

***Mariniradius saccharolyticus* gen. nov., sp. nov., a member of the family *Cyclobacteriaceae* isolated from marine aquaculture pond water and emended descriptions of the genus *Aquiflexum* and *Aquiflexum balticum* are also proposed**

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Running title

*Mariniradius saccharolyticus* gen. nov., sp. nov.,

Subject category

New taxa (*Cytophagia*)

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A novel marine, Gram-staining-negative, oxidase- and catalase- positive, rod-shaped bacterium, designated strain AK6<sup>T</sup>, was isolated from marine aquaculture pond water collected in Andhra Pradesh, India. The fatty acids were dominated by iso-C<sub>15:0</sub>, iso-C<sub>17:1</sub> ω<sub>9c</sub>, iso-C<sub>15:1</sub> G, iso-C<sub>17:0</sub> 3OH and anteiso-C<sub>15:0</sub>. Strain AK6<sup>T</sup> contained MK-7 as the sole respiratory quinone and phosphatidylethanolamine, one unidentified aminophospholipid, one unidentified phospholipid and six unidentified lipids as polar lipids. The DNA G + C content of strain AK6<sup>T</sup> was 45.6 mol%. Phylogenetic analysis showed that strain AK6<sup>T</sup> formed a distinct branch within the family *Cyclobacteriaceae* and clustered with *Aquiflexum balticum* DSM 16537<sup>T</sup> and other members of the family *Cyclobacteriaceae*. 16S rRNA gene sequence analysis confirmed that *Aquiflexum balticum* DSM 16537<sup>T</sup> was the nearest neighbor, with pairwise sequence similarity of 90.1%, while sequence similarity with the other members of the family was < 88.5%. Based on differentiating phenotypic characteristics and phylogenetic inference, strain AK6<sup>T</sup> is proposed as a representative of a new genus and species of the family *Cyclobacteriaceae* as *Mariniradius saccharolyticus* gen. nov., sp. nov. The type strain is AK6<sup>T</sup> (= MTCC 11279<sup>T</sup> = JCM 17389<sup>T</sup>). Emended descriptions of the genus *Aquiflexum* and *Aquiflexum balticum* are also proposed.

Members of the family *Cyclobacteriaceae* (order *Cytophagales*, within the phylum *Bacteroidetes*) are Gram-staining-negative, aerobic, chemo-organoheterotrophic, straight, ring/circle or horse-shoe shaped rods, usually non-motile (members of the genus *Echinicola* are motile by gliding), producing pink, red or orange pigments and containing predominantly branched and saturated fatty acids (e.g. iso-C<sub>15:0</sub>), menaquinone 7 (MK-7) and phosphatidylethanolamine (Nedashkovskaya & Ludwig, 2011). Members of the family are widely distributed in diverse habitats such as hot spring (Kämpfer *et al.*, 2010), soda lake (Anil Kumar *et al.*, 2010a, b; Anil Kumar *et al.*, 2012; Yang *et al.*, 2012), soil (Yoon *et al.*, 2006), fresh water lake (Liu *et al.*, 2009), various marine habitats such as marine surface water, marine organisms, salty water lagoon, sediment of an oilfield, and solar saltern (Brettar *et al.*, 2004a and b; Nedashkovskaya *et al.*, 2004, 2006; Copa-Patiño *et al.*, 2008; Ying *et al.*, 2006; Yoon *et al.*, 2005) and polar habitats (Bowman *et al.*, 2003; Van Trappen *et al.*, 2004). At the time of writing, the family *Cyclobacteriaceae* comprises 10 validly named genera: *Algoriphagus* (Bowman *et al.*, 2003), *Aquiflexum* (Brettar *et al.*, 2004b), *Belliella* (Brettar *et al.*, 2004a), *Cecemia* (Anil Kumar *et al.*, 2012), *Cyclobacterium* (Raj *et al.*, 1990), *Echinicola* (Nedashkovskaya *et al.*, 2006), *Fontibacter* (Kämpfer *et al.*, 2010), *Indibacter* (Anil Kumar *et al.*, 2010), *Mongoliitalea* (Yang *et al.*, 2012) and *Nitritalea* (Anil Kumar *et al.*, 2010). However, the genera *Litoribacter* and *Rhodonellum*, members of the family *Cytophagaceae*, cluster with members of family *Cyclobacteriaceae* in phylogenetic trees (All-Species Living Tree; Release LTPs106; Schmidt *et al.*, 2006). In the present study, we focused on the characterization and classification of the strain AK6<sup>T</sup>, by using a polyphasic approach (Vandamme *et al.*, 1996). From the results of phylogenetic and phenotypic analyses, the strain was assigned to a new genus and species in the family *Cyclobacteriaceae*.

Strain AK6<sup>T</sup> was isolated from seawater of a fish culture pond at Bheemli (GPS Positioning 17° 53'N 83° 26'E), Andhra Pradesh, India. The sample (1 ml) was serially diluted (10 fold dilutions) in 2% NaCl (w/v) solution and 100 µl of each dilution was plated on ZoBell marine agar (MA, HIMEDIA, India) and incubated at 30°C. A reddish-orange colored colony observed after five days of incubation was selected and characterized in the present study. Sub-cultivation of the isolate was carried out on MA at 30°C. Stock cultures were preserved at -80°C in marine broth (MB, HIMEDIA) with 10% glycerol.

DNA isolation, 16S rRNA gene amplification and sequencing were done as described previously (Srinivas *et al.*, 2011; Surendra *et al.*, 2011). The 16S rRNA gene was sequenced using primers 530f and 907r (Johnson, 1994) in addition to the PCR primers and the dideoxy chain-termination method (Pandey *et al.*, 2002). The 16S rRNA gene sequence of the isolate was subjected

to BLAST sequence similarity search (Altschul *et al.*, 1990) to identify the nearest taxa. The 16S rRNA gene sequences of closely related validly named taxa belonging to the family *Cyclobacteriaceae* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL\_X program (Thompson *et al.*, 1997) and the alignment was then corrected manually by deleting gaps and missing data. There were a total of 1258 positions in the final dataset. Phylogenetic trees were constructed using the maximum likelihood method using the PhyML program (Guindon *et al.*, 2005) and the neighbor-joining method (Saitou & Nei, 1987) using MEGA5 (Tamura *et al.*, 2011) and the tree topologies were evaluated by bootstrap analysis based on 100 and 1000 replicates, respectively (Felsenstein, 1989). The evolutionary distances (expressed in number of base substitutions per site) were computed using the Kimura 2-parameter method (Kimura, 1980).

The closest phylogenetic neighbor of strain AK6<sup>T</sup> was *Aquiflexum balticum* DSM 16537<sup>T</sup> with pair-wise sequence similarity of 90.1 %. *Cecembia lonarensis* LW9<sup>T</sup> (88.4 %), *Indibacter alkaliphilus* LW1<sup>T</sup> (88.1 %) and several *Algoriphagus* species (87.8-87.6 %) were the next closest relatives of strain AK6<sup>T</sup>. The neighbour-joining tree (Fig. 1) confirmed the clear affiliation of strain AK6<sup>T</sup> to the family *Cyclobacteriaceae*; the new isolate clustered with *Aquiflexum balticum* DSM 16537<sup>T</sup> and these two organisms clustered with the type strains of *Cecembia lonarensis*, *Fontibacter flavus* and *Indibacter alkaliphilus*. The topology of the maximum-likelihood tree was essentially the same (data not shown).

Colony morphology was studied after 48 h on MA at 30 °C. Cell morphology was investigated by phase contrast microscopy (Olympus BX51; X 1000). The Gram reaction was determined by using the HIMEDIA Gram Staining Kit according to manufacturer's protocol. Endospore formation was determined after malachite-green staining of the isolate grown on R2A (HIMEDIA) plates for a week. Flagellar motility was assessed on Motility-Indole-Lysine HiVeg<sup>TM</sup> medium (HIMEDIA) with 2 g l<sup>-1</sup> of agar, by phase contrast microscopy and gliding motility was determined by previously described method (Bernardet *et al.*, 2002).

Growth of strain AK6<sup>T</sup> was assessed (i) on MA, nutrient agar (NA; HIMEDIA) and Tryptone Soya agar (TSA; HIMEDIA); (ii) at 4, 10, 18, 25, 30, 35, 37, 42 and 45 °C in MB; (iii) in the presence of 0, 1, 2, 3, 4, 5, 6, 8 and 10% (w/v) NaCl in nutrient broth initially devoid of NaCl; and (iv) at pH 4, 5, 6, 7, 7.5, 8, 8.5, 9, 10, 11 and 12 in MB buffered with acetate buffer (pH 4-6), 100 mM NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 7-8), 100 mM NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> buffer (pH 9-10) or 100 mM Na<sub>2</sub>CO<sub>3</sub>/NaOH buffer (pH 11-12). Anaerobic growth was assessed by incubating agar slants under nitrogen atmosphere and by inoculating broth cultures in tightly closed tubes in the presence of

ascorbic acid. Activity of catalase, oxidase, lysine decarboxylase, and ornithine decarboxylase; nitrate reduction; carbon source assimilation; acid production from carbohydrates; H<sub>2</sub>S production; and the sensitivity to 18 different antibiotics [using the disc diffusion method with commercially available discs (HIMEDIA)] were determined by previously described methods (Lányi, 1987; Smibert & Krieg, 1994) except that all media used contained 2.0 % (w/v) NaCl. Strain AK6<sup>T</sup> was also tested in the Vitek 2 GN system (bioMérieux), according to the manufacturer's protocol, except that a 2.0 % (w/v) NaCl sterile solution was used to prepare the inoculum.

Phenotypic characteristics of strain AK6<sup>T</sup> are given in Table 1 and in the genus and species descriptions.

For cellular fatty acid analysis, strains AK6<sup>T</sup> and *Aquiflexum balticum* DSM 16537<sup>T</sup> were grown on TSA with 2% NaCl (w/v) at 30 °C. The physiological age of the two strains was standardised using the protocol ([http://www.microbialid.com/PDF/TechNote\\_101.pdf](http://www.microbialid.com/PDF/TechNote_101.pdf)) given by Sherlock Microbial Identification System (MIDI, USA). The two strains were collected after two days of incubation. Cellular fatty acid methyl esters (FAMES) were obtained by saponification, methylation and extraction following the standard protocol of the Sherlock Microbial Identification System. Cellular FAMES were separated by Gas Chromatography (Agilent 6890 series GC system), identified and quantified with the Sherlock Microbial Identification System Software (version.6.0, using the aerobic TSBA6 method and the TSBA6 database). Polar lipids and respiratory quinones were analysed on freeze-dried cells. The polar lipids of strains AK6<sup>T</sup> and *Aquiflexum balticum* DSM 16537<sup>T</sup> were extracted from cells grown on MA at 30 °C for 3 days under aerobic condition (Bligh & Dyer, 1959) and analyzed by two dimensional thin layer chromatography followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987). Isoprenoid quinones were extracted as described by Collins *et al.* (1977) and analyzed by high performance liquid chromatography (Groth *et al.*, 1997). The solvent was acetonitrile:isopropanol (65:35) and the flow rate was 1 ml min<sup>-1</sup>. The DNA of strain AK6<sup>T</sup> was isolated according to the procedure of Marmur (1961) and the G + C content was determined from melting point ( $T_m$ ) curves (Sly *et al.*, 1986) obtained by using a Lambda 2 UV-Vis spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package (Perkin Elmer). The DNA of *Escherichia coli* strain DH5- $\alpha$  was used as a standard.

The cellular fatty acid composition of strain AK6<sup>T</sup> was dominated by branched saturated and hydroxy fatty acids especially iso-C<sub>15:0</sub>, iso-C<sub>17:1</sub> *ω*9c, iso-C<sub>15:1</sub> G, iso-C<sub>17:0</sub> 3OH and anteiso-C<sub>15:0</sub>, this profile was quite different from that of *Aquiflexum balticum* DSM 16537<sup>T</sup> (Table 2). The sole respiratory quinone of strain AK6<sup>T</sup> was menaquinone 7 (MK-7) in line with all other members of the family *Cyclobacteriaceae* (Nedashkovskaya & Ludwig, 2011). The polar lipids of strain AK6<sup>T</sup>

consisted of phosphatidylethanolamine, one unidentified aminophospholipid and phospholipid and six unidentified lipids (Fig. 2). The polar lipids of *Aquiflexum balticum* DSM 16537<sup>T</sup> were phosphatidylethanolamine, the same unidentified aminophospholipid, phospholipid as strain AK6<sup>T</sup> and four unidentified lipids (three of which were shared with strain AK6<sup>T</sup> and one additional lipid). Strain AK6<sup>T</sup> had a DNA G + C content of 45.6 mol%, clearly distinct from that of *Aquiflexum balticum* DSM 16537<sup>T</sup> (38.4 mol%; Brettar *et al.*, 2004b) (Table 1).

Hence, phenotypic distinctiveness and phylogenetic inference warrant the description of a new genus and species to accommodate strain AK6<sup>T</sup>, for which the name *Mariniradius saccharolyticus* gen. nov., sp. nov. is proposed. On the basis of new data obtained in this study, emended descriptions of the genus *Aquiflexum* and *Aquiflexum balticum* are also proposed.

#### **Description of *Mariniradius* gen. nov.**

*Mariniradius* (Ma.ri.ni.ra'di.us. L. adj. *marinus*, of the sea, marine; L. masc. n. *radius*, a staff, rod; N.L. masc. n. *Mariniradius*, a marine rod, isolated from marine aquaculture pond water)

Cells are Gram-staining-negative, non-spore-forming, strictly aerobic, straight rods devoid of flagellar and gliding motility. Oxidase- and catalase- positive. The cells contain carotenoids but no flexirubin type of pigments. The major fatty acids ( $\geq 13$  %) are iso-C<sub>15:0</sub> and iso-C<sub>17:1</sub> *ω*9c. MK-7 is the sole respiratory quinone. The predominant polar lipids are phosphatidylethanolamine, one unidentified aminophospholipid and one unidentified lipid. The DNA G + C content of the type strain of the type species is 45.6 mol%. A member of the family *Cyclobacteriaceae* (class *Cytophagia*, phylum *Bacteroidetes*). The type species is *Mariniradius saccharolyticus*.

#### **Description of *Mariniradius saccharolyticus* sp. nov.**

*Mariniradius saccharolyticus* (sac.cha.ro.ly'ti.cus. Gr. n. *sakchâr*, sugar; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*), able to loose, able to dissolve; N.L. masc. adj. *saccharolyticus*, breaking up polysaccharides)

Exhibits the following properties in addition to those given in the genus description. Cells are 0.5-1  $\mu$ m in diameter and 4-6  $\mu$ m in length. Colonies on marine agar are circular, 2–3 mm in diameter, smooth, reddish-orange in colour, opaque and convex with entire margin. Grows at 25-42 °C (optimum, 30-37 °C) and at pH 7-8 (optimum, pH 7.5). Growth occurs in the presence of 0-4 % (w/v) NaCl (optimum, 2 %). H<sub>2</sub>S is not produced. Urea is not hydrolysed. In the Vitek 2GN system phosphatase, Ala-Phe-Pro-arylamidase,  $\beta$ -galactosidase,  $\beta$ -N-acetylglucosaminidase, glutamyl arylamidase,  $\beta$ -glucosidase,  $\beta$ -xylosidase, L-proline arylamidase, tyrosine arylamidase,  $\gamma$ -glutamyl-

transferase,  $\alpha$ -glucosidase,  $\alpha$ -galactosidase and glu-gly-arg-arylamidase activities are present; lipase, L-pyrrolydonyl-arylamidase,  $\beta$ -alanine arylamidase,  $\beta$ -*N*-acetyl galactosaminidase,  $\beta$ -glucuronidase, glycine arylamidase, lysine decarboxylase and ornithine decarboxylase activities are absent; negative for L-lactate and succinate alkanisation, ELLMAN reaction, fermentation of glucose, resistance to the vibriostatic compound 0/129 and utilization of adonitol, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, D-trehalose, citrate, malonate, 5-keto-D-gluconate, coumarate, L-malate, L-lactate and L-histidine. Acid is produced from sucrose, glucose, raffinose, fructose, mannose, arabinose, xylose and inulin, but not from inositol, dulcitol, rhamnase, galactose, maltose, melibiose, cellobiose, salicin, adonitol, trehalose, lactose, mannitol and sorbitol after 3 weeks of incubation. Susceptible to ( $\mu$ g per disc unless indicated) polymyxin-B (300), tetracyclin (30), bacitracin (8 units), spectinomycin (100), ciprofloxacin (10), vancomycin (30), chloramphenicol (30), cefadroxil (30), novobiocin (30), amoxycillin (30), chlortetracycline (30), ampicillin (25) and lincomycin (2) and resistant to novobiocin (30), kanamycin (30), penicillin-G (2 units), ceftazidime (30) and gentamycin (10). In addition to the major fatty acids listed above, intermediate amounts (>6%) of iso-C<sub>15:1</sub> G, iso-C<sub>17:0</sub> 3OH, anteiso-C<sub>15:0</sub> and significant amounts (>2%) of summed feature 3 (comprising iso-C<sub>15:0</sub> 2OH and/or C<sub>16:1</sub>  $\omega$ 7c), iso-C<sub>15:0</sub> 3OH, C<sub>16:0</sub> 3OH, iso-C<sub>16:1</sub> G, C<sub>16:1</sub>  $\omega$ 5c, iso-C<sub>16:0</sub>, C<sub>17:1</sub>  $\omega$ 6c and C<sub>16:0</sub> are also present. The complete cellular fatty acid composition is given in Table 2. In addition to the major polar lipids listed above, significant amounts of one unidentified phospholipid and five unidentified lipids are also present. The type strain, AK6<sup>T</sup> (= MTCC 11279<sup>T</sup> = JCM 17389<sup>T</sup>) was isolated from marine water collected from the sea shore at Visakhapatnam, Andhra Pradesh, India

#### **Emended description of the genus *Aquiflexum* Brettar *et al.* 2004**

The description is as given previously (Brettar *et al.*, 2004b) with the following modifications. MK-7 is the sole respiratory quinone. The predominant polar lipids are phosphatidylethanolamine and two unidentified lipids. In addition to the predominant polar lipids cited above, detectable amounts of one unidentified phospholipid, one unidentified aminophospholipid and two one unidentified polar lipids are also present.

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## Legends to Figures

- Fig. 1.** Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain AK6<sup>T</sup> and representatives of the families *Cyclobacteriaceae*, *Flammeovirgaceae* and *Cytophagaceae*. The grouped taxa include *Flammeovirgaceae* (8 type strains) and the genera *Echinicola* (4 type strains), *Algoriphagus* (22 type strains) and *Cyclobacterium* (4 type strains). Numbers at the nodes are bootstrap values >70%. *Rhodothermus marinus* DSM 4252<sup>T</sup> was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
- Fig. 2.** Two-dimensional thin-layer chromatogram of the total polar lipids of strain AK6<sup>T</sup> (a) and *Aquiflexum balticum* DSM 16537<sup>T</sup> (b) revealed by 5% ethanolic molybdato-phosphoric acid. PE, phosphatidylethanolamine; APL, unidentified aminophospholipid; L1-7, unidentified lipids; PL, unidentified phospholipid.

**Table 1.** Features that distinguish strain AK6<sup>T</sup> from *Aquiflexum balticum* DSM 16537<sup>T</sup>

All data from the present study except the DNA G+C content of *A. balticum* DSM 16537<sup>T</sup> (taken from Brettar et al., 2004b). Both strains were Gram-staining-negative non-motile rods, positive for catalase, oxidase, phosphatase, Ala-Phe-Pro-arylamidase,  $\beta$ -galactosidase,  $\beta$ -N-acetylglucosaminidase, glutamyl arylamidase,  $\beta$ -glucosidase,  $\beta$ -xylosidase, L-proline arylamidase, tyrosine arylamidase,  $\alpha$ -glucosidase,  $\alpha$ -galactosidase and glu-gly-arg-arylamidase activities; acid production from sucrose, glucose, fructose and inulin. Both strains were negative for lipase, urease, lysine decarboxylase, ornithine decarboxylase, L-pyrrolydonyl-arylamidase,  $\beta$ -alanine arylamidase,  $\beta$ -N-acetylgalactosaminidase and  $\beta$ -glucuronidase activities; L-lactate and succinate alkalisation; resistance to 0/129; ELLMAN reaction; acid production from dulcitol, maltose, melibiose, cellobiose, salicin, adonitol, trehalose, lactose, mannitol and sorbitol; utilization of adonitol, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, D-trehalose, citrate, malonate, 5-keto-D-gluconate, coumarate, L-malate, L-lactate and L-histidine. Both strains were susceptible to ( $\mu$ g per disc unless indicated) polymyxin-B (300), tetracyclin (30), bacitracin (8 units), spectinomycin (100), ciprofloxacin (10), vancomycin (30), chloramphenicol (30), cefadroxil (30), novobiocin (30), amoxicillin (30), chlortetracycline (30), ampicillin (25) and lincomycin (2) and resistant to kanamycin (30), ceftazidime (30) and gentamycin (10). +, positive; -, negative; R, resistant; S, sensitive.

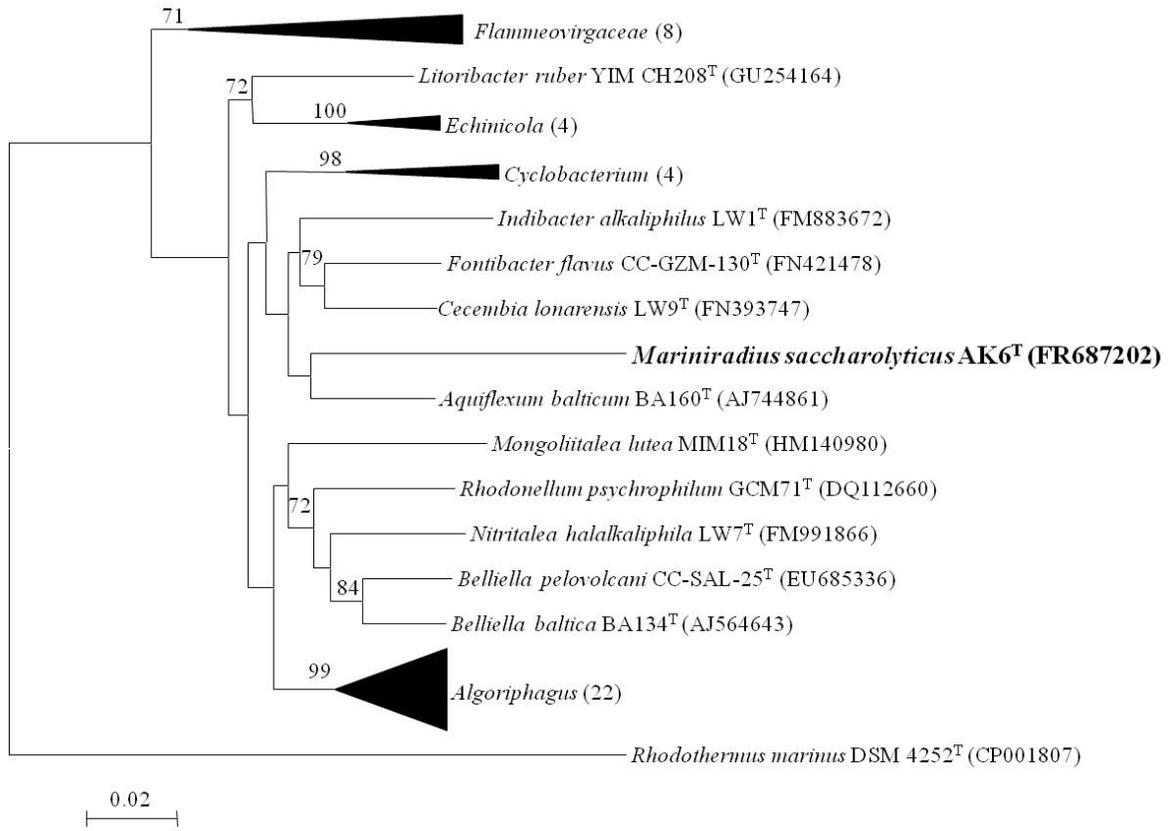
| Characteristic                               | <i>Mariniradius saccharolyticus</i><br>AK6 <sup>T</sup> | <i>Aquiflexum balticum</i><br>DSM 16537 <sup>T</sup> |
|--|---|--|
| Cell size ( $\mu$ m)(MA, 30°C, 48 h)         | 0.5-1 x 4-6   | 0.3-0.7 x 1-4  |
| Colony pigmentation                          | Reddish-orange  | Red  |
| Temperature growth range (°C)                | 25-42   | 4-40   |
| Optimum growth temperature (°C)              | 30-37   | 30   |
| NaCl concentration range (% <sub>w/v</sub> ) | 0-4   | 0-6  |
| pH growth range                              | 7-8   | 7-9  |
| Nitrate reduction                            | -   | +  |
| H <sub>2</sub> S production                  | +   | -  |
| $\gamma$ -glutamyl-transferase activity      | +   | -  |
| Glycine arylamidase activity                 | -   | +  |
| <b><i>Acid production from:</i></b>          |   |  |
| Inositol, rhamnose, galactose                | -   | +  |
| Raffinose, mannose, arabinose, xylose        | +   | -  |
| <b><i>Susceptibility to:</i></b>             |   |  |
| Novobiocin (30 $\mu$ g)                      | R   | S  |
| Penicillin-G (2 units)                       | R   | S  |
| DNA G+C content (mol%)                       | 45.6  | 38.4   |

**Table 2.** Fatty acid composition of strain AK6<sup>T</sup> and *Aquiflexum balticum* DSM 16537<sup>T</sup>

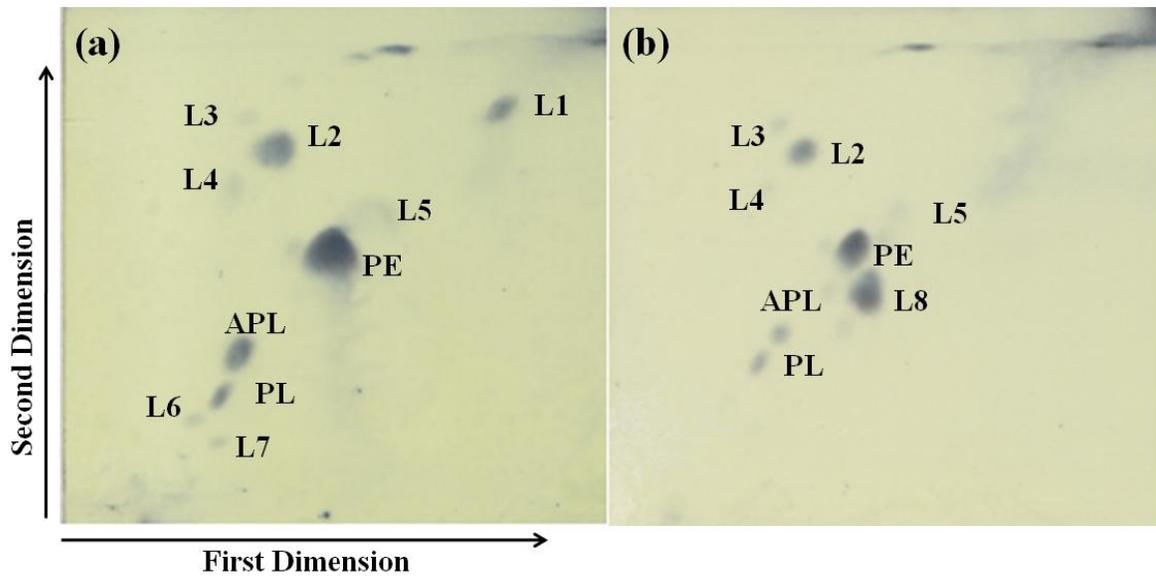
Results are percentage of the total fatty acids. Fatty acids amounting to <1% in the two strains are not shown. Fatty acids amounting to 5% or more of the total fatty acids are in bold. All data from the present study. ND, not detected. The strains were grown on Trypticase Soy agar with 2% (w/v) NaCl at 30°C for two days.

| Fatty acid                    | <i>Mariniradius saccharolyticus</i> AK6 <sup>T</sup> | <i>Aquiflexum balticum</i> DSM 16537 <sup>T</sup> |
|-------------------------------|--|---|
| iso-C <sub>14:0</sub>         | ND   | 1.9   |
| Unknown 13.6                  | 1.1  | ND  |
| iso-C <sub>15:0</sub>         | <b>33.1</b>  | <b>24.9</b>                                       |
| iso-C <sub>15:0</sub> 3OH     | 3.9  | 2.6   |
| anteiso-C <sub>15:0</sub>     | <b>6.9</b>   | <b>20.3</b>                                       |
| iso-C <sub>15:1</sub> G       | <b>9.0</b>   | 4.7   |
| C <sub>16:0</sub>             | 2.2  | ND  |
| C <sub>16:0</sub> 3OH         | 3.3  | ND  |
| iso-C <sub>16:0</sub>         | 2.5  | <b>7.2</b>  |
| iso-C <sub>16:0</sub> 3OH     | 1.8  | 2.3   |
| iso-C <sub>16:1</sub> G       | 3.2  | <b>10.8</b>                                       |
| C <sub>16:1</sub> ω5c         | 3.1  | 1.6   |
| C <sub>17:0</sub> 2OH         | 1.3  | 2.0   |
| iso-C <sub>17:0</sub> 3OH     | <b>8.9</b>   | <b>5.0</b>  |
| C <sub>17:1</sub> ω6c         | 2.4  | 2.2   |
| iso-C <sub>17:1</sub> ω9c     | <b>13.0</b>  | <b>5.8</b>  |
| <sup>#</sup> Summed feature 3 | 4.4  | <b>5.0</b>  |
| <sup>#</sup> Summed feature 4 | ND   | 3.7   |

<sup>#</sup>Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains iso-C<sub>15:0</sub> 2OH and/or C<sub>16:1</sub> ω7c; Summed feature 4 contains iso-C<sub>17:1</sub> I and/or anteiso-C<sub>17:1</sub> B.



**Fig. 1.**



**Fig. 2**