Chelativorans multitrophicus gen. nov., sp. nov. and *Chelativorans oligotrophicus* sp. nov., aerobic EDTA-degrading bacteria

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Two previously isolated strains (DSM 9103^T and LPM-4^T) able to grow with EDTA (facultatively and obligately, respectively) as the source of carbon, nitrogen and energy were investigated in order to clarify their taxonomic positions. The strains were strictly aerobic, Gram-negative, asporogenous and non-motile rods that required biotin for growth. Reproduction occurred by binary fission. The strains were mesophilic and neutrophilic. Their major fatty acids were summed feature 7 (consisting of C_{18:1}ω7c, C_{18:1}ω9t and/or C_{18:1}ω12t) and C_{19:0} cyclo ω8c. The polyamine pattern revealed homospermidine as a major polyamine. Predominant polar lipids were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidyl-dimethylethanolamine, phosphatidylmonomethylethanolamine and diphosphatidylglycerol.

Mesorhizobium-specific ornithine lipid was absent. The predominant isoprenoid quinone was Q-10. The DNA G + C values were 60.8 and 63.1 mol% (T_m) for strains LPM-4^T and DSM 9103^T, respectively. The level of 16S rRNA gene sequence similarity between these EDTA-utilizers was 99.3% while the DNA–DNA hybridization value was only 37%. Both strains were phylogenetically related to members of the genera *Aminobacter* and *Mesorhizobium* (95–97% sequence similarity). However, DNA–DNA hybridization values between the novel EDTA-degrading strains and *Aminobacter aminovorans* DSM 7048^T and *Mesorhizobium loti* DSM 2626^T were low (10–11%). Based on their genomic and phenotypic properties, the new alphaproteobacterial strains are assigned to a novel genus, *Chelativorans* gen. nov., with the names *Chelativorans multitrophicus* sp. nov. (type strain DSM 9103^T=VKM B-2394^T) and *Chelativorans oligotrophicus* sp. nov. (type strain LPM-4^T=VKM B-2395^T=DSM 19276^T).

EDTA is a chelating agent from the group of aminopolycarboxylic acids that are able to form stable, water-soluble

complexes with many metal ions. At present, the amount of EDTA utilized worldwide amounts to some 103 000 tonnes per year. It is used in many diverse fields including the photographic and galvanic industries, in textile and paper manufacturing, for decontamination of nuclear power installations, as a component of industrial cleaners, as an additive in cosmetics and food products, in gas scrubbing for the removal of hydrogen sulfide from waste gases and in agricultural applications to improve the uptake of micro-nutrients to correct trace metal deficiencies in plants (Potthoff-Karl *et al.*, 1996; Weilenmann *et al.*, 2004). The predominantly water-based use of EDTA and the recalcitrance of EDTA to biodegradation have resulted in high concentrations of this agent in surface waters. The extensive environmental EDTA pollution that has been observed in

Abbreviations: APL, aminophospholipid; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PDE, phosphatidyl-dimethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PHB, poly-β-hydroxybutyrate; PL, phospholipids; PME, phosphatidylmonomethylethanolamine.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains DSM 9103^T and LPM-4^T are EF457243 and EF457242, respectively.

The TLC for the polar lipid analysis and four additional phylogenetic trees are available with the online version of this paper. A comparison of the major fatty acid contents of the novel strains and those of related genera is presented as a supplementary table.

recent years has become a cause for concern, in particular because of its possible contribution to the mobilization of heavy and toxic metal ions deposited in sediments and soils and also due to the contamination of drinking water (Sillanpaa, 1997; Bucheli-Witschel & Egli, 2001). In order to develop appropriate biotechnological processes to deal with EDTA pollution, the isolation and detailed characterization of micro-organisms able to mineralize EDTA is required.

So far, only a few EDTA-degrading alphaproteobacterial strains have been isolated as pure cultures, i.e. *Agrobacterium radiobacter* sp. ATCC 55002 (Lauff *et al.*, 1990; Palumbo *et al.*, 1994), subsequently reclassified as *Rhizobium radiobacter* (Young *et al.*, 2001), and the Gram-negative strains BNC1 (=DSM 6780; Nörtemann, 1992), DSM 9103^T (Weilenmann *et al.*, 2004), LPM-4^T (Chistyakova *et al.*, 2005) and ANP11 (Nörtemann, 2005). The four latter strains have several features in common, for example growth with EDTA and no growth with iron-complexed EDTA. The present study focused on the taxonomic description and identification of strains DSM 9103^T and LPM-4^T.

Strains DSM 9103^T and LPM-4^T were grown on a mineral medium, 'E', containing (l^{-1}): 1.0 g Na₄EDTA; 1.0 g MgSO₄·7H₂O; 0.26 g KH₂PO₄; 0.4 g CaCl₂·2H₂O and 4 g NaHPO₄·12H₂O at pH 7.0. Medium E was supplemented with 2 ml Widdel's trace element solution (Pfennig *et al.*, 1981) and 1 ml vitamin solution (Egli *et al.*, 1988) per litre.

Physiological tests were performed according to Gerhardt *et al.* (1994). Utilization of a wide range of growth substrates was determined after the strains had been cultivated on mineral medium E for 2 weeks with EDTA replaced by 1% (NH₄)₂SO₄ and various carbon compounds (more than 60 were tested). The ability of the strains to undergo autotrophic growth on H₂ + CO₂ + O₂ or under CH₄ + O₂ was tested as previously described by Doronina *et al.* (2000). Carbohydrates, organic acids and amino acids were added at concentrations of 0.05–0.3% (w/v), while alcohols were added at concentrations of 0.2–0.5% (v/v). The novel bacterial strains were stored in liquid mineral medium E for 1 month, on agar slants at 4 °C for 2 weeks or in a freeze-dried state with a protectant (skimmed milk) for over a year.

Cell morphology was examined using batch cultures grown for 24 h on medium E for both strains and on medium supplemented with fumarate and ammonium chloride (0.5 g l^{-1}) for strain DSM 9103^T. An aliquot of cell suspension was mounted on a Formvar-coated copper grid and stained with 0.2% (w/v) phosphotungstic acid (pH 7.2). For thin sectioning, cells were collected by centrifugation and pre-fixed with 1.5% (v/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) and washed three times with 1% (w/v) OsO₄ in 0.05 M cacodylate buffer (pH 7.2) for 3 h at 20 °C. After dehydration in a series of alcohols, cells were embedded in Spurr epoxy resin and sectioned with a microtome (2128 Ultratome; LKB). Ultrathin sections were mounted on copper grids and double-stained with uranyl acetate and lead citrate (Fig. 1). Negatively stained preparations and thin sections were viewed with a transmission electron microscope (JEM-100B; JEOL) at operating voltages of 60 kV and 80 kV, respectively. Cells of the novel isolates were Gram-negative, non-motile, asporogenous rods that were 0.5–1.0 × 0.7–2.0 µm in size. Cells of both strains multiplied by binary fission. When grown on medium E agar, colonies of both strains were white and 0.1–0.3 mm in diameter after 7 days incubation at 30 °C. The metabolic features of the EDTA-degrading strains have been described previously (Witschel *et al.*, 1997; Doronina *et al.*, 2006).

Sensitivity to antibiotics was examined by placing Difco discs on cells spread on agar plates. The discs contained the following antibiotics (µg): gentamicin (10), neomycin (30), streptomycin (10), ampicillin (10), nalidixic acid (30), lincomycin (2), oxacillin (10), neomycin (30) and novobiocin (5). The effect of antibiotics on cell growth was assessed after 2 weeks.

The poly-β-hydroxybutyrate (PHB) content in the cell biomass was determined by reverse-phase HPLC (Korotkova *et al.*, 1997).

Fatty acids were extracted from cells in the exponential growth phase [medium E containing 1 g l^{-1} EDTA and trypticase soy broth supplemented with 1.5% (w/v) agar]. A 200 µl aliquot of a 5.4 M solution of anhydrous HCl in methanol was added to 30 mg dry biomass and the mixture was heated at 70 °C for 2 h. The methyl esters of fatty acids and aldehyde derivatives

Fig. 1. Electron micrographs of ultrathin sections of EDTA-grown cells of strain LPM-4^T (a) and strain DSM 9103^T (b) showing electron-dense polyphosphate inclusions. Bars, 0.5 µm.

obtained were extracted twice with 100 µl hexane. The extract was dried and silylated in 20 µl *N,O*-bis(trimethylsilyl)tri-fluoroacetamide for 15 min at 65 °C. A 1 µl sample of the reaction mixture was analysed with a GC-MS system (model HP-5985B; Hewlett Packard) equipped with a capillary column (25 × 0.25 mm) consisting of fused quartz containing an Ultra-1 non-polar methylsilicone phase. The temperature program was run from 150 °C (2 min isotherm) to 250 °C at 5 °C min⁻¹ and then from 250 to 300 °C at 10 °C min⁻¹. Data processing was carried out with a computer (HP-1000; Hewlett Packard) by using the standard programs of the GC-MS system (Hewlett Packard) as described by Doronina *et al.* (2003). The fatty acid profile of strain DSM 9103^T was composed of summed feature 7 (C_{18:1}7c, C_{18:1}9t and/or C_{18:1}12t, 67.63 %) and C_{19:0} cyclo ω8c (23.05 %). Strain LPM-4^T had rather similar profile containing summed feature 7 (67.31 %) and C_{19:0} cyclo ω8c (20.72 %) (see Supplementary Table S1 in IJSEM Online). Species of the genera *Aminobacter*, *Pseudaminobacter* and *Mesorhizobium* also contain summed feature 7, summed feature 4 (C_{16:1}7c and/or C_{15:0} iso 2-OH) and C_{16:0}, C_{17:0} iso and C_{18:0} fatty acids. The hydroxy fatty acid C_{12:0} 3-OH is also present in species of the genus *Aminobacter*. Fatty acids C_{17:1}ω8c and C_{20:1}ω9t were not detected in strains DSM 9103^T and LPM-4^T. The absence of fatty acids C_{15:0} iso 3-OH, C_{17:1}ω8c and C_{20:1}ω9t distinguished the novel strains from species of the genus *Pseudaminobacter*. In contrast to the novel strains, species of the genus *Mesorhizobium* contained high amounts of fatty acids C_{16:0} and C_{18:0} and low levels of summed feature 7. The absence of 11 methyl C_{18:1}ω7c and C_{17:0} cyclo fatty acids distinguished novel strains DSM 9103^T and LPM-4^T from the members of the genus *Mesorhizobium*.

For analysis of the cellular phospholipids, the novel strains were grown in minimal medium E containing 1 g l⁻¹ EDTA in batch culture at 28 °C. Cells of strains LPM-4^T and DSM 9103^T in the exponential growth phase (4 days) were harvested by centrifugation at 5000 g for 20 min. Cellular phospholipids were extracted using chloroform:methanol (1:2, v/v). The suspension was stirred for 1 h in an ice bath and was then centrifuged (5000 g, 20 min) to separate the two phases. The extraction procedure was performed twice and the upper phases were combined. Chloroform (2 ml) and distilled water (2 ml) were added to the supernatant with careful shaking for 15 min in an ice bath. The mixture was then centrifuged and three phases were separated. The lower phase containing the polar lipids was evaporated at 30 °C and dissolved in 200 µl chloroform. Polar lipids were separated by two-dimensional TLC (Kieselgel 60, 10 × 10 cm, Merck) using the solvent systems chloroform:methanol:water (65:25:4) for the first dimension, followed by chloroform:methanol:acetic acid:water (85:12:15:4) for the second dimension. Polar lipids were analysed as described by Tindall (1990). Non-specific detection of total lipids on the two-dimensional TLC plates was performed with iodine vapours. To detect lipids carrying free amino groups, the plates were sprayed with 0.2 % (w/v) ninhydrin dissolved in acetone followed by heating of the plates at 120 °C. To detect

phospholipids, two reagents were used: (i) 5 ml 60 % w/v perchloric acid, 10 ml 1 M HCl and 25 ml 4 % (w/v) ammonium molybdate (Skidmore & Entenmann, 1962); (ii) 10 % CuSO₄ in 8 % H₃PO₄ (Andreev & Galchenko, 1983) as seen in Supplementary Fig. S1 (available at IJSEM Online).

The polar lipids of strains DSM 9103^T and LPM-4^T were phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidyl-dimethylethanolamine (PDE), phosphatidylmonomethylethanolamine (PME) and diphosphatidylglycerol (DPG) (see Supplementary Fig. S1). This polar lipid profile was similar to those reported for species of the related genera *Aminobacter*, *Pseudaminobacter* and *Mesorhizobium*. The presence of the unknown phospholipids (PL1–PL3) and the unidentified aminophospholipid (APL) distinguished strains DSM 9103^T and LPM-4^T from members of the genera *Aminobacter* and *Pseudaminobacter*. Strains DSM 9103^T and LPM-4^T did not contain the *Mesorhizobium*-specific ornithine lipid, which was not reported to contain a phosphate group (Choma & Komaniecka, 2002).

Polyamines of strains LPM-4^T and DSM 9103^T were extracted according to the methods of Tkachenko *et al.* (2006). The dansylated polyamines were separated by using HPLC (Waters) equipped with two model 510 HPLC pumps, a U6K injector and a reversed-phase column (Hypersil ODS RP 18, 250 × 4.6 mm, 5 µm particles) as described by Altenburger *et al.* (1996). The polyamine patterns for strains DSM 9103^T and LPM-4^T were similar and consisted of large amounts of triamine *sym*-homospermidine [30.2 µmol (g dry wt)⁻¹], putrescine [6.5 µmol (g dry wt)⁻¹] and spermidine [5.3 µmol (g dry wt)⁻¹]. The genera *Aminobacter* and *Mesorhizobium* are characterized by the presence of *sym*-homospermidine as the predominant compound, high amounts of putrescine and low amounts of spermidine. The major polyamines of the genus *Pseudaminobacter* are spermidine [32.2 µmol (g dry wt)⁻¹], *sym*-homospermidine [18.6 µmol (g dry wt)⁻¹] and putrescine [15.6 µmol (g dry wt)⁻¹] (the level of polyamines given for the type species *Pseudaminobacter salicylatoxidans* BN12^T; Kämpfer *et al.*, 1999). Strains DSM 9103^T and LPM-4^T could be distinguished from members of the genus *Pseudaminobacter* by the minor amounts of putrescine and spermidine.

Ubiquinones were extracted and purified according to Collins (1985). Analysis was performed by MS (MX-1310; Finnigan). The quinone system of strains DSM 9103^T and LPM-4^T comprised major amounts of Q-10 (98 %) and minor amounts of Q-9. This pattern was in agreement with the ubiquinone pattern reported for the type species of the genera *Aminobacter* (Urakami *et al.*, 1992), *Pseudaminobacter* (Kämpfer *et al.*, 1999) and *Mesorhizobium* (Jarvis *et al.*, 1982, 1997).

DNA was isolated and purified according to Marmur (1961). DNA–DNA relatedness was determined as described previously (Doronina *et al.*, 2000). DNA–DNA relatedness between strains DSM 9103^T and LPM-4^T was 37 %. The DNA–DNA relatedness value between strains LPM-4^T and

BNC1 was 51%, indicating that the strains belong to separate species.

Other EDTA-degrading strains appear to be very closely related or even identical to strain DSM 9103^T (Nörtemann, 1999, 2005; Nörtemann *et al.*, 2004). In particular, strain BNC1, described formerly as *Mesorhizobium* sp. BNC1 and for which the genome has already been sequenced by the Joint Genome Institute (see <http://cmr.jcvi.org/tigr-scripts/CMR/GenomePage.cgi?org=ntms05>), exhibited 100% relatedness in DNA–DNA hybridizations with strain DSM 9103^T. This result means that the possibility that these organisms are identical cannot be excluded. A more detailed comparative study of strains DSM 9103^T, BNC1 and ANP11 is in progress.

The DNA–DNA relatedness values of strains DSM 9103^T and LPM-4^T with *Aminobacter aminovorans* DSM 7048^T and *Mesorhizobium loti* DSM 2626^T were low (10–11%). Hence, the results of the DNA–DNA hybridizations demonstrated that strains DSM 9103^T, BNC1 and LPM-4^T were clearly separated from representatives of the genera *Aminobacter* and *Mesorhizobium*.

DNA G+C content was determined by using the thermal denaturation (T_m) method with a spectrophotometer (DU-8B; Beckman) at a heating rate of 0.5 °C min⁻¹. The calculation was performed according to Owen & Lapage (1976) using the equation: mol G+C = ($T_m \cdot 2.08$) – 106.4. The DNA of *Escherichia coli* K-12 was used as a standard. The DNA G+C contents of strains LPM-4^T and DSM 9103^T were found to be 60.8 and 63.1 mol% (T_m), respectively.

The 16S rRNA genes of the novel strains were amplified and sequenced (Lane, 1991). The 16S rRNA gene sequences were aligned against those of representative taxa of the phylum *Proteobacteria* obtained from the GenBank database by using CLUSTAL W software (<http://www.genebee.msu.su/clustal>). Evolutionary distances were calculated by pairwise comparison of the aligned sequences (Jukes & Cantor, 1969), excluding all positions where there was a gap in any sequence in the alignment. In total, 1360 nucleotides for strains LPM-4^T and DSM 9103^T were used in the analysis. Phylogenetic relationships were determined by the neighbour-joining method and the programs from the TREECON software package (version 1.3b; Van de Peer & De Wachter, 1994), the PHYLIP package (v. 3.63), using the SEQBOOT, DNAML programs with bootstrap analysis of 100 trees, and MEGA version 4.0 (Tamura *et al.*, 2007). The phylogenetic trees were constructed by using various functions implemented in the TREECON program package (Fig. 2) and TreeView (Win32) version 1.6.6. (<http://taxonomy.zoology.gla.ac.uk/rod/rod.html>) (see Supplementary Fig. S2 in IJSEM Online) and MEGA4 (see Supplementary Figs S3–S5).

According to 16S rRNA gene sequence analysis, strains DSM 9103^T and LPM-4^T showed 99.3% similarity. They were closely related to members of the genera *Aminobacter* (94.8–95.9%), *Mesorhizobium* (94.2–96.9%) and *Pseuda-*

minobacter (95.0–95.7%) (Fig. 2 and Supplementary Fig. S2). The genera *Aminobacter* and *Mesorhizobium* had the highest sequence similarity (94.6–98.2%) followed by *Aminobacter* and *Pseudaminobacter* (96.4–97.0%) and *Mesorhizobium* and *Pseudaminobacter* (93.1–97.2%). The 16S rRNA gene sequence of strain DSM 9103^T was most similar (99.9% similarity) to that of strain BNC1 (Fig. 2 and Supplementary Fig. S2). Both of these strains could be included as members of the same species as suggested previously on the basis of the morphological, cytophysiological and biochemical data for strain BNC1 (Nörtemann, 1992; Bohuslavsek *et al.*, 2001). In the phylogenetic trees (see Fig. 2 and Supplementary Figs S2–S4), the novel EDTA-degrading bacteria represented a separate branch within the class *Alphaproteobacteria*.

Based on phenotypic and genotypic characteristics, strains DSM 9103^T and LPM-4^T should be placed in a separate new genus for which the name *Chelativorans* gen. nov. is proposed. Strains DSM 9103^T and LPM-4^T represent novel species within this genus for which the names *Chelativorans multitrophicus* gen. nov., sp. nov. and *Chelativorans oligotrophicus* sp. nov. are proposed, respectively. The key properties that differentiate the new genus from related genera of the class *Alphaproteobacteria* are listed in Table 1. Members of the genus *Chelativorans* gen. nov. can be distinguished from the genus *Aminobacter* by their inability to grow on methylated amines due to the absence of the appropriate C₁ enzymes, by the phospholipid and fatty acid profiles, by reproduction by binary fission, by the requirement for biotin and by the absence of PHB granules. Members of the genus *Chelativorans* gen. nov. can be distinguished from those of the genus *Mesorhizobium* by physiological differences, by some differences in the cellular fatty acids, by the absence of PHB granules and the presence of aminophospholipid (APL) in the polar lipid profile. Members of the genus *Mesorhizobium* are able to invade the root hairs of a wide range of plants and instigate the production of root nodules where atmospheric nitrogen can be reduced. However, a PCR amplification test using primers specific for the *nifH* gene (Fedorov *et al.* 2008) was negative in the novel strains, suggesting that they were not diazotrophs. In addition, representatives of the genera *Mesorhizobium* and *Aminobacter* are commonly pleomorphic and motile. Due to poor phenotypic descriptions for the genera *Aquamicrobium* and *Defluviobacter*, it is difficult to compare them with members of the genus *Chelativorans* gen. nov. However, these taxa are clearly distant phylogenetically (94.6–95.2% gene sequence similarity). Organisms that share 16S rRNA gene sequence similarities of lower than 95% are usually regarded as belonging to separate bacterial genera (Stackebrandt & Goebel, 1994). Formal descriptions of the new genus and the two novel species are given below.

Description of *Chelativorans* gen. nov.

Chelativorans (Che.la'ti.vor.ans. N.L. n. *chelatum* a chelate; L. part. adj. *vorans* devouring; N.L. masc. n. *Chelativorans* a bacterium digesting metal chelates).

Fig. 2. Phylogenetic position of *Chelativorans multitrophicus* DSM 9103^T and *Chelativorans oligotrophicus* LPM-4^T among related micro-organisms based on 16S rRNA gene sequences. All the sequences were aligned using the CLUSTAL W program. The similarity values were calculated with the Jukes–Cantor coefficient. The neighbour-joining method was used for construction of the phylogenetic tree and 100 subsamples were generated for bootstrap analysis using the TREECON program (1.3b). *Escherichia coli* 0157:H7 (AY513502) was used as an outgroup. Bar, 0.05 substitutions per base position.

Gram-negative, asporogenous, non-pleomorphic, rod-shaped and often found as pairs of cells. Non-motile. Multiply by binary fission. Form small white colonies (0.1–0.3 mm in diameter) on EDTA/mineral salt agar incubated for 7 days at 30 °C. Electron-dense inclusions found in EDTA-grown cells are thought to represent intracellular precipitates consisting of calcium and magnesium phosphates which are absent in fumarate-grown cells. PHB granules are absent. Oxidase- and catalase-positive. Do not reduce nitrate to nitrite. Do not fix atmospheric nitrogen. Mesophilic and neutrophilic; optimal pH and temperature for growth are 6.5–7.5 and 25–35 °C, respectively. Strictly aerobic with respiratory metabolism. Facultative or obligate utilizers of EDTA as the carbon, nitrogen and energy source. Do not utilize methanol and methylated amines as the carbon, nitrogen and energy source. Incapable of autotrophic or methylotrophic growth. Biotin is required as a growth factor. Oxidize EDTA by monooxygenase consuming flavin mononucleotide (FMN) H₂ delivered by

a NADH₂:FMN oxidoreductase. The major cellular fatty acids are summed feature 7 (consisting of C_{18:1}ω7c, C_{18:1}ω9t and/or C_{18:1}ω12t) and C_{19:0} cyclo ω8c. Hydroxy fatty acids such as C_{12:0} 3-OH, C_{13:0} 3-OH and C_{15:0} iso 3-OH are absent. The major ubiquinone is Q-10. Predominant polar lipids are PC, PG, PE, PDE, PME and DP. *Mesorhizobium*-specific ornithine lipid is absent. The major polyamine is sym-homospermidine and small amounts of spermidine and putrescine are detected. The DNA G + C content is 60–64 mol% (T_m). The genus is a member of the class *Alphaproteobacteria*. The type species is *Chelativorans multitrophicus*.

Description of *Chelativorans multitrophicus* sp. nov.

Chelativorans multitrophicus (mu.lti.tro'phi.cus. L. adj. *multus* many; Gr. adj. *trophikos* nursing, tending; N.L. masc. adj. *multitrophicus* utilizing many growth substrates).

Table 1. Main characteristics that differentiate the genera *Chelativorans*, *Aminobacter*, *Mesorhizobium*, *Pseudaminobacter*, *Aquamicrobium* and *Deffluviobacter*

Taxa: 1, *Chelativorans* gen. nov. (data from this study); 2, *Aminobacter* (Urakami *et al.*, 1992; Kämpfer *et al.*, 2002); 3, *Mesorhizobium* (Jarvis *et al.*, 1982, 1997; Tighe *et al.*, 2000); 4, *Pseudaminobacter* (Kämpfer *et al.*, 1999); 5, *Aquamicrobium* (Bambauer *et al.*, 1998); 6, *Deffluviobacter* (Fritsche *et al.*, 1999). +, Positive; –, negative; +/– variable; ND, not determined or data are not available for the taxon.

Characteristic	1	2	3	4	5	6
Reproduction by budding	–	+	–	–	–	–
Flagellation	–	+	+	+	+/–	+
Pleomorphism	–	+	+	–	–	–
Nitrate reduction	–	–	–	ND	+	–
Fix atmospheric N ₂	–	–*	+	ND	ND	ND
PHB	–	+	+	ND	ND	ND
Indole production	+	+	ND	ND	ND	–
Polyamines	<i>sym</i> -Homospermidine, spermidine, putrescine	<i>sym</i> -Homospermidine, putrescine, spermidine*	<i>sym</i> -Homospermidine, putrescine†	Spermidine, <i>sym</i> -homospermidine, putrescine, spermine, cadaverine, 1,3 diaminopropane‡	ND	Spermidine
Aminophospholipid	+	–	–	–	ND	ND
Fatty acids:						
C _{12:0} 3-OH	–	+	+	–	ND	ND
C _{13:0} iso 3-OH	–	–	+	–	ND	ND
C _{15:0} iso 3-OH	–	–	–	+	ND	ND
C _{17:1} ω8c	–	+	+	+	ND	ND
C _{20:1} ω9t	–	+	+	+	ND	ND

*Data from this study.

†Data from Hamana *et al.*, 1990.

‡Data are for *Pseudaminobacter salicylatoxidans* BN12^T (Kämpfer *et al.*, 1999).

Exhibits the following properties in addition to those given in the genus description. Facultative EDTA-utilizing rods of 0.5–1.0 × 0.7–2.0 µm. Grows on glucose, lactate, glutamate, fumarate, succinate, acetate, nitrilotriacetate, iminodiacetate, *N,N'*-ethylenediaminediacetate, and ethylenediaminedisuccinate as the sole carbon and energy sources. No growth on diethylenetriaminepentaacetate or hydroxyethylethylenediaminetriacetate. The generation time with EDTA as the source of carbon, nitrogen and energy at pH 7.0 and 30 °C is 14 h. Good, but slow growth on complex media at low concentrations of organic compounds, such as Plate Count Broth at one third of the normal strength. No growth in the presence of 3% (w/v) NaCl. Able to degrade uncomplexed EDTA and Ca²⁺–, Ba²⁺–, Mg²⁺–, Mn²⁺–, Pb²⁺–, Cu²⁺– or Zn²⁺–EDTA complexes, but not Fe³⁺–EDTA complex. Resistant to oxacillin, ampicillin and lincomycin, but sensitive to gentamicin, streptomycin, novobiocin, nalidixic acid and neomycin. Shows α-ketoglutarate dehydrogenase activity.

The type strain, DSM 9103^T (=VKM B-2394^T), was isolated from a mixture of soil extracts and activated sludge samples taken from various industrial wastewater treatment plants (Switzerland). The DNA G + C content of the type strain is 63.1 mol% (*T*_m).

Description of *Chelativorans oligotrophicus* sp. nov.

Chelativorans oligotrophicus (o.li.go.tro'phicus. Gr. adj. *oligos* little; Gr. adj. *trophikos* nursing, tending; N.L. masc. adj. *oligotrophicus* utilizing only a few growth substrates).

Exhibits the following properties in addition to those given in the genus description. Obligate EDTA-utilizing rods of 0.5–1.0 × 0.7–2.0 µm. The generation time of the strain grown with EDTA as the source of carbon, nitrogen and energy at pH 7.0 and 32–34 °C is 7 h. Able to degrade uncomplexed EDTA and Mn²⁺–, Ca²⁺–, Mg²⁺– and Ba²⁺–EDTA and slowly degrade Zn²⁺–EDTA, but not Pb²⁺–, Cu²⁺– or Fe³⁺–EDTA. Does not grow on peptone/yeast extract medium, nutrient broth, nutrient agar or in medium containing carbohydrates (D-glucose, D-fructose) or amino acids, or ethanol or methanol or organic acids (acetate, fumarate, succinate and citrate). Resistant to oxacillin, ampicillin, novobiocin, lincomycin, nalidixic acid, neomycin, but sensitive to gentamicin and streptomycin. Has multiple enzymic lesions in the central carbon metabolism. The tricarboxylic acid cycle is deficient in α-ketoglutarate dehydrogenase activity. The activities of 6-phosphofructokinase (ATP/PPi) are also absent.

The type strain, LPM-4^T (=VKM B-2395^T=DSM 19276^T), was isolated from municipal sewage sludge samples taken near Pushchino, Moscow region, Russia. The DNA G+C content of the type strain is 60.8 mol% (T_m).

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