Prolixibacter denitrificans sp. nov., an iron-corroding, facultatively aerobic, nitrate-reducing bacterium isolated from crude oil, and emended descriptions of the genus Prolixibacter and Prolixibacter bellariivorans

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Metallic iron (Fe⁰) and stainless steel corrosion causes a severe economic burden. Under aerobic conditions, molecular oxygen-mediated corrosion may be predominant, whereas under anaerobic conditions such as oil field, including oil reservoirs and pipelines, microbiologically influenced corrosion (MIC) is believed to be a major cause of corrosion-related failures (Javaherdashti, 2008). In our previous study (Iino et al., 2015), we isolated a novel non-hydrogenotrophic, Fe⁰-corroding, nitrate-reducing bacterium (NRB) designated MIC1-1T from a crude-oil sample collected at an oil well in Akita Prefecture, Japan on 16 December 2004. Under anaerobic conditions, this strain corroded Fe⁰ concomitantly with nitrate reduction, and microscopic crystals of FePO₄ and a layer of FeCO₃ developed on the surface of the Fe⁰ foils. The oxidation to ferric ion by MIC under anaerobic conditions is unusual because ferrous compounds have generally been detected as corrosion products in sulfate-reducing bacteria (SRB)-and methanogen-mediated MIC (Dinh et al., 2004; Uchiyama et al., 2010). NRB-assisted Fe⁰ corrosion has been reported mainly in association with the remediation of nitrate-contaminated groundwater using granular iron (Fe⁰)- and a hydrogenotrophic NRB such as Paracoccus denitrificans ATCC 17741T or an NRB-containing microbial consortium (De Windt et al., 2003; Ginner et al., 2004; Kielmoees et al., 2000; Till et al., 1998; Fan et al., 2009; Xu et al., 2013). Unlike these NRBs, strain MIC1-1T is not hydrogenophilic.

Phylogenetically, strain MIC1-1T is a member of the genus Prolixibacter in the family Prolixibacteraceae of the order Bacteroidales with 97.5 % sequence similarity to Prolixibacter bellariivorans JCM 13498T, which represents the single species of the genus (Holmes et al., 2007; Iino et al., 2015). To date, methanogens from the phylum Euryarchaeota, SRB and iron-oxidizing bacteria from the phylum Proteobacteria are most frequently associated with MIC (Dinh et al., 2004; Enning et al., 2012; McBeth et al., 2011; Uchiyama et al., 2010; Venzlaff et al., 2013). To our knowledge, Prolixibacter sp. strain MIC1-1T is the first Fe⁰-corroding representative from the phylum Bacteroidetes. This paper describes characterization of the non-hydrogenotrophic, Fe⁰-corroding, nitrate-reducing Prolixibacter sp. strain MIC1-1T and shows that this strain represents a novel species of the genus Prolixibacter. In addition, we also provide emended descriptions of the genus Prolixibacter and Prolixibacter bellariivorans according to our study.

Prolixibacter sp. strain MIC1-1T was maintained in HXSw medium supplemented with 0.2 % (w/v) yeast extract.

Abbreviations: MIC, microbiologically influenced corrosion; NRB, nitrate-reducing bacteria; PE, phosphatidylethanolamine; SRB, sulfate-reducing bacteria

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MIC1-1T is AB986195.
The culture of strain MIC1-1T in liquid medium was salmon pink, and stained Gram-negative by conventional Gram staining. Strain MIC1-1T was a facultatively aerobic bacterium that grew better under anaerobic conditions comprising N2/CO2 (4:1, v/v) atmosphere than aerobic conditions on air. Catalase and oxidase reactions were negative. The growth temperature for strain MIC1-1T ranged from 15 to 40 °C, with an optimum of 35–37 °C. No growth was observed at 10 °C or 45 °C. The pH range for growth was pH 4.5–8.0, with optimum at pH 6.5. No growth was observed at pH 4.0 or pH 8.5. Growth occurred in medium supplemented with 1–13 % (w/v) NaCl, with an optimum of 2 % (w/v) NaCl. No growth was observed without the addition of NaCl or with 14 % (w/v) NaCl. Strain MIC1-1T and Prolixibacter bellariivorans JCM 13498T grew fermentatively on L-arabinose, D-xylene, D-fructose, D-glucose, D-mannose, cellobiose, lactose, maltose, sucrose, trehalose (all at 10 mM) and soluble starch (0.2 %, w/v). No growth occurred on D-ribose, D-sorbose, D-mannitol, D-sorbitol (all at 10 mM) or CM-cellulose (0.2 %, w/v). Strain MIC1-1T produced succinic acid (93.3 %) as the major end-product from D-glucose fermentation; acetic acid (6.7 %) was also produced as a minor end-product. Strain MIC1-1T and Prolixibacter bellariivorans JCM 13498T were susceptible to ampicillin, chloramphenicol, rifampicin, tetracycline and vancomycin (all at 100 μg ml−1), but resistant to bacitracin, gentamicin, kanamycin and streptomycin (all at 100 μg ml−1). Prolixibacter bellariivorans JCM 13498T grew using oxygen (5 %, w/v) as the electron acceptor in the presence of L-cysteine (2 mM) as the electron donor. Sulfate (10 mM), sulfite (2 mM), thiosulfate (5 mM), elemental sulfur (1 %, w/v), nitrate (10 mM), nitrite (2 mM) and iron (III) oxide (2 mM) were not utilized as alternative electron acceptors in the presence of L-cysteine. Moreover, H2/CO2 (4:1, v/v), sulfide (2 mM), elemental sulfur (1 %, w/v), thiosulfate (5 mM), sulfite (2 mM), ammonium (10 mM) and nitrite (2 mM) were not utilized as alternative electron donors in the presence of oxygen (5 %, w/v). The utilization of iron (Fe3+) granule and ferrous chloride could not be determined accurately because oxygen oxidized them chemically.

The major isoprenoid quinone of strain MIC1-1T and Prolixibacter bellariivorans JCM 13498T was menaquinone-7 (MK-7), determined by the HPLC method described by Komagata & Suzuki (1987). The polar lipid pattern of strain MIC1-1T and Prolixibacter bellariivorans JCM 13498T mainly comprised phosphatidylethanolamine (PE), one or two unknown phospholipids and an unknown lipid, as determined by using two-dimensional TLC and spraying with 5 % ethanolic molybdophosphoric acid, ninhydrin, Dittmer & Lester reagent, anisaldehyde reagent and Dragendorff’s reagent, described by Lechevalier et al. (1977) and Minnikin et al. (1984). The major cellular fatty acids of strain MIC1-1T were iso-C15:0 (49.8 %) and anteiso-C15:0 (12.7 %), as determined by the MIDI Microbial Identification System version 6 (Microbial ID; Agilent Technologies) based on the method described by Sasser (1990). iso-C16:0 (5.7 %) was also detected as a minor component. The genomic DNA G + C content of strain MIC1-1T was 45.0 mol%, determined by the HPLC method described by Tamaoka and Komagata (1984).
was higher than that of *Prolixibacter bellariivorans* F2T, and the NaCl range for growth of strain MIC1-1T was broader than that of *Prolixibacter bellariivorans* F2T. In addition, strain MIC1-1T reduced nitrate to nitrite, but the type strain of *Prolixibacter bellariivorans* did not.

For phylogenetic analysis, according to the method previously described (Iino *et al.*, 2010), the 16S rRNA gene sequences of 10 phylogenetically related bacteria in the family *Prolixibacteraceae* were selected after alignment with the ARB software (Ludwig *et al.*, 2004). Phylogenetic trees were reconstructed by the neighbour-joining method with CLUSTAL X software (Saitou & Nei, 1987; Thompson *et al.*, 1997) and the maximum-likelihood method with MORPHY version 2.3b3 (Felsenstein, 1981; Hasegawa & Fujiwara, 1993). The topologies of the trees generated by these two methods were identical. In the phylogenetic tree based on 16S rRNA gene sequences (Fig. 1), strain MIC1-1T formed a monophyletic cluster together with *Prolixibacter bellariivorans* JCM 13498T, and this cluster was apparently distinct from other genera of the family *Prolixibacteraceae* with high bootstrap values (99–100 %). The 16S rRNA gene sequence similarity of strain MIC1-1T with *Prolixibacter bellariivorans* JCM 13498T was 97.5 %. This observed similarity is lower than the cut-off value recommended for species differentiation (98.7–99.0 %; Stackebrandt & Ebers, 2006).

On the basis of the distinct phylogenetic position, morphology, and biochemical and physiological properties described above, strain MIC1-1T is a member of the genus *Prolixibacter* separate from *Prolixibacter bellariivorans*. Consequently, a novel species with the name *Prolixibacter denitrificans* sp. nov. is proposed for strain MIC1-1T. In addition, emended descriptions of the genus *Prolixibacter* and *Prolixibacter bellariivorans* are also proposed based on data obtained in this study.

**Emended description of the genus *Prolixibacter* Holmes *et al.* 2007**

Cells are single rods that are non-sporulating, non-motile, do not contain pili or flagella, are Gram-stain-negative, and are facultatively anaerobic to facultatively aerobic, mesophilic, neutrophilic, moderately halophilic and chemoorganoheterotrophic. Cells do not contain c-type cytochromes and are catalase- and oxidase-negative. The major respiratory quinone is MK-7. The major polar lipids are PE, unknown phospholipids and an unknown lipid. The major cellular fatty acids are iso-C<sub>15:0</sub> and anteiso-C<sub>15:0</sub>.

The type species is *Prolixibacter bellariivorans*.

**Emended description of *Prolixibacter bellariivorans* Holmes *et al.* 2007**

The following properties are additional to those given by Holmes *et al.* (2007) in addition to the genus description. Sulfite, elemental sulfur and nitrite are not used as alternative electron acceptors. L-Cysteine is used as an alternative electron donor. H<sub>2</sub>/CO<sub>2</sub> (4:1, v/v), sulfide, elemental

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**Fig. 1.** Phylogenetic tree of strain MIC1-1T and representatives of related species based on 16S rRNA gene sequences. The tree was inferred from an alignment of 1386 bp of 16S rRNA gene sequences and reconstructed by the neighbour-joining method. Numbers at nodes are bootstrap percentages derived from 1000 replications (neighbour-joining maximum-likelihood). Bar, 0.01 substitutions per nucleotide position.
sulfur, thiosulfate, sulfate, ammonium and nitrite are not used as alternative electron donors.

**Description of Prolixibacter denitrificans** sp. nov.

Prolixibacter denitrificans (de.ni.tri‘fi.cans. N.L. v. denitrifico to denitrify; N.L. part. adj. denitrificans denitrifying).

The following properties are given in addition to the genus description. Cells are 0.3–0.5 × 3.4–6.3 μm in size and the cell appearance is salmon pink. Growth occurs at 15–40 °C with optimum growth at 35–37 °C. The pH range for growth is 4.5–8.0 with an optimum around pH 6.5. The NaCl range for growth is 1–13% (w/v), with an optimum of 2% (w/v) NaCl. Nitrate and oxygen are used as alternative electron acceptors. Nitrite is the end-product of nitrate reduction. Sulfate, sulfate, thiosulfate, elemental sulfur, nitrite and iron (III) oxide are not used as alternative electron donors. Fermentative growth occurs on L-arabinose, D-xylose, D-fructose, D-glucose, D-mannose, cellobiose, lactose, maltose, sucrose, trehalose and soluble starch. Growth does not occur on D-ribose, D-sorbitose, D-mannitol and D-sorbitol or CM-cellulose. Succinic acid is the major end-product from D-glucose fermentation.

The type strain is MIC1-1^T (=JCM 18694^T=NBRC 102688^T=DSM 27267^T), which was isolated from a marine-sediment fuel cell. The G+C content of genomic DNA of the type strain is 45.0 mol% (determined by HPLC).

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**References**


