Chryseobacterium arachidiradicis sp. nov., isolated from the geocarposphere (soil around the peanut) of very immature peanuts (*Arachis hypogaea*)

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A yellow-pigmented bacterial strain, 91A-612^T, isolated from the geocarposphere (soil around the peanut) of very immature peanuts (*Arachis hypogaea*) in Alabama, USA, was studied for its taxonomic position. Cells of the isolate were rod-shaped and stained Gram-negative. A comparison of the 16S rRNA gene sequence with the sequences of the type strains of the most closely related species showed that the strain belongs to the genus *Chryseobacterium*, showing the highest sequence similarities to the type strains of *Chryseobacterium molle* (98.4 %), *C. pallidum* (98.3 %) and *C. hominis* (97.8 %). The 16S rRNA gene sequence similarities to the type strains of all other species of the genus *Chryseobacterium* were below 97.0 %. The fatty acid profile of strain 91A-612^T consisted of the major fatty acids iso-$C_{15:0}$, summed feature 3 (iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$) and iso-$C_{17:0}$ 3-OH. Major compounds in the polar lipid profile were phosphatidylethanolamine and several unidentified lipids, including two lipids that did not contain a sugar moiety, an amino group or a phosphate group (L3, L8), and an aminolipid (AL1). The quinone system was composed mainly of MK-6. The polyamine pattern contained *sym*-homospermidine as the major compound and moderate amounts of spermidine and spermine. DNA–DNA hybridizations between strain 91A-612^T and the type strains of *C. molle*, *C. pallidum* and *C. hominis* resulted in relatedness values well below 70 %. These data and the differentiating biochemical and chemotaxonomic properties showed that isolate 91A-612^T represents a novel species of the genus *Chryseobacterium*, for which we propose the name *Chryseobacterium arachidiradicis* sp. nov. (type strain 91A-612^T=LMG 27814^T=CCM 8490^T=CIP 110647^T).

Since the proposal of the genus *Chryseobacterium* by Vandamme *et al.* (1994), the genus has been one of the most rapidly growing genera, comprising many species from very different places of isolation. Within the last few years, several novel species of the genus *Chryseobacterium* have been proposed (e.g. Hugo *et al.*, 2003; Kämpfer *et al.*, 2003; 2009, 2010a, b, 2011; Li *et al.*, 2003; de Beer *et al.*, 2005; Kim *et al.*, 2005, 2008; Shen *et al.*, 2005; Shimomura *et al.*, 2005; Young *et al.*, 2005; Park *et al.*, 2006; Tai *et al.*, 2006; Behrendt *et al.*, 2007, 2008; Hantsis-Zacharov *et al.*, 2008; Herzog *et al.*, 2008; Szoboszlay *et al.*, 2008; Weon *et al.*, 2008; Cho *et al.* 2010), with more than 20 species proposed since 2013 (Bajerski *et al.*, 2013; Charimba *et al.*, 2013; Hoang *et al.*, 2013; Holmes *et al.*, 2013; Kirk *et al.*, 2013; Montero-Calasanz *et al.*, 2013, 2014; Nguyen *et al.*, 2013; Park *et al.*, 2013; Sang *et al.*, 2013; Wu *et al.*, 2013; Kämpfer *et al.*, 2014a, b, 2015; Kook *et al.*, 2014; Loch & Faisal, 2014; Venil *et al.*, 2014; Chen *et al.*, 2015; Zhao *et al.*, 2015). Among these species, several were found in close association with plants (Nguyen *et al.*, 2013; Sang *et al.*, 2013; Chen *et al.*, 2014; Kämpfer *et al.*, 2014a, 2015; Montero-Calasanz *et al.*, 2014; Venil *et al.*, 2014; Zhao *et al.*, 2015).

A yellow-pigmented isolate (91A-612^T), originating from the root, i.e. the geocarposphere (soil around the peanut), of very immature peanuts grown at the Wiregrass Research

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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 91A-612^T is KP271932.

and Extension Center in Headland (Henry county), AL, USA, and originally grown on tryptic soy agar (TSA; Oxoid), was further studied and subcultivated on nutrient agar (NA; Oxoid) at 30 °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 the type strains of those species most closely related to it on the basis of 16S rRNA gene sequence similarity.

Because of the high 16S rRNA gene sequence similarities to *Chryseobacterium molle* DW3^T, *C. pallidum* 26-3St2b^T and *C. hominis* NF802^T, which had been previously studied in detail in our laboratory (Vaneechoutte *et al.*, 2007; Herzog *et al.*, 2008), these strains were chosen for comparison of physiological and biochemical tests, fatty acid analysis and DNA–DNA hybridization.

Cultural and morphological characteristics were recorded from cultures grown on NA for 48 h at 30 °C. The Gram stain reaction was tested on the basis of a modified method of Gerhardt *et al.* (1994) and the motility test was done under a light microscope on cells grown for 3 days in nutrient broth (Oxoid) at 30 °C. The oxidase reaction was assessed with oxidase reagent (bioMérieux) according to the instructions of the manufacturer. Catalase activity was recorded by observation of gas formation after dropping H_2O_2 on fresh biomass grown for 48 h on NA. In addition, growth was studied at 4, 8 or 10, 30, 37, 45 and 50 °C on NA. Tolerance of NaCl was investigated in tryptic soy broth (TSB; Oxoid) supplemented with 0.5–8.0 % (w/v) NaCl and pH dependency in TSB adjusted to pH 4.5–12.5 (in increments of 1.0 pH unit) by the addition of HCl or NaOH.

Isolate 91A-612^T showed Gram-negative staining behaviour and formed visible (diameter about 2 mm) yellowish colonies within 48 h at 30 °C. The colonies were translucent and glistening and showed entire edges. A bright-yellow pigment of the flexirubin type (KOH method according to Reichenbach, 1989) was produced on NA. Strain 91A-612^T was positive for oxidase activity and non-motile, and microscopic examination showed non-spore-forming rods (approx. 1 μm wide and 2 μm long).

Good growth was recorded on NA, as well as on brain heart infusion agar, R2A agar and TSA, but no growth was observed on MacConkey agar (Oxoid). No growth was observed below 4 °C or above 45 °C; growth at 4 and 45 °C was very weak. Strain 91A-612^T grew in TSB at 28 °C in the presence of 1 % (w/v) NaCl, but not 2 %, and in TSB adjusted to pH 5.5–10.5 but not pH 4.5 or 11.5.

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, activities 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) and hydrolysis of casein, gelatin (plate method), starch and tyrosine (Smibert & Krieg, 1994). Isolate 91A-612^T utilized very few carbon sources, similar to all members of the genus *Chryseobacterium*, but was able to produce acid from D-glucose, sucrose and L-arabinose and to produce acid weakly from L-rhamnose, and was also able to hydrolyse many chromogenic substrates. The biochemical and physiological data are given in Table 1 and in the species description.

For phylogenetic identification, the nearly full-length 16S rRNA gene of strain 91A-612^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 1441 nt, spanning positions 8–1474 (according to the *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 most closely related type strains. Phylogenetic trees including the type strains of all species of the genus *Chryseobacterium* were reconstructed 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). Sequences not included in the database were added after the sequences were aligned with the SILVA Incremental Aligner (SINA version 1.2.11; Pruesse *et al.*, 2012). The alignment of all sequences used for tree reconstruction was checked manually considering secondary structure information of the 16S rRNA. A maximum-likelihood tree (Fig. 1) was generated using RAxML version 7.04 (Stamatakis, 2006) with

Table 1. Comparison of physiological characteristics of strain 91A-612^T with closely related members of the genus *Chryseobacterium*

Species/strains: 1, 91A-612^T; 2, *C. pallidum* 26-3St1a^T; 3, *C. molle* DW3^T; 4, *C. hominis* (n=11). All data were from this study, obtained under exactly the same conditions. +, All strains tested positive; (+), weakly positive; −, all strains tested negative.

Characteristic	1	2	3	4
Acid production from:				
Glucose	+	+	(+)	+
Sucrose	+	(+)	−	−
Arabinose	+	(+)	(+)	−
Maltose	−	(+)	(+)	+
L-Rhamnose	+	−	−	−
Trehalose	−	(+)	−	−
Nitrate reduction	−	+	+	−

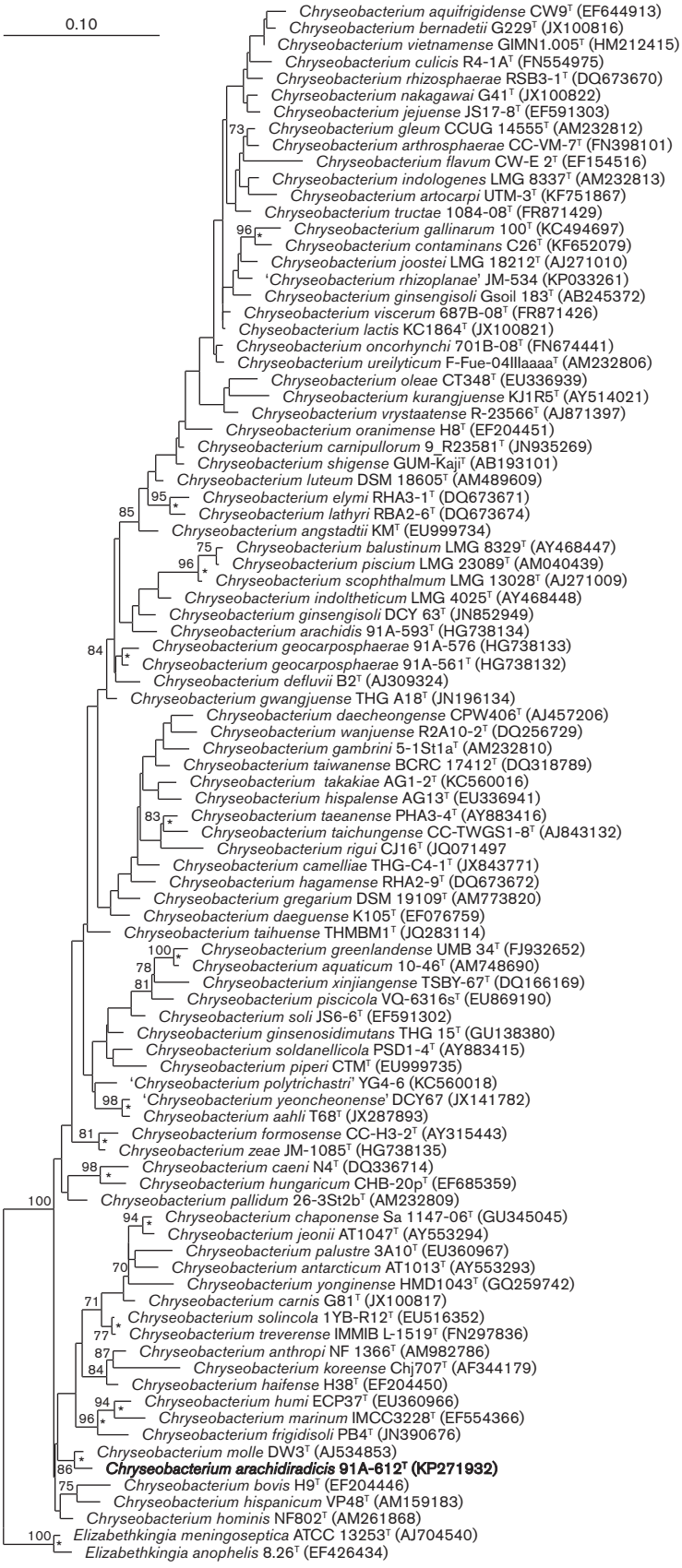

Fig. 1. Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic position of strain 91A-612^T and the type strains of the most closely related species of the genus *Chryseobacterium*. The tree was reconstructed in ARB using RAxML with rapid bootstrap analysis and 100 replications. Nucleotide positions 96–1394 (*E. coli* numbering; Brosius *et al.*, 1978) were included in the analysis. Bootstrap values $\geqslant$70 % are shown at branch nodes. Asterisks represent nodes with high bootstrap support, also present with high bootstrap support in the maximum-parsimony tree. The type strains of two species of the genus *Elizabethkingia* were used as an outgroup. Bar, 0.1 nucleotide substitutions per site.

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 sequence positions 96–1394 (according to Brosius *et al.*, 1978) and on 100 replications (bootstrap analysis; Felsenstein 1985).

The type strains of *C. molle* (98.4 % similarity), *C. pallidum* (98.3 %) and *C. hominis* (97.8 %) shared the highest 16S rRNA gene sequence similarity with strain 91A-612^T; the sequence similarity to the type strains of all other species of the genus *Chryseobacterium* was below 97.0 %. Both phylogenetic trees showed a clear placement of strain 91A-612^T within the genus *Chryseobacterium* and a distinct clustering with the type strain of *C. molle* (70 and 85 % bootstrap support in the maximum-parsimony and maximum-likelihood trees, respectively). A distinct clustering with type strains of other species of the genus *Chryseobacterium* was not observed; clustering with other species of the genus *Chryseobacterium* varied between the treeing methods.

DNA–DNA hybridization experiments were performed between strain 91A-612^T and the type strains of the three most closely related species of the genus *Chryseobacterium* according to the method of Ziemke *et al.* (1998) (except that, for nick translation, 2 μg DNA was labelled for 3 h at 15 °C). Strain 91A-612^T showed low DNA–DNA hybridization to all three type strains, *C. molle* DW3^T (33 %, reciprocal 25 %), *C. pallidum* 26-3St2b^T (40 %, reciprocal 13 %) and *C. hominis* NF802^T (45 %, reciprocal 26 %).

Analysis of cellular fatty acid profiles was performed as described previously (Kämpfer & Kroppenstedt, 1996) using an HP 6890 gas chromatograph with Sherlock MIDI software version 2.11 and TSBA peak-naming table version 4.1 and revealed the following most abundant fatty acids: iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{17:1}\omega9c$ and iso-$C_{15:0}$ 2-OH, detected as summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$) but shown in earlier studies to be clearly identified as iso-$C_{15:0}$ 2-OH (Vandamme *et al.*, 1994; Montero-Calasanz *et al.*, 2013).

The complete fatty acid pattern of 91A-612^T also showed slight, mainly quantitative differences from the reference type strains, and is shown in Table 2 in comparison with those of the type strains of the most closely related species of the genus *Chryseobacterium*.

Polyamines, quinones and polar lipids were extracted from biomass grown in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2). Biomass subjected to polyamine extraction was harvested at the late exponential growth phase, whereas biomass subjected to quinone and polar lipid extraction was harvested during the stationary growth phase. Polyamine analysis was carried out as described previously (Busse & Auling, 1988; Busse *et al.*, 1997). Quinones and polar lipids were extracted by an integrated procedure as described by Tindall (1990a, b) and Altenburger *et al.* (1996). HPLC analyses were carried out using the equipment described by Stolz *et al.* (2007). The polyamine pattern of strain 91A-612^T contained 37.4 μmol *sym*-homospermidine (g dry weight)$^{-1}$, 8.0 μmol spermidine (g dry weight)$^{-1}$, 6.2 μmol spermine (g dry weight)$^{-1}$ and traces [<0.1 μmol (g dry weight)$^{-1}$] of putrescine and cadaverine. The quinone system of strain 91A-612^T contained menaquinones MK-6 (95.5 %) and MK-5 (4.5 %). The polar lipid profile of strain 91A-612^T was composed of the major lipids phosphatidylethanolamine and several unidentified lipids, including an aminolipid (AL1) and two lipids that did not contain a sugar moiety, an amino group or a phosphate group (L3, L8), moderate amounts of lipid L1 and aminolipid AL3 and minor amounts of aminolipids AL2 and AL4, phospholipid PL1 and aminophospholipid APL1 (Fig. 2). Polyamine patterns and the quinone system

Table 2. Long-chain fatty acid composition of strain 91A-612^T and type strains of related species of the genus *Chryseobacterium*

Strains: 1, 91A-612^T; 2, *C. pallidum* 26-3St1a^T; 3, *C. molle* DW3^T; 4, *C. hominis* NF802^T. All data were obtained in this study under exactly the same conditions. –, Not detected.

Fatty acid	1	2	3	4
$C_{12:0}$	1.4	–	–	–
$C_{13:0}$	0.8	–	–	–
$C_{13:1}$ at 12–13	–	1.3	–	–
iso-$C_{13:0}$	0.8	1.5	1.8	3.7
Unknown 13.565*	4.9	4.6	2.0	6.3
$C_{14:0}$	2.3	–	–	–
$C_{15:0}$	1.2	–	–	–
iso-$C_{15:0}$	25.1	33.7	22.0	31.5
iso-$C_{15:0}$ 3-OH	2.3	3.2	4.0	6.1
$C_{15:0}$ 2-OH	–	1.0	1.6	–
anteiso-$C_{15:0}$	3.9	4.8	6.7	8.8
$C_{16:0}$	7.9	3.8	5.4	–
$C_{16:0}$ 3-OH	4.4	4.9	6.5	–
$C_{16:1}\omega5c$	1.6	1.6	1.5	–
$C_{17:0}$	1.1	–	–	–
iso-$C_{17:0}$ 3-OH	9.7	12.1	14.5	18.1
$C_{17:0}$ cyclo	–	–	–	–
iso-$C_{17:1}\omega9c$	1.7	2.3	5.2	15.6
Unknown 16.582*	–	1.0	–	–
$C_{18:0}$	2.4	–	–	–
$C_{19:0}$	1.1	–	–	–
$C_{20:0}$	2.2	–	–	–
Summed feature 4†	24.9	24.4	22.5	7.5
Summed feature 5†	–	–	–	2.4

*Unknown fatty acid; numbers indicate equivalent chain-length.

†Fatty acids that could not be separated by GC using the Microbial Identification System (Microbial ID) software were considered summed features. Summed feature 4 contains iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$ but, as shown before (Vandamme *et al.*, 1994; Montero-Calasanz *et al.*, 2013), could be identified clearly as iso-$C_{15:0}$ 2-OH. Summed feature 5 contains iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

are in agreement with the description of the genus *Chryseobacterium* (Vandamme *et al.* 1994; Kämpfer *et al.*, 2003), and a polar lipid profile consisting of the only known lipid phosphatidylethanolamine and several unidentified lipids has been described for numerous members of the genus *Chryseobacterium* such as *Chryseobacterium defluvii* B2^T, *C. gambrini* 5-1Sta^T, *C. molle* DW3^T, *C. ginsengisoli* DCY 63^T, *C. indoltheticum* LMG 4025^T and *C. gleum* ATCC 35910^T (Kämpfer *et al.*, 2003; Herzog *et al.*, 2008; Nguyen *et al.*, 2013).

On the basis of the results of our genotypic and phenotypic analyses, strain 91A-612^T warrants description as a representative of a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium arachidiradicis* sp. nov. is proposed.

Description of *Chryseobacterium arachidiradicis* sp. nov

Chryseobacterium arachidiradicis (a.ra′chi.di.ra′di.cis. N.L. n. *Arachis -idis* the generic name of the peanut plant; L. n. *radix -icis* root; N.L. gen. n. *arachidiradicis* of the root of *Arachis*).

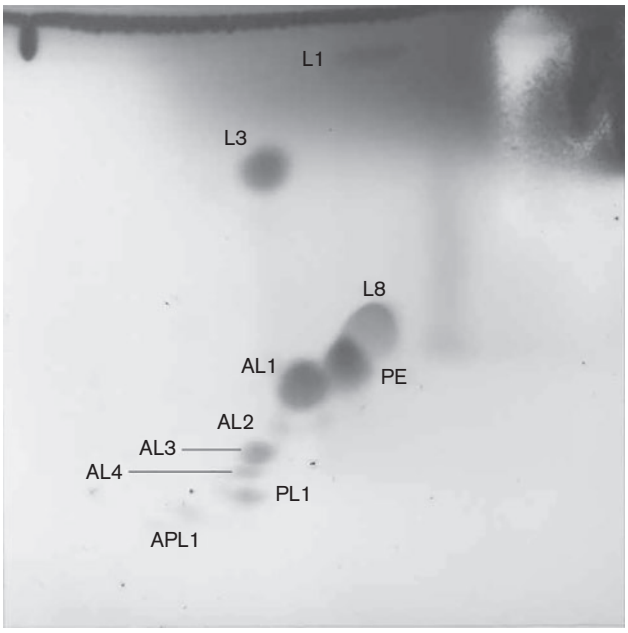

Fig. 2. Polar lipid profile of strain 91A-612^T after separation by two-dimensional TLC and detection using 5 % ethanolic molybdatophosphoric acid. PE, Phosphatidylethanolamine; AL1–4, unidentified aminolipids; PL1, unidentified phospholipid; APL1, unidentified aminophospholipid; L1, L3 and L8, unidentified polar lipids not detectable with any of the spray reagents specific for lipids containing a phosphate group, an amino group or a sugar moiety. Labels correspond to those used by Kämpfer *et al.* (2014a).

Cells show Gram-negative staining. They are non-motile, non-spore-forming rods, approx. 1 µm wide and 2 µm long. Aerobic, oxidase-positive and catalase-positive. Good growth occurs after 48 h on NA, brain heart infusion agar, TSA and R2A agar (all from Oxoid) but not on MacConkey agar (Oxoid) at 30 °C. Growth occurs on NA at 8–37 °C, but growth occurs only weakly at 4 and 45 °C, and not at 55 °C. Cells grow at 28 °C in the presence of 1.0 % NaCl (not at 2 %) as an additive ingredient of TSB and at pH 5.5–10.5 but not at pH 4.5 or 11.5. Colonies on NA are smooth, yellowish, circular, translucent and glistening with entire edges. Colonies become mucoid, and cannot be identified as single entities, after prolonged incubation. The yellow pigment of the flexirubin type is non-diffusible and non-fluorescent. Produces acid from D-glucose, L-arabinose, sucrose (weakly) and L-rhamnose. No acid is produced from adonitol, D-arabitol, dulcitol, erythritol, *myo*-inositol, lactose, D-mannitol, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose or D-xylose. Indole production and hydrolysis of casein, gelatin, starch, DNA and tyrosine are positive, while production of brown diffusible pigments on tyrosine agar, hydrogen sulphide production and activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and *β*-galactosidase (ONPG) are negative. The following compounds are utilized very weakly as sole sources of carbon: D-glucose, maltose, sucrose and L-rhamnose. The following compounds are not utilized as sole sources of carbon: acetate, propionate, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, gluconate, D-mannose, D-fructose, trehalose, glycerol, D-mannitol, maltitol, melibiose, D-ribose, salicin, D-xylose, adonitol, *myo*-inositol, D-sorbitol, putrescine, *cis*- and *trans*-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L-alanine, *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 (weakly), pNP *β*-D-galactopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, 2-deoxythymidine 2′-pNP phosphate, L-alanine *p*-nitroanilide (pNA), γ-L-glutamate pNA and L-proline pNA are hydrolysed. pNP *β*-D-xylopyranoside and pNP *β*-D-glucuronide are not hydrolysed. The major cellular fatty acids (>15 %) are iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, detected as summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso-$C_{17:1}\omega9c$ and iso-$C_{17:0}$ 3-OH. The polyamine pattern contains the major compound *sym*-homospermidine and moderate amounts of spermidine and spermine. The quinone system is predominantly menaquinone MK-6. The polar lipid profile is composed of the major lipids phosphatidylethanolamine and unidentified aminolipid AL1 and lipids L3 and L8. Furthermore, moderate amounts of unidentified lipid L1 and aminolipid AL3 and minor amounts of

aminolipids AL2 and AL4, phospholipid PL1 and aminophospholipid APL1 may be detectable.

The type strain is 91A-612^T (=LMG 27814^T=CCM 8490^T=CIP 110647^T), isolated from the root, i.e. the geocarposphere (soil around the peanut), of very immature peanuts (*Arachis hypogaea*) in Alabama, USA.

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