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Dechloromonas hortensis sp. nov. and strain ASK-1, two novel (per)chlorate-reducing bacteria, and taxonomic description of strain GR-1

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Recent studies on the occurrence of (per)chlorate-reducing bacteria have resulted in the characterization of strains capable of dissimilatory (per)chlorate reduction. Phylogenetic analysis has shown that these bacteria are members of the *Proteobacteria*. Strains have been isolated from polluted and pristine sites, but only strains from polluted sites have been characterized in detail and deposited in culture collections. Herein we describe the isolation and characterization of perchlorate-reducing bacterium strain MA-1^T and chlorate-reducing bacterium strain ASK-1, respectively isolated from a pristine and a chlorate-polluted site. Both isolates are members of the *Proteobacteria*. The 16S rRNA gene sequence similarity of MA-1^T to *Dechloromonas agitata* DSM 13637^T is 97·6 %, but the relatedness in DNA–DNA reassociation is only 37 %. Therefore, we propose to classify strain MA-1^T (=DSM 15637^T=ATCC BAA-776^T) as the type strain of a novel species, *Dechloromonas hortensis* sp. nov. Strain ASK-1 and a previously described strain GR-1 show 100 and 99 % 16S rRNA gene sequence similarity to *Pseudomonas chloritidismutans* DSM 13592^T and *Dechlorosoma suillum* DSM 13638^T, respectively. DNA–DNA hybridization studies indicated that strains ASK-1 and GR-1 are related at the species level to *P. chloritidismutans* DSM 13592^T (79 %) and *Dechlorosoma suillum* DSM 13638^T (85 %), respectively. As suggested previously, *Dechlorosoma suillum* appears to be a later heterotypic synonym of *Azospira oryzae*. Although strain ASK-1 is identified as *P. chloritidismutans*, its morphology and growth requirements are different from those of the type strain.

The occurrence of perchlorate and chlorate in natural environments is due mainly to human activities. Chlorate is used as a herbicide, as a catalyst in matches and for onsite production of chlorine dioxide (ClO_2), a bleaching agent employed in the paper and pulp industries. Perchlorate is used as rocket propellant in the defence and aerospace industries. In the United States, discharge of perchlorate-containing waste streams has been identified as the major source of perchlorate contamination in drinking water supplies (Renner, 1998; US Environmental Protection Agency, 2000). Chlorate and perchlorate are highly soluble and chemically stable under environmental conditions (Urbansky, 2002). Remediation strategies by chemical reduction or by adsorption to activated carbon are either too slow or incomplete (Urbansky, 1998). Recent research has shown that bioremediation may be the most economically feasible, fastest and easiest means of treating (per)chlorate-contaminated sites and water sources (Coates & Achenbach, 2004). (Per)chlorate-reducing bacteria are able to perform a

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Photographs of colonies and cell extracts of *P. chloritidismutans* and strain ASK-1 and a transmission electron micrograph of cells of strain GR-1 are available as supplementary material in IJSEM Online.

complete reduction of (per)chlorate to chloride using various carbon compounds or hydrogen as electron donor. (Per)chlorate is reduced to chlorite by a (per)chlorate reductase [EC 1.97.1.1] (Kengen *et al.*, 1999) and subsequently chlorite is converted to chloride and oxygen by a chlorite dismutase [EC 1.13.11.49] (van Ginkel *et al.*, 1996).

(Per)chlorate-reducing bacteria are widespread in nature (van Ginkel *et al.*, 1995; Coates *et al.*, 1999; Wu *et al.*, 2001) and have been isolated from chlorate- and perchlorate-contaminated sites. For example, '*Acinetobacter thermotoleranticus*' was isolated from a match-factory waste stream (Stepanyuk *et al.*, 1992). Previous studies on the ubiquity and diversity of (per)chlorate-reducing bacteria resulted in the description of *Dechloromonas* and *Dechlorosoma* species; to date, these appear to be the dominant (per)chlorate-reducing bacteria in the environment (Coates *et al.*, 1999). (Per)chlorate-reducing activity has been observed not only in chlorate- or perchlorate-polluted samples, but also in pristine sediments and soils (van Ginkel *et al.*, 1995; Coates *et al.*, 1999; Wu *et al.*, 2001). However, no isolate from a pristine setting has been characterized or deposited in culture collections. Here, we describe two novel strains, MA-1^T and ASK-1, isolated, respectively, from garden soil and the sludge of a bioreactor treating a bromate-/chlorate-polluted waste stream. We also performed phylogenetic analysis on a third, previously described strain, strain GR-1 (Rikken *et al.*, 1996). Strain GR-1 was included here because it represents one of the best studied (per)chlorate-reducing bacteria, especially concerning the biochemistry of the reduction process. However, a taxonomic description was as yet lacking.

Enrichment and cultivation of strains MA-1^T and ASK-1 were performed in anoxic medium as described previously for *Pseudomonas chloritidismutans* DSM 13592^T (Wolterink *et al.*, 2002) with the following modifications: The gas phase used was N_2/CO_2 (80 : 20) and, instead of 0·5 g Na_2S, 0·2 g Na_2SO_4 was used as a sulfur source (Wolterink *et al.*, 2002). For the isolation of both strains, chlorate (10 mM) and acetate (10 mM) were used as electron acceptor and electron donor, respectively. Batch cultures were incubated in the dark at 30 °C, pH 7·2, on an orbital shaker set at 100 r.p.m. For enrichment and isolation of strain ASK-1, sludge was taken from an anaerobic bioreactor treating chlorate-/bromate-polluted wastewater. Samples from this sludge were also used to isolate *P. chloritidismutans* DSM 13592^T (Wolterink *et al.*, 2002). Pure cultures of strain ASK-1 were obtained following repeated application of the roll-tube dilution method (Hungate, 1969). To isolate strain MA-1^T, approximately 2 g garden soil was added to 40 ml of the medium described above. Strain MA-1^T was isolated to purity on aerobically incubated nutrient agar plates. Enrichment cultures were monitored for chlorate reduction by analysing the growth medium by HPLC as described previously (Scholten & Stams, 1995). Oxygen levels were measured by GC as described by Stams *et al.* (1993). Substrates were added from 0·8 M stock solutions to give final concentrations of 10 mM. Use of the following electron donors (10 mM) was evaluated with chlorate (10 mM) as electron acceptor: acetate, propionate, glucose, maltose, mannitol, malate, lactate, arabinose, hydrogen, glycine, glycerol, formate, gluconate, ethanol, starch, citrate and succinate. The following electron acceptors (10 mM) were tested with acetate (10 mM) as electron donor: perchlorate, chlorate, chlorite, nitrate, bromate, sulfate and oxygen. All anions were supplied as sodium salts. For strain MA-1^T, Fe^{3+} reduction to Fe^{2+} [Fe^{3+} applied as iron(III) citrate] was monitored spectrophotometrically at 562 nm using the ferrozine assay described by Lovley & Phillips (1987). Different medium pH values were obtained by changing the CO_2 concentration appropriately in the gas phase, calculated using the Henderson–Hasselbach equation (Wolterink *et al.*, 2002). Growth was followed at the following temperatures: 10, 20, 30, 37 and 50 °C. Strain GR-1 (=DSM 11199) was grown in a medium described before (Rikken *et al.*, 1996) with modifications described by Kengen *et al.* (1999), *P. chloritidismutans* DSM 13592^T was grown in the medium described by Wolterink *et al.* (2002) and *Pseudomonas stutzeri* DSM 50227 was grown in the medium described by Matsubara *et al.* (1982) as modified by Coyle *et al.* (1985). Gram-type was determined by Gram staining using the protocol described by Plugge *et al.* (2000).

Almost full-length (approx. 95 %) 16S rRNA gene sequences for strains ASK-1, MA-1^T and GR-1 were determined at the DSMZ by direct sequencing of PCR-amplified 16S rRNA genes as described by Rainey *et al.* (1996). 16S rRNA gene sequences were analysed using ARB software (Ludwig & Strunk, 1996). 16S rRNA gene sequences of the three strains were compared to the following type strains: *P. chloritidismutans* DSM 13592^T (GenBank accession no. AY017341), *Dechloromonas agitata* DSM 13637^T (AF047462) and *Azospira oryzae* LMG 9096^T (AF011347). Also, *P. stutzeri* DSM 50227 (GenBank accession no. U26415), closely related to *P. chloritidismutans* DSM 13592^T (Wolterink *et al.*, 2002), and *Dechlorosoma suillum* DSM 13638^T (AF170348), closely related to *Azospira oryzae* LMG 9096^T, were used for 16S rRNA gene sequence comparison. Genomic DNA was isolated by chromatography on hydroxyapatite after the method of Cashion *et al.* (1977). DNA–DNA hybridization between strain GR-1 and *Dechlorosoma suillum* DSM 13638^T, strain MA-1^T and *Dechloromonas agitata* DSM 13637^T and strain ASK-1 and *P. chloritidismutans* DSM 13592^T was carried out as described by De Ley *et al.* (1970), as modified by Huß *et al.* (1983) and Escara & Hutton (1980). Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992). The G+C content of strain MA-1^T was determined using the HPLC method described by Mesbah *et al.* (1989); unmethylated lambda DNA (Sigma) was used as the standard. DNA–DNA hybridization studies as well as G+C analyses were conducted at the DSMZ.

Cell extracts were prepared in an anaerobic glovebox containing H_2/N_2 (4 : 96) gas (Wolterink *et al.*, 2002). Oxyanion reductase activities were determined spectrophotometrically,

as described previously by Kengen *et al.* (1999), by monitoring the oxidation of reduced methyl viologen at 578 nm and 30 °C. Chlorite dismutase activity was determined as described by Wolterink *et al.* (2002), by measuring O_2 production with a Clark-type oxygen electrode (Yellow Springs Instruments). We define 1 U activity as the amount of enzyme required to convert 1 µmol chlorite (or chlorate, bromate, nitrate) min^{-1}. The protein content of the cell extracts was determined according to the method of Bradford (1976) with BSA as the standard.

Cells of strain GR-1 were prepared for electron microscopy by fixation in 4 % formaldehyde in 0·01 M PBS (pH 7·6). Cells were subsequently washed, dispersed in PBS and adsorbed to glow-discharged, carbon-stabilized, Formvar-coated nickel grids, negatively stained using 2·0 % ammonium molybdate (pH 5·1) or 2·0 % potassium phosphotungstate (pH 6·0), and analysed in a Philips TECNAI 12 electron microscope at an operating voltage of 80 kV. Images were digitally stored and analysed using analySIS (Soft-imaging software). Images were printed after grey-value modification.

Strain ASK-1

The 16S rRNA gene sequence of strain ASK-1 was 100 % similar to those of *P. chloritidismutans* DSM 13592^T and *P. stutzeri* DSM 50227 (Wolterink *et al.*, 2002). DNA–DNA hybridization between strain ASK-1 and *P. chloritidismutans* DSM 13592^T showed 79 % relatedness. To differentiate two species, DNA–DNA similarity should be less than 60–70 % (Stackebrandt & Goebel, 1994). Therefore, strain ASK-1 is identified as a strain of *P. chloritidismutans*. Both bacteria are chlorate-reducing bacteria that can only grow with oxygen and chlorate as electron acceptors. Details of utilization of electron donors and acceptors are available in Table 1. Apparently, respiration with chlorate is the only energy-yielding process under anaerobic conditions. The observed doubling times for *P. chloritidismutans* DSM 13592^T and strain ASK-1 were respectively 1·5 and 10·6 h when chlorate and acetate were used as electron acceptor and electron donor. Respiration with chlorate is reflected in the observed chlorate reductase activity in cell-free extracts of ASK-1 (6·1 U mg^{-1}). Bromate reductase activity (4·5 U mg^{-1}) was also found, although no growth was observed on this compound. No reductase activity was found for ClO_4^-, NO_3^- or SO_4^{2-}. Similar results have been obtained for *P. chloritidismutans* DSM 13592^T, with chlorate and bromate reductase activities of 9·0 and 8·6 U mg^{-1}, respectively (Wolterink *et al.*, 2002). Despite the phylogenetic and physiological similarities, a morphological difference was seen when chlorate-grown cells were plated under aerobic conditions on nutrient agar plates. *P. chloritidismutans* DSM 13592^T formed yellow–brown circular colonies and strain ASK-1 formed white circular colonies. Remarkably, a cell extract of chlorate-grown cells of *P. chloritidismutans* DSM 13592^T (grown in strictly anaerobic medium) had a red–brownish colour, whereas a cell extract of chlorate-grown cells of strain ASK-1 (grown in anoxic medium) was white. This colour difference of chlorate-grown cells is most likely a result of the level of haem-containing chlorite dismutase in cell extracts, which was found to be respectively 134 and 6·3 U mg^{-1} for *P. chloritidismutans* and strain ASK-1. Pictures of these colonies and cell extracts are available as Supplementary Fig. S1 in IJSEM Online. The strain has been deposited as *P. chloritidismutans* strain ASK-1 (= DSM 15671 = ATCC BAA-775).

Strain GR-1

Strain GR-1 (= DSM 11199) was one of the first dissimilatory perchlorate-reducing bacteria to be described (Rikken *et al.*, 1996); moreover, the (per)chlorate reductase

Table 1. Electron donor and acceptor usage of the chlorate-reducing bacterium strain ASK-1, *P. chloritidismutans* DSM 13592^T and the perchlorate-reducing bacterium strain GR-1

For strain ASK-1, tests were performed in this study by measuring the electron acceptor concentration by HPLC. Data for *P. chloritidismutans* DSM 13592^T were taken from Wolterink *et al.* (2002) and those for strain GR-1 were taken from Rikken *et al.* (1996).

Donor/acceptor	Strain ASK-1	*P. chloritidismutans* DSM 13592^T	Strain GR-1
Electron donors			
Utilized	Acetate, glycerol, glycine, gluconate, glucose, mannitol, propionate	Acetate, propionate, glucose, maltose, gluconate, mannitol, ethanol, starch, glycerol, citrate	Acetate, propionate, caprionate, malate, succinate
Not utilized	Arabinose, citrate, ethanol, lactate, malate, maltose, starch, succinate	Malate, succinate, lactate, glycine, arabinose, H_2	Glycine, formate, glycolate, citrate, glucose, arabinose, mannose, mannitol, maltose, gluconate, *N*-acetylglucosamine, adipate, phenylacetate
Electron acceptors			
Utilized	ClO_3^-, O_2	ClO_3^-, O_2	ClO_3^-, O_2, ClO_4^-, NO_3^-, Mn(IV)
Not utilized	ClO_4^-, NO_3^-, BrO_3^-, SO_4^{2-}	ClO_4^-, NO_3^-, BrO_3^-, SO_4^{2-}	BrO_3^-, SO_4^{2-}, IO_4^-, SeO_4^{2-}, Fe(III)

and chlorite dismutase proteins were first purified and characterized from this strain. However, a complete taxonomic characterization of this perchlorate-reducing bacterium has not yet been done. Our data indicate that the 16S rRNA gene sequence of strain GR-1 is 99 % similar to that of *Dechlorosoma suillum* DSM 13638^T. DNA–DNA hybridization between these two strains revealed 85 % relatedness, indicating that strain GR-1 must be identified as *Dechlorosoma suillum*. Interestingly, the 16S rRNA gene sequences of *Dechlorosoma suillum* DSM 13638^T (GenBank accession no. AF170348) and *A. oryzae* LMG 9096^T (AF011347) are very similar (99·9 %), although the sequence of *A. oryzae* LMG 9096^T was not included in the description of *Dechlorosoma suillum* (Achenbach *et al.*, 2001). The 16S rRNA gene sequence of *A. oryzae* LMG 9096^T was already available in 1995 (Hurek & Reinhold-Hurek, 1995), and the name *A. oryzae* was validly published in 2000 (Reinhold-Hurek & Hurek, 2000). The DNA–DNA hybridization level of 90 % between *Dechlorosoma suillum* DSM 13638^T and *A. oryzae* LMG 9096^T is well above the suggested limit for species identity, supporting the conclusion of Tan & Reinhold-Hurek (2003) that *Dechlorosoma suillum* is a later heterotypic synonym of *A. oryzae*. Hence, we propose that strain GR-1 should be classified as a strain of *A. oryzae*.

A. oryzae LMG 9096^T and *Dechlorosoma suillum* DSM 13638^T share many phenotypic characteristics, including carbon source utilization preferences. Both bacteria are able to fix N_2, which is a distinguishing feature of *A. oryzae* LMG 9096^T. A *nifH* homologue has been detected in both strains, and Tan & Reinhold-Hurek (2003) observed that both can reduce acetylene (C_2H_2) to ethylene (C_2H_4), which confirms that the *nifH* gene is functional and nitrogenase is present. However, there is a key difference between these strains, the inability of *A. oryzae* LMG 9096^T to perform dissimilatory perchlorate reduction.

Details of electron donor and acceptor usage of strain GR-1 are available in Table 1. Supplementary Fig. S2 depicts an electron micrograph of cells of strain GR-1. The cells possess a single polar flagellum and are rod-shaped with dimensions of $1·8 \pm 0·2 \times 0·60 \pm 0·05$ μm. The micrograph reveals electron-transparent globules that vary in size and number among cells. These globules possibly contain poly-β-hydroxybutyrate or other kinds of poly-β-hydroxyalkanoates (Thalen *et al.*, 1999). We also found strain GR-1 to have fimbriae, by which it can presumably attach itself to surfaces (data not shown).

Strain MA-1^T

Strain MA-1^T showed 99·9 and 97·6 % 16S rRNA gene sequence similarity to *Dechloromonas* sp. strain SIUL and *Dechloromonas agitata* DSM 13637^T, respectively. The 16S rRNA gene sequence similarity between strain MA-1^T and *Ferribacterium limneticum* CdA-1^T was 97·5 %. Unfortunately, this strain is not available in culture collections and therefore could not be included in further studies. DNA–DNA hybridization between *Dechloromonas agitata* DSM 13637^T and strain MA-1^T showed only 37 % relatedness, indicating that strain MA-1^T represents a distinct species. The G+C content for strain MA-1^T is 63·6 mol%, which is similar to that of *Dechloromonas agitata* (63·6 mol%). Based on the 16S rRNA gene sequence data, strain MA-1^T resides in the *Dechloromonas* group (Achenbach *et al.*, 2001), which also contains the genera *Rhodocyclus* and *Ferribacterium*. However, representatives of the latter two genera are not capable of chlorate reduction nor do they dismutate chlorite to chloride and O_2. Moreover, *F. limneticum* is capable of Fe(III) reduction. Strain MA-1^T was not able to grow by Fe(III) reduction, similar to other chlorate-reducing bacteria (Coates *et al.*, 1999). It has already been concluded that *F. limneticum* is indeed a member of a separate genus distinct from *Dechloromonas* (Achenbach *et al.*, 2001). Taken together, these data imply that strain MA-1^T should be classified as a separate species within the genus *Dechloromonas*, and we propose the name *Dechloromonas hortensis* sp. nov.

Strain MA-1^T was unable to grow in anoxic medium reduced with sulfide, which indicated that sulfide cannot serve as an alternative electron donor, as was described for *Dechloromonas agitata* DSM 13637^T (Achenbach *et al.*, 2001). Results of the physiological characterization are given in the species description. The results of a physiological comparison of the three strains described above are available in Table 2. Optimal growth of strain MA-1^T was

Table 2. Characteristics of strain MA-1^T, *Ferribacterium limneticum* CdA-1^T and *Dechloromonas agitata* DSM 13637^T

For strain MA-1^T, tests were performed in this study by measuring the electron acceptor concentration by HPLC. Data for *F. limneticum* were taken from Cummings *et al.* (1999) and those for *Dechloromonas agitata* were taken from Achenbach *et al.* (2001).

Characteristic	Strain MA-1^T	*F. limneticum* CdA-1^T	*Dechloromonas agitata* DSM 13637^T
Electron donors	Acetate, propionate	Acetate, formate, lactate, benzoate	Acetate, propionate, butyrate, lactate, succinate, fumarate, malate, Fe^{2+}
Electron acceptors	ClO_4^-, ClO_3^-, O_2, NO_3^-	Fe^{3+}, NO_3^-, fumarate	ClO_4^-, ClO_3^-, O_2
Source	Garden soil	Mining-impacted lake sediment	Waste pulp, sludge from pulp and paper plant
Morphology	Rod-shaped, single or in flocs	Straight or slightly curved, rod-shaped, single or chains of two to four cells	Straight or slightly curved, rod-shaped

obtained at pH 7·2 and at a temperature of 30 °C. In cell extracts, the specific activity of chlorate reductase was 3·12 U mg^{-1}, while the chlorite dismutase had a specific activity of 155 U mg^{-1}. Strain MA-1^T was isolated from a garden soil with no known history of (per)chlorate contamination. Gram-staining showed that strain MA-1^T is a Gram-negative bacterium.

Description of *Dechloromonas hortensis* sp. nov.

Dechloromonas hortensis (hor.ten′sis. L. fem. adj. *hortensis* belonging to the garden).

Gram-negative, facultatively anaerobic, motile bacterium. Colonies on (aerobic) nutrient agar plates are circular and have a yellow colour. In anoxic medium, which is not reduced with sulfide, optimal growth is obtained at pH 7·2 and at a temperature of 30 °C. Growth occurs with acetate and propionate as electron donors. No growth is found with citrate, gluconate, glucose, mannitol, maltose, starch, ethanol, methanol or sulfide. Perchlorate, chlorate, nitrate and oxygen are used as electron acceptors. Cell extract contains perchlorate, chlorate, nitrate and bromate reductase activities. Chlorite is converted to chloride and oxygen. The specific activity of the chlorite dismutase in cell extracts is 155 U mg^{-1}. The species belongs to the '*Betaproteobacteria*'. The 16S rRNA gene sequence is 99·9 % similar to that of *Dechloromonas* sp. strain SIUL. The highest similarity to a species with a validly published name is 97·6 %, to *Dechloromonas agitata* DSM 13637^T. DNA–DNA hybridization between the type strain and *Dechloromonas agitata* DSM 13637^T shows 37 % relatedness. The G+C content of the type strain is 63·6 mol%.

The type strain, MA-1^T (=DSM 15637^T=ATCC BAA-776^T), was obtained from a garden soil.

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References

Achenbach, L. A., Michaelidou, U., Bruce, R. A., Fryman, J. & Coates, J. D. (2001). *Dechloromonas agitata* gen. nov., sp. nov. and *Dechlorosoma suillum* gen. nov., sp. nov., two novel environmentally dominant (per)chlorate-reducing bacteria and their phylogenetic position. *Int J Syst Evol Microbiol* **51**, 527–533.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Coates, J. D. & Achenbach, L. A. (2004). Microbial perchlorate reduction: rocket-fueled metabolism. *Nat Rev Microbiol* **2**, 569–580.

Coates, J. D., Michaelidou, U., Bruce, R. A., O'Connor, S. M., Crespi, J. N. & Achenbach, L. A. (1999). Ubiquity and diversity of dissimilatory (per)chlorate-reducing bacteria. *Appl Environ Microbiol* **65**, 5234–5241.

Coyle, C. L., Zumft, W. G., Kroneck, P. M., Korner, H. & Jakob, W. (1985). Nitrous oxide reductase from denitrifying *Pseudomonas perfectomarina*. Purification and properties of a novel multicopper enzyme. *Eur J Biochem* **153**, 459–467.

Cummings, D. E., Caccavo, F., Jr, Spring, S. & Rosenweig, R. F. (1999). *Ferribacterium limneticum*, gen. nov., sp. nov., an Fe(III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. *Arch Microbiol* **171**, 183–188.

De Ley, L., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.

Hungate, R. E. (1969). A roll tube method for cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.

Hurek, T. & Reinhold-Hurek, B. (1995). Identification of grass-associated and toluene-degrading diazotrophs, *Azoarcus* spp., by analyses of partial 16S ribosomal DNA sequences. *Appl Environ Microbiol* **61**, 2257–2261.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Jahnke, K.-D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.

Kengen, S. W. M., Rikken, G. B., Hagen, W. R., van Ginkel, C. G. & Stams, A. J. M. (1999). Purification and characterization of (per)chlorate reductase from the chlorate-respiring strain GR-1. *J Bacteriol* **181**, 6706–6711.

Lovley, D. R. & Phillips, E. J. P. (1987). Rapid assay for microbially reducible ferric iron in aquatic sediments. *Appl Environ Microbiol* **53**, 1536–1540.

Ludwig, W. & Strunk, O. (1996). ARB, a software environment for sequence data. http://www.arb-home.de/

Matsubara, T., Frunzke, K. & Zumft, W. G. (1982). Modulation by copper of the products of nitrite respiration in *Pseudomonas perfectomarinus*. *J Bacteriol* **149**, 816–823.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Plugge, C. M., Zoetendal, E. G. & Stams, A. J. M. (2000). *Caloramator coolhaasii* sp. nov., a glutamate-degrading, moderately thermophilic anaerobe. *Int J Syst Evol Microbiol* **50**, 1155–1162.

Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.

Reinhold-Hurek, B. & Hurek, T. (2000). Reassessment of the taxonomic structure of the diazotrophic genus *Azoarcus sensu lato* and description of three new genera and new species, *Azovibrio restrictus* gen. nov., sp. nov., *Azospira oryzae* gen. nov., sp. nov. and *Azonexus fungiphilus* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **50**, 649–659.

Renner, R. (1998). Perchlorate-tainted wells spur government action. *Environ Sci Technol* **32**, 210A.

Rikken, G. B., Kroon, A. G. M. & van Ginkel, C. G. (1996). Transformation of (per)chlorate into chloride by a newly isolated bacterium: reduction and dismutation. *Appl Microbiol Biotechnol* **45**, 420–426.

Scholten, J. C. M. & Stams, A. J. M. (1995). The effect of sulfate and nitrate on methane formation in a freshwater sediment. *Antonie van Leeuwenhoek* **68**, 309–315.

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.

Stams, A. J. M., van Dijk, J. B., Dijkema, C. & Plugge, C. M. (1993). Growth of syntrophic propionate-oxidizing bacteria with fumarate in the absence of methanogenic bacteria. *Appl Environ Microbiol* **59**, 1114–1119.

Stepanyuk, V. V., Smirnova, G. F., Klyushnikova, T. M., Kanyuk, N. I., Panchenko, L. P., Nogina, T. M. & Prima, V. I. (1992). New species of the *Acinetobacter* genus, *Acinetobacter thermotoleranticus* sp. nov. *Mikrobiologiia* **61**, 490–500 (in Russian).

Tan, Z. & Reinhold-Hurek, B. (2003). *Dechlorosoma suillum* Achenbach *et al.* 2001 is a later subjective synonym of *Azospira oryzae* Reinhold-Hurek and Hurek 2000. *Int J Syst Evol Microbiol* **53**, 1139–1142.

Thalen, M., van de IJsel, J., Jiskoot, W., Zomer, B., Roholl, P. J. M., de Gooijer, C., Beuvery, C. & Tramper, J. (1999). Rational medium design for *Bordetella pertussis*: basic metabolism. *J Biotechnol* **75**, 147–159.

Urbansky, E. T. (1998). Perchlorate chemistry: implications for analysis and remediation. *Bioremediat J* **2**, 81–95.

Urbansky, E. T. (2002). Perchlorate as an environmental contaminant. *Environ Sci Pollut Res Int* **9**, 187–192.

US Environmental Protection Agency (2000). *The effect of ammonium perchlorate on thyroids.* Pathology Working Group Report. Washington, DC: National Center for Environmental Assessment, US EPA. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=15262

van Ginkel, C. G., Plugge, C. M. & Stroo, C. A. (1995). Reduction of chlorate with various energy substrates and inocula under anaerobic conditions. *Chemosphere* **31**, 4057–4066.

van Ginkel, C. G., Rikken, G. B., Kroon, A. G. M. & Kengen, S. W. M. (1996). Purification and characterization of chlorite dismutase: a novel oxygen-generating enzyme. *Arch Microbiol* **166**, 321–326.

Wolterink, A. F. W. M., Jonker, A. B., Kengen, S. W. M. & Stams, A. J. M. (2002). *Pseudomonas chloritidismutans* sp. nov., a non-denitrifying, chlorate-reducing bacterium. *Int J Syst Evol Microbiol* **52**, 2183–2190.

Wu, J., Unz, R. F., Zhang, H. & Logan, B. E. (2001). Persistence of perchlorate and the relative numbers of perchlorate- and chlorate-respiring microorganisms in natural waters, soils, and wastewater. *Bioremediat J* **5**, 119–130.