Massilia brevitalea sp. nov., a novel betaproteobacterium isolated from lysimeter soil

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A Gram-negative, strictly aerobic bacterium, designated strain byr23-80^T, was isolated from lysimeter soil by using a high-throughput cultivation technique. Cells of strain byr23-80^T were found to be short rods that multiplied by binary fission and were motile by means of a single polar flagellum. Occasionally, two to three polar or lateral flagella were observed. The optimum growth temperature was 15 °C and the pH optimum was 7.0–7.5. The predominant cellular fatty acids were C_{16:1}ω7c (54.7 %) and C_{16:0} (21.4 %). In addition, the diagnostic fatty acids C_{10:0} 3-OH and C_{12:0} 2-OH were detected. Q-8 was the predominant respiratory quinone. The isolate was physiologically very versatile, using a wide range of sugars, organic acids and amino acids as single carbon and energy sources for growth. The G + C content of the genomic DNA was 65.3 mol%. Phylogenetic analyses supported the assignment of strain byr23-80^T to the genus *Massilia* within the family *Oxalobacteraceae* of the class *Betaproteobacteria*. Within the genus, strain byr23-80^T was most closely related to *Massilia aurea* DSM 18055^T, with a 16S rRNA gene sequence similarity of 98.3 %. However, DNA–DNA hybridization revealed a pairwise similarity for the genomic DNA of only 20.1 % between strain byr23-80^T and strain DSM 18055^T. The novel isolate could be distinguished from the existing species *Massilia timonae*, *Massilia dura*, *Massilia albidiflava*, *Massilia plicata*, *Massilia lutea* and *M. aurea* by its significantly lower temperature optimum for growth and by the absence of gelatinase, α-galactosidase and β-galactosidase activities. On the basis of these characteristics, strain byr23-80^T constitutes a novel species of the genus *Massilia*, for which the name *Massilia brevitalea* sp. nov. is proposed. The type strain is byr23-80^T (=DSM 18925^T=ATCC BAA-1465^T).

The genus *Massilia* comprises the following six species: *Massilia timonae* (La Scola *et al.*, 1998), *Massilia dura*, *Massilia albidiflava*, *Massilia plicata*, *Massilia lutea* (Zhang *et al.*, 2006) and *Massilia aurea* (Gallego *et al.*, 2006). *M. timonae* strains were isolated from human blood, cerebrospinal fluid and bone (La Scola *et al.*, 1998; Lindquist *et al.*, 2003). Bacteria of this species are straight rods that are motile by means of single polar flagellum or, more rarely, up to three polar or lateral flagella per cell. They are distinguishable from other aerobic, non-fermentative, Gram-negative rods on the basis of their 16S rRNA gene sequence, acid production from carbohydrates and positive reactions for catalase, oxidase, RNase, pectinase, chondroitinase, starch hydrolysis and aesculin hydrolysis. Other

typical characteristics are the presence of C_{16:0}, C_{16:1}ω7c, and C_{18:1}ω7c as the predominant fatty acids and the presence of C_{10:0} 3-OH and C_{12:0} 2-OH as diagnostic cellular fatty acids (Lindquist *et al.*, 2003). *M. dura*, *M. albidiflava*, *M. plicata* and *M. lutea* were recovered from farm soil contaminated with heavy metals and, with the exception of *M. lutea*, differed from *M. timonae* in their production of urease and their ability to reduce nitrate (Zhang *et al.*, 2006). *M. aurea* was isolated from drinking water and differed from the other species by the absence of β-glucosidase and α-galactosidase (Gallego *et al.*, 2006). Additional unidentified members of the genus *Massilia* utilize glucose (Padmanabhan *et al.*, 2003) and have the capacity to degrade phenanthrene (Bodour *et al.*, 2003) or other aromatic compounds (Khammar *et al.*, 2005).

Strain byr23-80^T was isolated during the investigation of bacterial communities in soil lysimeters (Zul *et al.*, 2007).

Abbreviation: TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the partial 16S rRNA gene sequence of strain byr23-80^T is EF546777.

The high-throughput MicroDrop technique (Bruns *et al.*, 2003) was employed to inoculate bacterial cells into microtitre plates containing soil solution equivalent (Angle *et al.*, 1991), which was buffered at a pH of 7.0 using 10 mM HEPES and supplemented with artificial root exudates, yeast extract (0.01 %, w/v) and inducers, as described previously (Bruns *et al.*, 2003; Zul *et al.*, 2007). Cultures were incubated at 15 °C for 6 weeks. Bacterial strains were isolated by streaking liquid cultures onto the medium described above, solidified with gellan gum (8 g l⁻¹; Sigma-Aldrich Chemie). One of the cultured betaproteobacteria, strain byr23-80^T, was selected for further characterization since it occurred in association with higher plants (Zul *et al.*, 2007). Subsequent physiological, biochemical and phylogenetic characterization revealed that the isolate represents a novel member of the genus *Massilia* within the family *Oxalobacteraceae* of the order *Burkholderiales*.

The shape and colour of colonies was examined on agar-solidified HD medium (1:10 diluted; consisting of 0.05 % casein peptone, 0.01 % glucose and 0.025 %, w/v, yeast extract) as well as on nutrient agar (Sigma). Colonies were pale white (young cultures) to yellowish (old cultures) on HD (1:10) agar plates and yellow on nutrient agar. Cells of strain byr23-80^T grown for 36 h in HD (1:10) were short

rods 1.5–2.0 µm in length and 0.7–1.0 µm in width (Fig. 1a); they divided by binary fission and were motile. In contrast, cells grown in undiluted HD medium became significantly elongated (up to 35 µm) and varied in shape (Fig. 1b). For scanning electron microscopy, drops of the sample were placed onto a glass slide, covered with a cover-slip and rapidly frozen with liquid nitrogen. The cover-slip was removed with a razor blade and the cells were immediately fixed with 2.5 % glutaraldehyde in 75 mM cacodylate buffer (pH 7.0), post-fixed with osmium tetroxide, dehydrated in a graded series of acetone solutions, critical-point dried with liquid CO₂, mounted on stubs and coated with 3 nm platinum with a magnetron sputter coater (BAE 121; Balzers). The specimens were examined with a field emission scanning electron microscope (S-4100; Hitachi) operated at 5 kV. Most cells exhibited a single polar flagellum (Fig. 2a). Occasionally, cells with two to three polar or lateral flagella were observed.

Cells stained Gram-negative, which was confirmed by transmission electron microscopy (TEM) of cells harvested in the exponential growth phase. Cells were fixed with 2.5 % glutaraldehyde in 75 mM sodium cacodylate and 2 mM MgCl₂ (pH 7.0) for 1 h at room temperature, rinsed several times in fixative buffer and post-fixed for 1 h with 1 % osmium tetroxide in fixative buffer at room

Fig. 1. (a) Phase-contrast photomicrograph of cells of strain byr23-80^T grown in 1:10-diluted HD medium at 15 °C for 36 h. (b) Morphology of cells grown in undiluted HD medium at 15 °C for 36 h. Bars, 10 µm.

Fig. 2. (a) Scanning electron micrograph of cells of strain byr23-80^T showing the monopolar, monotrichous flagellation and numerous protuberances of the cell envelope. (b) Transmission electron micrograph of an ultrathin section of cells, showing the cell envelope, typical of Gram-negative bacteria, and the protuberances of the outer membrane. Bars, 1 µm (a) and 500 nm (b).

temperature. After two washing steps with distilled water, the cells were stained *en bloc* with 1 % uranyl acetate in 20 % acetone for 30 min. Dehydration was performed with a graded acetone series. Samples were then infiltrated with, and then embedded in, Spurr's low-viscosity resin (Spurr, 1969); ultrathin sections (50 and 70 nm) were cut with a diamond knife and mounted on uncoated copper grids. The sections were post-stained with aqueous lead citrate (100 mM, pH 13.0). All micrographs were taken with a TEM (EM 912; Zeiss) equipped with an integrated OMEGA energy filter operated in zero-loss mode. Investigations of exponentially growing cells confirmed the presence of an outer membrane, a thin murein layer and a cytoplasmic membrane (Fig. 2b). A striking feature of the cells was the presence of numerous protuberances of various sizes, formed by the outer membrane, over the entire cell surface (Fig. 2a, b).

Catalase and oxidase tests were performed as described by Lányi (1987) and revealed that strain byr23-80^T was oxidase-negative and weakly catalase-positive. The temperature range and the optimum temperature for growth in HD (1:10) medium were 4–30 °C and 15 °C, respectively. While the temperature range for the novel strain was comparable with that of *M. aurea*, the optimum temperature for growth was unusual in that it was significantly lower than that of any recognized *Massilia* species (Table 1). Subsequent growth tests were therefore performed at 15 °C. The pH range for growth was 6.0–10.0, with an optimum value of 7.0–7.5. Salt tolerance was tested at NaCl concentrations between 0 and 10 % (w/v) and demonstrated that growth occurred at concentrations ≤ 2 % NaCl. Cells grew obligately aerobically and were capable of reducing NO₃⁻ to NO₂⁻, as tested on nitrate broth containing 0.2 % KNO₃ (Skerman, 1967). Hydrolysis of starch was tested on agar plates containing 1 % (w/v) peptone, 1 % yeast extract and 0.5 % soluble starch. Gelatinase production was determined on Luria–Bertani plates containing 3 % (w/v) gelatin. Hydrolysis of casein was examined on nutrient agar supplemented with 1.5 % skim milk (Sigma). Milk coagulation and peptonization were tested on 10.0 % (w/v) litmus milk medium. These assays demonstrated that the cells were capable of hydrolysing starch and casein but not gelatin. The inability of strain byr23-80^T to hydrolyse gelatin was a feature that clearly distinguished it from all recognized species of the genus *Massilia* (Table 1). Additional enzyme activities were determined by employing API ZYM galleries (bioMérieux) according to the protocol of the manufacturer and with incubation of the assays at 15 °C. Strain byr23-80^T gave strong positive reactions for acid phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase. In contrast to all other *Massilia* species tested, however, the novel isolate did not exhibit α -galactosidase or β -galactosidase activities. It also tested negative for arginine dihydrolase, ornithine decarboxylase and lysine decarboxylase activities. HD medium at a 1:10 dilution was used to analyse the methyl red and Voges–Proskauer reactions by

adding methyl red and Barritt's reagents, respectively. Indole production was examined in tryptophan broth, with detection using the addition of Kovács reagent. Urease production was determined in media supplemented with 0.5 % urea (Ederer *et al.*, 1971) and H₂S production was tested on Kligler's iron agar (Difco). Cells of strain byr23-80^T tested negative for the production of acetoin, indole and H₂S and did not exhibit urease activity.

Substrate utilization patterns were determined in microtitre plates filled with basal soil solution equivalent medium supplemented with individual carbon substrates (Gich & Overmann, 2006). Substrate utilization was evaluated by repeatedly measuring the optical density at 620 nm in each well using a microtitre plate reader (Tecan) over an incubation period of 6 weeks. Cellobiose, D-erythrose, L-erythrulose, D-galactose, glucose, glucose 1-phosphate, glucose 6-phosphate, maltose and N-acetylglucosamine were utilized as sole carbon and energy sources. In addition, the strain grew on 19 of 36 organic acids and on 13 of 22 amino acids tested, but did not utilize any of the eight alcohols tested (Table 1). The strain was also able to utilize α -ketoisocaproate, α -ketoglutarate, α -ketovaleate, Casamino acids, yeast extract and peptone.

Antibiotic susceptibilities were determined by using the disc-diffusion plate method, using HD (1:10) agar plates inoculated with strain byr23-80^T. The 16 antibiotics and antibiotic combinations tested were as follows (μ g per disc, unless otherwise stated): amikacin (30), ampicillin (25), bacitracin (10 U per disc), carbenicillin (100), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin monosulfate (30), novobiocin (5), penicillin G (10), rifampicin (5), streptomycin sulfate (30), sulfamethoxazole + trimethoprim (23.7 and 1.2), tetracycline (30), tobramycin (10) and vancomycin (30). Resistance was observed towards bacitracin, carbenicillin, chloramphenicol, erythromycin, gentamicin, novobiocin, and penicillin G.

The results of the chemotaxonomic analyses are given in Table 2 and the species description. For these determinations, cells were grown under optimum conditions on HD (1:10) at 15 °C for 36 h. Respiratory lipoquinones and polar lipids were extracted from, respectively, 2 and 0.1 g freeze-dried cell material and analysed according to Tindall (1990a, b). For the fatty acid analysis, a 40 mg (wet weight) sample of cells was scraped from Petri dishes and the fatty acid methyl esters were extracted according to the method of Miller (1982) and Kuykendall *et al.* (1988), using the Microbial Identification System (MIDI). These analyses were carried out by the Identification Services of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The major cellular fatty acids were C_{16:1}ω7c (54.7 %) and C_{16:0} (21.4 %). Similarly to members of the genus *Massilia* (Lindquist *et al.*, 2003), cells of strain byr23-80^T contained C_{10:0} 3-OH and C_{12:0} 2-OH as the diagnostic cellular fatty acids. The main quinone component was Q-8.

Table 1. Main morphological, biochemical and physiological characteristics of strain byr23-80^T in comparison with those of species within the genus *Massilia*

Strains: 1, byr23-80^T; 2, *M. timonae* CIP 105350^T (Lindquist *et al.*, 2003; La Scola *et al.*, 1998); 3, *M. dura* CCTCC AB 204070^T; 4, *M. albidiflava* CCTCC AB 204071^T; 5, *M. plicata* CCTCC AB 204072^T; 6, *M. lutea* CCTCC AB 204073^T (data for strains 3–6 from Zhang *et al.*, 2006); 7, *M. aurea* DSM 18055^T (Gallego *et al.*, 2006). All of the strains are Gram-negative, motile, non-spore-forming rods to short-rods, negative for arginine dihydrolase, ornithine decarboxylase, indole and H₂S production and grow in nutrient broth/agar. The carbon substrates tested and found not to be utilized by strain byr23-80^T are as follows: *N*-acetyl-D-galactosamine, acetoin, adonitol, D-arabinose, (+)-D-arabitol, (+)-L-arabitol, ascorbate, benzoate, γ -hydroxybenzoate, α -hydroxybutyrate, γ -hydroxybutyrate, isobutyrate, trimethoxybenzoate, citrate, isocitrate, α -cyclodextrin, dextrin, dulcitol, i-erythritol, formate, (+)-D-fucose, L-fucose, β -gentiobiose, D-gluconate, D-glucuronate, (+)-D-glucosamine, glycerol, glycogen, glycolate, glyoxylate, *myo*-inositol, β -D-lactose, D-lactose, lactulose, D-lyxose, malate, (+)-D-mannose, D-mannitol, (+)-D-melezitose, melibiose, D-psicose, (+)-D-raffinose, ribitol, D-ribose, shikimate, D-sorbitol, L-sorbose, D-tagatose, tartrate, turanose, xylitol and (+)-D-xylose, methanol, ethanol, propanol, 1,2-propanediol, butanol, 1,2-butanediol, 2,3-butanediol and ethylene glycol. Other carbon sources utilized by strain byr23-80^T are as follows: acetate, adipate, butyrate, β -hydroxybutyrate, caproate, hexanoate, caprylate, crotonate, D-erythrose, L-erythrulose, fumarate, glucose 1-phosphate, glucose 6-phosphate, isovalerate, lactate, malate, *N*-acetylglucosamine, oxaloacetate, propionate, protocatechuate, pyruvate, succinate and valerate. +, Positive; –, negative; w, weak; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Cell size (μm)	0.7–1.0 $\times$ 1.5–2.0	1.0 $\times$ 3.0	0.6–0.8 $\times$ 1.8–2.2	ND	0.6–0.7 $\times$ 1.8–2.2	ND	1.0 $\times$ 1.6–3.0
Growth characteristics:							
pH range	6.0–10.0	ND	6.5–8.5	6.5–8.5	6.5–8.5	6.5–8.5	4.0–9.0
pH optimum	7.0–7.5	ND	7.0–7.5	7.0–7.5	7.0–7.5	7.0–7.5	7.0–8.0
Temp. range ($^{\circ}\text{C}$)	4–30	25–35	10–45	10–45	10–45	10–45	4–30
Temp. optimum ($^{\circ}\text{C}$)	15	28	28–30	28–30	28–30	28–30	28
Flagellation	Polar or lateral	Polar or lateral	Lateral	Lateral	Lateral	Lateral	ND
Voges–Proskauer test	–	–	–	–	+	–	–
Methyl red test	–	–	+	–	–	–	–
Hydrolysis of:							
Starch	+	+	–	+	+	+	+
Casein	+	ND	+	+	+	+	+
Gelatin	–	+	+	+	+	+	+
Nitrate reduction	+	–	+	+	+	–	–
Urease	–	–	+	+	+	–	–
Oxidase	–	+	+	+	–	+	w
Catalase	w	+	+	+	+	+	+
Milk coagulation	–	ND	–	–	–	–	ND
Peptonization	–	+	–	–	–	–	ND
Enzyme activities:							
α -Chymotrypsin	–	ND	ND	ND	ND	ND	–
α -Fucosidase	–	ND	ND	ND	ND	ND	–
α -Galactosidase	–	ND	+	+	+	+	–
β -Galactosidase	–	ND	+	+	+	+	+
β -Glucuronidase	–	ND	+	+	–	+	–
α -Glucosidase	+	ND	+	+	+	+	+
β -Glucosidase	–	ND	+	+	+	+	–
Lipase	–	ND	–	+	+	+	–
<i>N</i> -acetylglucosaminidase	–	ND	+	+	–	+	–
Lysine decarboxylase	–	–	–	+	–	–	–
Utilization of:							
Glucose	+	–	+	+	+	+	+
Sucrose	–	–	+	+	+	+	–
D-Fructose	–	–	ND	ND	ND	ND	+
D-Galactose	+	–	ND	ND	ND	ND	+
L-Rhamnose	–	+	–	–	–	–	+
Trehalose	–	–	–	ND	–	ND	–
Malonate	–	+	–	ND	+	ND	ND
Maltose	+	+	–	ND	–	ND	+
Cellobiose	+	+	–	–	–	–	+
L-Arabinose	–	+	–	–	–	–	–
Galacturonate	–	+	–	–	–	–	ND
Isolate source	Soil	Blood	Soil	Soil	Soil	Soil	Drinking water
DNA G + C content (mol%)	65.3	62–67	65.9	65.3	65.1	63.3	66.0

Table 2. Cellular fatty acid content (%) of strain byr23-80^T and species of the genus *Massilia*

Strains: 1, byr23-80^T; 2, *M. timonae* CIP 105350^T (Lindquist *et al.*, 2003); 3, *M. dura* CCTCC AB 204070^T; 4, *M. albidiflava* CCTCC AB 204071^T; 5, *M. plicata* CCTCC AB 204072^T; 6, *M. lutea* CCTCC AB 204073^T (data for strains 3–6 from Zhang *et al.*, 2006); 7, *M. aurea* DSM 18055^T (Gallego *et al.*, 2006). tr, Trace.

Fatty acid	1	2	3	4	5	6	7
Saturated fatty acids							
C _{10:0}	0.5	1.0	0.6	1.3	1.4	0.5	0.4
C _{12:0}	5.1	8.0	5.6	6.0	7.1	2.8	4.6
C _{14:0}	2.1	tr	3.2	2.8	1.6	1.9	0.6
C _{15:0}	1.2	tr	–	–	–	–	–
C _{16:0}	21.4	25.0	25.5	22.8	25.1	22.8	36.8
Unsaturated fatty acids							
C _{16:1} ω7c	54.7	43.0	46.7	46.4	36.9	54.7	48.3
iso-C _{17:1} ω9c	0.4	–	–	–	–	0.7	–
C _{18:1} ω7c	7.5	10.0	5.9	7.4	11.7	10.0	2.5
Hydroxy fatty acids							
C _{10:0} 3-OH	4.3	8.0	8.3	9.6	10.1	4.6	4.8
C _{12:0} 2-OH	1.4	3.0	–	0.7	–	–	1.9
C _{14:0} 2-OH	–	–	3.4	3.0	6.1	2.0	–

The G+C content of the genomic DNA was calculated from the mid-point value (T_m) of the melting profile (Øvreas *et al.*, 2003) using the formula described by Mandel *et al.* (1970). Bacterial genomic DNA for the DNA G+C content determination (and for the analysis of the 16S rRNA gene sequence) was isolated using the method of Marmur (1961). The DNA G+C content was 65.3 mol%. The fatty acid profile, major respiratory quinone and DNA G+C content of strain byr23-80^T were all typical of those for species within the genus *Massilia* (Table 1).

For a higher resolution of the relationship between the novel strain and recognized species of the genus *Massilia*, the 16S rRNA gene sequence of byr23-80^T was amplified using primers 8f and 1492r (Lane, 1991) and subsequently sequenced using eight primers to cover the entire 16S rRNA gene (Gich *et al.*, 2005). The sequence of strain byr23-80^T and recently described species were retrieved from the GenBank database using BLAST 2.0.4 (Altschul *et al.*, 1997), imported into the ARB database and analysed using the ARB software package (Ludwig *et al.*, 2004). The FAST ALIGNER (version 1.03) tool from the ARB software package was used for automated sequence alignment with previously aligned sequences from the ARB database. The alignment was subsequently checked and corrected manually on the basis of secondary structure information. Maximum-likelihood and neighbour-joining methods were employed to construct phylogenetic trees and the sequence similarities were calculated using the ARB distance matrix. The robustness of the tree topologies was evaluated by calculating bootstrap values (Felsenstein, 1985) using 1000 resamplings.

On the basis of the phylogenetic tree obtained (Fig. 3), strain byr23-80^T fell within the group of existing lineages of the genus *Massilia*. Similar results were obtained using the maximum-likelihood method (not shown). The closest described relative of strain byr23-80^T was *M. aurea* DSM 18055^T, for which a similarity value of 98.3 % was calculated. The sequence similarities with respect to all other recognized *Massilia* species were ≤96.4 %. The 16S rRNA gene sequence divergence between strain byr23-80^T and *M. aurea* DSM 18055^T was within the range commonly used as a criterion for the separation of two bacterial species (Stackebrandt & Goebel, 1994). Therefore DNA–DNA hybridization was carried out to evaluate whether strain byr23-80^T should be considered as a separate novel species. DNA–DNA hybridization was performed as described by De Ley *et al.* (1970), with the modifications described by Huß *et al.* (1983). The chromosomal DNA for DNA hybridization was isolated using a French pressure cell (Thermo Spectronic) and purified by means of chromatography on hydroxyapatite (Cashion *et al.*, 1977). The similarity value between strain byr23-80^T and *M. aurea* DSM 18055^T was 20.1 % and hence significantly lower than the threshold value of 70 % that is commonly accepted for the definition of bacterial species (Wayne *et al.*, 1987).

On the basis of the phenotypic and genotypic data presented, strain byr23-80^T represents a novel species within the genus *Massilia*, for which the name *Massilia brevitalia* sp. nov. is proposed.

Description of *Massilia brevitalia* sp. nov.

Massilia brevitalia (bre.vi.ta'le.a. L. adj. *brevis* short; L. fem. n. *talea* a rod; N.L. fem. n. *brevitalia* a short-rod, referring to the shape of cells).

Cells are Gram-negative, short rods that are 0.7–1.0 µm wide and 1.5–2.0 µm long and motile by means of a single polar flagellum. Occasionally, cells with two or three polar or lateral flagella are observed. Cells are non-spore-forming, occur singly or in pairs (on 1:10-diluted HD medium at 15 °C after 36 h) and are obligately aerobic. Colonies are circular, entire, convex, opaque and pale white to yellow. Cells grow chemo-organotrophically on diluted HD (1:10) agar and on nutrient agar. Oxidase-negative and weakly positive for catalase. The temperature range for growth is 4–30 °C, with optimum growth occurring at 15 °C. The pH range for growth is 6.0–10.0, with optimum growth occurring at pH 7.0–7.5. Can grow at NaCl concentrations of ≤2 % (w/v). Tweens 20 and 80, casein and starch are hydrolysed, but gelatin is not hydrolysed. Positive for the following enzyme activities: acid phosphatase, alkaline phosphatase, α-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and urease. Distinguished from recognized species of the genus *Massilia* by an inability to hydrolyse gelatin and by the absence of α-galactosidase and β-galactosidase activities. Tests negative for milk coagulation, indole production,

Fig. 3. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain byr23-80^T and related bacteria within the family Oxalobacteraceae of the class Betaproteobacteria. The tree was rooted using *Ralstonia eutropha* ATCC 17697^T as an outgroup. Bootstrap percentages (based on 1000 bootstrap resamplings) >50% are given at branching points. Bar, 0.01 fixed point mutations per nucleotide.

hydrogen sulfide production and gives a negative result in the methyl red and Voges–Proskauer tests. Reduces NO₃⁻ to NO₂⁻. Cellobiose, D-erythrose, L-erythrulose, D-galactose, glucose, glucose 1-phosphate, glucose 6-phosphate, maltose and N-acetylglucosamine are utilized as sole carbon and energy sources. Growth occurs on acetate, adipate, butyrate, crotonate, hexanoate, caproate, caprylate, fumarate, β-hydroxybutyrate, isovalerate, α-ketoglutarate, α-ketoisohexanoate, α-ketovalerate, lactate, malate, oxaloacetate, propionate, protocatechuate, pyruvate, succinate, valerate and (slowly) levulinate. (+)-L-Alanine, L-alanyl-glycine, L-asparagine, (+)-L-asparagine, (+)-L-cysteine, (+)-L-glutamate, (+)-L-isoleucine, (+)-L-leucine, (+)-L-lysine, (+)-L-phenylalanine, (+)-L-serine, (+)-L-threonine and (+)-L-tyrosine are utilized. Growth occurs on Casamino acids, yeast extract and peptone as complex organic substrates. The DNA G+C content is 65.3 mol%. Q-8 is the predominant respiratory quinone and the major cellular fatty acids are C_{16:1ω7c} and C_{16:0}. In addition, the diagnostic fatty acids C_{10:0} 3-OH and C_{12:0} 2-OH are present.

The type strain, byr23-80^T (=DSM 18925^T=ATCC BAA-1465^T), was isolated from lysimeter soil from the Botanical Garden at the University of Bayreuth, Germany.

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