Corynebacterium humireducens sp. nov., an alkaliphilic, humic acid-reducing bacterium isolated from a microbial fuel cell

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A novel halotolerant, alkaliphilic, humic acid-reducing bacterium, designated MFC-5^T, was isolated from a microbial fuel cell that was fed continuously with artificial wastewater (pH 10.0). Cells were Gram-positive-staining, facultatively anaerobic, non-fermentative, non-motile rods and had a G+C content of 59.0 mol%. Microbial growth was observed with <13% (w/v) NaCl (optimum 10%), at pH 7.0–11.0 (optimum pH 9.0) and at 25–45 °C (optimum 37 °C). Strain MFC-5^T was active in the anaerobic reduction of a humic acid analogue, anthraquinone-2,6-disulphonate, with lactate, formate, acetate, ethanol or sucrose as the electron donor. The major cellular fatty acids were C_{18:1ω9c} (42.68%), C_{16:0} (33.69%), C_{18:0} (7.56%), C_{17:1ω8c} (5.14%) and C_{17:0} (3.39%). Phylogenetic analysis demonstrated that strain MFC-5^T displayed >3% 16S rRNA gene sequence divergence from its closest relatives. Based on phenotypic, genetic and phylogenetic analysis, a novel species, *Corynebacterium humireducens* sp. nov., is proposed. The type strain is MFC-5^T (=NBRC 106098^T =CGMCC 2452^T =DSM 45392^T).

Microbe-mediated humic substance reduction has been widely recognized, and quinone moieties have been shown to be the redox-active compounds in humic substances (Lovley *et al.*, 1996; Scott *et al.*, 1998; Straub *et al.*, 2005). Humic substance reduction may play a significant role in the anaerobic biotransformation of both organic and inorganic compounds. A wide variety of organic compounds, including priority pollutants, can be mineralized by micro-organisms under anaerobic conditions with humic substances serving as the terminal electron acceptor (Bradley *et al.*, 1998; Cervantes *et al.*, 2000, 2001; Finneran & Lovley, 2001). In addition, humic substances, once reduced, can shuttle electrons to insoluble electron acceptors (e.g. ferric oxides) and several distinct contaminants (e.g. azo dyes, chlorinated organic contaminants) by acting as a redox mediator (Bond & Lovley, 2002; Kappler *et al.*, 2004; Hong *et al.*, 2007; Wang *et al.*, 2009). Wang *et al.* (2009) found that the reduction rate of 2,4-dichlorophenoxyacetic acid (2,4-D) was increased significantly

by the addition of quinone, which was reduced microbially to the corresponding hydroquinone, which could then reduce 2,4-D chemically.

A large number of humic acid-reducing micro-organisms have been isolated from a broad range of environments (Field & Cervantes, 2005) and the humic acid reductions mediated by these micro-organisms are commonly studied at circumneutral pH. To the best of our knowledge, no studies have reported humic acid reduction under alkaline conditions or by an alkaliphilic humic acid-reducing bacterium. In this study, we successfully isolated a new alkaliphilic bacterium, strain MFC-5^T, from an alkaline microbial fuel cell and identified its ability to reduce humic acids (quinone) under alkaline conditions.

Strain MFC-5^T was isolated from the anode of a wastewater-fed microbial fuel cell in our laboratory that was continuously operated at pH 10.0. A piece of anode (0.5 cm³) was transferred to a 25.2 ml sterile bottle containing 20 ml sterilized mineral salts medium [MSM, containing (per litre deionized water) 0.6 g NaH₂PO₄, 0.25 g NH₄Cl, 0.1 g KCl, 0.2 g yeast extract, 10.0 ml vitamin stock solution and 10.0 ml mineral stock solution (Zachara *et al.*, 1998); pH 10.0] supplemented with 0.5 mM anthraquinone-2,6-disulphonate (AQDS; electron acceptor), 5 mM sodium lactate (electron donor) and

Abbreviations: AH₂DS, reduced anthraquinone-2,6-disulphonate; AQDS, anthraquinone-2,6-disulphonate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MFC-5^T is GQ421281.

Three supplementary figures are available with the online version of this paper.

10 mM carbonate buffer. The bottle was purged with O₂-free N₂/CO₂ (80:20, v/v) for 15 min and sealed with a butyl-rubber stopper and an aluminium cap. After 10 days of incubation, the colour of the contents of the bottle changed to red and the enriched population was serially diluted and plated onto MSM agar (pH 10.0) supplemented with (l⁻¹) 18 g agar, 1 mmol AQDS and 5 mmol sodium lactate. The plates were incubated in an anaerobic chamber (ShellLab; Sheldon Manufacturing) with N₂/CO₂ (80:20, v/v). Strain MFC-5^T formed red colonies owing to its ability to reduce AQDS.

To identify morphological features, strain MFC-5^T was cultivated aerobically on LB agar overnight at 30 °C. Cell morphology and dimensions were determined by scanning electron microscopy (ESEM-30; Philips). Cell motility was tested by the hanging-drop method. Physiological characteristics were determined by standard methods (Buchanan & Gibbons, 1974). Salt tolerance was determined in MSM supplemented with 0.5 mM AQDS and 5 mM sodium lactate and on LB agar supplemented with 0–15% (w/v) NaCl. The isolate was characterized biochemically using the API Coryne and API ZYM systems (bioMérieux), according to the manufacturer's instructions. Cellular fatty acids were extracted from dried cells and methylated and analysed as described by Kämpfer & Kroppenstedt (1996). Extraction of genomic DNA was performed according to Chun & Goodfellow (1995). The

G+C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989).

The 16S rRNA gene of the isolate was amplified by PCR using conserved primers that bind close to the 3' and 5' ends of the gene, as described by Finneran *et al.* (2003). The purified PCR product was sequenced using an automated sequencer (ABI 3730; Applied Biosystems). The sequence was aligned with related 16S rRNA gene sequences from GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Ribosomal Database Project (<http://rdp.cme.msu.edu/seqmatch>) using CLUSTAL X (Thompson *et al.*, 1997) and gaps at the 5' and 3' ends were removed. Phylogenetic trees were constructed with MEGA version 4.0 using the maximum-parsimony and neighbour-joining methods (Kumar *et al.*, 2004) according to Kimura's two-parameter model and bootstrap analyses were based on 1000 resamplings (Kimura, 1980; Saitou & Nei, 1987). 16S rRNA gene sequence similarities between strain MFC-5^T and its closest known relatives were calculated using the EzTaxon server.

To investigate AQDS reduction by strain MFC-5^T, batch experiments were conducted in a 25.2 ml serum bottle using cells grown aerobically in LB medium to late-exponential phase, harvested by centrifugation (8000 g at 4 °C for 10 min), washed twice and resuspended in sterilized MSM. To determine the pH range for AQDS reduction, 20 ml MSM (containing 1 × 10⁷ cells ml⁻¹,

Table 1. Characteristics that can be used to differentiate strain MFC-5^T from alkaliphilic and halotolerant corynebacteria

Strains: 1, *Corynebacterium humireducens* sp. nov. MFC-5^T; 2, *C. halotolerans* YIM 70093^T (data from Chen *et al.*, 2004); 3, *C. marinum* D7015^T (Du *et al.*, 2010); 4, *C. maris* Coryn-1^T (Ben-Dov *et al.*, 2009); 5, *C. matruchotii* (n=6; Barrett *et al.*, 2001); 6, *C. testudinoris* M935/96/4^T (Collins *et al.*, 2001); 7, *C. urealyticum* (n=6; Pitcher *et al.*, 1992). All strains were negative for aesculin hydrolysis, β-galactosidase and N-acetyl-β-glucosaminidase (no data available for *C. urealyticum*) and acid production from mannitol, xylose and lactose. ND, No data available.

Characteristic	1	2	3	4	5	6	7
Nitrate reduction	–	+	+	–	+	–	–
Hydrolysis of:							
Urea	–	+	–	ND	–	–	+
Gelatin	–	+	–	+	–	–	ND
Starch	–	–	+	ND	ND	ND	ND
Acid production from:							
Glucose	+	+	+	–	+	+	–
Maltose	–	–	+	–	+	+	–
Sucrose	–	–	+	–	+	+	–
Ribose	+	–	–	–	–	+	–
Enzyme activity							
Alkaline phosphatase	+	+	–	+	+	–	ND
Esterase	+	–	–	+	ND	–	ND
Esterase lipase	+	–	+	+	ND	–	ND
Lipase	+	+	–	+	ND	–	ND
Leucine arylamidase	–	–	+	+	–	+	ND
β-Glucuronidase	–	+	+	+	–	–	ND
α-Glucosidase	–	–	–	+	–	+	ND
Pyrazinamidase	+	–	+	+	+	+	ND
Pyrrolidonyl arylamidase	+	–	–	+	+	+	ND

5 mM lactate and 1 mM AQDS) was adjusted to pH 6.0–11.0 (at intervals of 1 pH unit). The pH was maintained using 20 mM phosphate buffer (for pH 6.0–8.0) (Pham *et al.*, 2003) or carbonate buffer (pH 9.0–11.0) (Ye *et al.*, 2004). To determine alternative electron donors for AQDS reduction, each bottle contained 1×10^7 cells ml^{-1} , 20 ml MSM with 1 mM AQDS and one of the following substrates (5 mM): formate, acetate, propionate, lactate, citrate, ethanol, glycerol and sucrose. For the treatment using lactate, cell numbers were determined by direct colony counts on LB agar. Strict anaerobic and sterile techniques were used throughout the reduction experiments, as described by Li *et al.* (2009). All treatments were tested in triplicate in the dark at 30 °C with two control assays: an abiotic set without cells and a biotic set without electron donor or acceptor. To determine the quantities of reduced AQDS (AH₂DS), triplicate bottles were sacrificed every 2 days, the samples were filter-sterilized (PVDF, 0.22 µm; Millipore) and AH₂DS was quantified using a UV-Vis spectrophotometer (TU1800-PC; PGeneral) at a wavelength of 408 nm (Liu *et al.*, 2007).

Vegetative cells of strain MFC-5^T were straight to curved rods, 1.0–2.0 µm long and 0.5–0.7 µm wide (Supplementary Fig. S1, available in IJSEM Online). Cells occurred singly or in pairs, were non-motile and stained Gram-positive during exponential and stationary growth. After 24 h on LB agar, colonies were uniformly round, 0.5–1.0 mm in diameter, yellowish, dry and rough with a distinct wrinkled morphology.

The temperature for growth of strain MFC-5^T was 25–40 °C (optimum 37 °C); no growth was observed at 45 or below 10 °C. Growth was observed at pH 7.0–11.0 (optimum pH 9.0) and with 0–12 % NaCl, but not with 13 % NaCl. The isolate was catalase-positive and oxidase- and methyl red-negative. With the API systems, strain MFC-5^T produced acid from glucose and ribose, but not from maltose, sucrose, mannose, xylose or lactose, and was positive for alkaline phosphatase, esterase, esterase lipase, lipase, pyrazinamidase and pyrrolidonyl arylamidase, but not for leucine arylamidase, *N*-acetyl-β-glucosaminidase, β-glucuronidase or β-galactosidase. In terms of overall phenotypic characteristics, the isolate resembled corynebacteria. Comparing the biochemical characteristics of strain MFC-5^T with representatives of alkaliphilic and halotolerant species of the genus *Corynebacterium* (Table 1) suggested that the isolate represented a novel species of the genus *Corynebacterium*.

Fig. 1(a) shows the reduction of AQDS by strain MFC-5^T using lactate as the electron donor at different pH. After 10 days, the AH₂DS concentration at pH 7, 8, 9, 10 and 11 was 0.23, 0.14, 0.48, 0.31 and 0.16 mM, respectively. Reduction of AQDS at pH 6.0 was negligible. In the abiotic control, <0.07 mM AH₂DS was formed (data not shown). Thus, the pH range for AQDS reduction by strain MFC-5^T was pH 7.0–11.0 and the optimum pH was pH 9.0. To the best of our knowledge, the reduction of humic acids and

quinone by alkaliphilic bacteria has not been reported, and the identification of strain MFC-5^T raises the upper pH limit for microbial quinone reduction. As shown in Fig. 1(b), AH₂DS was produced with formate, acetate, lactate, ethanol and sucrose as electron donors, but not with citrate, propionate or glycerol (not shown). The electron donors could be ranked according to the final concentration of AH₂DS, in descending order, as sucrose, acetate, lactate, ethanol and formate. In the abiotic control, almost no AH₂DS was detected (not shown). As shown in Fig. 1(c), significant microbial growth and AQDS reduction were observed with lactate as the electron donor over 10 days. Cell density in the active test approached a maximum of 13.7×10^7 cells ml^{-1} , which was approximately 14 times greater than the initial density. Data showed that AQDS reduction and the increase in cell numbers occurred at the same time throughout the

Fig. 1. Characteristics of AQDS reduction by strain MFC-5^T over 10 days. (a) Production of AH₂DS with lactate as electron donor at pH 6.0–11.0. (b) Production of AH₂DS with various electron donors (5 mM l⁻¹) at pH 9.0. (c) Cell growth with AQDS and lactate as electron donor at pH 9.0. Experiments were performed in MSM medium under anaerobic conditions at 30 °C with 1×10^7 cells ml^{-1} initial cell density. Error bars represent standard deviations ($n=3$).

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain MFC-5^T among its phylogenetic neighbours. Bootstrap values (>50%) based on 1000 resamplings are shown at branch nodes. Bar, 0.5% sequence divergence.

incubation. Cell growth was negligible in controls without AQDS or lactate, which indicated that strain MFC-5^T could not ferment lactate and only grew in the presence of both AQDS and lactate. As far as is known, this is the first report of a member of the genus *Corynebacterium* that can reduce extracellular quinones and harness energy for microbial growth by quinone reduction.

Examination of the long-chain cellular fatty acids of strain MFC-5^T showed that the non-hydroxylated long-chain cellular fatty acids were primarily straight-chain saturated and mono-unsaturated. The major acids were C_{14:0} (1.55%), C_{16:0} (33.69%), C_{17:0} (3.39%), C_{18:0} (7.56%), C_{17:1ω8c} (5.14%), C_{18:1ω9c} (42.68%) and C_{18:1ω7c} (1.71%), which were consistent with the genus *Corynebacterium* (Fernández-Garayzábal *et al.*, 2004). The DNA G+C content of strain MFC-5^T was 59.0 mol%, which fell within the range for the genus *Corynebacterium* (52.0–68.0 mol%). The presence of straight-chain saturated and mono-unsaturated fatty acids and the G+C content, along with the morphological and biochemical properties, strongly suggested that strain MFC-5^T was a member of the genus *Corynebacterium*.

A nearly complete 16S rRNA gene sequence (1401 nt) was obtained for strain MFC-5^T. The closest known relatives of strain MFC-5^T were *Corynebacterium marinum* D7015^T (96.9% 16S rRNA gene sequence similarity, 1393 bp considered), *C. testudinoris* CCUG 41823^T (96.8%, 1395 bp), *C. halotolerans* YIM 70093^T (96.4%, 1392 bp), *C. felinum* CCUG 39943^T (96.0%, 1383 bp), *C. singulare* CCUG 37330^T (96.0%, 1297 bp) and *C. freiburgense* 1045^T (95.9%, 1395 bp). A sequence alignment was created with sequences from the type strains of 98 species of the genus *Corynebacterium* and the results of phylogenetic analysis are shown in Supplementary Fig. S2. The neighbour-joining and

maximum-parsimony trees depicting the phylogenetic relationships between strain MFC-5^T and its nearest phylogenetic neighbours (95.3–96.9% 16S rRNA gene sequence similarity) are shown in Fig. 2 and Supplementary Fig. S3, respectively. The results suggested that strain MFC-5^T belongs to the genus *Corynebacterium*.

It is widely accepted that ≥3% 16S rRNA gene sequence divergence between bacterial strains indicates that they represent different genomic species (Stackebrandt & Goebel, 1994). Therefore, on the basis of phenotypic, genotypic and phylogenetic properties, strain MFC-5^T should be classified in a novel species of the genus *Corynebacterium*, for which the name *Corynebacterium humireducens* sp. nov. is proposed.

Description of *Corynebacterium humireducens* sp. nov.

Corynebacterium humireducens [hu.mi.re.du'cens. L. n. *humus* soil, used to refer to humic substances; L. part. adj. *reducens* leading back (to a reduced oxidation state); N.L. part. adj. *humireducens* converting humic substances to a reduced oxidation state].

Cells are Gram-positive-staining, non-motile, facultatively anaerobic, straight to curved rods (1.0–2.0 μm long and 0.5–0.7 μm wide). Colonies on LB agar are uniformly round, 0.5–1.0 mm in diameter, yellowish, dry and rough, with a distinct wrinkled morphology after 24 h. Optimum growth occurs at 37 °C, at pH 9.0 and with 10% NaCl (aerobic conditions). Positive for catalase but negative for oxidase, urease, reduction of nitrate and hydrolysis of urea, gelatin and tyrosine. Produces acid from glucose and ribose, but not from arabinose, fructose, galactose, lactose, maltose, mannitol, mannose, raffinose, rhamnose, salicin, sucrose, trehalose or xylose. Positive for alkaline phosphatase.

tase, esterase, esterase lipase, lipase, pyrazinamidase and pyrrolidonyl arylamidase, but not for leucine arylamidase, *N*-acetyl- β -glucosaminidase, β -glucuronidase or β -galactosidase. Grows with AQDS as the sole terminal electron acceptor at pH 7.0–11.0 (optimum pH 9.0) under anaerobic conditions. Available electron donors include formate, acetate, ethanol, lactate and sucrose, but not propionate, glycerol or citrate. The major cellular fatty acids (>5%) are C_{18:1} ω 9c, C_{16:0}, C_{18:0}, C_{17:1} ω 8c and C_{17:0}. The DNA G+C content of the type strain is 59.0 mol%.

The type strain, MFC-5^T (=NBRC 106098^T =CGMCC 2452^T =DSM 45392^T), was isolated from a microbial fuel cell fed with artificial wastewater and operated continuously at pH 10.0.

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References

- Barrett, S. L., Cookson, B. T., Carlson, L. C., Bernard, K. A. & Coyle, M. B. (2001). Diversity within reference strains of *Corynebacterium matruchotii* includes *Corynebacterium durum* and a novel organism. *J Clin Microbiol* **39**, 943–948.
- Ben-Dov, E., Ben Yosef, D. Z., Pavlov, V. & Kushmaro, A. (2009). *Corynebacterium maris* sp. nov., a marine bacterium isolated from the mucus of the coral *Fungia granulosa*. *Int J Syst Evol Microbiol* **59**, 2458–2463.
- Bond, D. R. & Lovley, D. R. (2002). Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinones. *Environ Microbiol* **4**, 115–124.
- Bradley, P. M., Chapelle, F. H. & Lovley, D. R. (1998). Humic acids as electron acceptors for anaerobic microbial oxidation of vinyl chloride and dichloroethene. *Appl Environ Microbiol* **64**, 3102–3105.
- Buchanan, R. E. & Gibbons, N. E. (editors) (1974). *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore: Williams & Wilkins.
- Cervantes, F. J., van der Velde, S., Lettinga, G. & Field, J. A. (2000). Quinones as terminal electron acceptors for anaerobic microbial oxidation of phenolic compounds. *Biodegradation* **11**, 313–321.
- Cervantes, F. J., Dijkstra, W., Duong-Dac, T., Ivanova, A., Lettinga, G. & Field, J. A. (2001). Anaerobic mineralization of toluene by enriched sediments with quinones and humus as terminal electron acceptors. *Appl Environ Microbiol* **67**, 4471–4478.
- Chen, H.-H., Li, W.-J., Tang, S.-K., Kroppenstedt, R. M., Stackebrandt, E., Xu, L.-H. & Jiang, C.-L. (2004). *Corynebacterium halotolerans* sp. nov., isolated from saline soil in the west of China. *Int J Syst Evol Microbiol* **54**, 779–782.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Collins, M. D., Hoyles, L., Hutson, R. A., Foster, G. & Falsen, E. (2001). *Corynebacterium testudinoris* sp. nov., from a tortoise, and *Corynebacterium felinum* sp. nov., from a Scottish wild cat. *Int J Syst Evol Microbiol* **51**, 1349–1352.
- Du, Z.-J., Jordan, E. M., Rooney, A. P., Chen, G.-J. & Austin, B. (2010). *Corynebacterium marinum* sp. nov. isolated from coastal sediment. *Int J Syst Evol Microbiol* **60**, 1944–1947.
- Fernández-Garayzábal, J. F., Vela, A. I., Egido, R., Hutson, R. A., Lanzarot, M. P., Fernández-García, M. & Collins, M. D. (2004). *Corynebacterium ciconiae* sp. nov., isolated from the trachea of black storks (*Ciconia nigra*). *Int J Syst Evol Microbiol* **54**, 2191–2195.
- Field, J. A. & Cervantes, F. J. (2005). Microbial redox reactions mediated by humus and structurally related quinones. In *Use of Humic Substances to Remediate Polluted Environments: from Theory to Practice*, pp. 343–352. Edited by I. V. Perminova, K. Hatfield & N. Hertkorn. Dordrecht: Springer.
- Finneran, K. T. & Lovley, D. R. (2001). Anaerobic degradation of methyl *tert*-butyl ether (MTBE) and *tert*-butyl alcohol (TBA). *Environ Sci Technol* **35**, 1785–1790.
- Finneran, K. T., Johnsen, C. V. & Lovley, D. R. (2003). *Rhodoferrax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). *Int J Syst Evol Microbiol* **53**, 669–673.
- Hong, Y. G., Guo, J., Xu, Z. C., Xu, M. Y. & Sun, G. P. (2007). Humic substances act as electron acceptor and redox mediator for microbial dissimilatory azoreduction by *Shewanella decolorationis* S12. *J Microbiol Biotechnol* **17**, 428–437.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kappler, A., Benz, M., Schink, B. & Brune, A. (2004). Electron shuttling via humic acids in microbial iron(III) reduction in a freshwater sediment. *FEMS Microbiol Ecol* **47**, 85–92.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Li, X. M., Zhou, S. G., Li, F. B., Wu, C. Y., Zhuang, L., Xu, W. & Liu, L. (2009). Fe(III) oxide reduction and carbon tetrachloride dechlorination by a newly isolated *Klebsiella pneumoniae* strain L17. *J Appl Microbiol* **106**, 130–139.
- Liu, C. X., Zachara, J. M., Foster, N. S. & Strickland, J. (2007). Kinetics of reductive dissolution of hematite by bioreduced anthraquinone-2,6-disulfonate. *Environ Sci Technol* **41**, 7730–7735.
- Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, F. J. P. & Woodward, J. C. (1996). Humic substances as electron acceptors for microbial respiration. *Nature* **382**, 445–448.
- Mesbah, M., Premachandran, U. & Whitman, B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Pham, C. A., Jung, S. J., Phung, N. T., Lee, J., Chang, I. S., Kim, B. H., Yi, H. & Chun, J. (2003). A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. *FEMS Microbiol Lett* **223**, 129–134.
- Pitcher, D., Soto, A., Soriano, F. & Valero-Guillén, P. (1992). Classification of coryneform bacteria associated with human urinary tract infection (group D2) as *Corynebacterium urealyticum* sp. nov. *Int J Syst Bacteriol* **42**, 178–181.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Scott, D. T., Mcknight, D. M., Blunt-Harris, E. L., Kolesar, S. E. & Lovley, D. R. (1998). Quinone moieties act as electron acceptors in the

reduction of humic substances by humics-reducing microorganisms. *Environ Sci Technol* **32**, 2984–2989.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Straub, K. L., Kappler, A. & Schink, B. (2005). Enrichment and isolation of ferric-iron- and humic-acid-reducing bacteria. *Methods Enzymol* **397**, 58–77.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Wang, Y. B., Wu, C. Y., Wang, X. J. & Zhou, S. G. (2009). The role of humic substances in the anaerobic reductive dechlorination of 2,4-dichlorophenoxyacetic acid by *Comamonas koreensis* strain CY01. *J Hazard Mater* **164**, 941–947.

Ye, Q., Roh, Y., Carroll, S. L., Blair, B., Zhou, J., Zhang, C. L. & Fields, M. W. (2004). Alkaline anaerobic respiration: isolation and characterization of a novel alkaliphilic and metal-reducing bacterium. *Appl Environ Microbiol* **70**, 5595–5602.

Zachara, J. M., Fredrickson, J. K., Li, S. M., Kennedy, D. W., Smith, S. C. & Gassman, P. L. (1998). Bacterial reduction of crystalline Fe³⁺ oxides in single phase suspensions and subsurface materials. *Am Mineral* **83**, 1426–1443.