Pseudogracilibacillus auburnensis gen. nov., sp. nov., isolated from the rhizosphere of *Zea mays*

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A Gram-positive-staining, aerobic, endospore-forming bacterium, strain P-207^T, was isolated from a rhizosphere soil sample in Auburn, AL, USA. On the basis of 16S rRNA gene sequence comparisons, strain P-207^T was grouped in the vicinity of representatives of the genera *Virgibacillus*, *Ornithinibacillus*, *Cerasibacillus*, *Lentibacillus* and *Oceanobacillus*, but could not be assigned clearly to any of these genera. The highest similarity was found to the sequence of *Virgibacillus carmonensis* LMG 20964^T (94.4 %); however, the 16S rRNA gene sequence similarity to the type strain of the type species of *Virgibacillus*, *Virgibacillus pantothenticus*, was only 92.9 %. The quinone system of strain P-207^T consisted predominantly of menaquinone MK-7. The polar lipid profile exhibited the major lipids diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine and moderate to minor amounts of several unidentified phospholipids, glycolipids and phosphoglycolipids, an aminophospholipid and an aminolipid. The diagnostic diamino acid of the peptidoglycan was *meso*-diaminopimelic acid and the polyamine pattern contained predominantly spermidine and spermine. The major fatty acids were anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$ and iso-$C_{15:0}$. The G+C content of the genomic DNA was 34 mol%. Because of the low sequence similarity of strain P-207^T to all representatives of *Virgibacillus*, *Ornithinibacillus*, *Cerasibacillus*, *Lentibacillus* and *Oceanobacillus*, which was always <95 %, and its unique lipid pattern, we propose that strain P-207^T represents a novel species in a new genus, for which the name *Pseudogracilibacillus auburnensis* gen. nov., sp. nov. is proposed. The type strain of *Pseudogracilibacillus auburnensis* is P-207^T (=CCM 8509^T=LMG 28212^T=CIP 110797^T).

The number of genera within the family *Bacillaceae* is growing rapidly as it is becoming obvious that the diversity of endospore-forming aerobic and facultatively anaerobic bacteria of the *Firmicutes* is far higher than earlier supposed. In addition, the number of species with validly published names (http://www.bacterio.net) isolated from various sources is growing tremendously; among them are halophilic, halotolerant, alkaliphilic and/or alkalitolerant representatives (Ash *et al.*, 1991; Nielsen *et al.*, 1994; Ventosa *et al.*, 1998; Arahal & Ventosa, 2002; Romano *et al.*, 2005; Lim *et al.*, 2006b, c; Carrasco *et al.*, 2007; Yumoto, 2007; Aino *et al.*, 2008; Chen *et al.*, 2009b, 2011; Liu *et al.*, 2009). Within the *Bacillaceae*, several species of the genus *Bacillus* were isolated from rhizosphere soil, e.g. *Bacillus rhizosphaerae* (Madhaiyan *et al.*, 2011), *Bacillus methylotrophicus* (Madhaiyan *et al.*, 2010), *Bacillus koreensis* (Lim *et al.*, 2006a) and *Bacillus patagoniensis* (Olivera *et al.*, 2005).

A cultivation-dependent analysis of rhizosphere soil from Auburn, AL, USA, indicated a high diversity of as-yet unknown bacterial taxa, several of which have been assigned to proposed novel species, *Cohnella rhizosphaerae* (Kämpfer *et al.*, 2014a), *Chryseobacterium zeae*, *Chryseobacterium arachidis* and *Chryseobacterium geocarposphaerae* (Kämpfer *et al.*, 2014b). During these studies, strain P-207^T was isolated from one of the rhizosphere soil samples on nutrient agar (NA; Oxoid) at 30 °C. The organism was maintained on NA after incubation at 30 °C for 48 h.

Phylogenetic analyses based on nearly full-length 16S rRNA gene sequences were performed in ARB release 5.2 (Ludwig

Abbreviation: Dpm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P-207^T is KJ490639.

et al., 2004) using the All-Species Living Tree Project (LTP; Yarza *et al.*, 2008) database release LTPs111 (February 2013). Sequences not included in the LTP database were aligned with SINA (version 1.2.11) according to the SILVA seed alignment (http://www.arb-silva.de; Pruesse *et al.*, 2012) and implemented in the ARB database, where the alignment including all sequences necessary for phylogenetic calculations was checked manually based on secondary structure information. Phylogenetic trees were reconstructed with the maximum-likelihood method using RAxML version 7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis, the neighbour-joining method with the Jukes–Cantor correction (Jukes & Cantor, 1969) and the maximum-parsimony method using DNAPARS version 3.6 (Felsenstein, 2005). Phylogenetic trees were calculated with 100 resamplings (bootstrap analysis; Felsenstein, 1985) and based on 16S rRNA gene sequences between positions 132 and 1427 according to the *Escherichia coli* numbering (Brosius *et al.*, 1978). The reconstruction of phylogenetic trees based on different treeing methods showed that strain P-207^T always grouped separately and distinctly from representatives of the closest related genera *Virgibacillus*, *Ornithinibacillus*, *Cerasibacillus*, *Lentibacillus* and *Oceanobacillus* (Fig. 1). Pairwise sequence similarities were calculated in the ARB neighbour-joining tool without using an evolutionary substitution model. The novel strain showed very low sequence similarities (<94 %) to the type strains representing species of related genera.

Cell morphology, abundance and localization of endospores and motility were investigated at × 1000 magnification with a Zeiss light microscope using cells that were grown for 3 days at 25 °C on tryptic soy agar (TSA; Oxoid). Gram-staining was performed by the modified Hucker method according to Gerhardt *et al.* (1994). Salt- and pH-dependent growth was tested in tryptic soy broth (TSB; Oxoid) containing 0–12 % (w/v) NaCl increasing in 1 % NaCl concentration steps and at pH 4.5–12.5, tested in steps of 1.0 pH unit. The pH was adjusted by adding HCl and NaOH. Temperature-dependent growth was tested at 4, 10, 15, 20, 25, 28, 30, 36, 45, 50 and 55 °C on TSA. Physiological characterization including substrate utilization, acid production and enzyme activity was done according to the methods described by Kämpfer *et al.* (1991) and Kämpfer (1990). In addition, the presence of urease was tested on urea agar (Merck) supplemented with 2 % urea according to Christensen (1946). Production of indole and sulphide was tested on SIM agar (sulfate-indole-motility medium) according to the instructions of the manufacturer (Merck). Strain P-207^T grew on TSA up to 1 % (w/v) NaCl (but not at 2 % or above), at pH 5.5–10.5 (optimal pH for growth pH 7–8) and at 10–36 °C, but not at 4 or 45 °C. The restriction of growth to low salt concentrations clearly distinguishes strain P-207^T from the type strains of species of related genera, which all grow at salt concentrations above 10 % (w/v) NaCl. A detailed summary of the results is listed in the species description and in Table 1.

Prior to testing the diagnostic peptidoglycan diamino acid, polyamines, quinone system and polar lipids, biomass of bacterial cells was grown in 3.3 × PYE broth (1 % peptone from casein, 1 % yeast extract, pH 7.2) at 28 °C. Cells were harvested at the late exponential growth phase as recommended for extraction of polyamines (Busse & Auling, 1988) or at the stationary growth phase for extraction of peptidoglycan diamino acid, quinones and polar lipids. The diagnostic diamino acid of the peptidoglycan was determined from purified cell-wall fractions according to Schleifer (1985). Quinones and polar lipids were extracted and analysed applying the integrated procedure reported by Tindall (1990a, b) and Altenburger *et al.* (1996). Polyamines were extracted and analysed according to Altenburger *et al.* (1997). HPLC analyses were carried out using the equipment described by Stolz *et al.* (2007). Fatty acids were extracted and analysed as described by Kämpfer & Kroppenstedt (1996). Biomass of strain P-207^T for fatty acid analysis was grown on TSA at 28 °C for 48 h. Fatty acids were identified with the Sherlock System version 2.11, TSBA40 revision 4.1. *meso*-Diaminopimelic acid (Dpm) was identified to be the diagnostic diamino acid of the peptidoglycan. The quinone system consisted of menaquinones MK-7 (94.2 %), MK-5 (3.2 %), MK-8 (1.4 %) and MK-6 (1.3 %). The combination of the major quinone MK-7 and diagnostic peptidoglycan *meso*-Dpm is found in numerous aerobic endospore-formers including the type species *Bacillus subtilis*, *Virgibacillus pantothenticus*, *Cerasibacillus quisquiliarum* and *Lentibacillus salicampi*. The polyamine pattern of strain P-207^T consisted predominantly of spermidine [7.3 μmol (g dry weight)$^{-1}$] and spermine [4.2 μmol (g dry weight)$^{-1}$], with minor amounts of cadaverine [0.2 μmol (g dry weight)$^{-1}$] and traces of putrescine and 1,3-diaminopropane [<0.1 μmol (g dry weight)$^{-1}$]. The spermidine/spermine ratio (approx. 1 : 0.6) is clearly different from those of representative species of the genus *Bacillus*, including *Bacillus subtilis*, *Bacillus cereus* and *Bacillus megaterium*, where the spermidine content is 9-fold higher than the spermine content (Hamana *et al.*, 1989).

The polar lipid profile of strain P-207^T was composed of the major lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and a lipid not detectable with any specific spray reagent (L1), moderate amounts of two unidentified phospholipids (PL2, PL3) and a phosphoglycolipid (PGL2) and minor amounts of two phospholipids (PL1, PL4), an aminophospholipid (APL1), a phosphoglycolipid (PGL1) and two glycolipids (GL1, GL2) (Fig. 2). The most distinctive traits in the polar lipid profile of strain P-207^T are the presence of the two phosphoglycolipids not reported for any representative of the related genera *Gracilibacillus*, *Paraliobacillus*, *Ornithinibacillus*, *Oceanobacillus*, *Virgibacillus*, *Cerasibacillus* or *Lentibacillus*. Furthermore, the presence of glyco- and phosphoglycolipids (GL1, GL2, PGL1, PGL2) and phospholipid PL3 in the polar lipid profile of strain P-207^T and the absence of the glycolipids present in *B. subtilis* distinguished strain P-207^T from *Bacillus subtilis* the type species of the genus *Bacillus* (Kämpfer *et al.*, 2006).

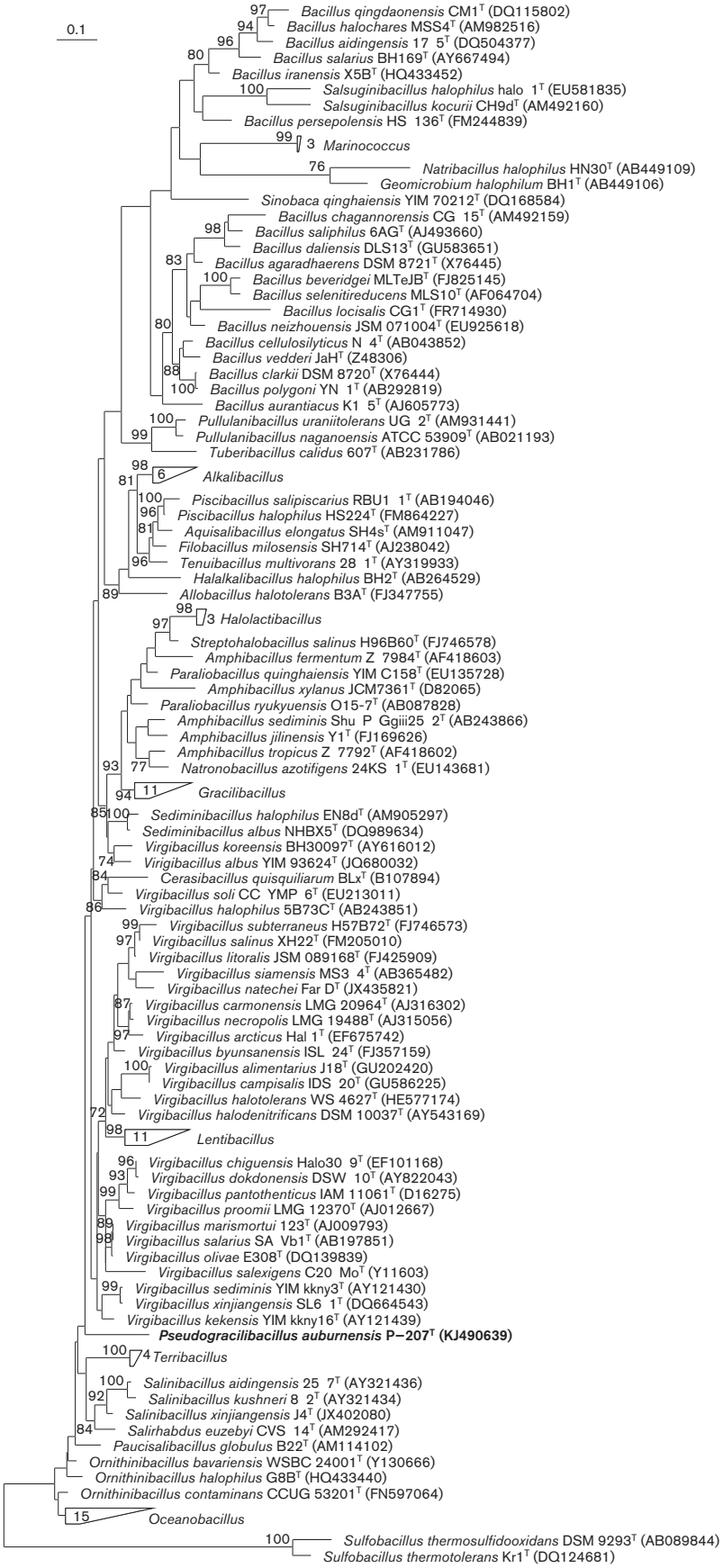

Fig. 1. Maximum-likelihood tree showing the phylogenetic position of strain P-207^T among type strains of species from the closest related genera of the *Bacillaceae*. The tree was reconstructed in ARB using RAxML (GTR-GAMMA, rapid bootstrap analysis, 100 bootstraps) and based on 16S rRNA gene sequences between positions 132 and 1427 (*E. coli* numbering; Brosius *et al.*, 1978). GenBank accession numbers are given in parentheses. Numbers at branch nodes refer to bootstrap values >70 % (100 replicates). The type strains of two species of the genus *Sulfobacillus* were used as an outgroup. Bar, 0.10 substitutions per site.

Table 1. Characteristics of strain P-207^T that distinguish it from related genera with *meso*-Dpm in the peptidoglycan

Taxa: 1, Strain P-207^T (*Pseudogracilibacillus* gen. nov.); 2, *Gracilibacillus*; 3, *Paraliobacillus*; 4, *Ornithinibacillus*; 5, *Oceanobacillus*; 6, *Virgibacillus*; 7, *Cerasibacillus*; 8, *Lentibacillus*. Unless indicated otherwise, data for *Gracilibacillus* were taken from Wainø *et al.* (1999), for *Paraliobacillus* from Chen *et al.* (2009a), for *Ornithinibacillus* from Mayr *et al.* (2006), for *Oceanobacillus* from Lu *et al.* (2001), for *Virgibacillus* from Heyrman *et al.* (2003) and Yoon *et al.* (2004), for *Cerasibacillus* from Nakamura *et al.* (2004) and for *Lentibacillus* from Jeon *et al.* (2005) and Jung *et al.* (2010). +, Present or positive for acid production; −, not present or negative for acid production; +/−, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
iso-$C_{15:0}$/anteiso-$C_{15:0}$ ratio	<1	<1	<1	>1	<1	0.05–1.7	>1	<1
Peptidoglycan diamino acid*	*meso*-Dpm	*meso*-Dpm	*meso*-Dpm	L-Orn	*meso*-Dpm*	*meso*-Dpm*	*meso*-Dpm	*meso*-Dpm
Phosphatidylglycerol content	Major	Major	−‡	Minor	Moderate	Moderate to major	ND	Major
Presence of:								
Phosphatidylethanolamine	+	+/−	−	−	+/−	+/−	ND	−
Glycolipids	+	+/−	−	+/−	+/−	+/−	ND	+/−
Phosphoglycolipids	+	−	−	−	−	−	ND	−
Upper limit of NaCl tolerance** (%, w/v)	1	15–30	20–22	10–12	>20	>10–25	7.5	15–30
Growth temperature range (°C)**	10–36	4–55	4–50	10–45	15–42†	5–50	30–55	10–50
pH range for growth**	5.5–10.5	5–10	5.5–10.0	7–10	6.5–10	ND (opt. 7.0)	7.5–10	6–8.5
Catalase activity	−	+	+§	+	+	+	+	+
Acid production from:								
D-Mannose	−	+	ND	−	+†	+/−	−	+/−
Trehalose	−	+	ND	+	−†	+/−	−	+/−
DNA G+C content (mol%)**	34	35.3–40.1	35.6–39.5	36–41	36†	36–42	35.5	41.6–49

*Determined in the study of Mayr *et al.* (2006) for *Oceanobacillus iheyensis* and *Virgibacillus picturae*. *V. pantothenticus* contains *meso*-Dpm direct (Claus & Berkeley, 1986) and *Virgibacillus halodenitrificans* and *Virgibacillus salexigens* contain *meso*-Dpm (Yoon *et al.*, 2004).
†Data taken Lu *et al.* (2001) by Mayr *et al.* (2006).
‡Determined for *Paraliobacillus quinghaiensis* by Chen *et al.* (2009a).
§If aerobic: otherwise negative (Ishikawa *et al.*, 2002).
**Data for all current type strains of respective genera were checked for redundancy, otherwise data were adjusted respectively.

The fatty acids comprised mainly iso- and anteiso-branched fatty acids. The detailed fatty acid profile obtained from cells grown on TSA for 72 h at 28 °C is as follows: iso-$C_{15:0}$ (8.0 %), anteiso-$C_{15:0}$ (49.9 %), $C_{16:0}$ (2.7 %), anteiso-$C_{17:0}$ (22.9 %), iso-$C_{16:0}$ (9.1 %), iso-$C_{17:0}$ (2.3 %), iso-$C_{14:0}$ (3.0 %) and $C_{15:0}$ (2.0 %).

For determination of the G+C content of the genomic DNA of strain P-207^T, DNA was extracted by the method of Pitcher *et al.* (1989) and the DNA G+C content was determined as described previously (Glaeser *et al.*, 2013) using the DNA melting temperature method described by Gonzalez & Saiz-Jimenez (2002). The G+C content of the genomic DNA of strain P-207^T was 34 mol%, which was slightly below the genomic DNA G+C content for known representatives of the closest related genera (Table 1).

Based on the low 16S rRNA gene sequence similarities, phylogenetic distinctness, polar lipid profiles and phenotypic traits (see Table 1), we conclude that strain P-207^T should not be assigned to a novel species within any of the pre-existing closely related genera *Virgibacillus*, *Ornithinibacillus*, *Cerasibacillus*, *Lentibacillus* or *Oceanobacillus*. We here propose the novel genus *Pseudogracilibacillus* gen. nov., with the type species *Pseudogracilibacillus auburnensis* sp. nov., to accommodate strain P-207^T.

Description of *Pseudogracilibacillus* gen. nov.

Pseu.do.gra.ci′li.ba.cil′lus. (Gr. adj. *pseudês* false; N.L. masc. n. *Gracilibacillus* a bacterial genus; N.L. masc. n. *Pseudogracilibacillus* the false *Gracilibacillus*).

Cells are Gram-stain-positive or Gram-variable rods (0.5–1.0 × 4–5 μm). They bear spherical to ellipsoidal endospores that are located terminally and sometimes subterminally. Spore formation can be very sparse. Colonies are circular and slightly irregular, smooth, glossy or sometimes matte, flat, butyrous, creamy to pinkish white and almost opaque on nutrient-rich media. Members of the genus are facultatively anaerobic and catalase-negative. They show a low salt tolerance: growth is positive with NaCl concentrations up to 1 % (w/v), but not at 2 % (w/v) in TSB. The polyamine pattern of the type species consists predominantly of spermidine and spermine. The quinone system consists predominantly of menaquinone MK-7, with smaller amounts of MK-5. The polar lipid profile is composed of the major compounds diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Glycolipids are

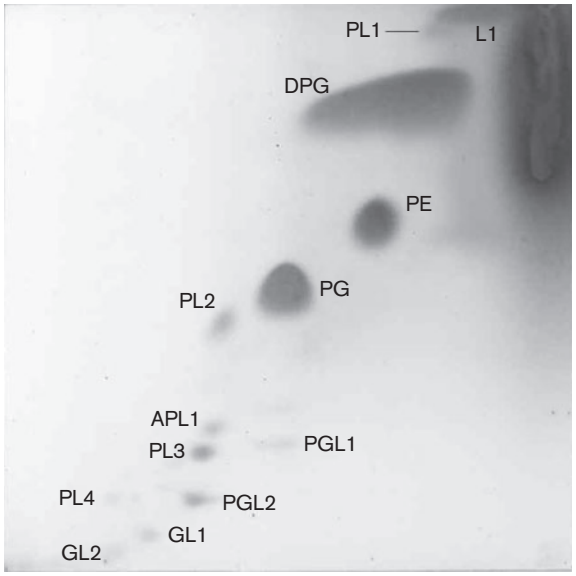

Fig. 2. Total polar lipid profile of strain P-207^T after two-dimensional TLC and staining with molybdatophosphoric acid. DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; APL1, unidentified aminophospholipid; PL1–PL4, unidentified phospholipids; PGL1, PGL2, unidentified phosphoglycolipids; GL1, GL2, unidentified glycolipids; L1, unidentified polar lipid not stainable with reagents specific for detection of phosphate, amino and sugar moieties.

present. The fatty acid profile comprises mainly iso- and anteiso-branched fatty acids. The diagnostic diamino acid of the peptidoglycan is *meso*-Dpm. The genomic DNA G+C content of the type strain of the type species is 34 mol%. The type species is *Pseudogracilibacillus auburnensis*.

Description of *Pseudogracilibacillus auburnensis* sp. nov.

Pseudogracilibacillus auburnensis (au.bur.nen′sis. N.L. masc. adj. *auburnensis* of or pertaining to Auburn, named after the place of origin of the type strain, Auburn, AL, USA).

Displays the following properties in addition to those described for the genus. No chains and filaments can be observed after growth on TSA at 28 °C for 48 h. Cells show no motility. Terminally located endospores are present in slightly swollen sporangia. No other cell inclusions can be found. Colonies grown on TSA after 48 h of incubation are convex and creamy to pinkish appearance with a mean diameter of 2–3 mm. Good growth on NA (Oxoid) and R2A agar (Oxoid) at 28 °C. Optimum temperature for growth is 28–30 °C; growth occurs at 10–36 °C but not at 4 or 45 °C. Optimal growth at pH 7–8; growth occurs at pH 5.5–10.5. Test for oxidase activity is negative. Tests for production of indole and urease are positive; production of sulphide, activities of gelatinase, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase and hydrolysis of gelatin, starch and casein are negative. Acid is not produced from D-glucose, D-xylose, lactose, sucrose, D-mannitol, dulcitol, salicin, D-adonitol, D-sorbitol, *myo*-inositol, L-arabinose, raffinose, L-rhamnose, maltose, trehalose, erythritol, melibiose or D-arabitol. The following carbon sources are utilized weakly according to the method of Kämpfer *et al.* (1991): D-glucose, maltose, ribose, trehalose, D-adonitol, acetate and DL-lactate. Negative for the utilization of *cis*- and *trans*-aconitate, citrate, fumarate, glutarate, L-malate, pyruvate, *N*-acetyl-D-glucosamine, L-arabinose, arbutin, cellobiose, D-fructose, D-galactose, gluconate, L-rhamnose, sucrose, salicin, trehalose, D-xylose, *myo*-inositol, D-maltitol, D-mannitol, D-mannose, D-sorbitol, melibiose, putrescine, adipate, 4-aminobutyrate, azelate, itaconate, 2-oxoglutarate and mesaconate. Negative for the hydrolysis of *p*-nitrophenyl (pNP) α-D-glycopyranoside, pNP β-D-glucuronoside, pNP β-D-glycopyranoside, bis-pNP phosphate, pNP phenylphosphonate, pNP phosphorylcholine, 2-deoxythymidine-5-pNP phosphate, *o*-nitrophenyl β-D-galactopyranoside, pNP β-D-xylopyranoside, L-alanine *p*-nitroanilide (pNA), L-glutamate γ-carboxy-pNA and L-proline pNA. In addition to the major compounds described for the genus, the polar lipid profile comprises minor amounts of an unidentified aminophospholipid (APL1) and moderate to minor amounts of four unidentified phospholipids (PL1–PL4), two glycolipids (GL1, GL2) and two phosphoglycolipids (PGL1, PGL2). Major fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$.

The type strain, P-207^T (=CCM 8509^T=LMG 28212^T=CIP 110797^T), was isolated from the rhizosphere of a corn plant (*Zea mays*) in Auburn, AL, USA.

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