Marinomonas mangrovi sp. nov., isolated from mangrove sediment

De-Chao Zhang1 and Rosa Margesin2

1Institute of Oceanology, Chinese Academy of Sciences, 266071, Qingdao, PR China
2Institute of Microbiology, University of Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria

The genus Marinomonas was proposed by Van Landschoot & De Ley (1983), members of which have been found distributed in different marine environments (Sanchez-Amat & Solano, 2005). At the time of writing, the genus Marinomonas comprised 23 recognized species, 11 of which have been described since 2009, namely Marinomonas arenicola (Romanenko et al., 2009), M. balearica and M. pollencensis (Espinosa et al., 2010), M. brasiliensis (Chimetto et al., 2011), M. alcarazii, M. rhizomae, M. fioriarn, M. posidonica and M. aquiplantarum (Lucas-Elío et al., 2011), M. hwangdonensis (Jung et al., 2012) and M. fungiae (Kumari et al., 2014). In this study, we report the characterization of a novel bacterium of the genus Marinomonas isolated from the rhizosphere soil of mangrove forest at Beigang Island in Hainan Province, China.

Strain B20-1T was isolated by the standard dilution plating technique on marine agar 2216 (MA; Difco) at 25 °C. The strain was routinely cultured on MA at 25 °C and stored as a suspension in skimmed milk (10 %, w/v) at −80 °C. The type strains of Marinomonas dokdonensis (KCTC 12394T; Yoon et al., 2005), M. rhizomae (CECT 7377T; Lucas-Elío et al., 2011) and M. fioriarn (CECT 7731T; Lucas-Elío et al., 2011) were routinely grown on MA at 25 °C and used as reference strains.

DNA was extracted and purified as described by Sambrook et al. (1989). The 16S rRNA gene was amplified by PCR with a pair of universal primers (Zhang et al., 2006). The PCR product was purified by using a QIAquick PCR Purification kit (Qiagen). The purified 16S rRNA gene PCR product was sequenced using dyeoxy chain termination/cycle sequencing, an ABI 3730XL sequencer (Applied Biosystems) and version 3.1 of the ABI Big Dye Terminator kit (Applied Biosystems) as recommended by the manufacturer. On the basis of pairwise comparisons of the 16S rRNA gene sequences using the recent version of the EzTaxon program (Kim et al., 2012), strain B20-1T was found to share highest sequence similarities with M. rhizomaeIVIA-Po-145T (97.6 %), M. dokdonensisDSW10-10T (97.0 %) and Marinomonas fioriarnIVIA-Po-155T (96.9 %). The predominant cellular fatty acids of strain B20-1T were C10 : 0 3-0H, C18 : 1ω7c, summed feature 3 (C16 : 1ω7c and/or iso-C15 : 0 2-0H) and C16 : 0. Phosphatidylethanolamine and phosphatidylglycerol were identified as the predominant phospholipids. The predominant ubiquinone was Q-8. The genomic DNA G + C content of strain B20-1T was 46.6 mol%. On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness, a novel species, Marinomonas mangrovi sp. nov., is proposed with B20-1T (=DSM 28136T=LMG 28077T) as the type strain.
microscopy (Zeiss Libra 120 EFTEM) of cells grown on MA at 25 °C. Motility was examined by microscopy (× 1000) and by observing the presence of spreading growth on MA soft agar (containing 0.3 % agar) plates (Süßmuth et al., 1987). Gram-staining was tested by using the bioMérieux Gram-stain kit. Catalase activity was determined by bubble production in 3 % (v/v) H₂O₂ and cytochrome c oxidase activity was determined using 1 % (w/v) N,N,N',N'-tetramethyl-p-phenylenediamine. Physiological and biochemical characteristics and enzyme activities were determined as currently described for strains from saline habitats (Zhang & Margesin, 2014). API 20 NE [supplemented with sea salts (S9883; Sigma) for assimilation tests], API 20 E and API ZYM (bioMérieux) test strips were

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**Fig. 1.** NJ tree, based on 16S rRNA gene sequence data, showing the phylogenetic position of strain B20-1ᵀ, recognized members of the genus *Marinomonas* and representatives of some related taxa. Numbers at nodes represent percentage levels of bootstrap support based on an NJ analysis of 1000 resampled datasets. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Bar, 1 % sequence divergence.
incubated at 25 °C. Oxidative/fermentative metabolism of glucose was determined as described by Süssmuth et al. (1987) on Hugh and Leifson’s OF basal medium (1% glucose, 0.2% peptone, 0.1% yeast extract, 0.5% NaCl, 0.02% K2HPO4, 0.008% bromothymol blue, 0.3% agar) supplemented with sea salts (S9883; Sigma). Aerobic growth on different media was assessed at 25 °C on MA, tryptase soy agar (TSA; 1.5% casein peptone, 0.5% soy peptone, 0.5% NaCl, 1.5% agar; pH 7) and R2A agar. Growth under anaerobic conditions was examined after 4 days of incubation at 25 °C in an anaerobic jar [containing Anaerocult A (Merck)] to produce anaerobic conditions on MA and on MA supplemented with 10 mM KNO3. Growth under microaerophilic conditions was investigated at 25 °C on MA and on MA supplemented with 10 mM KNO3 after incubation in a microaerophilic atmosphere containing 8–10% (v/v) CO2 and 5–7% (v/v) O2; this atmosphere was generated in sealed jars containing Anaerocult C (Merck). Growth at 1, 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C was assessed on MA and in marine broth with shaking at 150 r.p.m. Growth at −5 °C was assessed in marine broth (unshaken). Growth at pH 5, 6, 7, 8, 9 and 10 was determined at 25 °C in buffered marine broth in microtitre plates; growth was evaluated by measuring OD600. Salt tolerance was assessed at 25 °C as described by Zhang & Margesin (2014) on plates containing 0.5% peptone, 0.1% yeast extract, 1.5% agar, and 0, 2, 4, 6, 8 and 10% (w/v) NaCl. All tests were carried out simultaneously with strain B20-1T and the reference strains M. dokdonensis KCTC 12394T, M. rhizomae CECT 7377T and M. foliarum CECT 7731T. The morphological, physiological and biochemical characteristics of strain B20-1T are given in the species description and the features that differentiate it from the reference strains are given in Table 1.

For fatty acid methyl ester analysis, strain B20-1T and M. dokdonensis KCTC 12394T, M. rhizomae CECT 7377T and M. foliarum CECT 7731T were grown on MA at 25 °C for 1 day. All four strains shared similar growth behaviour and a sufficient amount of cells of comparable physiological age could be harvested from the third streak quadrant of the MA plates after cultivation under the applied conditions. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (MIDI; version 6.1) (Sasser, 1990) using the database TSBA40 for calculation. Fatty acid analyses were carried out by the Identification Service of the DSMZ, Braunschweig, Germany.

The predominant cellular fatty acids (>10% of the total) of strain B20-1T were C10:0 3-OH (27.9%), C18:1ω7c (25.1%), summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH) (19.7%) and C16:0 (13.3%). Thus, the fatty acid profile of strain B20-1T resembled those of other species of the genus Marinomonas (Yoon et al., 2005; Chimetto et al., 2011; Lucas-Elio et al., 2011; Jung et al., 2012). However,

| Table 1. Phenotypic characteristics that differentiate strain B20-1T from M. dokdonensis KCTC 12394T, M. rhizomae CECT 7377T and M. foliarum CECT 7731T |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Characteristic                  | 1               | 2               | 3               | 4               |
| Isolation source               | Mangrove forest | Seawater        | Seagrass        | Seagrass        |
| Cell shape                     | Curved rods     | Straight rods   | Curved rods     | Curved rods     |
| Growth on/in:                  |                 |                 |                 |                 |
| MA at 40 °C                    | +               | −               | −               | −               |
| TSA                            | +               | −               | +               | +               |
| Salt-free medium at 6% (w/v) NaCl | +             | −               | +               | +               |
| Nitrate reduction to nitrile   | −               | +               | −               | −               |
| Enzyme activities              |                 |                 |                 |                 |
| Valine arylamidase             | −               | −               | w               | w               |
| Acid phosphatase               | +               | −               | +               | +               |
| α-Glucosidase                  | +               | −               | w               | +               |
| β-Galactosidase                | +               | −               | −               | +               |
| Tryptophan deaminase           | −               | −               | −               | +               |
| Aesculin hydrolysis            | +               | −               | +               | +               |
| Assimilation of:               |                 |                 |                 |                 |
| L-Arabinose                    | +               | −               | −               | +               |
| N-Acetyl glucosamine           | −               | −               | −               | +               |

Strains: 1, B20-1T; 2, M. dokdonensis KCTC 12394T; 3, M. rhizomae CECT 7377T; 4, M. foliarum CECT 7731T (all data from this study). +, Positive; −, negative; w, weak. All strains are positive for aerobic and microaerophilic growth on MA, motility, catalase, cytochrome c oxidase, alkaline phosphatase, esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and assimilation of D-glucose, D-mannose, D-mannitol, maltose, potassium gluconate, adipic acid, malic acid and phenylacetic acid. All strains are negative for aerobic growth on MA in the absence of NaCl, aerobic growth on MA, aerobic growth on R2A agar, urease, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, gelatinase, α-galactosidase, β-glucoronidase, ω-mannosidase, α-fucosidase, arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, H2S production, indole production from tryptophan, assimilation of capric acid and glucose fermentation.
strain B20-1T contained higher amounts of C10:0 3-OH and lower amounts of C18:1ω7c compared with the other *Marinomonas* species. Details of the fatty acid profiles of strain B20-1T and the reference strains are given in Table S1.

Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu et al., 1989), using *M. dokdonensis* KCTC 12394T as a reference. The predominant ubiquinone was Q-8, which is in agreement with the description of the genus *Marinomonas* (Yoon et al., 2005; Jung et al., 2012).

The polar lipid profile was analysed according to Tindall (1990a, b). The polar lipid profile of strain B20-1T contained phosphatidylethanolamine (PE), phosphatidylglycerol (PG), two unidentified aminophospholipids, two unidentified phospholipids and two unidentified polar lipids (Fig. S3). The predominant phospholipids of strain B20-1T (PE and PG) are in accordance with the data reported for *Marinomonas pontica* (Ivana et al., 2005) and *M. hwangdongensis* (Jung et al., 2012).

The DNA G+C content was determined using the thermal denaturation method (Marmur & Doty, 1962). *Escherichia coli* K-12 was selected as the reference strain for these analyses, and the formula G+C mol% unknown strain = G+C mol% reference strain + 2.08 x (T_m unknown strain – T_m reference strain) was used to calculate the G+C content from the known T_m value (Owen & Pitcher, 1985). DNA–DNA hybridizations were performed using the liquid renaturation method (De Ley et al., 1970) as modified by Huss et al. (1983). DNA–DNA hybridizations were carried out in 2 x SSC at 70 °C and each determination was carried out in triplicate. Both experiments were carried out at 260 nm with a model Lambda 35 UV/VIS spectrometer equipped with a Peltier System (PTP 1 + 1) (Perkin–Elmer). The genomic DNA G+C content of strain B20-1T was 46.6 mol%. This value was higher than those of *M. foliarum* CECT 7377T (46.2 %), *M. rhizomae* CECT 7377T (45.5 %) and *M. dokdonensis* KCTC 12394T (45.3 %). The DNA–DNA hybridization experiments revealed that strain B20-1T shared 17 % DNA relatedness with *M. dokdonensis* KCTC 12394T and 23 % with *M. foliarum* CECT 7377T. All these values were well below the 70 % cut-off point recommended for the assignment of strains to the same genospecies (Wayne et al., 1987).

The data presented in this study demonstrate that strain B20-1T is a member of the genus *Marinomonas*, and is able to grow in the presence of up to 8 % (w/v) NaCl. Like other representatives of this genus, strain B20-1T requires Na+ for growth, which reflects the conditions from where it was isolated. Strain B20-1T could be differentiated from its three closest phylogenetic neighbours, *M. dokdonensis* KCTC 12394T, *M. rhizomae* CECT 7377T and *M. foliarum* CECT 7731T, by its ability to grow well at 40 °C and by a number of other physiological properties (Table 1). Compared with *M. dokdonensis* KCTC 12394T, strain B20-1T had a different cell shape, was unable to reduce nitrate and was able to grow on TSA and in the presence of 6 % (w/v) NaCl, to assimilate L-arabinose and to produce a number of enzymes. In comparison with *M. rhizomae* CECT 7377T, strain B20-1T was able to produce β-galactosidase, while in comparison with *M. foliarum* CECT 7731T it was unable to produce tryptophan deaminase. Based on the phenotypic, phylogenetic and genomic evidence, strain B20-1T was identified as a representative of a novel species of the genus *Marinomonas*, for which the name *Marinomonas mangrovi* sp. nov. is proposed.

**Description of *Marinomonas mangrovi* sp. nov.**

*Marinomonas mangrovi* (man.gro′vi. N.L. gen. n. mangrovi of a mangrove).

Cells stain Gram-negative, are curved rods, 0.5–0.6 x 1.5–2.5 μm in size after 3 days at 25 °C on MA and motile (polar flagellation, Fig. S4). Colonies on MA are creamy, convex, glossy, smooth and circular with an entire margin. Grows under aerobic and microaerophilic conditions on MA, but unable to grow under anaerobic conditions on MA and on MA supplemented with nitrate. Grows well at 10–40 °C on MA and in marine broth, growth at 1–5 °C is delayed and growth at 1 °C is weak. Grows in buffered marine broth at pH 6–9; growth is delayed at pH 6 and growth is weak at pH 9. Grows at 25 °C in salt-free medium supplemented with 2–6 % (w/v) NaCl, growth is delayed in the presence of 8 % (w/v) NaCl and is absent in the presence of 10 % (w/v) NaCl. No growth occurs in the absence of NaCl and on R2A agar. Positive for cytochrome c oxidase, catalase, alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase (aesculin hydrolysis), and assimilation of D-glucose, D-mannose, D-mannitol, maltose, potassium gluconate, adipic acid, malic acid and phenylacetic acid. Negative for nitrate reduction, urease, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, gelatinase, α-galactosidase, β-gluconidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, tryptophan deaminase, H2S production, indole production, assimilation of N-acetylgalosamine, capric acid and glucose fermentation. The predominant cellular fatty acids are C10:0 3-OH, C18:1ω7c summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH) and C16:0. Phosphatidylethanolamine and phosphatidylglycerol are the predominant phospholipids. The predominant ubiquinone is Q-8.

The type strain is B20-1T (= DSM 28136T = LMG 28077T) and was isolated from soil of the root system of a mangrove forest of Beigang Island in Hainan Province, China. The genomic DNA G+C content of the type strain is 46.6 mol%.

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