Pelobacter seleniigenes sp. nov., a selenate-respiring bacterium

Priya Narasingarao and Max M. Häggblom

Strain KM\textsuperscript{T} is a novel bacterium with the unique metabolic abilities of being able to respire selenate as the electron acceptor using acetate as the carbon substrate and possessing the ability to grow fermentatively on short-chain organic acids such as lactate, citrate and pyruvate. Strain KM\textsuperscript{T} was isolated from a sediment enrichment culture of a highly impacted wetland system in New Jersey, USA. Strain KM\textsuperscript{T} is able to reduce selenate as well as selenite to elemental selenium. The unique metabolic capabilities of strain KM\textsuperscript{T} include the respiration of nitrate, poorly crystalline Fe(III) and anthraquinone disulfonate. Phylogenetic analysis of the 16S rRNA gene of the novel isolate indicates that strain KM\textsuperscript{T} groups within the family Geobacteraeae in the class Deltaproteobacteria with approximately 96–97 % 16S rRNA gene sequence similarity to the closest known organisms Malonomonas rubra Gra Mal 1\textsuperscript{T}, Pelobacter acidigallici Ma Gal 2\textsuperscript{T} and species of the genus Desulfuromusa. Recognized species of the genera Malonomonas and Pelobacter cannot use any inorganic electron acceptors, while strains of the genus Desulfuromusa do not ferment organic substrates. This contrasts with the ability of strain KM\textsuperscript{T} to ferment organic compounds as well as to couple selenate reduction to acetate utilization. Based on 16S rRNA gene phylogeny and metabolic properties, strain KM\textsuperscript{T} represents a novel species for which the name Pelobacter seleniigenes sp. nov. (type strain KM\textsuperscript{T} = DSM 18267\textsuperscript{T} = ATCC BAA-1388\textsuperscript{3}) is proposed. Based on the phylogenetic grouping of species of the genus Pelobacter within the Desulfuromusa cluster, it is suggested that Malonomonas rubra Gra Mal 1\textsuperscript{T} should also be included in this group.

Dissimilatory selenate reduction is the process where selenate is used as a terminal electron acceptor for anaerobic respiration and is sequentially reduced to selenite and further to insoluble elemental selenium. A variety of micro-organisms reduce selenate and selenite to elemental selenium. We previously demonstrated that selenate-respiring bacteria appear to be ubiquitous in aquatic sediments and are phylogenetically diverse (Narasingarao & Häggblom, 2007). Five novel micro-organisms, which included two species of the class Gammaproteobacteria and a member of each of the classes Derrirribacteres, Chrysiogenetes and Deltaproteobacteria, capable of dissimilatory selenate reduction were isolated from different sediments. Here, we provide a detailed description of strain KM\textsuperscript{T} which was isolated for its selenate-respiring capability from wetland sediment, Kearny Marsh, NJ, USA.

Strain KM\textsuperscript{T} groups within the family Geobacteraeae in the class Deltaproteobacteria. The class Deltaproteobacteria houses a number of genera which play a highly significant role in the environment, such as the Fe(III)-respiring members of the genus Geobacter and mainly fermentative members of the genus Pelobacter. The Geobacteraeae family has recently been grouped into three phylogenetic clusters, Desulfuromusa, Desulfuromonas and Geobacter (Holmes et al., 2004), based on a number of conserved genes, including recA, gyrB, rpoB, nifA and fusA. Within this family, the genus Pelobacter comprises a group of organisms fermenting unusual substrates (Schink, 2005, 2006). Species of the genus Pelobacter are highly diverse and are scattered in the three phylogenetic clusters. Since strain KM\textsuperscript{T} shares similar characteristics with Malonomonas rubra Gra Mal 1\textsuperscript{T} and only two of the Pelobacter species, we compared the physiological properties of strain KM\textsuperscript{T} with closely related organisms and investigated the taxonomic position of strain KM\textsuperscript{T} and related species.

Strain KM\textsuperscript{T} is a dissimilatory selenate-respiring bacterium isolated from an enrichment culture of sediment from Kearny Marsh, a wetland system in New Jersey, USA (Narasingarao & Häggblom, 2007). After sequential transfers into fresh media, strain KM\textsuperscript{T} was isolated using strict anaerobic techniques in soft agar (0.4 % Noble agar; Difco) shake tubes with selenate (10 mM) as the electron acceptor and

**Abbreviation:** AQDS, anthraquinone disulfonate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Pelobacter seleniigenes sp. nov. KM\textsuperscript{T} is DQ991964.
pyruvate (20 mM) as the electron donor. Strain KM\textsuperscript{T} was cultivated and maintained in a minimal salts medium (Fennell et al., 2004) with pyruvate as the carbon source and selenate as the electron acceptor under a head space of N\textsubscript{2}/CO\textsubscript{2} (30\% : 70\%, v/v).

Strain KM\textsuperscript{T} was grown with selenate (10 mM) and pyruvate or fermentatively with only pyruvate (10 mM) to examine cell morphology. Transmission electron microscopy and scanning electron microscopy were performed as described previously (Narasingarao & Häggblom, 2006). Strain KM\textsuperscript{T} is a rod-shaped bacterium, approximately 2 × 0.5 μm (Fig. 1a). The cells formed large aggregates when grown in the presence of selenate and formed clumps along with precipitated elemental selenium (Fig. 1b) that settled at the bottom of the flask as a slimy mat. Strain KM\textsuperscript{T} is a Gram-negative, motile bacterium that formed bright red colonies in soft agar shake tubes when grown on selenate (10 mM) due to the precipitation of red elemental selenium. Cells of selenate-respiring cultures produce abundant elemental selenium granules which are closely associated with the cells. Strain KM\textsuperscript{T} has unique physiological and taxonomic characteristics that distinguish it from closely related genera and thus warrants classification as a novel species.

A range of electron acceptors and donors (listed in Table 1) was used to test the metabolic capabilities of strain KM\textsuperscript{T}. Strain KM\textsuperscript{T} respires selenate stoichiometrically to elemental selenium with acetate as the electron donor and the carbon source. The utilization of 5.4 mM acetate was accompanied by reduction of 7.3 mM selenate to 4.6 mM selenite and 2.7 mM elemental selenium (estimated from the difference in total and soluble selenium measured). Selenate reduction was followed by a transient accumulation of selenite and this was finally reduced to elemental selenium, as evidenced by a bright red precipitate and further confirmed using X-ray absorption near edge structure analysis (XANES; Narasingarao & Häggblom, 2007). Strain KM\textsuperscript{T} can also grow fermentatively on short-chain organic acids, such as pyruvate, citrate and lactate. Most dissimilatory selenate-reducing bacteria known so far are facultative or strictly anaerobic respiratory microorganisms, unknown for their fermentative capability (Stolz & Oremland, 1999).

The salt tolerance of strain KM\textsuperscript{T} was determined by growing the cells under a range of NaCl concentrations. It was interesting to note that strain KM\textsuperscript{T} was highly versatile and could grow in media without any added NaCl and also in media that contained up to 3.5\% NaCl (0, 0.5\%, 2.3\% and 3.5\%, w/v), under fermentative conditions with pyruvate as the sole carbon and energy source.

The fatty acid content of strain KM\textsuperscript{T} was determined after growth under fermentative conditions with pyruvate (10 mM) as the sole carbon and energy source at 28 °C using the method described by Narasingarao & Häggblom (2006). The bulk of the fatty acids (65\%) consisted of straight-chain fatty acids, such as C\textsubscript{15}, C\textsubscript{16} and C\textsubscript{17} (Table 2). Strain KM\textsuperscript{T}, being Gram-negative, possessed, as expected, some 3-hydroxy fatty acids from the lipopolysaccharide of the outer membrane. Unsaturated fatty acids, predominantly C\textsubscript{16}:1\textsuperscript{ω7c} and C\textsubscript{17}:1\textsuperscript{ω8c}, were found in small amounts. A fairly high amount (9.5\%) of C\textsubscript{17}:0 cyclo was also detected. The fatty acid content of strain KM\textsuperscript{T} is substantially different from that of Desulfuromusa ferrireducens (Vandieken et al., 2006), the only closely related species for which this information is available at the time of writing.

The G+C content (mol\%) of the genomic DNA was determined using the method described by Mesbah et al. (1989) with the following modifications. A Synergi 4U Fusion-RP 80A C\textsubscript{18} reverse-phase column (Phenomenex) was used in a HPLC (1100; Agilent) system. The solvent system consisted of eluent A, 20 mM ammonium acetate (pH 4.5) and eluent B, acetonitrile. With a 1 ml min\textsuperscript{-1} flow rate, a gradient was established starting with 95\% eluent A, gradually decreasing to 60\% over 10 min. The nucleosides were detected at a wavelength of 260 nm. Salmon sperm DNA was used for calibration and Sedimenticola selenatireducens AK4OH\textsubscript{T} was used as a

**Fig. 1.** Morphology of cells of strain KM\textsuperscript{T}. (a) Transmission electron micrograph of rod-shaped cells grown fermentatively without any selenate; (b) Scanning electron micrograph of cells as aggregates along with selenium granules. Bars, 0.5 μm (a) and 1 μm (b).
control for verification. The G+C content of strain KM<sup>T</sup> was found to be 54.1 ± 1.1 mol%.

The 16S rRNA gene sequence analysis was performed using primers described in Narasingarao & Häggblom (2006). The sequence data were compiled in Contig Express (Vector NTI Suite; Informax). The 16S rRNA gene sequences of related micro-organisms were identified using a BLAST search (Altschul et al., 1997) and downloaded from GenBank. The sequences were aligned using CLUSTAL_X (Thompson et al., 1997). A similarity matrix was constructed using the Ribosomal Database Project version 8.1 small-subunit similarity matrix calculator (http://rpdb8.cme.msu.edu/html/analyses.html). Phylogenetic trees were constructed using the neighbour-joining, maximum-parsimony and maximum-likelihood methods with PHYLIP (Felsenstein, 1989) and PHYML (Guindon et al., 2005). Bootstrap analysis was performed for all completed trees.

Strain KM<sup>T</sup> falls into the family Geobacteraceae within the class Deltaproteobacteria, based on the nearly complete 16S rRNA gene sequence. The 16S rRNA gene of strain KM<sup>T</sup> was 97.3 % similar to that of Malonomonas rubra Gra Mal 1<sup>T</sup>, 96.1 % to Pelobacter acidigallici Ma Gal 2<sup>T</sup>, 96.2 % to Pelobacter massiliensis HHQ7<sup>T</sup> (Schnell et al., 1991); 5, Desulfuromusa kysingii Kysw2<sup>T</sup> (Liesack & Finster, 1994). For strain KM<sup>T</sup>, electron acceptors were tested with pyruvate as electron donor and electron donors were tested with selenate as electron acceptor. +, Positive; −, negative; ND, not determined.

<table>
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<td>Requires 1 %</td>
<td>Requires 0.87 %</td>
<td>Requires 1 %</td>
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*Motile in young cultures.
†Lactate, pyruvate, citrate to acetate, propionate.
‡Gallic acid, 2,4,6-trihydroxybenzoic acid, phloroglucinol to acetate and CO<sub>2</sub>.
§Fumarate/malate fermented to succinate and CO<sub>2</sub>.
||Hydroxyhydroquinones, pyrogallol, phloroglucinol, gallic acid.
¶Selenite reduction occurs only during selenate reduction.
**Vandieken et al. (2006).
Pelobacter massiliensis and 96.4–96.6% similar to that of species of the genus Desulfuromusa, but highly dissimilar to other species of the genus Pelobacter (<90% in some cases), reflecting their complex taxonomy. The family Geobacteraeae has recently been grouped into three clusters, Desulfuromusa, Desulfuromonas and Geobacter (Holmes et al., 2004), based on sequence analysis of a number of genes such as nifD, recA, gyrB, rpoB and fusc, along with the 16S rRNA gene. A phylogenetic tree (Fig. 2) of the family Geobacteraeae constructed incorporating the 16S rRNA gene sequence of strain KM\textsuperscript{T} showed the same topology as that described by Holmes et al. (2004), with strain KM\textsuperscript{T} grouping within the Desulfuromusa cluster. Strain KM\textsuperscript{T} is most closely related to *M. rubra* Gra Mal 1\textsuperscript{T}, along with two of the *Pelobacter* species, *P. acidigallici* Ma Gal 2\textsuperscript{T} and *P. massiliensis*, and a group of species of the genus *Desulfuromusa*. This distinct grouping is also supported by neighbour-joining and maximum-parsimony methods (not shown).

Table 1 shows a comparison of the metabolic characteristics of strain KM\textsuperscript{T} with closely related genera. The ability of strain KM\textsuperscript{T} to ferment short-chain fatty acids such as pyruvate, citrate and lactate is not shared by any of the closely related genera, although members of the genera *Malonomonas* and *Pelobacter* are strict fermenters. This also contrasts with species of the genus *Desulfuromusa* that are primarily respiratory and ferment only fumarate and malate (Liesack & Finster, 1994). Furthermore, the fermentative capability of members of the genera *Malonomonas* and *Pelobacter* is restricted to only a very narrow range of compounds. *P. acidigallici* Ma Gal 2\textsuperscript{T} can ferment only gallic acid, 2,4,6-trihydroxybenzoic acid and phloroglucinol (Schink & Pfennig, 1982). *P. massiliensis* can ferment only hydroquinones (Schnell et al., 1991) and *M. rubra* Gra Mal 1\textsuperscript{T} can ferment only malonate, fumarate and malate (Dehning & Schink, 1989). The 16S rRNA gene sequence of *M. rubra* Gra Mal 1\textsuperscript{T} is about 96% similar to those of the two species of the genus *Pelobacter* and about 95% to the species of the genus *Desulfuromusa*. Thus, reclassifying *M. rubra* Gra Mal 1\textsuperscript{T} as a species of genus *Pelobacter* would result in better grouping as opposed to grouping with species of the genus *Desulfuromusa*, as suggested by Vandieken et al. (2006). This is further supported by 16S rRNA phylogeny (Fig. 2) in which, *M. rubra* Gra Mal 1\textsuperscript{T} groups within this cluster consistently with strain KM\textsuperscript{T}. This is affiliation is further supported by phylogenetic analysis of other conserved genes (Holmes et al., 2004).

In terms of its electron acceptor utilization, strain KM\textsuperscript{T} is very versatile in its ability to reduce nitrate, fumarate, selenate, anthraquione disulfonate (AQDS), Fe(III) and elemental sulfur, along with its fermentative capability, which is in contrast to species of the genera *Malonomonas* and *Pelobacter*. Furthermore, *P. acidigallici* Ma Gal 2\textsuperscript{T} and *P. massiliensis* do not possess any cytochromes (Schnell et al., 1991). Species of the genus *Malonomonas* can however use Fe(III), as recently shown by Nevin et al. (2003), and also possess high amounts of periplasmic cytochromes (Kolb et al., 1998). The enzymes of the citric acid cycle were detected in *M. rubra* Gra Mal 1\textsuperscript{T} with a possible explanation that it could be involved in assimilatory metabolism. Unfortunately none of these strains have been tested for growth on selenate as electron acceptor, which could be one of the distinguishing characters. Strain KM\textsuperscript{T} can grow at a wide range of NaCl concentrations (0.5–3.5%, w/v) whereas all other related strains have a strict requirement for NaCl, primarily due to their marine origin.

Prior to the 16S rRNA gene sequencing era, species of the genus *Pelobacter* were grouped in the same genus because of their unique ability to ferment a narrow range of compounds. However, based on 16S rRNA gene phylogeny (Fig. 2), it is clear that species of the genus *Pelobacter* are scattered throughout the phylogenetic tree of the family *Geobacteraeae*. This heterogeneity is further supported by other conserved genes (Holmes et al., 2004) and evidence arising from DNA–DNA hybridization [J. P. Touzel & B. Schink, unpublished data as cited in Schink (2005)]. Thus, *P. acidigallici* Ma Gal 2\textsuperscript{T} and *P. massiliensis*, which group in the *Desulfuromusa* cluster, should be clearly distinguished from the other species of the genus *Pelobacter*.

Based on the priority of publication, *P. acidigallici* Ma Gal 2\textsuperscript{T}, the type species of the genus (Schink & Pfennig, 1982), should retain the original genus name and the other species of the genus *Pelobacter* not belonging to the *Desulfuromusa* cluster may need to be reclassified. Since *M. rubra* Gra Mal 1\textsuperscript{T} and strain KM\textsuperscript{T} group with the genus *Pelobacter sensu strictu* (*P. acidigallici* Ma Gal 2\textsuperscript{T} and *P. massiliensis*), it is suggested that the genus *Malonomonas* and its only recognized species, *Malonomonas rubra*, could be included.
in the genus Pelobacter. As the type strain of Malonomonas rubra, Gra Mal 1T, is currently only available in one culture collection (as DSM 5091T), it is not possible to make a formal proposal for this reclassification at this time. Strain KMT is classified as representing a novel species, for which the name Pelobacter seleniigenes sp. nov. is proposed.

**Description of Pelobacter seleniigenes sp. nov.**

Pelobacter seleniigenes (sel.en’ii.gen.es. N.L. n. selenium selenium; Gr. v. gennao produce; N.L. part. adj. seleniigenes selenium-producing).

Gram-negative, motile rod-shaped bacterium approximately 0.5 × 2 μm. Strictly anaerobic. Ferments pyruvate, citrate and lactate and can respire selenate to elemental selenium coupled to acetate utilization. Is able to respire other inorganic electron acceptors such as Fe(III), nitrate, AQDS and elemental sulfur. In agar shake tubes, colonies are round, about 5 mm in diameter and bright red coloured due to the elemental selenium formed during selenate reduction. Tolerates a wide range of NaCl concentrations (0.5–3.5 %, w/v) and grows robustly. The predominant cellular fatty acids are penta, hexa- and heptadecanoic acid and their corresponding monounsaturated fatty acids, C17 : 0cyclo fatty acid and 3-hydroxyl fatty acids. Groups within the family Geobacteraceae of the class Deltaproteobacteria. The genomic DNA G + C content of the type strain is 54.1 ± 1.1 mol%.

The type strain, strain KMT (=DSM 18267T = ATCC BAA-1388T) was isolated for its selenate-respiring capability from a freshwater wetland system in New Jersey, USA.

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References


