Chromobacterium rhizoryzae sp. nov., isolated from rice roots

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A novel facultatively anaerobic, rod-shaped bacterium, designated LAM1188T, was isolated from the roots of rice (Oryza sativa) in Hubei Province. Cells of LAM1188T were Gram-stain-negative and motile. The temperature and pH ranges for growth were 15–40 °C (optimum: 30 °C) and pH 5–10 (optimum: pH 7), respectively. The strain did not require NaCl for growth but tolerated up to 3.5 % NaCl (w/v). Analysis of the 16S rRNA gene sequence indicated that the isolate represented a member of the genus Chromobacterium, and was most closely related to Chromobacterium haemolyticum MDA0585T and Chromobacterium aquaticum CC-SEYA-1T with 98.7 % and 97.3 % sequence similarity, respectively. The values of DNA–DNA hybridization between LAM1188T and C. haemolyticum JCM 14163T and C. aquaticum CCUG 55175T were 54.0±2.1 % and 44.0±1.2 %, respectively. The major cellular fatty acids were C16:0 and summed feature 3 (C16:1ω6c and/or C16:1ω7c). The main polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, four unidentified aminolipids and four unidentified lipids. The respiratory quinone was ubiquinone Q-8. The DNA G+C content was 64.1 mol% as determined by the Tm method. On the basis of its phenotypic, chemotaxonomic and phylogenetic characteristics, strain LAM1188T is suggested to represent a novel species of the genus Chromobacterium, for which the name Chromobacterium rhizoryzae sp. nov. is proposed. The type strain is LAM1188T (=ACCC 19900T=JCM 31180T).

The genus Chromobacterium belongs to the family Neisseriaceae (Betaproteobacteria) and was first described by Bergonzini a century ago. At the time of writing, the genus Chromobacterium comprised eight recognized species: Chromobacterium violaceum as the type species, Chromobacterium subtsugae (Martin et al., 2007), Chromobacterium aquaticum (Young et al., 2008), Chromobacterium haemolyticum (Han et al., 2008), Chromobacterium piscineae, Chromobacterium pseudoviolaceum (Kämpfer et al., 2009), Chromobacterium vaccini (Soby et al., 2013) and Chromobacterium amazonense (Menezes et al., 2015). The members of the genus are aerobic, rod-shaped, motile and Gram-stain-negative, the DNA G+C content ranges from 62.3 to 68.7 mol%, the major fatty acids are C16:0 and C16:1ω6c, the production of violet pigment is strain-dependent.

Rice (Oryza sativa) is one of the most important food crops in the world and widely planted in China. Rice is susceptible to a number of serious diseases, including the rice blast caused by the widespread foliar fungal pathogen Magnaporthe oryzae. There are currently no effective means to control the pathogen, the use of biological control agents may be an alternative method of crop protection (Spence et al., 2014). In order to obtain microbes with the capacity of suppressing rice blast pathogen, we have begun to isolate bacteria from the roots of rice planted at the Experimental Demonstration Base at Huazhong Agricultural University.

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Abbreviation: ACCC, Agricultural Culture Collection of China.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LAM1188T is KT951843.

Three supplementary figures and one supplementary table are available with the online Supplementary Material.
Wuhan city, PR China. The rice samples were washed thoroughly with sterilized water to get rid of the soil clinging to the surface of the roots. Then the detached roots of rice were surface sterilized for 2 min in ethanol (75%) followed by 10 min in a NaClO solution (3%). Treated samples were washed thoroughly with sterilized water, and the last wash was saved and spread on to nutrient agar medium (BD/Difco 212000) to evaluate the efficiency of the sterilization procedure. After confirmation that there were no bacterial colonies, the sample segments were crushed in 4% sucrose solution with a sterilized mortar and pestle. Aliquots were 10-fold serially diluted on tryptic soy agar (TSA) plates (BD/Difco 236950) at 30 °C. Subcultivation was done on TSA plates at 30 °C for 24 h (technique modified from that of Jha & Kumar, 2007). The antagonistic activity of bacterial strains was determined by measuring the inhibition zone of mycelial growth of the fungal pathogens on potato dextrose agar (PDA) medium using in vitro confrontation bioassay (Bibi et al., 2012). Among these bacteria, a Chromobacterium-like strain, designated LAM1188T, showed a good inhibitory effect on Magnaporthe oryzae (Fig. S1, available in the online Supplementary Material). The taxonomic position of the strain was determined by a polyphasic taxonomic approach, the results imply that LAM1188T represents a novel species of the genus Chromobacterium.

LAM1188T was isolated from the roots of rice (Oryza sativa) plants. The reference type strain C. haemolyticum JCM 14163T was obtained from Japan Collection of Microorganisms (JCM, Japan), C. aquaticum CCUG 55175T was courtesy of Professor Kämpfer from Justus Liebig Universität Giessen, Germany. The reference type strains were cultured under the same conditions as strain LAM1188T for comparative analyses.

Cultural and morphological characteristics were determined from cultures grown on TSA medium using a light microscope (model 80i; Nikon) and a transmission electron microscope (model 7500; Hitachi). Growth at different temperatures (4, 10, 15, 20, 25, 30, 35, 40 and 45 °C) and pH values (pH 3–11 at intervals of 0.5 pH units) were determined in TSB (BD/Difco 211825) medium. The medium was adjusted to the desired pH by using HCl (pH 3.0–4.0), sterile solutions of citric acid/Na2HPO4 (pH 4.0–5.0), Na2HPO4/NaH2PO4 buffer (pH 6.0–8.0), NaHCO3/Na2CO3 buffer (pH 9.0–10.0) and Na2HPO4/NaOH buffer (pH 11.0). NaCl concentrations (0–12% at intervals of 0.5, w/v) were determined both in mineral medium (0.2 g MgSO4·7H2O l–1, 1 g KH2PO4 l–1, 1 g KH2PO4 l–1, 1 g NH4NO3 l–1, 0.8 g C6H12O6 l–1, 0.05 g FeSO4 l–1, 0.02 g CaCl2 l–1, pH 7.0–7.2) and TSB medium (pH 7) but varying the addition of NaCl. Growth rates were determined by monitoring the increase in OD660. All tests were independently performed in duplicate. The Gram reaction was performed as described by Smibert & Krieg (1994). Cellular motility was tested using a semi-solid agar medium (Tittsler & Sandholzer, 1936). Growth under anaerobic condition was performed in TSB medium in Hungate tubes filled with oxygen-free N2 at 30 °C (Ruan et al., 2014). Catalase activity was determined by observing bubble formation in 3% (v/v) hydrogen peroxide solution. Oxidase activity was detected by using 1% (w/v) tetramethyl-p-phenylenediamine (Shin et al., 2012). Lecithinase, lipase and proteolytic activity were tested by egg yolk agar (BD/Difco 233471). The haemolysis assay, poly-β-hydroxybutyrate and fluorescence testing were performed as described by Young et al. (2008). Antibiotic sensitivity was tested on TSA plates at 30 °C for 48 h with different concentrations of ampicillin, carbenicillin, polymyxin B, kanamycin, tetracycline, chloromycetin, streptomycin, gentamicin, neomycin, tetracycline, minocycline, levofoxacin, erythromycin, clindamycin, nitrofurantoin, vancomycin, teicoplanin, rifampicin, fusidic acid and cotrimoxazole. LAM1188T was incubated in the presence of 0, 10, 20, 50, 100, 200 and 300 mg of each antibiotic ml–1. Antibiotic susceptibility was determined by measuring the zone of inhibition and comparing the bacteriostatic diameter with the data as described by Dong & Cai (2001). Soil carbon source assimilation was performed using Biolog GN2 MicroPlate (Biolog). Enzyme activities, utilization of various substrates as sole carbon and energy sources were also evaluated by using API ZYM, API 20 E and API 20 NE strips (BioMérieux) according to the manufacturer’s instructions.

Cells of strain LAM1188T were Gram-stain-negative, facultatively anaerobic, had a single polar flagellum, were motile, non-fluorescent and rod-shaped (1.6–2.2 µm in length and 0.4–0.5 µm in width) as shown in Fig. 1. Colonies of the strain on TSA medium were tan-colored and smooth after 24 h at 30 °C. The isolate could grow at temperature of 15–40 °C (optimum: 30 °C) and at pH 5–10 (optimum: pH 7). The strain did not require NaCl for growth but tolerated up to 3.5% NaCl (w/v) both in mineral medium and TSB medium (pH 7). Growth at 30 °C was also observed on nutrient agar (BD/Difco), MacConkey agar (Oxoid) and R2A agar (Oxoid). LAM1188T was found to hydrolyse casein and produce lecithinase and lipase on egg-yolk agar.
Poly-β-hydroxybutyrate granule accumulation on TSA plates was examined by light microscopy and a clear zone around the colony on TBAB (Difco) with 5% sheep blood was observed. The results of testing for susceptibility to antibiotics are as follows, with the antibiotic concentrations in µg ml⁻¹: The strain was sensitive to polymyxin B (10), kanamycin (10), tetracycline (20), chloromycetin (10), streptomycin (10), gentamicin (10), neomycin (10), tetracycline (50), minocycline (10), levofloxacin (20), erthyromycin (20), clindamycin (20) and nitrofurantonin (10); resistant to ampicillin (10), carbenicillin (10), vancomycin (20), cline (50), minocycline (10), levofloxacin (20), erythromycin (10), teicoplanin (20), rifampicin (10), fusidic acid (10) and cotrimoxazole (10). The detailed phenotypic characteristics of LAM1188 are given in the species description. LAM1188⁷, C. haemolyticum JCM 14163⁷ and C. aquaticum CCUG 55175⁷ are all positive for haemolytic activity (5% sheep blood), Poly-β-hydroxybutyrate, lecithinase and lipase production, but negative for fluorescence. The comparison of the physiological characteristics between LAM1188 and closely related type strains of species of the genus Chromobacterium is shown in Table 1.

Genomic DNA for PCR amplification was extracted and purified according to the method described by Marmur (1961). The 16S rRNA gene was amplified by PCR with universal primers 27F and 1492R (Weisburg et al., 1991) and purified using a PCR purification kit (TianGen) according to the manufacturer’s instruction. The purified PCR products were cloned into pMD19-T vector and sequenced by the Life Technologies Company (Shanghai, China). EzTaxon-e server (Kim et al., 2012) and CLUSTAL W (Thompson et al., 1994) were applied to analyze the 16S rRNA gene sequence similarities and multiple sequence alignment. Phylogenetic trees were reconstructed with the neighbor-joining method (Saitou & Nei, 1987), maximum-parsimony method (Fitch, 1971) and maximum-likelihood method (Felsenstein, 1981) by using the MEGA 6 program package (Tamura et al., 2013). Evolutionary distances were calculated according to the algorithm of the Kimura’s two-parameter model (Kimura et al., 1980) for the neighbour-joining method. Phylogenetic trees were evaluated with 1000 resamplings bootstrap analysis (Felsenstein, 1985). DNA–DNA hybridization was performed between LAM1188⁷ and C. haemolyticum JCM 14163⁷ and C. aquaticum CCUG 55175⁷ by measuring the renaturation rates of the denatured DNAs as described by De Ley et al. (1970) and Huss et al. (1983). The genomic DNA G+C content was determined by the thermal-renaturation method (Marmur & Doty, 1962) using a Beckman DU 800 spectrophotometer (Beckman Coulter), with Escherichia coli K-12 as the reference strain. The experiments were carried out in quadruplicate. A nearly complete 16S rRNA gene sequence of strain LAM1188⁷ was obtained (1445 nt, GenBank accession number KT951843). Comparative 16S rRNA gene sequence analysis with the EzTaxon-e server showed that LAM1188⁷ shared the highest sequence similarity with C. haemolyticum.

**Fig. 2.** Neighbour-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequences of strain LAM1188⁷ and its closest relatives and several outgroup strains. All bootstrap values, based on 1000 replications, are shown as percentages at the nodes. Branching nodes supported by the maximum-likelihood and maximum-parsimony algorithms are indicated by filled circles. GenBank accession numbers are shown in parentheses. Bar, 0.01 nucleotide substitutions per nucleotide position.
Table 1. Comparison of phenotypic, genotypic and physiological characteristics of LAM1188<sup>T</sup> and its relatives of the genus Chromobacterium

<table>
<thead>
<tr>
<th>Characteristics</th>
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<td>Tan</td>
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<td>62.6</td>
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<td>64.5</td>
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<td>Adipic acid</td>
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<td>Phenylactic acid</td>
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<td>3.5% (w/v) NaCl</td>
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MDA0585<sup>T</sup> (98.7%) followed by C. aquaticum CC-SEYA-1<sup>T</sup> (97.3%), C. piscinae CCUG 53329<sup>T</sup> (96.5%), C. pseudoviolaceum CCM 2076<sup>T</sup> (96.3%), C. subtsgue PRAA4-1<sup>T</sup> (96.2%), C. vaccinii MWU205<sup>T</sup> (96.1%), C.amazonense CBMAI 310<sup>T</sup> (96.0%) and C. violaceum ATCC 12472<sup>T</sup> (95.2%). The phylogenetic tree reconstructed by the neighbor-joining (Fig. 2) method clearly showed that LAM1188<sup>T</sup> was located within the monophyletic cluster of the genus Chromobacterium and formed a distinct subcluster with C. haemolyticum MDA0585<sup>T</sup> and C. aquaticum CC-SEYA-1<sup>T</sup>, the filled circles indicate branches that were also found in maximum-likelihood and maximum-parsimony trees. The DNA G+C content of LAM1188<sup>T</sup> was 64.1 mol% (T<sub>m</sub>), which was in the range reported for the genus Chromobacterium. The DNA–DNA hybridization values of LAM1188<sup>T</sup> with C. haemolyticum JCM 14163<sup>T</sup> and C. aquaticum CCUG 55175<sup>T</sup> were 54.0±2.1% and 44.0±1.2% (mean percentage reassocation±SD, n=4), respectively. The low DNA–DNA relatedness values (<70%) between the novel isolate and its closest relatives precludes genomic relatedness and supports the designation of strain LAM1188<sup>T</sup> as the representative of a novel species within the genus Chromobacterium (Stackebrandt & Goebel, 1994).

Chemotaxonomic characterization of LAM1188<sup>T</sup> and the reference strains included analyses of cellular fatty acids, respiratory quinones and polar lipids. All strains were incubated with TSB medium at 30°C. Cells were harvested in the late exponential phase for analysis. The fatty acid profiles were generated using the MIDI Sherlock Microbial Identification System (MIDI Sherlock software package, version 6.0; Agilent 6890N). Analysis of cellular fatty acids was performed according to the manufacturer’s instructions as described by Sakamoto et al. (2002). HPLC analyses of respiratory quinones were carried out using the equipment described by Komagata & Suzuki (1987). Further analysis was carried out as described by Minnikin et al. (1984) and Xu et al. (2011). Polar lipids were extracted from lyophilized biomass using the two-stage method described by Tindall (1990a, b) and separated by two-dimensional silica gel TLC. The total polar lipids were detected by spraying the
plates with 5% phosphomolybdic acid hydrate ethanol solution followed by heating, while head groups were detected using specific staining reagents as previously described by Tindall (1990a, b). The result was analyzed as described by Fang et al. (2012).

The fatty acid profile mainly comprised C16:0 and summed feature 3 (C16:1ω6c and/or C16:1ω7c). The fatty acids were similar to those of C. haemolyticum JCM 14163T and C. aquaticum CCUG 55175T, while differences existed in their content and other minor compositions. The proportion of C16:0 of LAM1188T was found to be much higher than that of C. haemolyticum JCM 14163T and C. aquaticum CCUG 55175T, and iso-C17:0 3-OH was only detected in LAM1188T. The proportion of C12:0 of LAM1188T was found to be much lower than that of C. haemolyticum JCM 14163T and C. aquaticum CCUG 55175T, and a lower proportion of C10:0 3-OH was also found in LAM1188T compared with those of C. haemolyticum JCM 14163T and C. aquaticum CCUG 55175T. The fatty acids profile comparison between LAM1188T, C. haemolyticum JCM 14163T and C. aquaticum CCUG 55175T is shown in Table S1. The respiratory quinone of LAM1188T was ubiquinone Q-8 which was consistent with the phenotypes of C. haemolyticum JCM 14163T and C. aquaticum CCUG 55175T. The major polar lipids were diphosphatidylglycerol, phosphatidyglycerol and phosphatidylethanolamine, which were found to be identical to the major polar lipids of C. haemolyticum JCM 14163T (Fig. S2) and C. aquaticum CCUG 55175T (Fig. S3). Four unidentified lipids and four unidentified aminolipids were also detected in LAM1188T (Fig. 3).

On the basis of the phenotypic, phylogenetic and genotypic characteristics, it is suggested that LAM1188T represents a novel species within the genus Chromobacterium, for which the name Chromobacterium rhizoryzae sp. nov. is proposed. The type strain is LAM1188T (=ATCC 1990T=JCM 31180T).

**Description of Chromobacterium rhizoryzae sp. nov.**

*C. rhizoryzae* (rhiz.o.ry'zae. Gr. n. rhiza root; L. gen. n. oryzae of rice; N.L. gen. n. rhizoryzae of rice roots).

Cells are Gram-stain-negative, facultatively anaerobic, oxidase-negative, catalase-positive, rod-shaped (1.6–2.2 µm in length and 0.4–0.5 µm in width) and motile by means of a single polar flagellum. Colonies are tan-colored and smooth on TSA medium after 24 h at 30°C. Growth occurs on nutrient agar, MacConkey agar and R2A agar. Positive for haemolytic activity (5% sheep blood), lecitinase and lipase production. Negative for fluorescein. Growth occurs at 15°C–40°C (optimum: 30°C) and pH 5–10 (optimum: 7). Does not require NaCl for growth but tolerates up to 3.5% NaCl (w/v) both in mineral medium and TSB medium (pH 7). Positive for the utilization of dextrin, glycogen, Tween 40, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, D-fructose, α-D-glucose, myo-inositol, D-mannitol, D-psicose, D-trehalose, pyruvic acid methyl ester, acetic acid, cis-aconitic acid, citric acid, D-gluconic acid, β-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, D,L-lactic acid, propionic acid, bromosuccinic acid, L-alanine, L-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyI-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-serine, L-threonine, urocanic acid, uridine, thymidine, putrescine, adipic acid, trisodium citrate, phenylacetic acid, glyceraldehyde, D,L-α-glyceraldehyde phosphate, α-D-glucose-1-phosphate and D-glucose-6-phosphate and weakly positive from D-arabitol, succinic acid monomethyl ester, maltose, D-mannose, formic acid, α-ketovaleric acid, L-proline, γ-aminobutyric acid, potassium gluconate, capric acid, malic acid, N-acetylglucosamine and inosine as sole carbon source, but negative for α-cyclodextrin, adonitol, D-cellobiose, D-sorbitol, i-erythritol, L-fucose, D-galactose, gentiobiose, α-D-lactose, lactulose, D-melibiose, β-methyl-D-glucoside, D-raffinose, L-rhamnose, sucrone, turanose, xyitol, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, malonic acid, quinic acid, sebacic acid, succinic acid,
glucuronamide, D,L-carnitine, phenylethylamine, 2-aminoethanol, 2,3-butanediol and amygdalin. Positive for alkaline phosphatase, butyrate esterase (C4), caprylate esterase (C8) (weakly), myristate lipase (C14) (weakly), valine arylamidase (weakly), α-chymotrypsin, acid phosphatase, napthol-AS-BI-phosphorylhydrolase, arginine dihydrolase, gelatinase and N-acetyl-β-glucosaminidase, but negative for cystine arylami-
dase, trypsin, α-galactosidase, β-galactosidase, β-glucuronid-
ase, α-glucosidase, β-glucosidase, α-mannosidase, lysine decarboxylase, urease, tryptophan deaminase and α-fucosi-
dase. Positive for the reduction of nitrate to nitrite, but nega-
tive for H2S production, indole production and acetoin production. The major fatty acids are C16:0 and summed feature 3 (C16:0ω6c and/or C16:0ω7c). The respiratory quinone is ubiquinone Q-8. The main polar lipids are diphosphatidylglyc-
cerol, phosphatidylglycerol, phosphatidylethanolamine, four unidentif-
ed lipids and four unidentified aminolipids.

The type strain is LAM1188T (=ACC 1990T=JCM 31180T), which was isolated from the roots of rice planted in the Experimental Demonstration Base at Huazhong Agricultural University, Wuhan city, China. The DNA G+C content of the type strain is 64.1 mol% (Tm).

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References

Bibi, F., Yasir, M., Song, G.-C., Lee, S.-Y. & Chung, Y.-R. (2012). Diver-

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measure-


Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maxi-


ne sp. nov., and Chromobacterium pseudoviolaceum sp. nov., from environ-


