Vibrio rhizosphaerae sp. nov., a red-pigmented bacterium that antagonizes phytopathogenic bacteria

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Two novel red-pigmented *Vibrio* strains, MSSRF3^T and MSSRF10, with antibacterial activity against phytopathogens were isolated from the rhizosphere region of mangrove-associated wild rice (*Porteresia coarctata* Tateoka), in Pichavaram, India. The cells were Gram-negative, facultatively anaerobic and rod-shaped and were motile by means of single polar flagella. The two strains were catalase-positive and oxidase-negative, and were able to grow in 0.1–10% NaCl (with optimum growth in 2% NaCl) and at temperatures of 20–42 °C (optimum growth at 25–30 °C). Both strains produced acid and gas from D-glucose under anaerobic conditions and utilized a wide range of compounds as sole carbon and energy sources. The DNA G+C contents determined were 51.3 mol% for strain MSSRF3^T and 51.0 mol% for strain MSSRF10.

Phylogenetic analysis based on 16S rRNA, *rpoA*, *recA* and *pyrH* gene sequences showed that strains MSSRF3^T and MSSRF10 belong to the genus *Vibrio* and are very closely related to *Vibrio ruber* JCM 11486^T, with which they share 98.3–98.5% (16S rRNA), 98.3–99.7% (*rpoA*), 90.2–99.8% (*recA*) and 91.3–99.4% (*pyrH*) gene sequence similarities, respectively. Levels of DNA–DNA relatedness were 44% between strains MSSRF3^T and MSSRF10, 80% between strain MSSRF10 and *V. ruber* JCM 11486^T and 45% between strain MSSRF3^T and *V. ruber* JCM 11486^T. Strain MSSRF3^T was phenotypically similar to *V. ruber* JCM 11486^T. However, the inability to reduce nitrate to nitrite, the ability to grow in 0.1% NaCl and the presence of caseinase were characteristics that allowed differentiation between *V. ruber* JCM 11486^T and strain MSSRF3^T. In addition, strain MSSRF3^T could be differentiated from strain MSSRF10 and its closest relative *V. ruber* JCM 11486^T with respect to its genomic fingerprinting analysis (random amplified polymorphic DNA, GTG₅, BOX, PCR-restriction fragment length polymorphism and ribotyping). Therefore, based on phenotypic, genotypic, phylogenetic and DNA–DNA hybridization analyses, strain MSSRF3^T (=LMG 23790^T=DSM 18581^T) should be classified as representing the type strain of a novel species of the genus *Vibrio*, for which the name *Vibrio rhizosphaerae* sp. nov. is proposed.

The genus *Vibrio* has a complex taxonomy and, at the time of writing, comprised 64 recognized species (Thompson

Abbreviations: RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MSSRF3^T and MSSRF10 are DQ847123 and DQ273663, respectively, and those for the *recA*, *rpoA* and *pyrH* gene sequences of strains MSSRF3^T, MSSRF10 and *Vibrio ruber* JCM 11486^T are EF523232–EF523240.

Figures showing the colony and cell morphologies of strain MSSRF3^T, phylogenetic trees constructed using maximum-likelihood and maximum-parsimony and based on partial 16S rRNA gene sequences, neighbour-joining trees based on partial *recA*, *rpoA* and *pyrH* gene sequences and results of PCR-based fingerprinting analysis using RAPD, GTG₅, BOX, PCR-RFLP and ribotyping are available with the online version of this paper.

et al., 2005), which were isolated from various environments such as aquatic, estuarine and coastal, and from marine sediments (Thompson *et al.*, 2004). Several cultivation-dependent and -independent studies have shown that vibrios appear at particularly high densities in and/or on marine organisms, e.g. corals, fish, molluscs, seagrass, sponges, shrimp and zooplankton (Thompson *et al.*, 2004). Some species are found as symbionts in specialized luminous organs of marine fish and invertebrates, whereas a number of other species are well-known pathogens of humans or marine animals (Thompson *et al.*, 2004). To date, relatively few studies have shown that vibrios can produce antimicrobial substances (Hjelm *et al.*, 2004; Long & Azam, 2001; Sugita *et al.*, 1997). However, these vibrios were not identified at the species level. Herein we report the isolation and polyphasic taxonomic study of

two novel *Vibrio* strains, MSSRF3^T and MSSRF10, which inhibit phytopathogenic bacteria.

This study was conducted in Pichavaram mangroves, on the south-east coast of India, situated about 250 km south of Chennai (11° 27' N 79° 47' E). The soil salinity was about 25–30 ppt and the temperature was about 28–32 °C. Strains MSSRF3^T and MSSRF10 were isolated from the rhizosphere region of mangrove-associated wild rice (*Porteresia coarctata* Tateoka) as part of a research programme, the main objective of which was to isolate and screen plant-growth-promoting bacteria, in particular for biocontrol of bacterial phytopathogens. Briefly, the rhizosphere soil samples were obtained by shaking the roots gently to remove loosely attached soil. The soil adhering to the roots was then rinsed with 10 ml sterile water. The resulting rinse solution containing bacteria from the rhizosphere was serially diluted and plated onto half-strength trypticase soy agar (TSA; Difco), supplemented with 2 % NaCl (TSA+NaCl). Colonies that appeared on half-strength TSA+NaCl plates after 1–7 days incubation at 28 °C were selected and purified; two strains, designated MSSRF3^T and MSSRF10, were found to have antimicrobial activity against *Xanthomonas oryzae*, *Erwinia carotovora* and *Pseudomonas syringae*, and were chosen for further study. Both strains were maintained on TSA+NaCl at 4 °C or stored at –80 °C in trypticase soy broth (TSB; Difco) containing 2 % NaCl (TSB+NaCl) with 15 % (v/v) glycerol.

Strains MSSRF3^T and MSSRF10 were grown in TSB+NaCl at 28 °C with aeration. Cell-free culture fractions were recovered by centrifugation (5000 g for 30 min at 4 °C) of cultures grown in TSB+NaCl for 48 h at 28 °C in flasks without shaking. The cell-free spent culture medium was decanted and sterilized by passing it through a 0.22 µm-pore-size filter, and was maintained at 4 °C for short-term storage or at –80 °C for long-term storage. The antimicrobial activity of the cultures or cell-free culture fractions was determined by using a spot-on-lawn assay. The bacterial phytopathogens *X. oryzae*, *E. carotovora* and *P. syringae* were grown separately in TSB for 72 h at 28 °C. From these cultures, 100 µl (at OD 600 nm, approximately 10⁶ c.f.u. ml⁻¹) was pipetted onto TSB containing 1.5 % (w/v) agar and spread plated. After the plates had been dried for 3 h at room temperature, 5 µl of 24 h cultures of strains MSSRF3^T and MSSRF10 was spotted on the target organism lawn, and the spots were allowed to dry. Similarly, a 3 mm well was made in plates containing the target organism; to these wells 100 µl filter-sterilized, cell-free culture supernatants obtained from strains MSSRF3^T and MSSRF10 were added. The plates were incubated at 28 °C and examined each day for the appearance of zones of inhibition of microbial growth. The diameter (in mm) of the zones of inhibition was used as a measure of antimicrobial activity.

Strains MSSRF3^T and MSSRF10 inhibited a wide range of bacterial phytopathogens (Fig. 1 and Table 1). The antibacterial activity of both strains did not require the

Fig. 1. Spot-on-lawn assay of strain MSSRF3^T showing antibiotic-like inhibition against the phytopathogens *X. oryzae* (a) and *E. carotovora* (b).

presence of living cells, because antibacterial activity was demonstrated using filter-sterilized, cell-free culture fractions of both strains grown in TSB+NaCl, as shown in Table 1, indicating that the antibacterial compound produced by both strains was extracellular. Purification and characterization of the antibacterial substance produced by strains MSSRF3^T and MSSRF10 has still to be undertaken.

Extraction and amplification of genomic DNA for 16S rRNA gene sequence analysis were carried out as described previously (Loganathan & Nair, 2004) and the *recA*, *pyrH* and *rpoA* genes were amplified and sequenced as described by Thompson *et al.* (2005). The sequences of these genes were compared against sequences available from GenBank using the BLAST program (Altschul *et al.*, 1997) and were aligned manually using CLUSTAL W version 1.6 (Thompson *et al.*, 1994). Distance matrices for the aligned sequences were determined using the Kimura two-parameter method (Kimura, 1980). The distance matrices were then used to reconstruct phylogenetic trees using neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein,

Table 1. Antibacterial activities of cultures and cell-free culture filtrates of strains MSSRF3^T and MSSRF10 against bacterial phytopathogens

Values given are the mean of three replicates.

Phytopathogen	Inhibition zone diameter (mm)			
	Strain MSSRF3 ^T		Strain MSSRF10	
	Culture	Cell-free filtrate	Culture	Cell-free filtrate
<i>Xanthomonas oryzae</i>	20	15	18	13
<i>Erwinia carotovora</i>	17	12	15	12
<i>Pseudomonas syringae</i>	16	12	14	10

1981) and maximum-parsimony (Felsenstein, 1996). The robustness of individual branches was estimated by bootstrapping with 1000 replicates (Felsenstein, 1985).

Nearly complete 16S rRNA gene sequences for strains MSSRF3^T (1424 bp) and MSSRF10 (1392 bp) were obtained. Comparison with 16S rRNA gene sequences of recognized *Vibrio* species held in GenBank indicated that the two strains were related phylogenetically to members of this genus. Phylogenetic trees based on almost-complete sequences and using neighbour-joining, maximum-likelihood and maximum-parsimony methods were all in agreement and showed that strains MSSRF3^T and MSSRF10 are closely related to *V. ruber* JCM 11486^T (98.3 and 98.5 % similarity, respectively) and *Vibrio gazogenes* LMG 19540^T (95.7 and 95.5 % similarity, respectively) (Fig. 2; see also Supplementary Fig. S1a, b, available in IJSEM Online). The level of 16S rRNA gene sequence similarity between strains MSSRF3^T and MSSRF10 was 98.0 %, and below 95.7 % with other *Vibrio* species. The values obtained from the 16S rRNA gene sequence analysis for strains MSSRF3^T and MSSRF10 were above the cut-off value of 97 % for the definition of bacterial species (Stackebrandt & Goebel, 1994) and, hence, as limitations of 16S rRNA gene sequencing for differentiating closely related species have been documented (Fox *et al.*, 1992), we analysed other housekeeping genes that are useful for

differentiation of *Vibrio* species as described previously (Thompson *et al.*, 2005).

Analysis of the *pyrH* gene supported the phylogenetic position of strains MSSRF3^T and MSSRF10 within the genus *Vibrio*, as the results showed 91.3–99.4 % sequence similarity with respect to *V. ruber* JCM 11486^T, 92.6–92.0 % similarity with *V. gazogenes* LMG 19540^T, 90.2 % similarity between each other, and less than 90.0 % similarity with other *Vibrio* species (Supplementary Fig. S2a, in IJSEM Online). The *recA* gene has been also proposed as an alternative marker for species discrimination in the genus *Vibrio* (Thompson *et al.*, 2005). A pairwise analysis of the *recA* sequence of strain MSSRF3^T showed low levels of similarity with that of strain MSSRF10 (91.2 %), *V. ruber* JCM 11486^T (90.2 %), *V. gazogenes* LMG 19540^T (88.5 %) and less than 88.5 % with other *Vibrio* species. Nevertheless, strain MSSRF10 showed high *recA* sequence similarity with *V. ruber* JCM 11486^T (99.8 %) (Supplementary Fig. S2b, in IJSEM Online). This result is in agreement with those obtained from the *pyrH* gene sequence analysis and the lower similarity values found between these two genes (*recA* and *pyrH*) of *V. ruber* JCM 11486^T and strain MSSRF3^T confirm that this strain does not belong to *V. ruber* whereas strain MSSRF10 does belong to *V. ruber*, according to Thompson *et al.* (2005).

In addition to the *pyrH* and *recA* genes, the *rpoA* gene has also been proposed as an alternative marker for bacterial classification and as a chronometer in the family *Vibrionaceae* (Thompson *et al.*, 2005). Sequence comparisons of the *rpoA* sequence of strain MSSRF3^T showed 98.3 % similarity with *V. ruber* JCM 11486^T, 98.4 % with *V. gazogenes* LMG 19540^T and 98.0 % with strain MSSRF10. However, strain MSSRF10 shared high *rpoA* sequence similarity with *V. ruber* JCM 11486^T (99.7 %) (Supplementary Fig. S2c, in IJSEM Online). Strains MSSRF3^T and MSSRF10 showed less than 91.0 % similarity with other *Vibrio* species. This result correlates well with the 16S rRNA gene analysis and shows that *rpoA* gene sequences were slightly less discriminatory than *recA* and *pyrH* sequences, supporting the fact that species with >98 % *rpoA* sequence similarity will have <97 % *recA* and *pyrH* gene sequence similarities (Thompson *et al.* 2005).

Based on sequence analysis of four different genetic loci, it is evident that analysis of *recA* and *pyrH* gene sequences was more discriminatory than analysis based on *rpoA* and 16S rRNA gene sequences, similar to results obtained by other workers, indicating that *rpoA* and 16S rRNA gene sequences have similar discriminatory powers (Thompson *et al.*, 2004, 2005). Therefore, considering the results of the overall phylogenetic analysis, the genealogical positions of strains MSSRF3^T and MSSRF10 were further examined together with *V. ruber* JCM 11486^T by using a polyphasic approach, as described by Thompson *et al.* (2004) and Stackebrandt *et al.* (2002).

Restriction analysis of the 16S rRNA gene was performed according to Behrendt *et al.* (1999). 16S rRNA genes were

Fig. 2. Evolutionary distance phylogenetic tree based on 16S rRNA gene sequences constructed using neighbour-joining, showing the phylogenetic placement of strains MSSRF3^T and MSSRF10 and related members of the genus *Vibrio*. Bootstrap percentages (based on 1000 replicates) are indicated at branch points. Bar, 0.01 substitutions per nucleotide.

amplified using primers described previously (Loganathan & Nair, 2004), resulting in amplification of a single fragment of approximately 1500 bp. Digestion of the PCR products was performed using a set of endonucleases (*AccI*, *AluI*, *DdeI* and *RsaI*), which were selected on the basis of the 16S rRNA gene sequence of *V. ruber* JCM 11486^T using NEBcutter version 2.0 software. Ribotyping was performed with the enzymes *HindIII* and *HaeIII*. The electrophoresis conditions, blotting procedures, hybridization with a ³²P-labelled 16S rRNA probe of *V. ruber* JCM 11486^T using a *rediprime* DNA-labelling kit (Amersham) at 65 °C and detection procedures were carried out as described by Sambrook *et al.* (1989). Random amplified polymorphic DNA (RAPD) analysis was performed using two random 10-mer universal primers, OPD5 (5'-TGAGCGGACA-3') and OPD6 (5'-ACCTGAACGG-3'), as described previously (Loganathan *et al.*, 1999). Repetitive extragenic palindromic PCR (rep-PCR) fingerprinting using GTG₅ and BOX primers was performed as described by Ben-Haim *et al.* (2003). *V. ruber* JCM 11486^T was used as a reference strain in studies related to PCR-based fingerprinting analysis.

From the 16S rRNA gene restriction analysis, it was found that, using the enzymes *AccI*, *AluI* and *RsaI*, strain MSSRF3^T could be differentiated from strain MSSRF10 and *V. ruber* JCM 11486^T, whereas use of the enzyme *DdeI* enabled differentiation of *V. ruber* JCM 11486^T from strains MSSRF3^T and MSSRF10 (Supplementary Fig. S3a, in IJSEM Online), suggesting that strain MSSRF10 was very closely related to *V. ruber* JCM 11486^T when compared with strain MSSRF3^T at the 16S rRNA gene level. These results were in good agreement with the phylogenetic analysis of the 16S rRNA gene, showing that *V. ruber* JCM 11486^T and strain MSSRF10 form a distinct cluster and that, within that cluster, strain MSSRF3^T represents a separate branch, as shown in Fig. 2.

Ribotype patterns obtained with *HaeIII* and *HindIII* and fingerprints obtained with RAPD primers (OPD5, OPD6) and with BOX and GTG₅ primers clearly indicate that strains MSSRF3^T, MSSRF10 and *V. ruber* JCM 11486^T are distinct from each other (see Supplementary Fig. S3b, c, in IJSEM Online). From these results, it is suggested that strains MSSRF3^T and MSSRF10 are not clonal in origin, that they should be recognized as a distinct genotype and are different from *V. ruber* JCM 11486^T.

Cells of strains MSSRF3^T and MSSRF10 were grown in TSB+NaCl for 24 h and their morphology and motility were examined using phase-contrast and electron microscopy. For electron microscopy, fresh bacterial cells were transferred to a 300-mesh, carbon-coated copper grid, stained with 2% (w/v) phosphotungstic acid solution (pH 6.5) for 1 min and then observed with a JEM-1200EX electron microscope (JOEL). Classical phenotypic tests were performed as described by Baumann *et al.* (1984), Farmer & Hickman-Brenner (1992), Leifson (1963) and Shieh *et al.* (2000). The ability to utilize various carbon

compounds as the sole carbon source was investigated by testing 0.5% carbon compounds in 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 recorded after 7 days incubation at 28 °C, with *V. ruber* JCM 11486^T being used as a reference. For *in vitro* pigment analysis, strains MSSRF3^T, MSSRF10 and *V. ruber* JCM 11486^T were grown aerobically on TSA+NaCl at 28 °C, extracted with acetone/methanol (7:2, v/v) and analysed spectrophotometrically (Shieh *et al.*, 2003).

According to the data, strains MSSRF3^T and MSSRF10 were characterized as mesophilic, halophilic, facultative anaerobic, Gram-negative rods that are motile by means of single polar flagella (Supplementary Fig. S4a, in IJSEM Online), indicating that the strains probably represent a species of *Vibrio* in the family *Vibrionaceae* (Baumann *et al.*, 1984). Both strains produced non-diffusible, cellular red pigments, regardless of the presence of light (see Supplementary Fig. S4b, in IJSEM Online). Acetone/methanol extracts of the red pigments produced by strains MSSRF3^T, MSSRF10 and *V. ruber* JCM 11486^T showed maximum absorption at about 535 nm, which is identical to the absorption spectrum of prodigiosin (Allen, 1967). Strain MSSRF3^T was phenotypically related to strain MSSRF10, and could be differentiated from it by not being able to reduce nitrate. Unusually, we observed that strains MSSRF3^T and MSSRF10 could utilize substrates (trehalose, adonitol, dulcitol, inositol, sorbitol, L-alanine, L-arginine, L-lysine, L-ornithine, L-threonine and L-glycine) very well when they were grown on minimal salt base agar medium rather than in liquid medium. Interestingly, negative results were obtained for *V. ruber* JCM 11486^T for utilization of the above substrates when grown on minimal salt base agar medium and in minimal salt base liquid medium, under the same conditions as for strains MSSRF3^T and MSSRF10. Differential phenotypic characteristics of strain MSSRF3^T, the phylogenetically closest species *V. ruber* JCM 11486^T and other phenotypically and phylogenetically related *Vibrio* species are shown in Table 2. Additional phenotypic characteristics of strain MSSRF3^T are given in the species description.

The DNA G+C content was determined by the Microbial Type Culture Collection (MTCC), India, using the thermal denaturation method (Marmur & Doty, 1962), as already reported (Delorme *et al.*, 2002). The DNA G+C content of strain MSSRF3^T was 51.3 mol%, which is slightly higher than the values reported for other species of the genus *Vibrio*, and 51.0 mol% for strain MSSRF10, which was in good agreement with values for the genus *Vibrio* (Baumann *et al.*, 1984) but was considerably higher than the value of 45.8 mol% for *V. ruber* JCM 11486^T (Shieh *et al.*, 2003).

To confirm the results obtained from the biochemical, genotyping and phylogenetic analyses, DNA-DNA hybridization was performed between strains MSSRF3^T, MSSRF10 and their closest relative *V. ruber* JCM 11486^T,

Table 2. Phenotypic characteristics that differentiate strain MSSRF3^T (*Vibrio rhizosphaerae* sp. nov.) and *V. ruber* JCM 11486^T and phenotypically and phylogenetically related *Vibrio* species

Taxa: 1, strain MSSRF3^T; 2, *V. ruber* JCM 11486^T; 3, *V. gazogenes* (data from Baumann *et al.*, 1984); 4, *Vibrio aerogenes* (Shieh *et al.*, 2000); 5, *Vibrio metschnikovii* (Baumann *et al.*, 1984); 6, *Vibrio vulnificus* (Baumann *et al.*, 1984); +, Positive; –, negative; v, variable; ND, not determined. All taxa are positive for amylase, gelatinase and lipase.

Characteristic	1	2	3	4	5	6
Pigment	Pink–red	Red	Red	–	–	–
Oxidase	–	–	–	–	–	+
Nitrate reduction	–	+	–	+	–	+
Gas from glucose	+	+	+	+	–	–
Growth in 0.1 % NaCl	+	–	ND	–	ND	ND
Growth in 10 % NaCl	+	+	–	–	–	ND
Caseinase	+	–	+	+	+	+
Utilization of*:						
Sorbitol	+	–	+	–	v	–
Dulcitol	+	–	ND	–	ND	ND
Raffinose	+	–	ND	ND	ND	ND
Inositol	+	–	–	+	v	–
Trehalose	+	–	–	–	+	+
L-Ornithine	+	–	–	+	+	–
L-Lysine	+	–	ND	+	ND	ND
L-Alanine	+	–	–	+	+	+
L-Proline	+	+	+	ND	+	+
L-Arginine	+	–	–	+	+	+

*Substrate utilization was performed using minimal base agar medium.

by using the membrane filter method (Tourova & Antonov, 1987) as described previously (Shivaji *et al.*, 1992; Reddy *et al.*, 2000). Hybridizations were performed on four replicates. Each DNA–DNA relatedness value is the mean of reciprocal and non-reciprocal reactions. The results showed that, at the DNA–DNA level, there was 44 % relatedness between strains MSSRF3^T and MSSRF10, 80 % between strain MSSRF10 and *V. ruber* JCM 11486^T and 45 % between strain MSSRF3^T and *V. ruber* JCM 11486^T. These values are the means of four replicate experiments, which did not differ by more than 2 %. These results indicate that strain MSSRF3^T does not belong to *Vibrio ruber* JCM11486^T, whereas strain MSSRF10 is considered to be a member of *Vibrio ruber* JCM11486^T when the recommendation of a threshold value of 70 % DNA–DNA relatedness for the definition of species is considered (Wayne *et al.*, 1987).

In conclusion, strain MSSRF3^T can be differentiated from strain MSSRF10 and its closest relative *V. ruber* JCM 11486^T with respect to its genomic fingerprinting analysis (RAPD, GTG₅, BOX, PCR-restriction fragment length polymorphism and ribotyping), phylogenetic analysis

(16S rRNA, *recA*, *pyrH*, *rpoA*), phenotypic characteristics, high DNA G + C content and the fact that it shows <60 % relatedness at the DNA–DNA level with these species. Thus, based on phenotypic, genotypic and phylogenetic characteristics, strain MSSRF3^T represents the type strain of a novel species of the genus *Vibrio*, for which the name *Vibrio rhizosphaerae* sp. nov. is proposed.

Description of *Vibrio rhizosphaerae* sp. nov.

Vibrio rhizosphaerae (rhi.zo'sp.hae.rae. Gr. fem. n. *rhiza* root; L. fem. n. *sphaera* -ae ball, any globe, sphere; N.L. gen. n. *rhizosphaerae* of the rhizosphere).

Cells are Gram-negative rods (approx. 1.8–3.2 µm long and 0.4–0.5 µm wide) and motile by means of single polar flagella. Catalase-positive and oxidase-negative. Colonies on agar media are red–pink, non-luminescent and circular, with entire margins. Swarming is not detected. Can inhibit a range of bacterial phytopathogens. Facultative anaerobe capable of both aerobic and anaerobic fermentative growth. Acid and gas are produced from fermentation of glucose. Other carbohydrates such as cellobiose, galactose, lactose, mannose, sucrose, xylose, mannitol and salicin are also fermented. Trehalose, dulcitol, sorbitol and inositol are not fermented. Voges–Proskauer, amylase, gelatinase, caseinase and lipase tests are positive, but arginine dihydrolase, lysine and ornithine decarboxylase tests are negative. Indole is not produced. Nitrate is not reduced. Optimum growth occurs at 25–30 °C and pH 7 and in 2 % NaCl. Growth occurs between 20 and 42 °C, but not at 4 or 45 °C. Growth occurs in 0.1–10 % NaCl; no growth occurs in the absence of NaCl. Grows in mineral medium containing glucose and NH₄Cl. Cellobiose, galactose, glucose, lactose, mannose, melibiose, sucrose, xylose, mannitol, acetate, citrate, fumarate, pyruvate, L-aspartate, L-glutamate, L-serine and L-cysteine are utilized as sole carbon and energy sources. Utilizes trehalose, adonitol, dulcitol, inositol, sorbitol, proline, L-alanine, L-arginine, L-lysine, L-ornithine, L-threonine and L-glycine as sole sources of carbon and energy, only when grown on minimal salt agar medium, not in liquid medium. The DNA G + C content of the type strain is 51.3 mol%.

The type strain, MSSRF3^T (=LMG 23790^T=DSM 18581^T), was isolated from the rhizosphere of mangrove-associated wild rice (*Porteresia coarctata* Takeoka).

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