Vibrio porteresiae sp. nov., a diazotrophic bacterium isolated from a mangrove-associated wild rice (*Porteresia coarctata* Tateoka)

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Two facultatively anaerobic, nitrogen-fixing bacteria (strains MSSRF30^T and MSSRF31) were isolated from a mangrove-associated wild rice (*Porteresia coarctata* Tateoka). These strains were determined to be nitrogen-fixers using the acetylene reduction assay and by PCR detection of a *nifH* gene amplicon. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the novel strains were most closely related to *Vibrio fluvialis* LMG 7894^T (96.8 % gene sequence similarity), *Vibrio furnissii* LMG 7910^T (96.8 % sequence similarity) and *Vibrio tubiashii* CIP 102760^T (96.7 % sequence similarity). Further multilocus sequence analysis using *recA*, *pyrH*, *rpoA* and *nifH* genes also showed low levels of sequence similarities (83–93 %) with all species of the genus *Vibrio* with validly published names. A multigene phylogenetic tree using concatenated sequences of the four genes (16S rRNA, *rpoA*, *recA* and *pyrH*) showed that strains MSSRF30^T and MSSRF31 occupied a distinct phylogenetic position, forming a long branching that was not clustered with any other recognized *Vibrio* species. The fatty acid profile also suggested that the novel strains belonged to the genus *Vibrio*. The results of physiological and biochemical tests, genomic fingerprinting and DNA–DNA hybridization analyses clearly differentiated both novel strains from their closest phylogenetic neighbours, *Vibrio cholerae* IID6019, *Vibrio mimicus* LMG 7896^T, *V. fluvialis* LMG 7894^T and *V. furnissii* LMG 7910^T. Several phenotypic traits enabled the differentiation of strain MSSRF30^T from other species of the genus *Vibrio*. The DNA G + C content of strain MSSRF30^T was 44.4 ± 3.1 mol%. Based on genotypic, phenotypic, chemotaxonomic, phylogenetic and DNA–DNA hybridization analyses, the name *Vibrio porteresiae* sp. nov. (type strain MSSRF30^T=LMG 24061^T=DSM 19223^T) is proposed for this novel taxon.

The genus *Vibrio* encompasses a genetically diverse group of heterotrophic marine bacteria that are commonly found in aquatic habitats and in association with marine organisms (Thompson *et al.*, 2004a). Members of the genus *Vibrio* may account for nearly 10 % of the culturable

Abbreviation: NfM, nitrogen-free medium.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MSSRF30^T and MSSRF31 are EF488079 and EF488080, respectively. The accession numbers for the *recA*, *pyrH*, *rpoA* and *nifH* gene sequences of strains MSSRF30^T and MSSRF31 are EF547199, EF554361, EF547200, EF554362 and EU072027–EU072030, inclusive.

Three supplementary neighbour-joining phylogenetic trees based on partial *rpoA*, *recA* and *pyrH* gene sequences and the results of PCR genomic fingerprinting analyses are available as Supplementary Figures S1–S4 with the online version of this paper. A supplementary table detailing the amplification and sequencing primers used in this study is also available.

marine bacteria (Thompson *et al.*, 2004b). They include several species that are pathogenic for humans and marine animals and some that are facultative symbiotic (Thompson *et al.*, 2004a). To date, relatively few studies have demonstrated that vibrios are capable of N₂-fixation; this characteristic has so far been confirmed for *Vibrio diazotrophicus* (Guerinot *et al.*, 1982), *Vibrio natriegens*, *Vibrio pelagius* and *Vibrio cincinnatiensis* (Urdaci *et al.*, 1988), *Vibrio anguillarum* and *Vibrio campbellii* (Holguin *et al.*, 1992).

During a study of diazotrophic bacteria from mangrove-associated wild rice, numerous isolates that could fix atmospheric nitrogen were isolated. Based on the preliminary characterization, these isolates were tentatively identified as members of the genera *Swaminathania*, *Vibrio* and *Serratia*. In the present work, we describe the isolation and taxonomic characterization of two novel

diazotrophs (strains MSSRF30^T and MSSRF31) belonging to the genus *Vibrio*.

Strains MSSRF30^T and MSSRF31 were isolated from a wild rice collected from Pichavaram mangroves, India. The plant roots were washed with sterilized distilled water, surface-sterilized with sodium hypochlorite (4%) for 5 min and then washed several times using sterile distilled water. The surface-sterilized root samples were macerated in a blender, serially diluted and 100 µl aliquots were inoculated into 30 ml test tubes containing 10 ml of semi-solid N-free medium (NfM) (Gyaneshwar *et al.*, 2001) consisting of (l⁻¹): 5 g sucrose; 3 g mannitol; 0.8 g K₂HPO₄; 0.2 g KH₂PO₄; 0.2 g MgSO₄·7H₂O; 20 g NaCl; 0.02 g CaCl₂; 28 mg Na₂FeEDTA; 25 mg Na₂MoO₄·2H₂O; 2 ml 0.5% bromothymol blue in 0.2 N KOH and 2 g agar. The final pH was adjusted to 7.0 by KOH. The NfM was modified by adding 0.02 g yeast extract (NfM+Y), as it has already been reported that a trace amount of fixed nitrogen is required for the isolation of most diazotrophs from the rhizosphere of rice (Gyaneshwar *et al.*, 2001). The samples were incubated at 28 °C for 4–5 days. Tubes showing a fine subsurface pellicle were transferred to fresh NfM+Y for further incubation and observation of pellicle formation. Further purification was performed by repeatedly streaking the isolates on plates of NfM+Y agar followed by tryptone soy agar (TSA; HiMedia) supplemented with 1.5% NaCl (TSA+NaCl). Both strains were maintained on TSA+NaCl at 4 °C or were stored frozen in tryptone soy broth (TSB; HiMedia) supplemented with 1.5% NaCl (TSB+NaCl) with 15% glycerol at –80 °C.

Genomic DNA was extracted from strains MSSRF30^T and MSSRF31 as described by Ausubel *et al.* (1987). The 16S rRNA gene was amplified and sequenced as described previously by Saha *et al.* (2005) and the *recA*, *rpoA* and *pyrH* genes were amplified and sequenced according to Thompson *et al.* (2005). The primer sequences that were used for amplification and sequencing for the 16S rRNA, *recA*, *rpoA* and *pyrH* genes are listed in Supplementary Table S1 (see IJSEM Online). The sequences of these genes were compared with the sequences available from GenBank using the BLASTN program (Altschul *et al.*, 1990) and were aligned using CLUSTAL_X software (Thompson *et al.*, 1997). Distances were calculated according to Kimura's two-parameter correction (Kimura, 1980). Phylogenetic trees were inferred using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was based on 1000 resamplings. The MEGA3 package (Kumar *et al.*, 2004) was used for all analyses.

The partial 16S rRNA gene sequences comprising 1359 nucleotides were determined for strains MSSRF30^T and MSSRF31. A comparison with the 16S rRNA gene sequences of all of the recognized members of the genus *Vibrio* held in GenBank indicated that both strains were phylogenetically related to members of this genus (Fig. 1). Strains MSSRF30^T and MSSRF31 were identical when the pairwise 16S rRNA gene sequence similarity was deter-

mined. The gene sequence similarities between strain MSSRF30^T and *V. fluvialis* LMG 7894^T, *V. furnissii* LMG 7910^T and *V. tubiashii* CIP 102760^T were 96.8%, 96.8% and 96.7%, respectively, but were less than 96.7% when compared with other species of the genus *Vibrio*. Gene sequence similarity values of not more than 93.0% were found when strain MSSRF30^T was compared with other genera in the family *Vibrionaceae*. The node to which strains MSSRF30^T and MSSRF31 belonged was also supported in phylogenetic trees generated with the maximum-likelihood and maximum-parsimony algorithms (data not shown). These gene sequence similarity values are below the cut-off value of 97.0%, the level normally judged sufficient to justify the proposal of a novel bacterial species (Stackebrandt & Goebel, 1994).

Other housekeeping genes previously found to be useful in the differentiation of members of the genus *Vibrio* and related species (Thompson *et al.*, 2004a, 2005; Kumar & Nair, 2007) were also analysed. Analysis of the *rpoA* gene sequence also supported the phylogenetic position of strains MSSRF30^T and MSSRF31 within the genus *Vibrio*. The novel strains showed 100% *rpoA* gene sequence similarity with each other, 93.0% similarity with *V. fluvialis* LMG 7894^T and 92.3% similarity with *V. furnissii* LMG 7910^T (see Supplementary Fig. S1 in IJSEM Online). The remaining species of the genus *Vibrio* showed less than 92.3% similarity with the novel strains. This result was in agreement with the 16S rRNA gene sequence analysis and coincided with those obtained by other workers. This indicates that *rpoA* gene sequence analyses correlate well with those based on 16S rRNA gene sequences (Kumar & Nair, 2007; Thompson *et al.*, 2005) and that the *rpoA* gene evolves as slowly as the 16S rRNA gene (Zeigler, 2003).

The *recA* and *pyrH* genes have also been proposed as an alternative marker for the family *Vibrionaceae* and their sequences seem to be more discriminatory than those of the *rpoA* and 16S rRNA genes for the genus *Vibrio* (Kumar & Nair, 2007; Thompson *et al.*, 2005). A pairwise analysis of the *recA* sequences of strains MSSRF30^T and MSSRF31 revealed 100% sequence similarity between the two strains. Low levels of gene sequence similarity were found with several species from the genus *Vibrio*. For example, the analysis pointed to 87.3% gene sequence similarity with *V. diazotrophicus* LMG 7893^T, 86.6% with *V. rotiferianus* LMG 21460^T, 84.8% with *V. furnissii* LMG 7910^T and 84.0% with *V. fluvialis* LMG 7894^T (see Supplementary Fig. S2 in IJSEM Online). The *pyrH* gene sequence analysis also supported the phylogenetic position of strain MSSRF30^T within the genus *Vibrio* as this strain showed low levels of similarity, 83.6–86.6%, with respect to all of the recognized species of the genus *Vibrio*. A phylogenetic tree showing this relationship is available as Supplementary Fig. S3 (available in IJSEM Online).

The low gene sequence similarity values found between the four genes for recognized species of the genus *Vibrio* and strains MSSRF30^T and MSSRF31 confirmed that the novel

Fig. 1. Neighbour-joining phylogenetic tree based on partial 16S rRNA gene sequences of *Vibrio porteresiae* sp. nov. MSSRF30^T and other related organisms of the family Vibrionaceae. Bar, 0.005 substitutions per nucleotide position.

strains did not belong to the currently recognized *Vibrio* species (Thompson *et al.*, 2005). The position of isolates MSSRF30^T and MSSRF31 varied between the trees

constructed from the different genetic loci, suggesting that recombination/gene conversion events in the *rpoA*, *recA* and *pyrH* genes might have occurred among these *Vibrio*

species (Thompson *et al.*, 2005). These recombination events hampered the exact identification and positioning of isolates MSSRF30^T and MSSRF31 in the genus *Vibrio*. In order to overcome the effects of gene conversion and recombination, multigene phylogenetic trees were constructed for strains MSSRF30^T and MSSRF31 using the four genes. The sequences of the *pyrH* (445 bp), *recA* (652 bp), *rpoA* (834 bp) and 16S rRNA genes (1272 bp) of 62 representative type strains (Thompson *et al.*, 2005; Sawabe *et al.*, 2007b), including strain MSSRF30^T, of the family *Vibrionaceae* were retained and used to reconstruct a concatenated network tree based on SPLITSTREE version 4.6 (Huson & Bryant, 2005). In the SPLITTS decomposition analysis using nine gene loci, at least 14 distinctive clades were recognized for vibrios (Sawabe *et al.*, 2007b). Even in the four-gene-concatenated network tree, the clade was likely to be retained. Strains MSSRF30^T and MSSRF31 occupied a distinct phylogenetic position, forming a long branching that was not clustered with *V. cholerae*, *V. mimicus*, *V. furnissii*, *V. fluvialis* and *V. diazotrophicus* or with any other recognized *Vibrio* species. Strains MSSRF30^T and MSSRF31 may belong to the *V. cholerae*

clade (Fig. 2), as defined by Sawabe *et al.* (2007b), which is strong evidence to propose these two new isolates as a novel taxon in the genus *Vibrio*.

Repetitive extragenic palindromic PCR (Rep-PCR) fingerprinting using the GTG₅ primer was performed as described previously (Ben-Haim *et al.*, 2003) using *V. cholerae* IID6019, *V. mimicus* LMG 7896^T, *V. fluvialis* LMG 7894^T and *V. furnissii* LMG 7910^T as references. The results showed that no similarity was observed between the fingerprints of strains MSSRF30^T and MSSRF31 and those of the reference strains (see Supplementary Fig. S4 available in IJSEM Online). Thus, the GTG₅-generated PCR fingerprints were in agreement with the findings above and indicated that the two new isolates are members of a single novel species.

Fatty acid methyl ester (FAME) analysis for strain MSSRF30^T was performed at the Belgian Coordinated Collections of Micro-organisms Laboratorium voor Microbiologie (Universiteit Gent, Belgium) according to the recommendations of the commercial MIDI identification system (Microbial Identification System, Inc.). For

Fig. 2. Concatenated split network tree based on four gene loci. The *pyrH*, *recA*, *rpoA* and 16S rRNA gene sequences (3203 bp) from 62 taxa were concatenated and reconstructed by SPLITSTREE4 (version 4.6). Distances were corrected with the Jukes–Cantor correlation. Strain MSSRF31 had identical 16S rRNA and protein-coding gene sequences and clustered with strain MSSRF30^T. Bar, 1 nucleotide substitution per 100 nucleotide positions.

fatty acids analyses, strain MSSRF30^T was cultivated for 24 h at 28 °C on TSA medium supplemented with 1.5 % NaCl. The results of the analysis revealed that strain MSSRF30^T possessed the main chemotaxonomic features that are typical for the genus *Vibrio* (Bertone *et al.*, 1996; Lambert *et al.*, 1983). The predominant fatty acids (accounting for >80 % of the total cellular fatty acid content) were summed feature 3 (comprising C_{16:1}ω7c and/or C_{15:0} iso 2-OH), C_{16:0}, C_{18:1}ω7c, C_{14:0} and C_{12:0}. The results of the FAME analyses are given in the species description.

DNA of strain MSSRF30^T was prepared according to the procedures of Marmur (1961), with minor modifications. The G+C content of the DNA was determined by HPLC (Tamaoka & Komagata, 1984) as a mean value of triplicate samples. The DNA G+C content was 44.4 ± 3.1 mol% for strain MSSRF30^T, which was in good agreement with the values previously found for members of the genus *Vibrio* (Baumann *et al.*, 1984).

Cells of strains MSSRF30^T and MSSRF31 were grown in TSB+NaCl for 24 h and were examined for morphology and motility using a phase-contrast microscope. Classical phenotypic tests were performed as described previously (Baumann *et al.*, 1984; Farmer & Hickman-Brenner, 1992; Leifson, 1963). The ability of the cultures to utilize various carbon compounds as the sole carbon source was investigated by testing 0.5 % carbon compound in a minimal base medium containing 2.0 % (w/v) NaCl, 1.0 % (w/v) K₂HPO₄, 0.45 % (w/v) KH₂PO₄, 0.14 % (w/v) CaCl₂, 0.15 % (w/v) MgCl₂, 0.075 % (w/v) KCl, 0.1 % (w/v) (NH₄)₂SO₄ and 1.5 % (w/v) agar. The results were noted after 3 days of incubation at 28 °C. Growth in different salt concentrations was monitored in tubes of 1 % tryptone broth pH 7.5 (with NaCl concentrations of 0 to 10%) incubated for 7 days at 28 °C and growth in thiosulfate citrate bile salts medium (TCBS; from three manufacturers, Oxoid, Merck and Eiken) was monitored for 2 days of incubation at 28 °C. *V. fluvialis* LMG 7894^T and *V. furnissii* LMG 7910^T were used as reference strains. The results showed that strains MSSRF30^T and MSSRF31 possessed the main phenotypic features characteristic for the genus *Vibrio*; they were straight to slightly curved rods, motile, facultatively anaerobic, Gram-negative, oxidase- and catalase-positive and their growth was stimulated by NaCl. The phenotypic characteristics of strain MSSRF30^T are given in the species description below. Strains MSSRF30^T and MSSRF31 possessed some phenotypic characteristics that are quite uncommon among the vibrios, including production of gas from glucose, resistance to O/129 and no growth in TCBS. The species *V. gazogenes*, *V. aerogenes*, *V. ruber*, *V. rhizosphaerae* and *V. furnissii* are the only other *Vibrio* species that have been previously reported to produce gas from glucose (Shieh *et al.*, 2000, 2003; Kumar & Nair, 2007). However, *V. gazogenes*, *V. ruber* and *V. rhizosphaerae* produce red colonies on plate media. Moreover, these strains are negative for the oxidase reaction (Shieh *et al.*, 2000, 2003; Kumar & Nair, 2007). Colonies produced by

strain MSSRF30^T, on the other hand, were white and were positive for the oxidase reaction. The only species of the genus *Vibrio* previously reported to show no growth in TCBS is *V. pectenicida* (Lambert *et al.*, 1998). In addition, strain MSSRF30^T could be differentiated from *V. cholerae* and *V. mimicus* on the basis of many biochemical and physiological properties such as no growth at 0 % NaCl, no growth in TCBS, resistance to O/129, production of gas from glucose, positive utilization of D-xylose, cellobiose, lactose, salicin and L-rhamnose, and negative results in tests for nitrate reduction and amylase. Furthermore, the novel strain could be differentiated from all other *Vibrio* species by the following combination of properties: no growth in TCBS, negative in tests for arginine dihydrolase and ornithine decarboxylase, positive in tests for nitrogen fixation, production of gas from glucose and for the utilization of D-xylose and lactose. The novel strain gave a negative result in tests for nitrate reduction, was unable to utilize trehalose and was resistant to O/129. Differential features between the novel strains and closely related members of the genus *Vibrio* are presented in Table 1.

The acetylene reduction assay (ARA) was used to test the isolates grown on semi-solid (NfM+Y) medium for potential nitrogen fixation. The amount of ethylene produced was measured using 10 % (v/v) acetylene according to the method of Li & MacRae (1992) using a Hewlett Packard 4890 GC equipped with a Poropack N column. Strains MSSRF30^T and MSSRF31 were able to reduce acetylene to ethylene and fixed 39.68 ± 1.42 and 38.2 ± 2.0 (nmoles of ethylene ml⁻¹ h⁻¹), respectively. The strains were subjected to a *nifH*-specific PCR amplification using the primers of Ueda *et al.* (1995) and the expected 390 bp amplification product was observed in both isolates and negative results were observed with *V. fluvialis* LMG 7894^T and *V. furnissii* LMG 7910^T (Fig. 3). The amplified 390 bp fragments of strains MSSRF30^T and MSSRF31 were sequenced and were found to show 100 % sequence similarity. The 390 bp fragment showed 83.0 % sequence similarity with those of *V. diazotrophicus* and *V. natriegens*; however, similarities with other *nifH* sequences from the NCBI database were 76.0 to 90.0 %. Thus, the results of the ARA and the presence of the *nifH* gene confirmed the nitrogen-fixing ability of strains MSSRF30^T and MSSRF31.

To confirm the above results obtained from biochemical, genotyping and phylogenetic analyses, DNA–DNA hybridization was performed between strain MSSRF30^T and its phylogenetic closest relatives, *V. cholerae* IID6019, *V. mimicus* LMG 7896^T, *V. fluvialis* LMG 7894^T, *V. furnissii* LMG 7910^T and *V. diazotrophicus* LMG 7893^T, in microdilution wells by using a fluorometric direct binding method (Ezaki *et al.*, 1988, 1989) as described previously (Sawabe *et al.*, 1998, 2004, 2007a). Genomic DNA of strain MSSRF30^T was labelled with photoprobe biotin (Vector Laboratories). Strain MSSRF30^T exhibited low DNA–DNA relatedness with the phylogenetically related type strains of *V. cholerae* IID6019 (13 %), *V. mimicus* LMG 7896^T (12 %),

Table 1. Features useful for differentiating *Vibrio porteresiae* sp. nov. MSSRF30^T from closely related species of the genus *Vibrio*

Species: 1, *Vibrio porteresiae* sp. nov. ($n=2$); 2, *V. fluvialis* (data from Macián *et al.*, 2004); 3, *V. furnissii* (from Macián *et al.*, 2004); 4, *V. tubiashii* (Alsina & Blanch, 1994); 5, *V. hepatarius* (Thompson *et al.*, 2003); 6, *V. vulnificus* (Baumann *et al.*, 1984); 7, *V. fortis* (Thompson *et al.*, 2003); 8, *V. cholerae* (Farmer & Hickman-Brenner, 1992); 9, *V. mimicus* (Farmer & Hickman-Brenner, 1992); 10, *V. diazotrophicus* (Farmer & Hickman-Brenner, 1992). +, Positive; –, negative; ND, no data; v, variable.

Biochemical characteristic	1	2	3	4	5	6	7	8	9	10
Arginine dihydrolase	–	+	+	v	–	–	–	+	+	+
Voges–Proskauer	+	–	–	–	+	–	v	+	–	–
Nitrate reduction	–	+	+	+	+	+	v	+	+	+
Amylase	–	+	+	+	ND	+	ND	+	ND	+
Caseinase	–	+	+	ND	ND	+	ND	+	+	ND
NaCl required for growth	+	+	v	+	–	+	+	–	–	+
Growth in TCBS	–	+	+	+	+	+	+	+	+	+
Utilization of:										
L-Arabinose	–	+	+	–	–	–	–	–	–	+
Cellobiose	+	–	–	+	+	+	+	–	–	+
Trehalose	–	+	+	+	+	+	+	+	+	+
Lactose	+	–	–	v	–	v	–	–	–	+
Rhamnose	+	–	–	–	–	–	–	–	–	–
D-Xylose	+	–	–	–	–	–	–	–	–	+
L-Ornithine	–	+	–	ND	+	–	+	+	+	ND
L-Arginine	–	+	+	v	–	–	–	+	+	+

V. fluvialis LMG 7894^T (14%), *V. furnissii* LMG 7910^T (13%) and *V. diazotrophicus* LMG 7893^T (9.9%).

Fig. 3. PCR amplification of the *nifH* gene from two novel diazotrophs of the genus *Vibrio*. Lanes: 1, 100 bp marker; 2, MSSRF30^T; 3, MSSRF31; 4, *V. fluvialis* LMG 7894^T; 5, *V. furnissii* LMG 7910^T; 6, *Swaminathanian salitolerans* PA51^T (positive control).

Based on phenotypic, phylogenetic (multilocus sequence analysis using 16S rRNA, *recA*, *pyrH*, *rpoA* and *nifH* genes), genotypic (genomic fingerprinting using the GTG₅ primer), chemotaxonomic and DNA–DNA hybridization analyses presented here, it is concluded that strain MSSRF30^T should be classified as representing a novel species of the genus *Vibrio*. The name *Vibrio porteresiae* sp. nov. is proposed.

Description of *Vibrio porteresiae* sp. nov.

Vibrio porteresiae (por.te.re'si.ae. N.L. gen. fem. n. *porteresiae* of porteresia, because the organism was isolated from *Porteresia coarctata*, a wild relative of rice growing in mangroves).

Cells are Gram-negative, straight to slightly curved rods (0.9×1.0 – 2.0 μm) which occur singly or in pairs and are motile. Colonies on TSA supplemented with 1.5% (w/v) NaCl are circular, smooth, and white with exopolysaccharide production within 2 days at 28 °C. Swarming is not detected. Able to fix atmospheric nitrogen. Positive for oxidase and catalase. The DNA G + C content of the type strain is 44.4 ± 3.1 mol%. Positive result in the Voges–Proskauer reaction and in tests for aesculin hydrolysis and citrate utilization. Negative result in tests for indole production, ONPG and H₂S production. Does not produce arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, caseinase or amylase. Negative for the reduction of nitrate to nitrite and no growth is observed in TCBS. Grows in the presence of NaCl concentrations of up to 5% (w/v), but not at 8% NaCl. Optimum growth occurs in the presence of 1–2% (w/v) NaCl at 28 °C. No

growth is observed in 0% NaCl. Growth occurs at 20–37 °C (optimal growth occurs at 28 °C), but no growth occurs at 4 or 45 °C, and at pH 5.5–9.0 (optimal growth occurs at pH 7). Resistant to the vibriostatic agent O/129 (10 or 150 µg per disc). Facultative anaerobe capable of both aerobic and anaerobic fermentative growth. Acid and gas are produced from fermentation of glucose. Other carbohydrates such as lactose, maltose, fructose, glucose, cellobiose, galactose, rhamnose, sucrose, xylose, mannitol and salicin are also fermented. Trehalose, dulcitol, raffinose, melibiose, sorbose, melezitose, inulin, glycerol, sorbitol, adonitol and inositol are not fermented. Grows in a mineral medium containing glucose and NH₄Cl. Utilizes cellobiose, lactose, sucrose, mannitol, salicin, rhamnose, xylose, fructose, glucose, maltose, mannitol, L-serine, and L-proline as sole carbon sources. Utilization of melibiose is weak. Does not use inulin, trehalose, inositol, dulcitol, arabinose, raffinose, sorbitol, mannose, L-alanine, L-choline, L-ornithine, L-arginine, L-lysine, L-valine, L-methionine or L-threonine as carbon sources. The most abundant fatty acids are summed feature 3 (27.53%; comprising C_{16:1}ω7c and/or C_{15:0} iso 2-OH), C_{16:0} (18.75%), C_{18:1}ω7c (19.20%), C_{14:0} (8.15%), C_{12:0} (6.84%), summed feature 2 (6.81%; comprising an unidentified fatty acid with an equivalent chain-length of 10.928 and/or C_{12:0} ALDE, C_{16:1} iso I and/or C_{14:0} 3-OH) and C_{12:0} 3-OH (6.40%). The following fatty acids are detected in small amounts; C_{10:0} (0.08%), C_{10:0} 3-OH (0.49%), C_{11:0} 3-OH (0.38%), C_{13:0} (0.25%), C_{12:0} 2-OH (0.22%), C_{12:1} 3-OH (0.25%), summed feature 1 (0.64%; comprising C_{13:0} 3-OH and/or C_{15:1} iso I/H), C_{15:1}ω8c (0.26%), C_{16:1}ω7c alcohol (0.26%), C_{16:1}ω5c (0.24%), C_{17:1}ω8c (0.58%), C_{17:0} (0.36%), C_{18:0} (0.58%), 11 methyl C_{18:1}ω7c (0.82%) and an unidentified fatty acid with an equivalent chain-length of 12.484 (0.94%).

The type strain, MSSRF30^T (=LMG 24061^T=DSM 19223^T), was isolated from *Porteresia coarctata*, a wild relative of rice growing in mangroves.

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