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Sphingomonas zeae sp. nov., isolated from the stem of *Zea mays*

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A yellow-pigmented bacterial isolate (strain JM-791^T) obtained from the healthy internal stem tissue of 1-month-old corn (*Zea mays*, cultivar 'Sweet Belle') grown at the Plant Breeding Unit of the E.V. Smith Research Center in Tallassee (Elmore county), Alabama, USA, was taxonomically characterized. The study employing a polyphasic approach, including 16S RNA gene sequence analysis, physiological characterization, estimation of the ubiquinone and polar lipid patterns, and fatty acid composition, revealed that strain JM-791^T shared 16S rRNA gene sequence similarities with type strains of *Sphingomonas paucimobilis* (98.3 %), *Sphingomonas pseudosanguinis* (97.5 %) and *Sphingomonas yabuuchiae* (97.4 %), but also showed pronounced differences, both genotypically and phenotypically. On the basis of these results, a novel species of the genus *Sphingomonas* is described, for which we propose the name *Sphingomonas zeae* sp. nov. with the type strain JM-791^T (=LMG 28739^T=CCM 8596^T).

The genus *Sphingomonas*, proposed by Yabuuchi *et al.* (1990) encompassed Gram-negative, non-fermentative rods, which can be chemotaxonomically characterized by the presence of ubiquinone Q-10, *sym*-homospermidine as the key polyamine, a lipid pattern consisting of phosphatidylethanolamine (exception: *Sphingomonas echinoides* DSM 1805^T; Denner *et al.*, 1999), phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid and phosphatidylcholine as major lipids, and the presence of 2-hydroxymyristic acid ($C_{14:0}$ 2-OH) and the absence of 3-hydroxy fatty acids in their fatty acid profiles (Busse *et al.*, 1999; Takeuchi *et al.*, 2001; Zhang *et al.*, 2005; Yoon *et al.*, 2006). Members of the genus have been isolated from roots or rhizosphere soil (Xie & Yokoto, 2006; Takeuchi *et al.*, 1995; Chung *et al.*, 2011), the phyllosphere (Talà *et al.*, 2013; Rivas *et al.*, 2004) and also from endophytic compartments of plants (Huang *et al.*, 2012) suggesting an important role of the genus for plant–microbe interaction.

In this study employing a polyphasic characterization, we describe a novel species of the genus *Sphingomonas*, which was isolated from the healthy internal stem tissue of 1-month-old corn (*Zea mays*, cultivar 'Sweet Belle') grown at the Plant Breeding Unit of the E.V. Smith Research Center in Tallassee (Elmore county), Alabama, USA.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JM-791^T is KP999966.

Strain JM-791^T was originally grown on Tryptic Soy Agar (TSA; Oxoid), and was further studied and sub-cultivated on nutrient agar (NA; Oxoid) at 28 °C for 48 h and subsequently analysed for its 16S rRNA gene sequence, fatty acid methyl ester composition of the whole-cell hydrolysate, further phenotypic features, and DNA–DNA relatedness to those species most closely related on the basis of 16S rRNA gene sequence similarities.

On NA at 28 °C the strain showed a yellow pigmentation. Cells of strain JM-791^T stained as Gram-negative with the modified Hucker method after Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000 magnification, with cells grown for 24 h at 28 °C on medium NA. Good growth occurred on NA, as well as on brain heart infusion agar, R2A agar and TSA, but no growth was observed on MacConkey agar (Oxoid). Very reduced growth was observed at 4 °C and 45 °C. The best growth was recorded at temperatures between 25 °C and 30 °C. Strain JM-791^T grew in TSB at 28 °C in the presence of 1 %, but not 2 % (w/v) NaCl and above, and in TSB adjusted to pH 5.5–9.5 but not at pH 4.5 or pH 10.5.

Detailed physiological characterization and biochemical tests were performed to assess the carbon source utilization pattern, acid formation from different sugars and/or sugar-related compounds, and hydrolysis of chromogenic substrates as described by Kämpfer *et al.* (1991). In addition, other biochemical tests were performed, such as production of hydrogen sulphide, indole reaction with

Ehrlich's and Kovacs' reagents, the activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, β-galactosidase (ONPG), and urease on Christensen's urea agar (all performed with the Micronaut E kit; Kämpfer, 1990). Hydrolysis of casein, gelatin (plate method), starch and tyrosine were performed according to Smibert & Krieg (1994). Isolate JM-791^T utilized many carbon sources, similar to all species of the genus *Sphingomonas*, and was able to produce acid from D-glucose, lactose (weak), sucrose, L-arabinose (weak), maltose, D-xylose,

Table 1. Differentiating characteristics of strain JM-791^T, and related *sym*-homospermidine-containing species of the genus *Sphingomonas*

1, JM-791^T; 2, *S. pseudosanguinis* G1-2^T; 3, *S. yabuuchiae* DSM 14562^T; 4, *S. sanguinis* NBRC 13937^T; 5, *S. pituitosa* EDIV^T; 6, *S. trueperi* ATCC 12417^T; 7, *S. paucimobilis* ATCC 29837^T; 8, *S. parapaucimobilis* JCM 7510^T; 9, *S. roseiflava* IAM 14823^T. Data for taxa 1 from this study; data for taxa 2–9 from Kämpfer *et al.* (2007), obtained under exactly the same conditions. All data were obtained with the same method (Kämpfer *et al.*, 1991). +, Positive; (+), weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9
Acid production from:									
Glucose	+	−	(+)	−	(+)	−	−	−	−
D-Mannitol	−	−	−	−	−	−	−	−	−
Salicin	−	−	−	−	−	−	+	−	−
Sorbitol	−	−	−	−	−	+	−	−	−
Rhamnose	−	−	−	−	−	−	−	(+)	−
Maltose	+	−	−	−	(+)	−	(+)	(+)	−
D-Xylose	+	−	−	−	(+)	−	+	(+)	−
Trehalose	+	−	−	−	−	−	−	−	−
Cellobiose	−	−	−	−	(+)	−	(+)	(+)	−
Methyl D-glucoside	−	−	−	−	−	−	+	−	−
Melibiose	+	−	−	−	−	−	(+)	(+)	−
D-Mannose	+	−	−	−	−	−	(+)	(+)	−
Hydrolysis of:									
Aesculin	+	+	+	−	+	−	(+)	(+)	+
pNP-β-D-glucuronide	−	−	(+)	+	+	+	−	+	−
pNP-phosphoryl-choline	+	+	−	+	+	+	−	+	−
L-Alanine-pNA	+	+	+	+	+	−	+	+	+
L-Proline-pNA	+	+	+	−	−	−	−	+	+
Assimilation of:									
D-Fructose	+	+	+	+[I]	−	+	−	−	+
Gluconate	−	−	+	−[I]	−	−	−	+	+
α-Melibiose	+	−	+	+	+	+	−	+	−
L-Rhamnose	−	−	−	−	−	−	−	(+)	−
Salicin	+	−	+	+	+	+	−	+	−
Maltitol	−	−	+	+	−	+	−	−	−
Acetate	+	−	+	+	+	+	−	+	−
Propionate	−	−	−	+	−	+	−	+	−
cis-Aconitate	−	−	+	−	−	−	+	+	+
trans-Aconitate	−	−	+	−	−	−	+	−	+
Citrate	+	+	+	−	−	−	−	+	−
Fumarate	+	+	+	+	+	+	+	−	+
Glutarate	+	+	+	−	−	+	−	+	−
DL-3-Hydroxybutyrate	−	+	+	+	+	+	−	+	+
DL-Lactate	+	−	+	+	−	+	−	+	−
Oxoglutarate	+	+	+	+	−	−	+	+	+
L-Alanine	−	+	+	+	+	+	−	+	(+)
L-Asparate	−	−	−	+	−	+	−	−	−
L-Leucine	−	+	−	+	−	+	−	+	(+)
L-Ornithine	−	−	−	+	−	−	−	−	−
L-Proline	−	+	+	+	+	−	−	−	+
L-Serine	−	−	−	−	−	+	−	−	−
4-Hydroxybenzoate	−	−	−	−	−	+	−	−	−

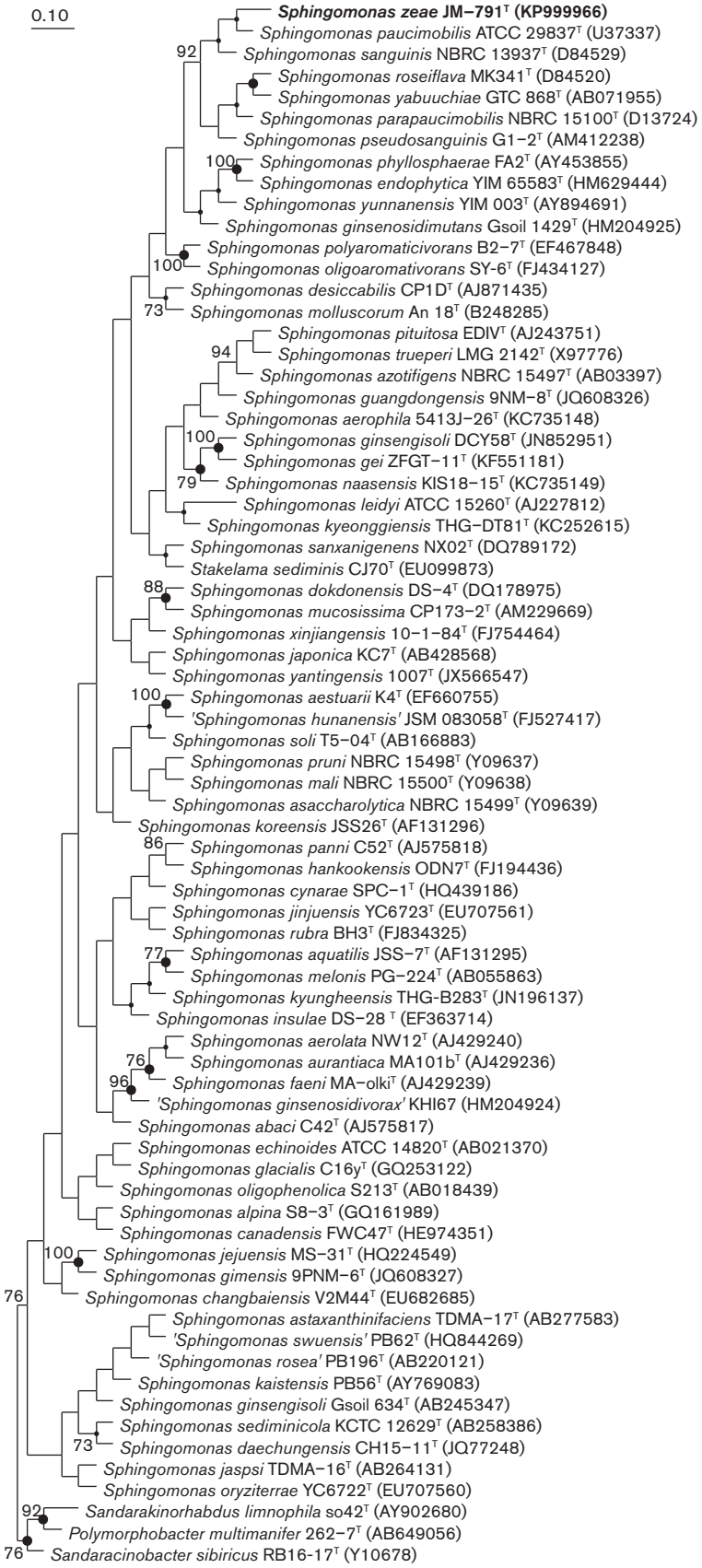

Fig. 1. Phylogenetic placement of strain JM-791^T within the genus *Sphingomonas*. The analysis is based on 16S rRNA gene sequences (gene termini: 84 to 1392) and was generated in ARB (LTP database) with the maximum-parsimony methods using DNAPARS. Numbers at nodes represent bootstrap values based on 100 replications (bootstrap-analysis); only bootstrap values >70 % are displayed. Filled circles indicate nodes which were also present in the phylogenetic tree generated with the maximum-likelihood method; larger filled circles represent nodes which were thereby supported by bootstrap values >70 %. Type strains of species of the genera *Sandaracinobacter* and *Polymorphobacter* were used as outgroup. Bar, 0.1 substitutions per nucleotide position.

trehalose and melibiose, and hydrolyse many chromogenic substrates. The biochemical/physiological data are given in Table 1 and in the species description.

For phylogenetic identification, the nearly full-length 16S rRNA gene of strain JM-791^T was amplified and sequenced using the universal primers 8F and 1492R (Lane, 1991). After manual sequence correction, the sequence used for analysis had a length of 1401 nt spanning gene termini 15 to 1470 (according to *Escherichia coli* numbering; Brosius *et al.*, 1978). The EzTaxon server (*http://www.ezbiocloud.net/eztaxon*; Kim *et al.*, 2012) was used to determine the 16S rRNA gene sequence similarity to the closest related type strains. Phylogenetic trees including type strains of all species of the genus *Sphingomonas* were calculated with the ARB software package release 5.2 (Ludwig *et al.*, 2004) and the 'All-Species Living Tree' Project (LTP; Yarza *et al.*, 2008) database release LTPs119 (November 2014). The sequence of strain JM-791^T was aligned with the SILVA Incremental Aligner (SINA; version 1.2.11; Pruesse *et al.*, 2012) and implemented into the LTP database. The final alignment used for tree reconstruction was checked manually considering the secondary structure information of the 16S rRNA. A maximum-likelihood tree was generated on the basis of sequences of the most closely related species using RAxML version 7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis (100 resamplings) and a maximum-parsimony tree was created using DNAPARS version 3.6 (Felsenstein, 2005). Both trees were based on 16S rRNA gene sequences between gene termini 84 to 1392 (according to Brosius *et al.*, 1978) and based on 100 replications (bootstrap analysis; Felsenstein, 1985).

Type strains of *Sphingomonas paucimobilis* (98.4 %), *Sphingomonas pseudosanguinis* (97.5 %) and *Sphingomonas yabuuchiae* (97.4 %) shared the highest 16S rRNA gene sequence similarities with strain JM-791^T; the sequence similarity to all other species of the genus *Sphingomonas* was below 97.0 %. Both phylogenetic trees showed a clear placement of strain JM-791^T within the genus *Sphingomonas*, clustering with the type strain of the type species *S. paucimobilis*, but not supported by high bootstrap values (Fig. 1).

Chemotaxonomic analyses were performed as follows: respiratory quinones and polar lipids were extracted from cells harvested at the stationary growth phase determined according to Tindall (1990a,b) and Altenburger *et al.* (1996), respectively; polyamines were extracted from cells harvested at the late exponential growth phase and analysed as described by Busse & Auling (1988) applying HPLC conditions described by Busse *et al.* (1997). HPLC apparatus used for analysis of quinones and polyamines was described by Stolz *et al.* (2007).

The detection of a quinone system consisting of ubiquinone Q-10 (98 %), Q-11 (1 %) and Q-9 (1 %) in strain JM-791^T corresponded with the characteristics of species of the genus *Sphingomonas sensu stricto* (Busse *et al.*, 1999; Kosako *et al.*, 2000). The polyamine pattern exhibited the predominance of *sym*-homospermidine [41.6 μmol (g dry weight)$^{-1}$], the key characteristic of *Sphingomonas sensu stricto* (Busse *et al.*, 1999; Takeuchi *et al.*, 2001), and minor amounts of spermidine [1.7 μmol μmol (g dry weight)$^{-1}$], spermine [0.3 μmol (g dry weight)$^{-1}$], and putrescine and cadaverine (eacg 0.1 μmol (g dry weight)$^{-1}$]. The polar lipid profile of strain JM-791^T contained the major compounds phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid, phosphatidylcholine, and a hydrophobic unidentified phospholipid (PL1). In addition, moderate to minor amounts of one unidentified aminolipid (AL1), one unidentified glycolipid (GL1), another unidentified phospholipid (PL2), an unidentified phosphoglycolipid (PGL1) and one lipid (L1) only detectable after total lipid staining were also present (Fig. 2). This profile shares the major characteristics of species of *Sphingomonas sensu stricto* (Busse *et al.*, 1999).

Gas chromatography on the basis of the MIDI system was used to analyse the fatty acid profiles of the strains, as described previously (Kämpfer & Kroppenstedt, 1996; Kämpfer *et al.*, 1997). Strain JM-791^T showed a fatty acid profile typical for members of this genus with $C_{14:0}$ 2-OH as the key hydroxylated fatty acid (Table 2).

Due to the relatively high similarity of strain JM-791^T to type strains of *S. paucimobilis*, *S. sanguinis* and *S. yabuuchiae*, DNA–DNA hybridization experiments were performed with the type strains of these three species using the method described by Ziemke *et al.* (1998), except that for nick translation, 2 μg DNA was labelled during a 3 h incubation at 15 °C. Strain JM-791^T showed relatively low DNA–DNA relatedness to *S. paucimobilis* DSM 1098^T (51 %; reciprocal 37 %), *S. pseudosanguinis* G1-2^T (60 %; reciprocal 53 %) and *S. yabuuchiae* DSM 14562^T (20 %; reciprocal 58 %).

On the basis of the 16S rRNA gene sequence analysis, DNA–DNA hybridization values and the phenotypic data,

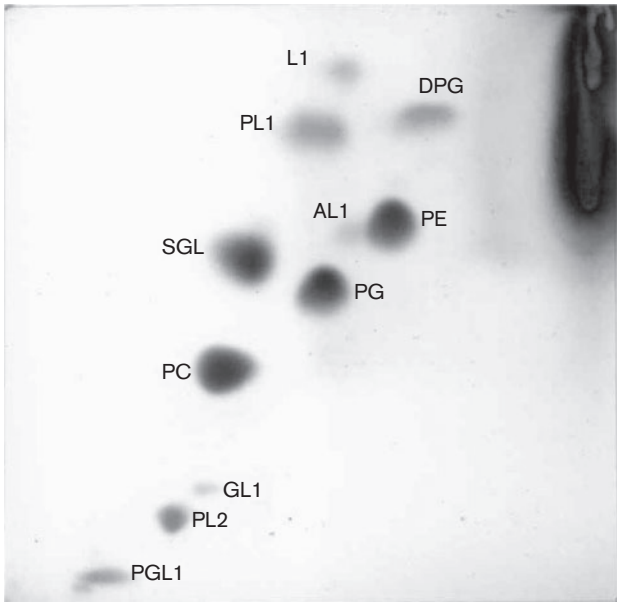

Fig. 2. Polar lipid profile of JM-791^T after two-dimensional thin layer chromatography and detection with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipid; PL1, 2, unidentified phospholipids; AL1, unidentified aminolipid; PGL1, unidentified phosphoglycolipid; GL1, unidentified glycolipid; L1, unidentified polar lipid.

Table 2. Fatty acid composition of strain JM-791^T and type strains of selected species of the genus *Sphingomonas*

Strains: 1, JM-791^T; 2, *S. pseudosanguinis* G1-2^T; 3, *S. sanguinis* NBRC 13937^T; 4, *S. yabuuchiae* A1-18^T; 5, *S. paucimobilis* ATCC 29837^T; 6, *S. parapaucimobilis* JCM 7510^T; 7, *S. trueperi* ATCC 12417^T. Data were taken from the present study and Kämpfer *et al.* (2007), obtained under exactly the same conditions. Values are percentages of total fatty acids. ECL, Equivalent chain-length; TR, trace (<1.0 %); −, not detected.

Fatty acid	1	2	3	4	5	6	7
$C_{14:0}$	−	1.0	1.0	1.3	1.4	1.0	−
$C_{14:0}$ 2-OH	12.4	5.4	6.7	8.7	6.4	5.0	6.7
$C_{15:0}$ 2-OH	1.5	−	−	−	−	−	−
$C_{15:0}$	−	−	−	−	−	1.1	−
$C_{16:0}$	13.3	12.9	9.6	9.9	8.7	13.6	9.8
$C_{16:1}\omega5c$	−	−	1.9	TR	−	1.0	−
Summed feature 4*	−	4.8	8.6	5.9	2.7	6.7	−
$C_{17:0}$	1.6	−	−	1.2	−	0.5	1.6
$C_{17:1}\omega6c$	4.7	0.8	3.8	5.1	3.0	2.4	13.6
$C_{18:0}$	1.3	1.6	−	TR	−	0.6	−
Summed feature 7*	63.8	72.0	65	62.5	74.6	64.6	64.2
$C_{18:1}\omega5c$	1.4	1.4	3.5	2.3	3.2	3.5	4.0

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system (Microbial ID). Summed feature 4 contained one or more of $C_{16:1}\omega7t$, $C_{15:0}$ iso 2-OH and $C_{16:1}\omega7c$; summed feature 7 contained one or more of $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$.

strain JM-791^T represents a novel species of the genus *Sphingomonas*, for which we propose the name *Sphingomonas zeae* sp. nov.

Description of *Sphingomonas zeae* sp. nov.

Sphingomonas zeae [L. gen. n. *zeae* of spelt, of *Zea mays*, referring to its isolation from the stem of corn (*Zea mays*)].

Cells are rod-shaped, 0.8–1.5 μm in length and 0.4–0.6 μm in diameter. Yellow colonies are formed. Acid is produced from D-glucose, sucrose, lactose (weak), maltose, D-xylose, trehalose and melibiose, but not from D-mannitol, salicin, dulcitol, adonitol, *i*-inositol, D-sorbitol, L-rhamnose, cellobiose, erythritol or D-arabitol. Uses *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, maltose, melibiose, sucrose, salicin, trehalose, D-xylose, acetate, citrate, fumarate, glutarate, DL-lactate and L-malate as substrate, but not gluconate, L-rhamnose, ribose, D-adonitol, maltitol, D-mannitol, D-sorbitol, propionate, DL-3-hydroxybutyrate, azelate, *cis*-aconitate, adipate, 4-aminobutyrate, pyruvate, L-alanine, L-leucine, L-aspartate, L-histidine, L-ornithine, L-phenylalanine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. Hydrolysesaesculin, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-β-D-galactopyranoside, bis-pNP-phosphate, pNP-phenyl phosphonate, 2-deoxythymidine-5′-pNP-phosphate, pNP-phosphoryl choline, L-alanine-pNA, L-glutamate-γ-3-carboxy-pNA and L-proline-pNA, but not pNP-β-D-glucuronide. The species is characterized by the major fatty acids $C_{18:1}$, $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$ (detected as summed feature 7) and $C_{16:0}$ and the major hydroxy-fatty acid $C_{14:0}$ 2-OH. The major polyamine is *sym*-homospermidine; minor polyamines are putrescine, cadaverine, spermidine and spermine. The quinone system is ubiquinone Q-10 with minor amounts of Q-9 and Q-11. The polar lipid profile is composed of the major lipids phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid, phosphatidylcholine, and an unidentified phospholipid (PL1). In addition, moderate to minor amounts of an unidentified aminolipid (AL1), one unidentified glycolipid (GL1), another unidentified phospholipid (PL2), an unidentified phosphoglycolipid (PGL1) and one lipid (L1) are present.

The type strain JM-791^T (=LMG 28739^T=CCM 8596^T), was isolated from the healthy internal stem tissue of 1-month-old corn (*Zea mays*, cultivar 'Sweet Belle') grown at the Plant Breeding Unit of the E.V. Smith Research Center in Tallassee (Elmore county), Alabama, USA.

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