Mucilaginibacter auburnensis sp. nov., isolated from a plant stem

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A yellow-pigmented, Gram-negative, rod-shaped, non-spore-forming bacterium (strain JM-1070^T) was isolated as a presumptive endophyte from internal stem tissue of a healthy corn stem. Phylogenetic analyses based on the 16S rRNA gene sequence placed strain JM-1070^T in the monophyletic cluster of the genus *Mucilaginibacter*, with closest affiliation to the type strains of *Mucilaginibacter composti* (98 % similarity) and *Mucilaginibacter calamicampi* (97.2 %). 16S rRNA gene sequence similarity to type strains of other species of the genus *Mucilaginibacter* was 93.4–96.0 %. DNA–DNA hybridizations between strain JM-1070^T and the type strains *M. composti* LMG 23497^T and *M. calamicampi* CCUG 63418^T showed low relatedness values of 13 % (reciprocal 18 %) and 52 % (reciprocal 54.4 %). Major respiratory quinones were menaquinones MK-6 and MK-7. The predominant fatty acids (>15 %) were iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ (measured as summed feature 3) and iso-$C_{17:0}$ 3-OH. Several other iso-branched and hydroxylated fatty acids were detected. The polar lipid profile was composed of the major components phosphatidylethanolamine and an unidentified aminophospholipid. The polyamine pattern contained predominantly *sym*-homospermidine. Characterization by 16S rRNA gene sequencing, physiological parameters and polyamine, ubiquinone, polar lipid and fatty acid compositions revealed that strain JM-1070^T represents a novel species of the genus *Mucilaginibacter*. For this reason, we propose the name *Mucilaginibacter auburnensis* sp. nov., with the type strain JM-1070^T (=CIP 110694^T=LMG 28078^T).

The genus *Mucilaginibacter*, which is grouped phylogenetically into the family *Sphingobacteriaceae*, was introduced by Pankratov *et al.* (2007). The genus description was subsequently updated and emended by Urai *et al.* (2008) and Baik *et al.* (2010). Members of the genus are characterized by the presence of menaquinone 7 (MK-7) as the major quinone type, straight-chain and branched saturated, and hydroxylated fatty acids as constituents of membranes and DNA G+C contents in the range of 42.4–47.0 mol% (Pankratov *et al.*, 2007; Urai *et al.*, 2008; Baik *et al.*, 2010).

At the time of writing, the genus *Mucilaginibacter* contained 28 species with validly published names (http://www.bacterio.net/mucilaginibacter.html; Euzéby, 1997), isolated from a wide variety of habitats such as peat bog, soil, rice straw, rice paddy, freshwater, the rhizosphere and decaying lichen, including *Mucilaginibacter paludis* (the type species) and *M. gracilis* (Pankratov *et al.*, 2007), *M. kameinonensis* (Urai *et al.*, 2008), *M. daejeonensis* (An *et al.*, 2009), *M. ximonensis* (Luo *et al.*, 2009), *M. oryzae* (Jeon *et al.*, 2009), *M. rigui* (Baik *et al.*, 2010), *M. gossypii* and *M. gossypiicola* (Madhaiyan *et al.*, 2010), *M. frigoritolerans*, *M. lappiensis* and *M. mallensis* (Männistö *et al.*, 2010), *M. dorajii* (Kim *et al.*, 2010a), *M. composti* (Cui *et al.*, 2011), *M. myungsuensis* (Joung & Joh, 2011), *M. boryungensis* (Kang *et al.*, 2011), *M. polysacchareus* (Han *et al.*, 2012), *M. angelicae* (Kim *et al.*, 2012a), *M. lutimaris* (Kim *et al.*, 2012b), *M. soli* (Jiang *et al.*, 2012), *M. litoreus* (Yoon *et al.*, 2012), *M. calamicampi* (Yoon *et al.*, 2013), *M. herbaticus* (Lee *et al.*, 2013), *M. gynuensis* (Khan *et al.*, 2013a), *M. jinjuensis* (Khan *et al.*, 2013b), *M. sabulilitoris* (Kang *et al.*, 2013), *M. soyangensis* (Joung *et al.*, 2014) and *M. defluvii* (Hwang *et al.*, 2014).

Strain JM-1070^T was isolated in July 1990 as an endophyte from the internal tissue of a healthy corn stem (*Zea mays*

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JM-1070^T is KF892548.

'Sweet Belle'), 10 weeks after planting in the field at the Plant Breeding Unit facility of the E. V. Smith Research Center, Tallassee, AL, USA. The strain showed single cells forming small, pale-yellow colonies (<0.5 mm) with a smooth surface after 48 h at 28 °C on tryptone soy agar (TSA; Oxoid). Cell morphology was investigated by phase-contrast microscopy for cells grown on TSA at 28 °C. During exponential growth, rod-shaped cells of strain JM-1070^T were 1.6±0.4 μm long and 0.8±0.2 μm wide and motile in the early exponential phase. Cells stained Gram-negative and were negative for cytochrome oxidase, as determined by using an oxidase test (Merck). Endospores could not be detected.

Phylogenetic analysis was performed in ARB release 5.2 (Ludwig *et al.*, 2004) using the All-Species Living Tree Project (LTP; Yarza *et al.*, 2008) database release s111 (February 2013). Sequences not included in the LTP database were aligned with SINA (version 1.2.9; Pruesse *et al.*, 2012) according to the SILVA seed alignment (http://www.arb-silva.de; Pruesse *et al.*, 2007) and implemented in the ARB database. The alignment was checked manually based on secondary structure information. Pairwise sequence similarities were calculated in ARB without the use of an evolutionary substitution model. Phylogenetic trees were reconstructed with the maximum-likelihood method using RAxML version 7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis and PhyML (without bootstrap analysis), the neighbour-joining method (ARB neighbour-joining) 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 125 and 1385 (according to *Escherichia coli* numbering; Brosius

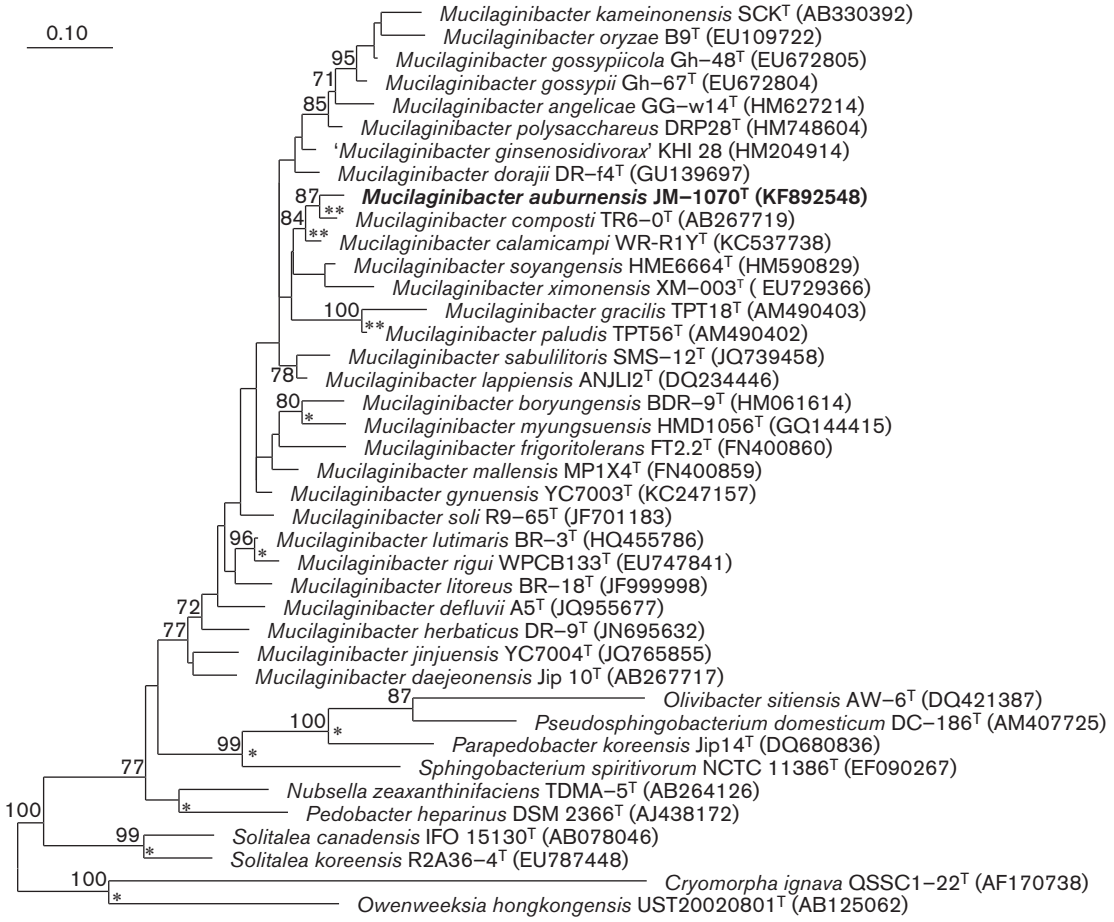

Fig. 1. Maximum-likelihood tree showing the phylogenetic position of strain JM-1070^T among type strains of the genus *Mucilaginibacter*. The tree was generated in ARB using RAxML (GTR-GAMMA, rapid bootstrap analysis, 100 bootstraps) and based on 16S rRNA gene sequences between positions 125 and 1385 (*E. coli* numbering; Brosius *et al.*, 1978). Numbers at branch nodes refer to bootstrap values >70 (100 replicates). The 16S rRNA gene sequences of *Cryomorpha ignava* QSSC1-22^T and *Owenweeksia hongkongensis* UST20020801^T were used as an outgroup. Single asterisks indicate nodes that were also obtained with high bootstrap support in either the neighbour-joining or the maximum-parsimony tree; two asterisks indicate nodes that had high bootstrap support in both trees. Bar, 0.10 substitutions per nucleotide site.

et al., 1978). Type strains of the type species of all other genera in the *Sphingobacteriaceae* were included in the phylogenetic analysis, and two type strains of the family *Cryomorphaceae* were used as outgroup. The phylogenetic trees were checked for consistency, and nodes that were conserved in all trees were marked by asterisks in the RAxML tree (Fig. 1).

The 16S rRNA gene sequence of strain JM-1070^T is a continuous stretch of 1467 unambiguous nucleotides (16S rRNA gene sequence positions 17–1467, according to *E. coli* numbering). Based on the 16S rRNA gene sequence analysis, strain JM-1070^T was placed into the monophyletic cluster of the genus *Mucilaginibacter*, with the highest 16S rRNA gene sequence similarity to *M. composti* TR6-0^T (98 %) and *M. calamicampi* WR-R1Y^T (97.2 %). The 16S rRNA gene sequence similarity to the type strains of other species of the genus *Mucilaginibacter* was 93.4–96.0 %. Phylogenetic trees showed that strain JM-1070^T formed a distinct cluster (bootstrap support >70 %) with *M. composti* TR6-0^T and *M. calamicampi* WR-R1Y^T (Fig. 1), clustering closest to *M. composti* TR6-0^T.

For more detailed genotypic differentiation, strain JM-1070^T, *M. composti* LMG 23497^T and *M. calamicampi* LMG 63418^T were compared by DNA–DNA hybridization. Genomic DNA was extracted by the method of Pitcher *et al.* (1989) and hybridized as described by Ziemke *et al.* (1998) except for a modification to nick translation: 2 µl genomic DNA was labelled for 3 h at 15 °C. DNA–DNA hybridization showed relatively low relatedness of the three strains at the genomic level, indicated by low relatedness of JM-1070^T with *M. composti* LMG 23497^T (13.3 %; reciprocal value 18.4 %) and *M. calamicampi* CCUG 63418^T (52 %; 54.4 %).

Further phenotypic characterization of strain JM-1070^T was performed with the substrate assimilation panel and enzyme tests with chromogenic substrates (*p*-nitrophenyl- and *p*-nitroanilide-linked substrates) described previously by Kämpfer *et al.* (1991). The results of comparative characterization with the most closely related type strains, *M. composti* LMG 23497^T and *M. calamicampi* CCUG 63418^T, are given in Table 1. The results showed that strain JM-1070^T can be distinguished clearly from the most closely related species of the genus *Mucilaginibacter*.

Fatty acids were analysed from biomass grown on TSA as described by Kämpfer & Kroppenstedt (1996). The dominant fatty acids of JM-1070^T were iso-$C_{15:0}$ (32.2 %), iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ (measured as summed feature 3; 31.8 %) and iso-$C_{17:0}$ 3-OH (15.1 %). In addition, $C_{16:1}\omega5c$ (4.0 %), $C_{16:0}$ (2.3 %) and iso-$C_{15:0}$ 3-OH (3.0 %) could be detected in smaller amounts. The detailed fatty acid profile of strain JM-1070^T is shown in Table 2.

For analysis of the peptidoglycan diamino acid, polyamines, quinones and polar lipids, cells were grown in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) and harvested at the stationary growth phase. Extractions of quinones and polar lipids were carried out by applying an integrated procedure and analysed according to Tindall (1990a, b) and Altenburger *et al.* (1996). Polyamines were extracted according to Busse & Auling (1988) and analysed by HPLC according to Busse *et al.* (1997). The HPLC apparatus applied was described by Stolz *et al.* (2007). The diagnostic diamino acid of the peptidoglycan was identified according to Schleifer (1985) and revealed the presence of *meso*-diaminopimelic acid. The quinone system was composed of 5.1 % MK-5, 25.1 % MK-6, 69.2 % MK-7 and 0.5 % MK-8. The polar lipid profile contained the major lipids phosphatidylethanolamine and the unidentified aminophospholipid APL2, and lipid L1, which was only visible after staining with molybdatophosphoric acid (Fig. 2). Minor amounts of unidentified aminolipid AL1 and aminophospholipids APL1 and APL3 were also detected. The polyamine pattern of strain JM-1070^T consisted of 34.6 µmol *sym*-homospermidine (g dry weight)$^{-1}$, 1.8 µmol spermidine (g dry weight)$^{-1}$, 0.2 µmol putrescine (g dry weight)$^{-1}$, 0.2 µmol spermine (g dry weight)$^{-1}$ and 0.1 µmol cadaverine (g dry weight)$^{-1}$. The quinone system with major amounts of MK-6 and MK-7 is in good agreement with the original genus description (Pankratov *et al.*, 2007). However, some species contain only MK-6. The polar lipid profile is not listed in either the original genus description or the emended descriptions (Pankratov *et al.*, 2007; Urai *et al.*, 2008; Baik *et al.*, 2010). However, it resembles those of the close relatives *M. calamicampi* and *M. sabulilitoris*, but lacks lipids with lower chromatographic motilities than APL3 reported to be present in the latter two species (Yoon *et al.*, 2013; Kang *et al.*, 2013). So far, polyamine patterns have not been reported for any species of the genus *Mucilaginibacter*. However, polyamine patterns with the predominant compound *sym*-homospermidine have been reported for other members of the *Sphingobacteriaceae*, in the genera *Sphingobacterium*, *Pedobacter* and *Parapedobacter* (Hamana & Matsusaki, 1991; Hamana & Nakagawa, 2001; Kim *et al.*, 2007, 2010b). Hence, it can be concluded that, like the major menaquinone MK-7, the presence of the major polyamine *sym*-homospermidine is characteristic of the family *Sphingobacteriaceae*.

A combination of the observed genotypic, chemotaxonomic and physiological differences warrant the proposal of a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter auburnensis* sp. nov. is proposed.

Description of *Mucilaginibacter auburnensis* sp. nov.

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

The species shares all characteristics of the genus as given by Pankratov *et al.* (2007), Urai *et al.* (2008) and Baik *et al.* (2010). Rod-shaped cells, 1.6 ± 0.4 µm long and 0.8 ± 0.2 µm wide, and motile in the early exponential phase. Growth is observed on PYE agar, nutrient agar, TSA and R2A agar. On TSA, circular yellow colonies are formed after incubation

Table 1. Differentiating characteristics of JM-1070^T and the type strains of related species of the genus *Mucilaginibacter*

Strains: 1, JM-1070^T; 2, *M. composti* LMG 23497^T; 3, *M. calamicampi* CCUG 63418^T; 4, *M. lappiensis* ANJLI2^T; 5, *M. rigui* WPCB133^T; 6, *M. dorajii* FR-f4^T. Data for JM-1070^T, *M. composti* LMG 23497^T and *M. calamicampi* CCUG 63418^T were obtained in this study unless indicated otherwise; other data were taken from Cui *et al.* (2011), Männistö *et al.* (2010), Baik *et al.* (2010) and Kim *et al.* (2010a). All strains were Gram-negative, non-motile and rod-shaped. All strains were positive for assimilation of D-glucose and L-arabinose. All strains were negative for nitrate reduction, indole production, hydrolysis of xylan and assimilation of D-mannitol, *N*-acetylglucosamine, caprate, citrate, phenylacetate, 4-hydoxybenzoate, L-rhamnose, inositol, itaconate, suberate, acetate, L-alanine, 5-ketogluconate, 3-hydroxybenzoate and L-serine. +, Positive; (+), weakly positive; −, negative; ND, no data available.

Characteristic	1	2	3	4	5	6
Isolation source	Plant	Compost[a*]	Soil[b]	Lichen	Wetland freshwater	Rhizosphere
Colony colour on R2A	Light yellow	Light yellow[a]	Yellow[b]	Light pink to reddish	Pale pink	Light yellow
Cell width (μm)	0.8–1.0	0.4–0.6[a]	0.2–0.4[b]	0.3–0.5	0.3–0.4	0.6–0.8
Cell length (μm)	1.6–2.0	1.8–3.0[a]	0.4–2.5[b]	1–3	1.0–1.7	1.1–1.8
Growth temperature (°C)	15–36	4–42[a]	4–30[b]	0–31	4–37	4–30
pH range	5.5–9.5	6.0–8.0[a]	ND	4.5–8.0	5.0–10.0	5.0–8.0
Tolerance of 1 % (w/v) NaCl	+	−[c]†	+[d]	+	−	+
Catalase	−	−[c]	−[d]	+	+	+
Arginine dihydrolase	−	+	−	+	+	−
β-Glucuronidase	(+)	−[c]	−	+	−	−
Urease	−	+[c]	−	+	+	−
Hydrolysis of starch	+	−[c]	−	−	−	+
Utilization of:						
Gluconate	+	−[c]	+[d]	+	−	−
Adipate	−	−[c]	−[d]	+	−	−
Malate	−	−[c]	−[d]	+	+	−
Salicin	−	+[c]	−[d]	−	−	+
Melibiose	−	−[c]	−[d]	+	+	+
D-Sorbitol	−	−[c]	−[d]	+	−	−
Propionate	−	−[c]	−[d]	+	−	−
Caprate	−	−[c]	−[d]	−	−	−
Valerate	−	−[c]	−[d]	+	−	−
L-Histidine	−	−[c]	−[d]	+	−	−
2-Ketogluconate	−	−[c]	−[d]	+	−	−
3-Hydroxybutyrate	−	−[c]	−[d]	+	−	−
L-Proline	−	−[c]	−[d]	+	+	−
D-Ribose	−	−[c]	−[d]	+	−	−
Sucrose	+	−[c]	−[d]	−	+	+
D-Mannose	+	−[c]	+[d]	+	+	+
Maltose	−	−[c]	−[d]	+	+	+
Malonate	−	−[c]	−[d]	+	−	−
Lactate	−	−[c]	−[d]	+	−	−
Glycogen	−	−[c]	−[d]	−	+	(+)
Menaquinone(s)	7, 6, 5	7[a]	7[b]	7, 6	7	7

*Data taken from: *a*, Cui *et al.* (2011); *b*, Yoon *et al.* (2013).
†Data identical to those reported by: *c*, Cui *et al.* (2011); *d*, Yoon *et al.* (2013).

for 2–3 days at 28 °C. Cells show Gram-negative staining and are negative for cytochrome oxidase. Endospores are not observed. On TSA, growth occurs at 15–36 °C, but not at 10 or 45 °C, and at pH 5.5–9.5, but not at pH 4.5 or 10.5. Produces acid from D-glucose, sucrose, L-arabinose, D-xylose and cellobiose (weakly), but not from lactose, maltose, D-mannose, melibiose, raffinose, L-rhamnose, trehalose, methyl α-D-glucoside, D-arabitol, erythritol, D-mannitol, dulcitol, salicin, D-adonitol, inositol or D-sorbitol. The following compounds are utilized as sole sources of carbon: L-arabinose, D-gluconate, D-glucose (weakly), D-mannose, sucrose and D-xylose. The following compounds are not utilized as sole sources of carbon: acetate, propionate, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arbutin, cellobiose, D-galactose, maltose, D-fructose, trehalose, glycerol, D-mannitol, maltitol, melibiose, L-rhamnose, D-ribose, salicin, D-xylose, adonitol, *myo*-inositol, D-sorbitol, putrescine, *cis*- and *trans*-aconitate, 4-aminobutyrate, adipate,

Table 2. Cellular fatty acid profiles of strain JM1070^T and type strains of related species of the genus *Mucilaginibacter*

Strains: 1, JM-1070^T; 2, *M. composti* LMG 23497^T [data in parentheses from Cui *et al.* (2011) for strain TR6-03^T]; 3, *M. calamicampi* CCUG 63418^T [data in parentheses from Yoon *et al.* (2103) for strain WR-RY1^T]; 4, *M. lappiensis* ANJLI2^T (data from Cui *et al.*, 2011); 5, *M. rigui* WPCB133^T (Cui *et al.*, 2011); 6, *M. dorajii* FR-f4^T (Kim *et al.*, 2010a). Data were obtained this study unless indicated. All strains were cultured on R2A agar for 2 days with MIDI version 6.0. −, Not detected; TR, trace amount (<0.5 %); ECL, equivalent chain-length. Some fatty acids that accounted for less than 0.5 % of the total fatty in all strains are excluded; therefore, the percentages do not add up to 100 %.

Fatty acid	1	2	3	4	5	6
Saturated						
$C_{14:0}$	3.2	− (0.9)	− (1.6)	0.6	0.6	0.6
$C_{15:0}$	−	− (−)	5.2 (−)	−	−	2.8
$C_{16:0}$	2.3	3.7 (8.2)	3.5 (14.3)	7.3	7.7	7.8
Unsaturated						
$C_{14:1}\omega5c$	−	− (3.2)	− (−)	1.4	2.4	−
$C_{15:1}\omega6c$	−	− (−)	− (−)	−	0.6	TR
$C_{16:1}\omega5c$	4.0	7.5 (9.5)	6.1 (2.7)	3.4	4.6	6.8
$C_{17:1}\omega8c$	−	− (0.5)	− (−)	−	0.9	0.6
Branched-chain						
iso-$C_{13:0}$	−	− (−)	− (−)	−	0.6	−
iso-$C_{15:0}$	32.1	25.0 (19.1)	26.8 (36.3)	23.2	25.6	15.0
iso-$C_{15:0}$ 3-OH	3.0	2.6 (3.1)	3.6 (4.1)	2.1	2.1	1.8
iso-$C_{16:0}$	−	− (−)	− (−)	0.5	1.2	TR
iso-$C_{17:0}$	−	− (1.4)	− (−)	0.8	1.0	TR
iso-$C_{17:0}$ 3-OH	15.1	13.0 (13.0)	12.1 (10.1)	14.9	9.9	7.0
anteiso-$C_{15:0}$	−	− (2.2)	− (1.0)	0.8	1.7	−
anteiso-$C_{17:0}$	−	− (1.7)	− (−)	0.8	1.4	−
iso-$C_{17:1}\omega9c$	−	− (4.1)	− (−)	4.2	4.6	1.8
Hydroxy						
$C_{15:0}$ 2-OH	−	− (−)	2.7 (−)	−	0.7	0.5
$C_{15:0}$ 3-OH	−	− (−)	− (−)	−	0.5	TR
$C_{16:0}$ 3-OH	−	− (−)	− (3.5)	2.1	0.8	1.6
Summed features						
Summed feature 3*	31.8	40.0 (32.8)	37.0 (25.5)	37.0	31.8	45.6
Unknown						
ECL 13.565	3.6	3.2 (−)	− (−)	−	−	1.2
ECL 16.582	−	− (−)	− (−)	−	−	0.5
ECL 18.810	−	− (−)	3.1 (−)	−	−	0.6

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 consists of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L- and β-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates *p*-nitrophenyl (pNP) α-D-glucopyranoside, pNP β-D-glucopyranoside

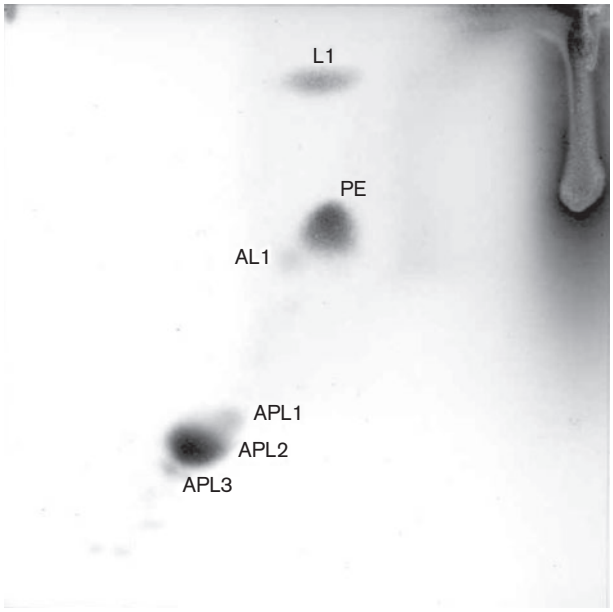

Fig. 2. Polar lipid profile of strain JM-1070^T after staining with molybdatophosphoric acid. PE, Phosphatidylethanolamine; APL1–3, unidentified aminophospholipids; AL1, unidentified aminolipid; L1, unidentified lipid only detectable with molybdatophosphoric acid.

(weakly) and pNP β-D-galactopyranoside are hydrolysed. pNP β-D-glucuronide, pNP β-D-xylopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, 2-deoxythymidine 2′-pNP, L-alanine *p*-nitroanilide (pNA), γ-L-glutamate pNA and L-proline pNA are not hydrolysed. The diagnostic diamino acid of the peptidoglycan is *meso*-diaminopimelic acid. The polar lipid profile consists of the major lipids phosphatidylethanolamine, an unidentified aminophospholipid and an unidentified lipid only visible in total lipid staining. The quinone system contains the major menaquinones MK-6 and MK-7 and minor amounts of MK-5. The major compound in the polyamine pattern is *sym*-homospermidine. The fatty acid profile consists of iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ (measured as summed feature 3) and $C_{17:0}$ iso 3-OH as major compounds. $C_{16:1}\omega5c$, $C_{16:0}$ and iso-$C_{15:0}$ 3-OH are detected in minor amounts.

The type strain, JM-1070^T (=CIP 110694^T=LMG 28078^T), was isolated in July 1990 as an endophyte from internal stem tissue of a healthy corn stem (*Zea mays* 'Sweet Belle'), 10 weeks after planting in the field at the Plant Breeding Unit facility of the E. V. Smith Research Center in Tallassee, AL, USA.

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