains NIO-S1^T and NIO-S2 within the family “*Cyclobacteriaceae*”. The results indicated that at the 16S rRNA gene sequence level, strains NIO-S1^T and NIO-S2 were very close and shared 99.99% sequence similarity and together were close to the phylogenetic neighbors *Fontibacter flavus*, *Cecembia lonarensis* and *Aquiflexum balticum* with pairwise sequence similarity of 94, 94 and 93 % respectively. In Parsimony tree constructed using ARB software package also strains NIO-S1^T and NIO-S2 were clustered with *Fontibacter flavus* (Data not shown). Phylogenetic analyses based on maximum-likelihood tree further indicated that strain NIO-S1^T and

NIO-S2 were clustered together and in turn clustered with *Fontibacter flavus*, *Cecembia lonarensis* and *Aquiflexum balticum* at a phylogenetic distance of 6.8, 9.0 and 12.2 % (93.2, 91.0 and 87.8 % similarity) respectively. Neighbour joining tree topology was similar to the ML tree (data not shown).

The characteristics that differentiate strains NIO-S1^T and NIO-S2 from the type strains of species *Fontibacter flavus*, *Cecembia lonarensis* and *Aquiflexum balticum* which belong to the same family are given in Table 1. The 16S rRNA gene sequence analysis showed a clear dissimilarity between *Shivajiella indica* (strains NIO-S1^T and NIO-S2) and its phylogenetic neighbors and other members of the family “*Cyclobacteriaceae*” (Fig. 1). Based on the 16S rRNA gene sequence phylogeny, the nearest taxa in the family “*Cyclobacteriaceae*” was *Fontibacter flavus*. For instance, strains NIO-S1^T and NIO-S2 differed from *Fontibacter flavus* CC-GZM-130^T with respect to colony colour, cell size, tolerance to salt, growth at 40 °C, pH growth range, some biochemical characteristics, utilization of various carbon sources, antibiotic susceptibility, fatty acid and polar lipid composition and DNA G + C content (Tables 1 and 2). Thus, the cumulative differences that strains NIO-S1^T and NIO-S2 exhibits from the *Fontibacter flavus* unambiguously support the creation of a new genus and species to accommodate strains NIO-S1^T and NIO-S2, for which the name *Shivajiella indica* gen. nov., sp. nov. is proposed.

Description of *Shivajiella* gen. nov.

Shivajiella [Shi.va.ji'.el'la. N.L. fem. dim. n. Shivajiella, named after Dr. Shivaji, an eminent Indian microbiologist who has made a significant contribution to our knowledge of heterotrophic bacteria from different habitats world over].

Cells are Gram-negative-staining, non-motile, strictly aerobic, rod shaped, oxidase and catalase positive. The major fatty acids are iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{17:0} 3OH and C_{16:1 ω7c}/C_{16:1 ω6c} (summed feature 3). MK-7 is the predominant respiratory quinone. The polar lipids include diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and three unidentified lipids (L). The genus is affiliated to the family “*Cyclobacteriaceae*”. The DNA G + C content is 41.2 to 41.3 mol%. The genus is a member of the family “*Cyclobacteriaceae*” of the class “*Sphingobacteriia*” of the phylum “*Bacteroidetes*”. The type species is *Shivajiella indica*.

Description of *Shivajiella indica* sp. nov.

Shivajiella indica (in'di.ca. L. fem. adj. *indica* of India, Indian, named after India the country in which the type strain was isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.5-0.8 μm wide and 2.0-5.0 μm long occurs singly. Colonies on marine agar are circular, 2-3 mm in diameter, smooth, orange, translucent and raised with entire margins. The colour of the strains is due to the presence of carotenoids but not flexirubin. Growth was observed at 25-37°C (optimum, 30°C) and at pH 7-11 (optimum, 7.5). Strains tolerated up to 3% NaCl (optimum, 1-2%). Strains grow without NaCl also. β -galactosidase activity is present. In Hi25™ *Enterobacteriaceae* identification kit it is negative for lysine decarboxylase, ornithine decarboxylase and phenylalanine deaminase activities. Nitrate is reduced but H₂S and indole are not produced. The methyl red and Voges-Proskauer's reactions are negative. Aesculin, casein, cellulose, starch, Tween 40 and urea are not hydrolysed. In VITEK GN ID card, strains are positive for β -galactosidase, β -*N*-acetylglucosaminidase, γ -glutamyl-transferase, β -xylosidase, L-proline arylamidase, tyrosine arylamidase, α -glucosidase, α -galactosidase, phosphatase, glycine arylamidase and glu-gly-arg-arylamidase activities but negative for L-Pyrrolydonyl-arylamidase, β -alanine arylamidase pNA, lipase, β -*N*-acetylgalactosaminidase and β -glucuronidase. Ala-Phe-Pro-arylamidase, glutamyl arylamidase pNA and β -glucosidase activities are variable (Vitek GN card system) (positive in strain NIO-S1^T). Utilizes D-cellobiose and D-trehalose, do not utilize L-arabitol, D-mannitol, D-mannose, palatinose, D-sorbitol, D-tagatose, 5-keto-D-gluconate, L-histidine, coumarate, L-malate and L-lactate and variable with D-glucose (positive in strain NIO-S1^T) and D-maltose (negative in strain NIO-S1^T) (Vitek GN card system). Negative for L-lactate and succinate alkalisation, fermentation of glucose and variable for ELLMAN reaction (positive in strain NIO-S1^T) and 0/129 resistance (negative in strain NIO-S1^T) (Vitek GN card system). Produces acid from cellobiose, galactose, lactose, malonate, mannose, melibiose, methyl α -D-glucoside, sucrose and trehalose (in the Hi25™ *Enterobacteriaceae* identification kit) and the new species is able to ferment the same sugars plus citrate when using the HiCarbohydrate™ kit. Both strains produce weakly acid from maltose and xylitol but only strain NIO-S1^T utilizes weakly methyl α -D-mannoside, raffinose and glucose. Do not utilizes adonitol, arabitol, L- or D-arabinose, dulcitol, erythritol, fructose, glycerol, inositol, inulin, mannitol, melezitose, rhamnose, salicin, sodium gluconate, sorbitol, sorbose and xylose. Susceptible to (μg per disc unless indicated) amoxicillin (20), ampicillin (10), azithromycin (15), bacitracin (10), cephalothin (30), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamycin (10), kanamycin (30), methicillin (5), nalidixic acid (30), neomycin (30), nitrofurantoin (300), norfloxacin (10), penicillin G (10), rifampicin (5), streptomycin (10), tetracycline (30), trimethoprim (5), tobramycin (10) and vancomycin (30). The cellular fatty acid composition is given in Table 2. The type strain, NIO-S1^T (= KCTC 19812^T = MTCC 11065^T) was isolated from a water sample of a pond adjacent to coast, Kakinada, Bay of Bengal, India and the reference strain NIO-S2 was isolated from a algal mat from a fish pond at Kakinada, Bay of Bengal, India.

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Legends to Figures

Fig. 1. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strains NIO-S1^T and NIO-S2 and other representatives of the family “*Cyclobacteriaceae*”. Filled circles indicate branching topologies that are common in the trees obtained using the neighbor-joining and maximum-likelihood methods. Numbers at the nodes are bootstrap values >50%. *Parapedobacter soli* DCY14^T was used as an out group. Bar, 0.02 substitutions per nucleotide position.

Supplementary Fig. S1. Phase contrast photograph of cells of strain NIO-S1^T. Bar, 5 μm.

Supplementary Fig. S2. Two-dimensional thin-layer chromatogram of polar lipids of the strain NIO-S1^T. Detection of total lipids by molybdato-phosphoric acid. Abbreviations: DPG, diphosphatidyl-glycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; L, unidentified lipid.

Supplementary Fig. S3. Characteristic rep-PCR fingerprint patterns of strains NIO-S1^T and NIO-S2. Lane 1, 1Kb Marker; lane 2, NIO-S1^T; and lane 3, NIO-S2.

Table 1. Features that distinguish strains NIO-S1^T, NIO-S2 from the closely related species of the genera *Fontibacter*, *Cecembia* and *Aquiflexum*

Data from the present study. All the strains were rod-shaped, positive for catalase activity, negative for phenylalanine deaminase activity, indole and H₂S production, methyl red and Voges Proskauer's reactions, negative for hydrolysis of cellulose, Tween 40 and urea, utilized malaonate and glucose, do not utilized arabitol, dulcitol, glycerol, inositol, L-arabinose, salicin and sorbitol. All strains were sensitive to (µg per disc) ampicillin (10), bacitracin (10), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamycin (10), nalidixic acid (30), norfloxacin (10), penicillin G (10), rifampicin (5), streptomycin (10), tetracycline (30) and vancomycin (30). +, positive; -, negative; w, weak; R, resistant; S, sensitive; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

Characteristic	<i>Shivajiella indica</i> NIO-S1 ^T	<i>Shivajiella indica</i> NIO-S2	<i>Fontibacter flavus</i> CC-GZM-130 ^T	<i>Cecembia lonarensis</i> LW9 ^T	<i>Aquiflexum balticum</i> BA160 ^T
Cell size (µm)	0.5-0.8 x 2.0-5.0	0.5-0.8 x 2.0-5.0	0.3-0.6 x 1.5- 3.2	0.5-0.6 x 1.5-3.0	0.3-0.5 x 1.5-4.5
Colony colour	Orange	Orange	Bright orange	Reddish- orange	Red
Salinity growth range (%)	0-3	0-3	0-3.5	0-4	0-6
Temperature growth range (°C)	25-37	25-37	25-40	10-40	4-40
Temperature optimum (°C)	30	30	30	30-37	30
pH growth range	7-11	7-11	7-9	7.5-10	7-10
Biochemical:					
Oxidase	+	+	+	-	+
Lysine decarboxylase	-	-	+	+	-
Ornithine decarboxylase	-	-	+	+	+
Citrate utilization	+	+	-	+	+
Nitrate reduction	+	+	-	-	+
Hydrolysis of:					
Aesculin	-	-	-	-	+
Gelatin	-	-	-	-	+
ONPG	+	+	-	-	+
Starch	-	-	-	-	+
Production of acid from:					
Adonitol	-	-	-	+	-
Cellobiose	+	+	-	+	-
D-arabinose	-	-	-	+	-
Erythritol	-	-	w	+	-
Fructose	-	-	w	+	+
Galactose	+	+	-	-	+
Inulin	-	-	w	-	-
Lactose	+	+	-	+	+
Maltose	w	w	w	+	-
Mannitol	-	-	-	-	+
Mannose	+	+	w	+	+
Melibiose	+	+	-	+	-
Melezitose	-	-	w	-	-

Characteristic	<i>Shivajiella indica</i> NIO-S1 ^T	<i>Shivajiella indica</i> NIO-S2	<i>Fontibacter flavus</i> CC-GZM-130 ^T	<i>Cecembia lonarensis</i> LW9 ^T	<i>Aquiflexum balticum</i> BA160 ^T
Methyl α-D-glucoside	+	+	W	-	-
Methyl α-D-mannoside	W	+	-	-	-
Raffinose	W	+	-	+	-
Rhamnose	-	-	+	+	-
Sodium gluconate	-	-	-	-	+
Sorbose	-	-	-	-	+
Sucrose	+	+	W	+	-
Trehalose	+	+	W	-	-
Xylitol	W	W	-	-	-
Xylose	-	-	W	+	+
Antibiotic susceptibility:					
Kanamycin	S	S	R	R	S
Neomycin	S	S	R	S	S
Nitrofurantoin	S	S	R	S	S
Tobramycin	S	S	R	R	S
Major fatty acids	iso-C ₁₅ : 0, anteiso-C ₁₅ : 0, iso-C ₁₇ : 0 3OH, SF3 [#]	iso-C ₁₅ : 0, anteiso-C ₁₅ : 0, iso-C ₁₇ : 0 3OH, SF3 [#]	iso-C ₁₅ : 0, anteiso-C ₁₅ : 0, iso-C ₁₇ : 0 3OH, SF3 [#]	iso-C ₁₅ : 0, anteiso-C ₁₅ : 0, iso-C ₁₆ : 0, iso-C ₁₆ : 1 H, iso-C ₁₇ : 0 3OH, iso-C ₁₇ : 1ω9c, SF3 [#]	iso-C ₁₅ : 0, anteiso-C ₁₅ : 0, iso-C ₁₅ : 1 G, iso-C ₁₆ : 1 H, iso-C ₁₇ : 0 3OH, iso-C ₁₇ : 1ω9c, SF3 [#]
*Phospholipids	DPG, PG, PE	DPG, PG, PE	PE	PE	PE
*DNA G + C content (mol%)	41.2	41.3	53.2	40.5	38.4
*Habitat	Coastal sea water	Algal mat of a fish pond	Hot spring	Soda lake	Baltic Sea

*Data from the present study; Kämpfer *et al.* [11], Anil Kumar *et al.* [4], Brettar *et al.* [8].

[#]SF3, summed feature 3. Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C₁₆:1ω7c/iso-C₁₅:0 2OH

Table 2. Fatty acid profile of strains NIO-S1^T, NIO-S2 and the closely related species of the genera *Fontibacter*, *Cecembia* and *Aquiflexum*.

Data from the present study. All five strains were grown on TSA plates with 2 % NaCl 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.

Fatty acid composition	<i>Shivajiella indica</i> NIO-S1 ^T	<i>Shivajiella indica</i> NIO-S2	<i>Fontibacter flavus</i> CC-GZM-130 ^T	<i>Cecembia lonarensis</i> LW9 ^T	<i>Aquiflexum balticum</i> BA160 ^T
iso-C _{14:0}	ND	ND	ND	1.9	3.2
C _{15:0} 2OH	ND	ND	ND	ND	1.5
C _{15:0} 3OH	ND	ND	ND	1.6	ND
iso-C _{15:0}	39.6	38.9	29.6	25.0	19.8
anteiso-C _{15:0}	9.9	9.7	7.4	5.1	14.8
iso-C _{15:0} 3OH	2.2	2.2	2.3	ND	1.3
iso-C _{15:1} G	2.6	2.6	2.3	4.1	8.3
C _{15:1} ω _{6c}	2.3	2.3	1.9	3.5	1.2
C _{16:0}	ND	ND	1.9	ND	ND
C _{16:0} 3OH	1.6	1.6	3.0	1.8	1.5
iso-C _{16:0}	3.1	3.1	2.9	10.0	3.3
iso-C _{16:0} 3OH	2.4	2.4	2.4	3.6	3.1
iso-C _{16:1} G	1.4	1.4	ND	ND	ND
iso-C _{16:1} H	ND	ND	1.8	7.5	8.3
C _{16:1} ω _{5c}	3.0	3.2	8.5	3.6	2.9
C _{17:0} 2OH	1.5	1.6	2.0	2.5	2.3
C _{17:0} 3OH	1.1	1.1	ND	ND	ND
iso-C _{17:0} 3OH	10.9	11.0	9.3	6.1	5.3
anteiso-C _{17:1} B	ND	ND	ND	ND	2.9
C _{17:1} ω _{6c}	2.0	2.0	2.1	4.3	4.3
iso-C _{17:1} ω _{9c}	ND	ND	ND	5.9	5.1
anteiso-C _{17:1} ω _{9c}	ND	ND	ND	ND	1.6
iso-C _{19:1} I	ND	ND	ND	ND	1.2
Summed Feature 3	5.7	5.7	11.5	6.9	7.6
Summed Feature 4	2.0	2.1	2.2	ND	ND
Summed Feature 9	3.5	3.7	4.4	ND	ND

#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_{16:1} ω_{7c}/iso-C_{15:0} 2OH; summed feature 4 contains iso-C_{17:1} I/anteiso-C_{17:1} B; anteiso-C_{17:1} B/ iso-C_{17:1} I; summed feature 9 contains iso-C_{17:1} ω_{9c}/C_{16:0} 10-methyl

Supplementary Table 1. Biochemical and enzymatic characteristics of strains NIO-S1^T and NIO-S2 revealed from the GN card of VITEK 2 system

Characteristic	<i>Shivajiella indica</i> NIO-S1 ^T	<i>Shivajiella indica</i> NIO-S2
Ala-Phe-Pro-arylamidase	+	-
L-pyrrolydonyl-arylamidase	-	-
L-arabitol	-	-
D-cellobiose	+	+
β -galactosidase	+	+
β -N-acetylglucosaminidase	+	+
Glutamyl arylamidase pNA	+	-
D-glucose	+	-
γ -glutamyl-transferase	+	+
Fermentation glucose	-	-
β -glucosidase	+	-
D-maltose	-	+
D-mannitol	-	-
D-mannose	-	-
β -xylosidase	+	+
β -alanine arylamidase pNA	-	-
L-proline arylamidase	+	+
Lipase	-	-
Palatinose	-	-
Tyrosine arylamidase	+	+
D-sorbitol	-	-
D-tagatose	-	-
D-trehalose	+	+
5-keto-D-gluconate	-	-
L-lactate alkalisation	-	-
α -glucosidase	+	+
Succinate alkalisation	-	-
β -N-acetylgalactosaminidase	-	-
α -galactosidase	+	+
Phosphatase	+	+
Glycine arylamidase	+	+
L-histidine	-	-
Courmarate	-	-
β -glucuronidase	-	-
O/129 resistance	-	+
glu-gly-arg-arylamidase	+	+
L-malate assimilation	-	-
ELLMAN	+	-
L-lactate assimilation	-	-

Supplementary- Table 2. Principles of different tests done by using Hi25™ *Enterobacteriaceae* Identification and HiCarbohydrate™ Kits

Characteristic	Principle
Hi25™ <i>Enterobacteriaceae</i> Identification Kit	
ONPG	Detects β -galactosidase activity
Lysine utilisation	Detects Lysine decarboxylation
Ornithine utilisation	Detects Ornithine decarboxylation
Urease	Detects Urease activity
Phenylalanine deamination	Detects Phenylalanine deamination activity after adding TDA reagent
Nitrate reduction	Detects Nitrate reduction after adding sulphanic acid and N,N-Dimethyl-1-Naphthylamine reagents
H ₂ S production	Detects H ₂ S production
Citrate utilisation	Detects capability of organism to utilize citrate as a sole carbon source
Voges Proskauer's reaction	Detects acetoin production after adding Baritt reagents A and B
Methyl red reaction	Detects acid production after adding Methyl red reagent
Indole	Detects deamination of tryptophan after adding Kovac's reagent
Malonate utilisation	Detects capability of organism to utilize sodium malonate as a sole carbon source
HiCarbohydrate™ Kit	
Lactose	Detects utilization – fermentation which results in acid production
Xylose	Detects utilization – fermentation which results in acid production
Maltose	Detects utilization – fermentation which results in acid production
Fructose	Detects utilization – fermentation which results in acid production
Dextrose	Detects utilization – fermentation which results in acid production
Galactose	Detects utilization – fermentation which results in acid production
Raffinose	Detects utilization – fermentation which results in acid production
Trehalose	Detects utilization – fermentation which results in acid production
Melibiose	Detects utilization – fermentation which results in acid production
Sucrose	Detects utilization – fermentation which results in acid production
L-Arabinose	Detects utilization – fermentation which results in acid production
Mannose	Detects utilization – fermentation which results in acid production
Inulin	Detects utilization – fermentation which results in acid production
Sodium gluconate	Detects utilization – fermentation which results in acid production
Glycerol	Detects utilization – fermentation which results in acid production
Salicin	Detects utilization – fermentation which results in acid production
Dulcitol	Detects utilization – fermentation which results in acid production
Inositol	Detects utilization – fermentation which results in acid production
Sorbitol	Detects utilization – fermentation which results in acid production
Mannitol	Detects utilization – fermentation which results in acid production
Adonitol	Detects utilization – fermentation which results in acid production
Arabitol	Detects utilization – fermentation which results in acid production
Erythritol	Detects utilization – fermentation which results in acid production
Methyl α -D-glucoside	Detects utilization – fermentation which results in acid production
Rhamnose	Detects utilization – fermentation which results in acid production
Cellobiose	Detects utilization – fermentation which results in acid production
Melezitose	Detects utilization – fermentation which results in acid production

Methyl α -D-mannoside	Detects utilization – fermentation which results in acid production
Xylitol	Detects utilization – fermentation which results in acid production
ONPG	Detects β -galactosidase activity
Esculin hydrolysis	Detects esculin hydrolysis
D-Arabinose	Detects utilization – fermentation which results in acid production
Citrate utilization	Detects capability of organism to utilize citrate as a sole carbon source
Malonate utilization	Detects capability of organism to utilize sodium malonate as a sole carbon source
Sorbose	Detects utilization – fermentation which results in acid production

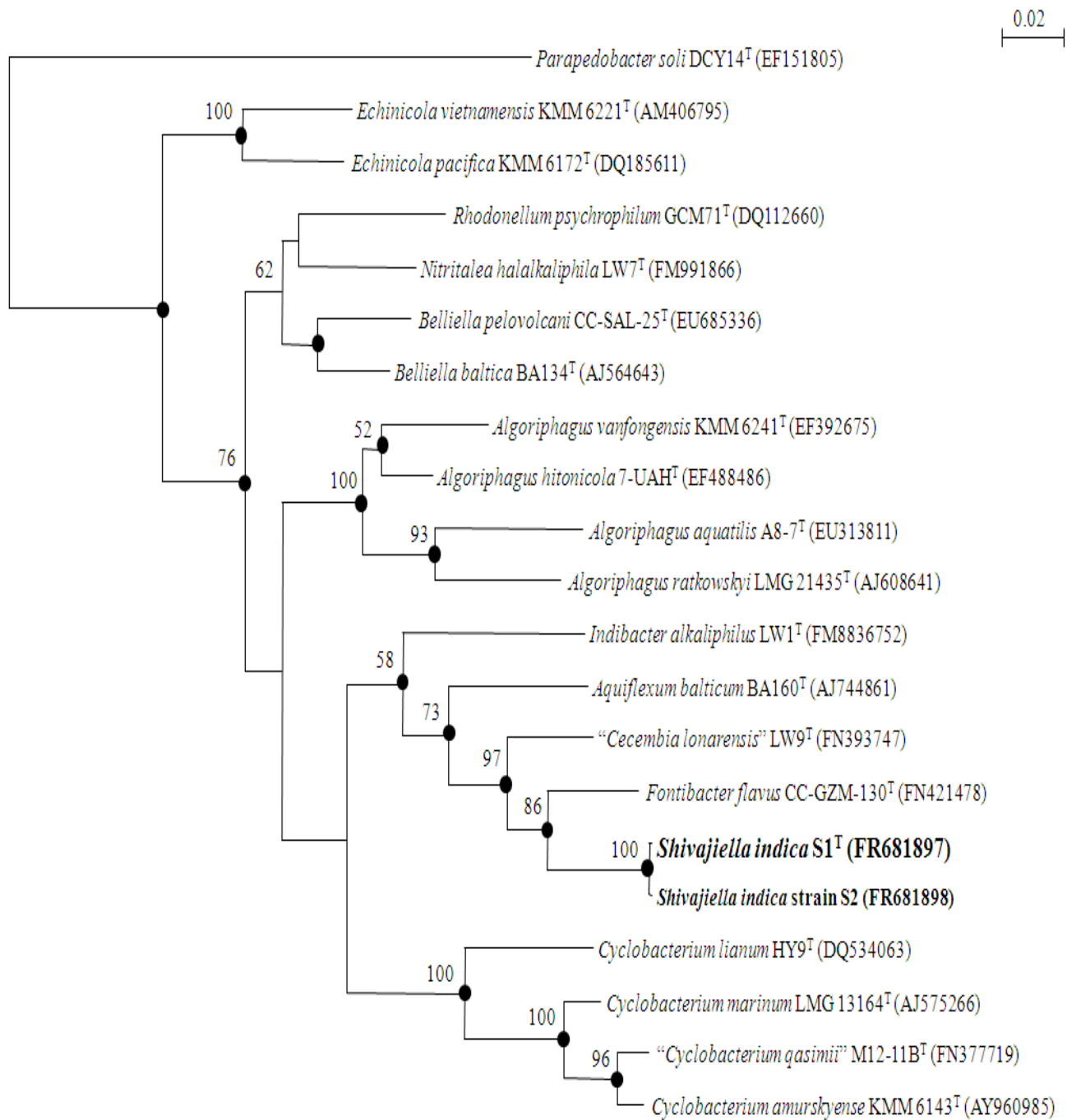
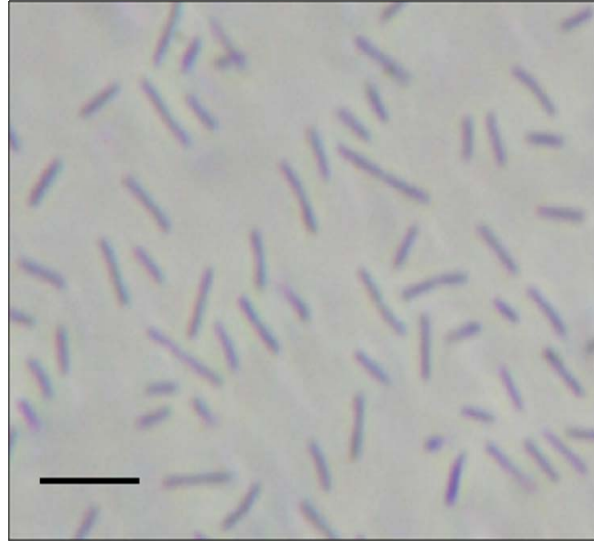
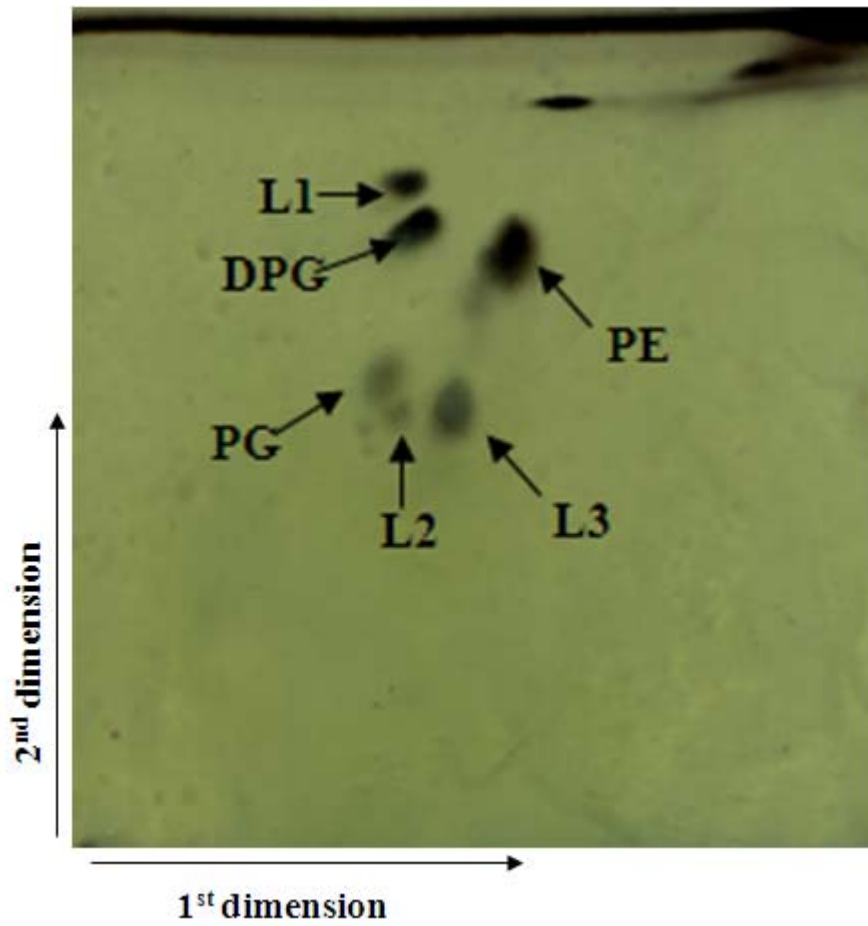


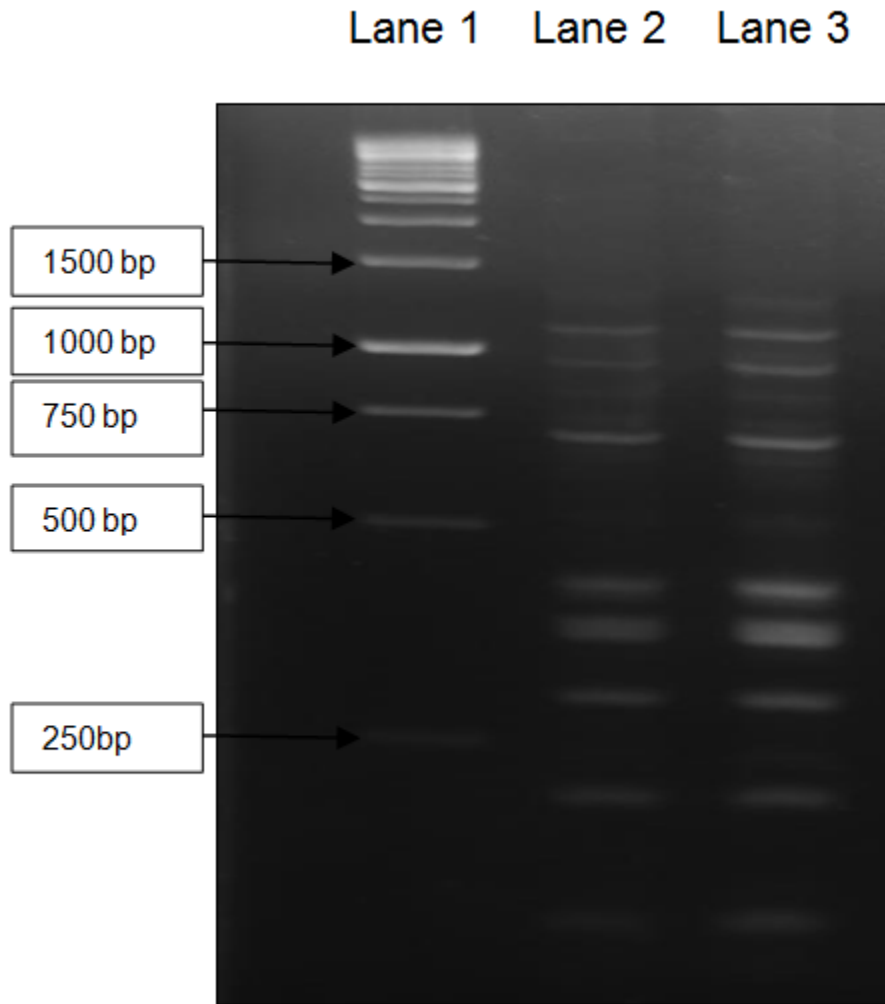
Fig. 1



Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Fig. S3