Terrimonas rubra sp. nov., isolated from a polluted farmland soil and emended description of the genus *Terrimonas*

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A salmon-red-pigmented bacterial strain, designated M-8^T, was isolated from a polluted farmland soil sample in China and was characterized in a taxonomic study using a polyphasic approach. Strain M-8^T was Gram-stain-negative, rod-shaped, non-motile and non-spore-forming. Growth occurred at 20–37 °C, at pH 5.0–10.0 and with 0–2 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain M-8^T belonged to the genus *Terrimonas*. 16S rRNA gene sequence similarity values between strain M-8^T and the type strains of the three recognized species of the genus *Terrimonas*, *Terrimonas ferruginea* KACC 11310^T, *Terrimonas aquatica* LMG 24825^T and *Terrimonas lutea* KACC 13047^T, were 97.1, 96.3 and 95.3 %, respectively. The predominant respiratory quinone was menaquinone-7 (MK-7) and the major fatty acids were iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1ω7c} and/or C_{16:1ω6c}). The DNA G + C content of strain M-8^T was 47.0 mol%. On the basis of genotypic and phenotypic data, strain M-8^T is considered to represent a novel species of the genus *Terrimonas*, for which the name *Terrimonas rubra* sp. nov. is proposed. The type strain is M-8^T (=CCTCC AB 2010401^T=KCTC 23299^T). An emended description of the genus *Terrimonas* is also presented.

The genus *Terrimonas* was proposed by Xie & Yokota (2006) with *Terrimonas ferruginea* as the type species. At the time of writing, the genus *Terrimonas* comprised three recognized species: *Terrimonas aquatica*, *Terrimonas lutea* and *T. ferruginea* (*T. ferruginea* was previously described as [*Flavobacterium*] *ferrugineum*) (Sheu *et al.*, 2010; Xie & Yokota, 2006). Members of the genus *Terrimonas* are generally characterized as strictly aerobic, Gram-negative, non-motile, non-gliding and non-filamentous bacteria that are oxidase-positive and weakly catalase-positive. These bacteria usually contain iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and summed feature 3 as major fatty acids, menaquinone-7 (MK-7) as the predominant respiratory quinone and have DNA G + C contents of 47.0–48.9 mol%.

During a project to investigate bacterial diversity from an imazethapyr (a weedkiller)-polluted farmland soil in Shandong Province, China, a salmon-red-pigmented bacterial strain, designated M-8^T, was isolated and studied using a polyphasic taxonomic approach. Initial 16S rRNA gene sequence analysis indicated that strain M-8^T was related most closely to *T. ferruginea* KACC 11310^T, *T. aquatica* LMG 24825^T and *T. lutea* KACC 13047^T (97.1, 96.3 and 95.3 % similarity, respectively). Strain M-8^T clustered closely with the above type strains, and the four strains formed a separate phylogenetic branch within the family *Chitinophagaceae*. To determine the taxonomic and phylogenetic position of strain M-8^T, *T. ferruginea* KACC 11310^T, *T. aquatica* LMG 24825^T and *T. lutea* KACC 13047^T were selected for comparison of morphological, physiological, biochemical and chemotaxonomic characteristics; and DNA–DNA hybridizations were also performed between strain M-8^T and its two closest phylogenetic neighbours

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M-8^T is JF803808.

A supplementary table is available with the online version of this paper.

(*T. ferruginea* KACC 11310^T and *T. aquatica* LMG 24825^T). Data from the present study indicate that strain M-8^T represents a novel species of the genus *Terrimonas*.

Strain M-8^T was isolated from a polluted soil sample after plating dilutions (distilled water) onto R2A agar and incubation at 30 °C for 3 days. Subcultivation was performed on R2A agar medium at 30 °C for 48–72 h. The pure culture of strain M-8^T was preserved at –80 °C in R2A broth with 20 % (v/v) glycerol. *T. ferruginea* KACC 11310^T and *T. lutea* KACC 13047^T were obtained from the Korean Agricultural Culture Collection (KACC) and *T. aquatica* LMG 24825^T was obtained from the BCCM/LMG Bacteria Collection. The type strains of the three recognized *Terrimonas* species were selected as reference strains for DNA–DNA hybridization experiments and for chemotaxonomic and physiological characterization.

Cell morphology was determined by transmission electron microscopy (H-7650; Hitachi). Gram staining was performed following the method described by Beveridge *et al.* (2007). Catalase activity was determined by assessing bubble production in 3.0 % (w/v) H₂O₂. Oxidase activity was tested by using oxidase reagent (bioMérieux) according to the manufacturer's instructions. The presence of

flexirubin-type pigments was detected by using 20 % KOH (Fautz & Reichenbach, 1980). The pH range for growth was determined by measuring the optical density (wavelength 600 nm) of R2A broth adjusted to pH 4–10 (at intervals of 1.0 pH unit) by using appropriate biological buffers (Chung *et al.*, 1995). The temperature range for growth was determined on R2A agar at 4, 10, 15, 20, 25, 30, 32, 37, 40 and 45 °C. Tolerance to NaCl was tested in modified R2A broth lacking NaCl and with 0.5 % and 1.0–5.0 % (w/v) NaCl (intervals of 1.0 %). Antibiotic susceptibility was examined by placing different antibiotic discs on R2A agar as described by Zhou *et al.* (2007). The antibiotic discs (Oxoid) tested were: ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (5 µg), rifampicin (5 µg), penicillin G (1 µg), streptomycin (10 µg), trimethoprim (5 µg) and tetracycline (30 µg). Additional biochemical tests were performed by using API ZYM and API 20NE kits (bioMérieux) and carbon source utilization was evaluated by using the Biolog GN2 microplate (Biolog) according to the manufacturers' instructions. Comparative phenotypic characteristics between strain M-8^T and the type strains of recognized *Terrimonas* species are given in Table 1 and Table S1 (available in IJSEM Online). The detailed

Table 1. Differential phenotypic characteristics between strain M-8^T and the type strains of species of the genus *Terrimonas*

Strains: 1, M-8^T; 2, *T. ferruginea* KACC 11310^T; 3, *T. aquatica* LMG 24825^T; 4, *T. lutea* KACC 13047^T. All data were obtained from the present study. +, Positive; –, negative; (+), weakly positive.

Characteristic	1	2	3	4
Colony pigmentation	Salmon red	Salmon red	Yellowish-orange	Yellow
Growth temperature range (°C)	20–37	10–37	10–37	10–37
Growth with 2 % NaCl	+	+	–	–
Enzyme activities				
Trypsinase	+	–	–	+
α-Chymotrypsin	+	–	–	+
α-Galactosidase	+	–	+	+
β-Galactosidase	–	–	+	+
β-Glucuronidase	–	–	(+)	–
α-Glucosidase	+	(+)	+	+
β-Glucosidase	–	(+)	+	+
α-Mannosidase	–	(+)	(+)	+
β-Fucosidase	+	–	+	(+)
Assimilation of:				
Glucose	+	–	+	–
Arabinose	+	(+)	+	–
Mannose	+	–	+	(+)
N-Acetylglucosamine	+	(+)	+	–
Maltose	+	(+)	+	–
Sensitivity to:				
Chloramphenicol	+	+	–	+
Gentamicin	–	–	+	+
Nalidixic acid	+	+	+	–
Roxithromycin	+	–	–	–
Streptomycin	–	+	+	+
Vancomycin	+	+	–	+

phenotypic characteristics of strain M-8^T are given in the species description below.

Genomic DNA was prepared for 16S rRNA gene amplification and DNA G + C content and DNA–DNA hybridization tests according to the method described by Marmur (1961). Bacterial universal primer set 27F and 1492R was selected for PCR amplification of the 16S rRNA gene (Lane, 1991). PCR amplification of the 16S rRNA gene sequence was performed as described by Li *et al.* (2007). The 16S rRNA gene sequence was manually aligned with reference sequences retrieved from the GenBank database following BLAST searches. A phylogenetic tree was constructed by using the MEGA version 4.0 software package (Tamura *et al.*, 2007) after multiple alignment of the sequence data by CLUSTAL_X (Thompson *et al.*, 1997). The corrected evolutionary distance was calculated according to Kimura's two-parameter model (Kimura, 1980, 1983) and clustering was based on the neighbour-joining and maximum-parsimony methods. Bootstrap analysis was used to evaluate the tree topology by performing 1000 resamplings (Felsenstein, 1985). The almost-complete 16S rRNA gene sequence (1418 nt) of strain M-8^T was obtained in the present study. 16S rRNA gene sequence analysis indicated that strain M-8^T belongs to the genus *Terrimonas* and was most closely related to *T. ferruginea* KACC 11310^T, *T. aquatica* LMG 24825^T and *T. lutea* KACC 13047^T. The four strains formed a distinct

phylogenetic branch within the family *Chitinophagaceae* (Fig. 1).

DNA–DNA hybridization experiments were performed to further clarify the level of relatedness between strain M-8^T and *T. ferruginea* KACC 11310^T and *T. aquatica* LMG 24825^T (Stackebrandt & Goebel, 1994). DNA–DNA hybridization experiments were carried out according to the method described by Ezaki *et al.* (1989). Levels of DNA–DNA relatedness between strain M-8^T and *T. ferruginea* KACC 11310^T and *T. aquatica* LMG 24825^T were 54.3 and 23.0 %, respectively, which were significantly lower than the threshold value of 70 % commonly accepted for definition of a novel bacterial species (Stackebrandt & Goebel, 1994).

Analysis of cellular fatty acids was performed as described by Sasser (1990) by using the Microbial Identification System (MIDI, Sherlock Version 6.1) and TSBA6 database. Bacterial biomass collected for analysis of cellular fatty acids was cultivated at 30 °C for 2 days on R2A agar plates and the cells were harvested from the third quadrant of the quadrant-streaked plate. The fatty acid profile of strain M-8^T was similar to those of the type strains of the three recognized species of the genus *Terrimonas* determined with the same cultivation and analysis conditions in the present study (Table 2). The major fatty acids of strain M-8^T were iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain M-8^T and related taxa in the family *Chitinophagaceae*. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. *Flavobacterium aquatile* DSM 1132^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 2. Cellular fatty acid profiles of strain M-8^T and the type strains of recognized species of the genus *Terrimonas*

Strains: 1, M-8^T; 2, *T. ferruginea* KACC 11310^T; 3, *T. aquatica* LMG 24825^T; 4, *T. lutea* KACC 13047^T. Values are percentages of the total fatty acids. All data were obtained from the present study under the same test conditions.

Fatty acid	1	2	3	4
C _{16:0}	1.7	2.1	1.2	1.5
iso-C _{15:0}	36.7	44.8	43.5	36.9
iso-C _{15:1}	8.4	19.8	15.1	18.1
iso-C _{15:0} 3-OH	2.9	1.8	4.5	2.6
C _{15:0} 2-OH	1.3	0.3	0.8	1.4
C _{15:0} 3-OH	1.1	0.1	0.4	1.1
C _{16:0} 3-OH	4.3	2.1	2.3	2.8
C _{17:0} 3-OH	0.7	0.2	0.4	1.1
iso-C _{17:0} 3-OH	12.4	14.2	15.3	13.1
Summed feature 3*	25.1	9.0	11.8	16.0

*Summed features are fatty acids that cannot be separated by GC with the Microbial Identification System (Microbial ID) software. Summed feature 3 comprised C_{16:1}ω6c and/or C_{16:1}ω7c.

(comprising C_{16:1}ω7c and/or C_{16:1}ω6c). Respiratory quinones were extracted from lyophilized cells and the extracts were purified and analysed by HPLC according to the method of Collins (1985). The predominant respiratory quinone of strain M-8^T was menaquinone-7 (MK-7), which is consistent with members of the genus *Terrimonas*. The DNA G+C content was determined by reversed-phase HPLC according to Mesbah *et al.* (1989). The DNA G+C content of strain M-8^T was 47.0 mol%, consistent with data for recognized *Terrimonas* species (47.0–48.9 mol%).

Phylogenetic analysis and chemotaxonomic characteristics (major fatty acids, predominant respiratory quinone, DNA G+C content) unequivocally supported placement of strain M-8^T within the genus *Terrimonas*. However, differences in phenotypic characteristics (Tables 1 and S1) between strain M-8^T and the type strains of the three species of the genus *Terrimonas* suggested that strain M-8^T cannot be assigned to any recognized species of this genus. Furthermore, strain M-8^T could be differentiated from recognized *Terrimonas* species based on differences in fatty acid composition (Table 2). Morphological, physiological and chemotaxonomic characteristics together with DNA–DNA hybridization results thus supported the suggestion that strain M-8^T represents a novel species of the genus *Terrimonas*, for which the name *Terrimonas rubra* sp. nov. is proposed.

In the present study, levels of iso-C_{15:1} G in strain M-8^T (8.4%) and summed feature 3 in *T. ferruginea* KACC 11310^T (9.0%) were lower than 10%, and thus should not be listed as major fatty acid components for the genus *Terrimonas*. Gliding ability was observed for strain M-8^T, but not for *T. ferruginea*, *T. aquatica* or *T. lutea*. Strain M-8^T was strictly positive for catalase activity. Additionally,

growth of strain M-8^T was observed in the presence of 2% (w/v) NaCl, and nitrate was not reduced; characteristics not previously described for the genus *Terrimonas*. Therefore, an emended description of the genus *Terrimonas* is proposed.

Emended description of the genus *Terrimonas*

Cells are strictly aerobic, Gram-stain-negative, non-motile, non-filamentous single rods. Catalase activity is positive (or weakly positive) and cells of some species are negative in tests for oxidase activity. Growth is moderately sensitive to NaCl [$<2\%$ (w/v)]. Positive result in tests for gelatin liquefaction, but negative result for urease activity and nitrate reduction. Major cellular fatty acids are iso-C_{15:0} and iso-C_{17:0} 3-OH. The predominant respiratory quinone is MK-7, with MK-6 as a minor component. The DNA G+C content is 47.0–48.9 mol%. The type species is *Terrimonas ferruginea*.

Description of *Terrimonas rubra* sp. nov.

Terrimonas rubra (ru'bra. L. fem. adj. *rubra* red, describing the colour of the cells).

Shows the following characteristics in addition to those given in the genus description above. Cells are non-spore-forming, rod-shaped (0.4–0.5 µm wide and 1.0–2.5 µm long) and lack flagella. Flexirubin-type pigments are present. Whole cells are covered by fimbriae (Fig. 2). Colonies are salmon-red, circular (1.0–2.0 mm in diameter) with entire edges and convex. Growth occurs at 20–37 °C (optimum, 30 °C), at pH 5.0–10.0 (optimum,

Fig. 2. Transmission electron micrograph of a negatively stained cell of strain M-8^T grown on R2A agar for 24 h. Bar, 0.5 µm.

pH 7.0) and with 0–2% (w/v) NaCl (optimum, 0%). Positive for catalase activity and negative for oxidase activities. Positive for activities of β -glucosidase and β -galactosidase, but negative result for arginine dihydrolase and urease. Hydrolyses gelatin. Negative result in tests for nitrate reduction, indole production and glucose acidification. Assimilates arabinose, glucose, mannose, maltose and *N*-acetylglucosamine, but not adipate, caprate, citrate, gluconate, phenyl-acetate, mannitol or malate (API 20NE tests). Positive for activities of alkaline phosphatase, C4 esterase, C8 esterase lipase, C14 lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, α -glucosidase, *N*-acetyl- β -glucosaminidase and β -fucosidase, but negative result for β -galactosidase, β -glucuronidase, β -glucosidase and α -mannosidase activity (API ZYM tests). The following compounds are utilized as sole carbon sources in the GN2 microplate: α -cyclodextrin, dextrin, glycogen, Tweens 40 and 80, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, α -D-glucose, maltose, melibiose, turanose, methyl pyruvate, acetic acid, D-galacturonic acid, β -hydroxybutyric acid, DL-lactic acid, propionic acid, succinic acid and glycyl-L-glutamic acid. The other substrates in the GN2 microplate are not utilized. Susceptible to chloramphenicol, rifampicin, vancomycin, novobiocin, tetracycline, roxithromycin and nalidixic acid, but resistant to gentamicin, streptomycin, kanamycin, ampicillin, spectinomycin, penicillin G and trimethoprim. The major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1} ω 7c and/or C_{16:1} ω 6c). The predominant respiratory quinone is MK-7.

The type strain, M-8^T (=CCTCC AB 2010401^T=KCTC 23299^T), was isolated from a polluted farmland soil sample in Shandong Province, China. The DNA G+C content of the type strain is 47.0 mol% (HPLC).

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