Mangrovibacter plantisponsor gen. nov., sp. nov., a nitrogen-fixing bacterium isolated from a mangrove-associated wild rice (Porteresia coarctata Tateoka)

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A facultatively anaerobic, nitrogen-fixing bacterium, strain MSSRF40T, was isolated from roots of mangrove-associated wild rice (Porteresia coarctata Tateoka). On the basis of 16S rRNA gene sequence similarities, strain MSSRF40T was shown to belong to the family Enterobacteriaceae, most closely related to Cronobacter muytjensii E603T (97.2 % sequence similarity), Enterobacter cloacae subsp. dissolvens LMG 2683T (97.1 %), E. radicincitans D5/23T (97.1 %) and E. ludwigii EN-119T (97.0 %). Sequence analysis of rpoB, gyrB and hsp60 genes showed that strain MSSRF40T had relatively low sequence similarity (<91, <84 and <90 %) to recognized species of different genera of the family Enterobacteriaceae and formed an independent phylectic lineage in all phylogenetic analyses using the 16S rRNA, rpoB, gyrB and hsp60 genes, clearly indicating that strain MSSRF40T could not be affiliated to any of the recognized genera within the family Enterobacteriaceae. The dominant cellular fatty acids were C16 : 0, C16 : 1ω7c and/or iso-C15 : 0 2-ОH and C18 : 1ω7c, similar to those of other members of the Enterobacteriaceae. The DNA G + C content was 50.1 mol%. Phylogenetic distinctiveness and phenotypic differences from its phylogenetic neighbours indicated that strain MSSRF40T represents a novel species and genus within the family Enterobacteriaceae, for which the name Mangrovibacter plantisponsor gen. nov., sp. nov. is proposed. The type strain of Mangrovibacter plantisponsor is strain MSSRF40T (=LMG 24236T =DSM 19579T).

The family Enterobacteriaceae is a large, heterogeneous group of Gram-negative, facultatively anaerobic, rod-shaped bacteria that do not form endospores and are catalase-positive and oxidase-negative (Brenner, 1984). Members of the family are distributed worldwide, with some being saprophytes and others being parasites of plants and animals. Many species of this family are of considerable economic importance due to their pathogenic effects on agriculture and livestock (Janda & Abbott, 2006). Currently, there are more than 35 genera described in this family (Dauga, 2002; Janda, 2006), including the recently described genus Cronobacter (Iversen et al., 2008), which was previously recognized as Farmer’s Enterobacter sakazakii biogroups 1–4, 7, 8, 11 and 13 (Iversen et al., 2007, 2008).

Several studies have shown that members of the Enterobacteriaceae may have beneficial effects on plant development when they are associated with the plants as rhizobacteria (Lodewyckx et al., 2002; Taghavi et al., 2009). They improve plant growth via nitrogen fixation, suppression of plant pathogens and production of phytohormones and enzymes involved in the metabolism of growth regulators such as ethylene, 1-aminocyclopropane 1-carboxylic acid (ACC), auxins and indole-3-acetic acid (IAA) (Gyaneshwar et al., 2001; Kämpfer et al., 2005; Taghavi et al., 2009).

During a study of diazotrophic bacteria from mangrove-associated wild rice, numerous isolates that are able to fix atmospheric nitrogen were isolated. Based on preliminary characterization, most isolates were tentatively identified as members of the genera Swaminithania, Vibrio and Serratia. In the present work, we describe the isolation and taxonomic characterization of a diazotroph, strain MSSRF40T, belonging to the family Enterobacteriaceae. On the basis of our results obtained from a polyphasic
approach, it can be concluded that isolate MSSRF40 T represents a new genus and species in the family Enterobacteriaceae.

Strain MSSRF40 T was isolated from mangrove-associated wild rice (Porteresia coarctata Tateoka) collected from the Pichavaram mangrove forest in India. The plant roots were washed with sterilized distilled water, surface-sterilized with sodium hypochlorite (4 %) for 5 min and washed several times with sterile distilled water. One gram of surface-sterilized roots was macerated in a blender and serially diluted, and 100 µl aliquots were inoculated into 30 ml test tubes containing 10 ml semi-solid N-free medium as described by Rameshkumar et al. (2008b). The samples were incubated at 28 °C for 4–5 days. Material from tubes showing a fine subsurface pellicle was transferred to fresh semi-solid N-free medium as described above and observed for pellicle formation. Further purification was done by repeatedly streaking the isolates on plates of NM + Y agar as described by Rameshkumar et al. (2008b) and then on tryptone soya agar (TSA; HiMedia). Isolates were maintained on TSA at 4 °C or stored frozen in tryptone soya broth (TSB; HiMedia) with 15 % glycerol at −80 °C.

Genomic DNA was extracted from isolate MSSRF40 T as described by Ausubel et al. (1987). The 16S rRNA gene was amplified and sequenced with primers as described by Rameshkumar et al. (2008b) and the rpoB, gyrB and hsp60 genes were amplified and sequenced as described by Mollet et al. (1997), Dauga (2002) and Iversen et al. (2004). The primer sequences for amplification and sequencing of the 16S rRNA, rpoB, gyrB and hsp60 genes are listed in Supplementary Table S1, available in IJSEM Online. For all the above-mentioned genes, PCR amplification was carried out in a total volume of 20 µl containing 2 µl template DNA (50–100 ng), 0.3 µl Taq DNA polymerase (3 U µl⁻¹), 2 µl 10 × Taq buffer, 2 µl dNTP mixture (2 mM) and 2 µl each of the forward and reverse primers (30 ng µl⁻¹) and brought to a final volume of 20 µl using double-sterilized distilled water. Amplification was carried out in an MJ Research minicycler. In all the reactions, water was used in place of DNA as a negative control. The sequences of these genes were compared with 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 (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods and bootstrap analysis was based on 1000 resamplings. The MEGA3 package (Kumar et al., 2004) was used for all analyses.

An almost-complete 16S rRNA gene sequence of strain MSSRF40 T comprising 1368 nt was determined and used for phylogenetic analysis. The analysis showed that strain MSSRF40 T was phylogenetically related to members of the family Enterobacteriaceae. A neighbour-joining tree based on these 16S rRNA gene sequences was constructed (Fig. 1) and showed that strain MSSRF40 T formed an independent phylogenetic lineage that was related most closely to the clade that includes the recently described genus Cronobacter (Iversen et al., 2008) and the type strains of two Enterobacter species (Enterobacter helveticus 513/05 T and Enterobacter pulvers 601/05 T). Although the stability of the branch was not confirmed by a high bootstrap resampling value (<55 %), the branching of the MSSRF40 T lineage separate from Cronobacter or any of the Enterobacter clusters was confirmed by maximum-parsimony analysis (Supplementary Fig. S1). Pairwise 16S rRNA gene sequence comparisons revealed that strain MSSRF40 T exhibited the highest similarity to Cronobacter muytjensii E603 T (97.2 %), followed by Enterobacter cloacae subsp. dissolvens LMG 2683 T (97.1 %), Enterobacter radicicinatans D5/23 T (97.1 %) and Enterobacter ludwigii EN-119 T (97.0 %). Furthermore, relatively high sequence similarity was also found to Enterobacter cancerogenus LMG 2693 T, Citrobacter farmeri CDC 2991-81 T and Enterobacter cloacae subsp. cloacae ATCC 13047 T, sharing 96.9 % sequence similarity, and Serratia ureilytica NiVa 51 T, sharing 96.8 % sequence similarity. These relatively high 16S rRNA gene sequence similarities between members of different genera and the partitioning of genera to several different phyla have been reported in other phylogenetic studies on the Enterobacteriaceae (Drancourt et al., 2001; Hoffmann & Roggenkamp, 2003; Rosenblueth et al., 2004; Kämpfer et al., 2005). Overall, no 16S rRNA gene sequence similarities above 97.2 % were found with any recognized species of different genera of the family Enterobacteriaceae, suggesting that strain MSSRF40 T could represent a novel genus, as it has been observed in previous studies that 98 % 16S rRNA gene sequence similarity is a reasonable cut-off value to delineate different genera in the family Enterobacteriaceae (Drancourt et al., 2001; Iversen et al., 2007, 2008).

Since the branches that represent the different genera of the family Enterobacteriaceae are not monophyletic and are not supported by high bootstrap values and since the highest similarity values of strain MSSRF40 T were found with strains of different genera, it can be concluded that, for the family Enterobacteriaceae, the similarity levels obtained reflect a high level of homoplasy in the 16S rRNA gene sequences. The results of 16S rRNA gene sequence analysis indicate that strain MSSRF40 T belongs to the family Enterobacteriaceae but, based on these results alone, the novel strain cannot be allocated unequivocally to a lower taxonomic rank.

As the usefulness of rpoB (encoding the RNA polymerase beta subunit) gene sequences for identification and species discrimination among members of the family Enterobacteriaceae has been reported previously by several authors (Mollet et al., 1997; Drancourt et al., 2001; Li et al., 2004; Kämpfer et al., 2005; Stephan et al., 2007, 2008), this approach was also used to determine the taxonomic position of strain MSSRF40 T. A 919 bp rpoB sequence of
strain MSSRF40T was compared with sequences from the databases. This analysis showed low levels of sequence similarity with different species of the family Enterobacteriaceae. For example, the analysis showed the highest similarity to Citrobacter braakii CDC 80-58T (GenBank accession no. EU010096; 91.2 %) and Citrobacter freundii ATCC 11012T (U77434; 89.9 %). A neighbour-joining tree based on these rpoB gene sequences was constructed (Supplementary Fig. S2) and showed that strain MSSRF40T formed a long separate branch that did not cluster with any species of the genera Enterobacter, Cronobacter, Citrobacter, Klebsiella, Salmonella or Kluyvera. Only similarities below 91 % were found to the rpoB sequences of all other strains shown in Supplementary Fig. S2. Similar results were obtained with the maximum-parsimony algorithm (not shown), supporting the view that strain MSSRF40T does not belong to any existing genus of the family Enterobacteriaceae, taking into account 6 % rpoB sequence dissimilarity as a reasonable limit to differentiate genera of the Enterobacteriaceae (Drancourt et al., 2001).

We used sequences of two further protein-encoding genes, gyrB (Dauga, 2002) and hsp60 (Harada & Ishikawa, 1997; Iversen et al., 2004), to describe the phylogenetic relationship of strain MSSRF40T with other members of the Enterobacteriaceae. The results of gyrB sequence analysis showed that strain MSSRF40T shared relatively low similarity (76.0–83.8 %) with all strains shown in Supplementary Fig. S3. According to the studies of Dauga (2002), similarities between gyrB nucleotide sequences from all Serratia species ranged from 84.8 to 97.3 %, and the range of variation within the genera Klebsiella, Enterobacter and Proteus was similar to that for Serratia. Strain MSSRF40T exhibited gyrB sequence similarities of less than 84 % to all its relatives, indicating that this strain does not belong to any existing genus in the family Enterobacteriaceae, supporting the results of rpoB sequence analysis. Furthermore, phylogenetic analysis of gyrB sequences also placed strain MSSRF40T as a separate branch that did not cluster with any of the species of the Enterobacteriaceae (Supplementary Fig. S3), and the same

![Fig. 1. Neighbour-joining tree constructed on the basis of 16S rRNA gene sequences of Mangrovibacter plantisponsor gen. nov., sp. nov. MSSRF40T and related members of the Enterobacteriaceae. Bootstrap values (percentages of 1000 replicates) ≥50 % are shown. Bar, 0.01 % nucleotide substitutions.](http://ijs.sgmjournals.org)
results were obtained when using the maximum-parsimony algorithm (not shown), indicating that strain MSSRF40T represents a novel genus.

Supporting these findings, the results of hsp60 gene analysis demonstrated that strain MSSRF40T had low sequence similarity (83.4–90.6 %) to the strains shown in Supplementary Fig. S4, and phylogenetic analysis of hsp60 also placed strain MSSRF40T as a separate branch that did not cluster with any of the species of the Enterobacteriaceae (Supplementary Fig. S4). The same results were obtained when using the maximum-parsimony algorithm (not shown).

Thus, based on phylogenetic analysis using 16S rRNA, rpoB, gyrB and hsp60 gene sequences, strain MSSRF40T could not be affiliated to any described genus. We therefore propose that strain MSSRF40T represents a taxonomic unit that deserves genus rank.

DNA base composition was determined by HPLC. Briefly, DNA was isolated after cell disruption with a French pressure cell and purified on hydroxyapatite, according toCashion et al. (1977). PI hydrolysis and nucleotide dephosphorylation with alkaline phosphatase were done as described byMesbah et al. (1989). HPLC conditions (LKB equipment with Shimadzu CR-3A integrator) on a Nucleosil 100-5C18 column were chosen according to Tamaoka & Komagata (1984). The DNA G+C content of strain MSSRF40T was 50.1 mol%, within the range given for the family Enterobacteriaceae (38–60 mol%; Farmer, 2006). However, the value was lower than the range given for the genus Cronobacter (56.7–57.0 mol%; Iversen et al., 2007) and slightly lower than the range given for the genus Enterobacter (52–60 mol%; Grimont & Grimont, 2005). The G+C contents of the Enterobacter species most closely related to MSSRF40T are not known except that for E. cloacae subsp. dissolvens, which is 54 mol% (Grimont & Grimont, 2005).

Physiological and biochemical tests were carried out at 28 °C. Conventional biochemical tests were performed according to standard methods (Smibert & Krieg, 1994). API 20E, API 20NE and API 50CH (medium E) strips (bioMérieux) were used according to the manufacturer’s instructions; results were read after 1 and 2 days. Salt tolerance tests were carried out as described byRameshkumar et al. (2008a). Data for reference type strains were obtained at the DSMZ under the same conditions as for MSSRF40T. Whole-cell fatty acid methyl ester analysis was performed according to Smibert & Krieg (1994). The analysis showed that strain MSSRF40T exhibited a fatty acid composition typical of the family Enterobacteriaceae, containing high levels of C16 : 0 (28.42 %), C18 : 1ω7c (28.04 %) and summed feature 3 (C16 : 1ω7c and/or iso-C15 : 0 2-OH; 23.65 %). The acids C12 : 0 (0.35 %), C14 : 0 (8.63 %), an unidentified fatty acid with an equivalent chain-length of 14.502 (0.72 %), C15 : 0 (0.36 %), C16 : 1ω5c (0.27 %), C17 : 0 (0.18 %), C17 : 0 cyclo (1.84 %), C18 : 0 (0.23 %) and summed feature 2 (iso-C16 : 1ω7c and/or C14 : 0 3-OH; 7.32 %) were also detected.

The acetylene reduction assay was used to test for nitrogen fixation by the novel isolate grown on semi-solid (NIM + Y) medium. The amount of ethylene produced was measured using 10 % (v/v) acetylene according to the method ofLi & MacRae (1992) using a Hewlett Packard 4890 GC equipped with a Poropack N column, as described previously (Rameshkumar, 2008; Rameshkumar et al., 2008b). Strain MSSRF40T was able to reduce acetylene to ethylene (32.68 ± 1.42 nmol ethylene ml⁻¹ h⁻¹), indicating that the isolate is able to fix atmospheric nitrogen.

In conclusion, based on the phenotypic, chemotaxonomic and phylogenetic data, it was clear that strain MSSRF40T was a member of the family Enterobacteriaceae. However, 16S rRNA gene sequence similarity values between strain MSSRF40T and all other described species within the family Enterobacteriaceae were ≤ 97.2 %. It was stated previously that 16S rRNA gene sequence similarity values between species of Klebsiella and Raoultella and of Cronobacter and Enterobacter are 97–98 % (Drancourt et al., 2001; Iversen et al., 2007, 2008) and that it can be assumed from these values that the threshold for the differentiation of genera within the family Enterobacteriaceae is at <98 % similarity (Drancourt et al., 2001; Iversen et al., 2007, 2008). Supporting this observation, strain MSSRF40T had very low rpoB gene similarity (<91 %) to related members of the family Enterobacteriaceae and formed a long, separate branch that did not cluster with any species of the genera.
Biochemical differentiation of strain MSSRF40\textsuperscript{T} from other members of the family Enterobacteriaceae

Cronobacter dublinensis DSM 18705\textsuperscript{T},
Cronobacter malonaticus DSM 18702\textsuperscript{T},
Cronobacter muytjensii,
Cronobacter sakazakii DSM 4485\textsuperscript{T},
Cronobacter turicensis DSM 18703\textsuperscript{T},
Enterobacter cancerogenus DSM 17580\textsuperscript{T},
Enterobacter cowanii DSM 18146\textsuperscript{T},
Enterobacter gergoviae,
Enterobacter helveticus,
Enterobacter hormaechei DSM 12409\textsuperscript{T},
Enterobacter ludwigii DSM 16688\textsuperscript{T},
Enterobacter pulveris DSM 17873\textsuperscript{T},
Citrobacter koseri,
Buttiauxella agrestis,
Edwardsiella tarda,
Hafnia alvei DSM 30163\textsuperscript{T},
Klebsiella pneumoniae DSM 30104\textsuperscript{T},
Leclercia adecarboxylata DSM 30081\textsuperscript{T},
Rahnella aquatilis DSM 4594\textsuperscript{T},
Raoultella terrigena DSM 2687\textsuperscript{T},
Salmonella enterica.

API 20E tests were carried out at the DSMZ.

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Enterobacter, Cronobacter, Citrobacter, Klebsiella, Salmonella or Kluyvera (Supplementary Fig. S2), demonstrating clearly that strain MSSRF40\textsuperscript{T} does not belong to any established genus of the family Enterobacteriaceae, taking into account 6% rpoB sequence dissimilarity as a reasonable limit to differentiate genera of the family Enterobacteriaceae (Drancourt et al., 2001). Additionally, strain MSSRF40\textsuperscript{T} formed an independent phylogenetic lineage in all phylogenetic analyses using sequences of the genes gyrB (Supplementary Fig. S3; <84% similarity) and hsp60 (Supplementary Fig. S4; <90% similarity), supporting the conclusion from the 16S rRNA gene and rpoB sequence analyses that it is difficult to allocate strain MSSRF40\textsuperscript{T} to any of the previously described genera in the family Enterobacteriaceae.

Some of the genera in the family Enterobacteriaceae have been distinguished mostly on the basis of differences at the molecular phylogenetic level. For instance, Cronobacter (Iversen et al., 2007, 2008) was delineated from Enterobacter (Farmer et al., 1980) on the basis of 98% 16S rRNA gene sequence similarity and its clinical importance (Iversen et al., 2007, 2008), though members of Cronobacter are phenotypically similar to members of Enterobacter (Iversen et al., 2007, 2008). The genus Raoultella (Drancourt et al., 2001) was delineated from Klebsiella (Orskov, 1984) at the level of 16S rRNA gene and rpoB sequence similarity (98% and 94%), though it exhibited phenotypic differences only with respect to growth at 10 °C and utilization of L-sorbose (Drancourt et al., 2001). Brenneria (Hauben et al., 1998) was delineated from Erwinia (Winslow et al., 1917) at the level of 16S rRNA gene and glyceraldehyde-3-phosphate dehydrogenase (gapDH) gene sequence similarity (Brown et al., 2000), though members of Brenneria are biochemically very similar to members of Pantoaea, Erwinia and Pectobacterium in that they fail to produce arginine dihydrolase and to decarboxylate amino acids such as ornithine and lysine (Lelliott & Dickey, 1984). Pectobacterium (Brenner et al., 1973) was delineated from Erwinia (Winslow et al., 1917) on the basis of 16S rRNA gene sequence similarity (Hauben et al., 1998) and distinct pathogenic and biochemical properties (Brenner et al., 1973).

Phenotypically, strain MSSRF40\textsuperscript{T} most closely resembles the genera Cronobacter and Enterobacter, but it can be distinguished from species of these genera by biochemical characteristics and G+C content. Strain MSSRF40\textsuperscript{T} differed from the type strains of the genus Cronobacter in negative reactions for the Voges–Proskauer reaction, ornithine decarboxylase, ascorbic acid hydrolysis and growth at 45 °C and positive reactions for acid production from sorbitol and the methyl red test. For several genera and species within the family Enterobacteriaceae, it has been decided that they should retain their taxonomic status not only on a genetic basis but also by virtue of their source and functional role. For instance, the genera Escherichia and Shigella are genetically indistinguishable; however, the
genus *Shigella* remains separate from *Escherichia* because of its unique clinical and public-health importance as the cause of bacillary dysentery and shigellosis (Janda, 2006). Similarly, the genera *Pectobacterium* and *Erwinia* are genetically closely related at the 16S rRNA gene sequence level (Hauben *et al*., 1998); however, the genus *Pectobacterium* remains separate from *Erwinia* on the basis of its distinct pathogenic properties (Brenner *et al*., 1973). Likewise, sources from which *Cronobacter* strains have been isolated include sterile body sites of children, infants and adults and these strains are involved in clinical disease, which was deemed sufficient to delineate the genus from the genus *Enterobacter* (Iversen *et al*., 2007, 2008). Strain MSSRF40<sup>T</sup> is associated with mangrove roots and fixes atmospheric nitrogen, supporting the fact that strain MSSRF40<sup>T</sup> should not be placed in the genus *Cronobacter*.

Similarly, strain MSSRF40<sup>T</sup> differs from the description of the genus *Enterobacter* (Grimont & Grimont, 2005) in that most members of the latter genus are negative for the methyl red test and positive for the Voges–Proskauer reaction. Furthermore, strain MSSRF40<sup>T</sup> differed from the genera *Cronobacter* and *Enterobacter* in its G+C content, which is 50.1 mol% compared with 56.7–57.0 mol% for *Cronobacter* (Iversen *et al*., 2007) and 52–60 mol% for *Enterobacter* (Grimont & Grimont, 2005).

Thus, based on the combination of phylogenetic information showing the formation of a distinct clade within the family *Enterobacteriaceae* (Fig. 1 and Supplementary Figs S2–S4) and low sequence similarity for the 16S rRNA (≤97.2 %), rpoB (<91 %), gyrB (<84 %) and hsp60 (<90 %) genes to related genera and phenotypic characters (Table 1), it is most appropriate to conclude that the diazotrophic strain MSSRF40<sup>T</sup> represents a novel genus and species of the family *Enterobacteriaceae*, for which the name *Mangrovibacter plantisponsor* gen. nov., sp. nov. is proposed.

### Description of Mangrovibacter plantisponsor gen. nov., sp. nov.


Cells are Gram-negative rods (1.0 × 1.2–4.0 μm) with peritrichous flagella, facultatively anaerobic, oxidase-negative and catalase-positive. Phylogenetically most closely related to members of the genera *Cronobacter*, *Enterobacter* and *Citrobacter*. Phenotypically similar to species of the genus *Cronobacter*, but distinguished by biochemical characteristics such as negative reactions for the Voges–Proskauer reaction, ornithine decarboxylase, aesculin hydrolysis and acid from inositol and positive reactions for acid from sorbitol and the methyl red test. No growth at or above 42 °C. Environmental isolates may be able to fix nitrogen. Major fatty acids are C<sub>16:0</sub>, C<sub>18:1</sub>ω7c and summed feature 3 (C<sub>16:1</sub>ω7c and/or iso-C<sub>15:0</sub> 2-OH). The DNA G+C content of the type strain of the type species is 50.1 mol%. The type species is *Mangrovibacter plantisponsor*.

### Description of Mangrovibacter plantisponsor sp. nov.

*Mangrovibacter plantisponsor* (plan. ti. spon.’sor. L. fem. n. *planta* plant; L. masc. n. *sponsor* sponsor, guarantor; N.L. masc. n. *plantisponsor* sponsor of plants, referring to the potentially plant-beneficial properties of the type strain).

Colonies on TSA (HiMedia) are circular, smooth, creamy white and 1–2 mm in diameter within 2 days at 28 °C; swarming is not detected. Growth occurs at 15–40 °C, optimally at 28–30 °C, but not at 5 °C. Growth occurs in the presence of 0–8 % NaCl; no growth at 10 % NaCl. Positive for nitrate reduction and citrate utilization. Negative reactions for urease, lysine decarboxylase, tryptophan deaminase, indole production, hydrogen sulphide production, denitrification and hydrolysis of Tween 80, DNA, starch, casein and gelatin. Produces acid from sucrose, raffinose, cellobiose, arabinose, sorbitol, glycerol, ribose, D-xylose, sucrose, galactose, glucose, fructose, mannose, rhamnose, mannitol, methyl 2,3-D-glucoside, N-acetylgalactosamine, arbutin, cellobiose, maltose, melibiose, sucrose, trehalose, raffinose, gentiobiose, L-fucose, 2-arabinose, L-arabinose, gluconate and 6-ketogluconate. Delayed acid production (48 h) in API CH50 test strips for salicin and amygdalin. Negative for acid production from erythritol, D-arabinose, 5-ketogluconate, adonitol, L-xylose, methyl β-D-xyloside, sorbose, dulcitol, inositol, methyl α-D-mannoside, lactose, inulin, melezitose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose and L-arabitol. Utilizes gluconate, 5-ketogluconate and malate as carbon sources. Does not utilize phenylacetate, caprate or adipate (API 20NE). Other characteristics are as described for the genus.

The type strain, MSSRF40<sup>T</sup> (=LMG 24236<sup>T</sup> =DSM 19579<sup>T</sup>), was isolated from mangrove-associated wild rice (*Porteresia coarctata* Tateoka) in Pichavaram, Tamil Nadu, India.

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### References


