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ORIGINAL PAPER

Okibacterium endophyticum sp. nov., a novel endophytic actinobacterium isolated from roots of *Salsola affinis* C. A. Mey

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Abstract A white bacterial strain, designated EGI 650022^T, was isolated from the roots of *Salsola affinis* C. A. Mey, collected from Urumqi City, Xinjiang, north-western China. The strain was found to be aerobic, Gram-stain positive, oxidase-positive and catalase-positive. Cells were non-motile and irregular rods. Growth occurred at NaCl concentrations between 0 and 7 % (w/v), temperatures between 5 and 45 °C, and pH 6.0–9.0. Phylogenetic analysis based on 16S rRNA gene sequence indicated that strain EGI 650022^T belongs to a clade with the genera *Okibacterium* and *Plantibacter* in the family *Microbacteriaceae*. The novel strain EGI 650022^T showed highest levels of 16S rRNA gene sequence similarity with members of the genera *Okibacterium* and *Plantibacter* (97.2–98.0 %). The cell-wall peptidoglycan contained glutamate, homoserine, glycine, alanine and lysine. The predominant menaquinones (MKs) were MK-11, MK-12 and MK-12 (H_4). The polar lipid pattern comprised phosphatidylglycerol, diphosphatidylglycerol, two unknown glycolipids and two unknown phospholipids. The major fatty acids were anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The DNA G+C content was 66.0 mol%. The DNA–DNA relatedness values of strain EGI 650022^T with *Okibacterium fritillariae* DSM 12584^T, *Plantibacter flavus* DSM 14012^T and *Plantibacter auratus* DSM 19586^T

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were 39.7, 19.7 and 22.0 %. Based on phylogenetic, phenotypic, chemotaxonomic and DNA–DNA hybridization data, strain EGI 650022^T is considered to represent a novel species of the genus *Okibacterium*, for which the name *Okibacterium endophyticum* sp. nov. is proposed; the type strain is EGI 650022^T (=JCM 30086^T = KCTC 29492^T).

Keywords *Okibacterium endophyticum* sp. nov. · Endophytic actinobacterium · Polyphasic taxonomy

Introduction

The genus *Okibacterium* was first proposed by Evtushenko et al. (2002) and comprises only one species, *Okibacterium fritillariae*, which was isolated from seeds of *Fritillaria ruthenica* Wikstr and *Clematis recta* L. *Okibacterium* strains contain lysine, alanine, glycine, glutamate and homoserine in their cell wall peptidoglycan, which enable them to be differentiated from members of the genus *Plantibacter* (Behrendt et al. 2002). The genus *Plantibacter* currently contains two recognized species and can be clustered with *Okibacterium* in the phylogenetic tree based on 16S rRNA gene sequence analysis (Lin and Yokota 2006; as shown in Fig. 1). In this study, we describe a bacterial strain, designated EGI 650022^T, which was isolated from surface-sterilized root of *Salsola affinis* C. A. Mey, collected from Urumqi City, Xinjiang. Comparative 16S rRNA gene sequence analysis indicated that strain EGI 650022^T is closely related to *O. fritillariae* JCM 12284^T, *Plantibacter flavus* DSM 14012^T and *Plantibacter auratus* DSM 19586^T (highest levels of 16S rRNA gene sequence similarity of 98.0, 97.5 and 97.2 %, respectively). The aim of this work was to determine the exact taxonomic position of strain EGI 650022^T by using a polyphasic approach.

Materials and methods

Isolation and maintenance of organism

The halophyte plant *S. affinis* C. A. Mey was collected from the suburban district of Urumqi, Xinjiang Province and used as a source for isolation of bacterial strains. Strain EGI 650022^T was isolated from the

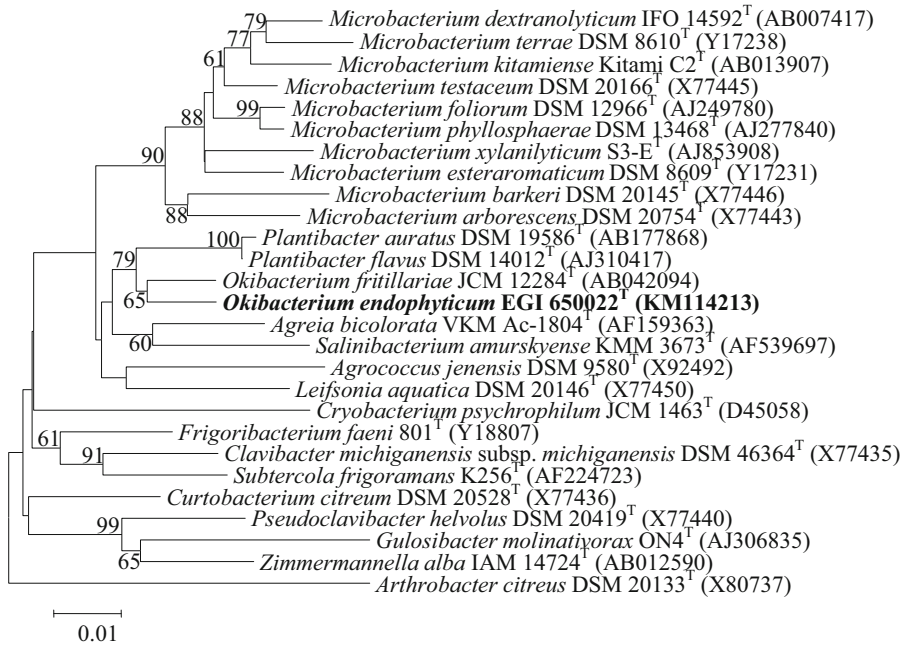

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of strain EGI 650022^T and related taxa. *Numbers* at the *nodes* indicate the level of bootstrap values (>50 %) based on 1,000 replications. *Bar* 0.01 changes per nucleotide position

roots of healthy halophyte plant *S. affinis* C. A. Mey. The healthy plant samples were washed in running tap water to remove adhered epiphytes and surface-sterilized according to the five-step sterilization procedure (Qin et al. 2008). The surface-sterilized roots were aseptically crumbled into smaller fragments using a commercial Joyoung blender (Qin et al. 2009), spread onto glycerol-asparagine agar (ISP 5; Shirling and Gottlieb 1966) amended with 3 % NaCl, and incubated at 30 °C for 4–6 weeks. The purified strain was maintained on yeast extract-malt extract agar (ISP 2; Shirling and Gottlieb 1966) slants at 4 °C for short-term preservation and as 25 % (v/v) glycerol suspension at −80 °C for long-term preservation. Strain EGI 650022^T has been deposited in the Korean Collection for Type Cultures (KCTC; South Korea) and the Japan Collection of Microorganisms (JCM; Japan) under the accession numbers KCTC 29492^T and JCM 30086^T, respectively.

Phenophytic characterization

Gram-stain was carried out by using the standard Gram stain procedure. The morphological, physiological and biochemical characteristics of strain EGI 650022^T were investigated using cultures grown on ISP 2 medium, at 30 °C for 5 days. Cell morphology was observed using light microscopy (BH-2; Olympus) and scanning electron microscopy (QUANTA200; FEI). Colours of colonies were determined by using colour chips from the ISCC-NBS colour charts standard (Kelly 1964). Cell motility was tested by monitoring the degree of turbidity on motility test medium as described by MacFaddin (1980). Growth at different temperatures (0, 5, 10, 15, 20, 25, 30, 37, 40, 45, 50, 55 and 60 °C), different NaCl concentrations (0–10 %, w/v) (at intervals of 1 %) was tested on ISP 2 medium at 30 °C for 14 days. The pH range (4.0–12.0 at intervals of 1.0 pH units) for growth was tested in ISP 2 liquid medium with the pH adjusted as described by Xu et al. (2005). Catalase and oxidase activities were determined in 3 % (v/v) H_2O_2 and 1 % (w/v) tetramethyl-*p*-phenylenediamine, respectively. Decomposition of test substances was performed by using the media and methods of Gonzalez et al. (1978). H_2S production was tested as described by Lee et al. (2001). Other biochemical tests including methyl red and indole tests were detected according to Goodfellow (1986). Nutritional features were determined using Biolog GN III MicroPlate according to manufacturer's instructions and the reactions were observed after incubating the plates at 30 °C for 12–48 h. Enzyme activities were examined using the API ZYM kit (bioMérieux) following the manufacturer's instructions. Other physiological and biochemical tests were performed using API 20NE and API 50CH.

O. fritillariae JCM 12284^T, *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T, which were used as reference strains for phenotypic characterization, fatty acid and DNA–DNA hybridization, were obtained from the JCM (Japan) and the Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ, Germany).

Chemotaxonomy

Cell biomass of strain EGI 650022^T for DNA extraction and for the analysis of menaquinones (MKs), cell wall peptidoglycan and polar lipids were obtained from cultures grown on ISP 2 medium at 30 °C for 5 days. MKs were extracted and analysed as described previously (Collins et al. 1977; Kroppenstedt 1982), using reversed-phase HPLC with an Agilent ZORBAX Eclipse XDB-C18 (150 × 4.6 mm) column. A purified cell-wall preparation was obtained and hydrolysed as described by Kim and Lee (2011). Amino acids in cell-wall hydrolysates were analysed by using precolumn derivatization with *o*-phthalaldehyde (OPA) by using HPLC as described by Tang et al. (2009). Polar lipids were extracted and separated by two-dimensional TLC following the method of Minnikin et al. (1984), and identified by spraying the plates with 10 % ethanolic molybdophosphoric acid, molybdenum blue, ninhydrin and α-naphthol reagents (Minnikin et al. 1984; Komagata and Suzuki 1987) and with Dragendorff's reagent (Sigma). For cellular fatty acid analysis, cell biomass of strain EGI 650022^T, *O. fritillariae* JCM 12284^T, *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T was harvested from TSA plates [tryptic soy agar (soybean-casein digest agar medium); BD] at 30 °C for 5 days. Cellular fatty acids analysis was performed as described by Sasser (1990) according to the standard protocol of the MIDI/Hewlett Packard microbial identification system (Sherlock Version 6.1; MIDI database TSBA6). For identification of the mycolic acids present in the cell wall, one-

dimensional TLC was carried out following the standard procedure by Minnikin et al. (1975).

Molecular analysis

Extraction of chromosomal DNA and the amplification of the 16S rRNA gene by PCR were performed as described by Li et al. (2007). The DNA G+C content of strain EGI 650022^T was determined by the method of Mesbah et al. (1989). The 16S rRNA gene was sequenced at Sangon Biotech (Shanghai) Co., Ltd. (http://www.sangon.com), and was identified using the Eztaxon-e server database (Kim et al. 2012). Alignment of sequences was carried out with CLUSTAL X 1.83 software (Thompson et al. 1997). Phylogenetic tree was constructed according to the neighbor-joining (Saitou and Nei 1987) methods by using MEGA version 5.0 (Tamura et al. 2011). Evolutionary distances were computed using the Kimura two-parameter method (Kimura 1983). Bootstrap analysis was used to evaluate reliability of tree topology of the neighbor-joining data by performing 1,000 replications (Felsenstein 1985). DNA–DNA hybridization tests were carried out by the fluorometric micro-well method (Ezaki et al. 1989; Christensen et al. 2000). Hybridization was performed with five replications for each example, and the two extreme values (highest and lowest) for each sample were excluded. DNA–DNA relatedness values are expressed by calculating the means of the remaining three values. The hybridization temperature was 46 °C.

Results and discussion

Phenotypic characteristics

Strain EGI 650022^T was found to be Gram-staining positive, aerobic, non-spore-forming, non-motile and irregular rod-shaped (Supplementary Fig. S2). Colonies on ISP 2 medium were observed to be smooth, circular, convex, translucent and white-coloured. Growth of strain EGI 650022^T was observed at 5–45 °C, pH 6.0–9.0 and 0–7 % NaCl (w/v), with optimal growth at 25–30 °C, 2–4 % NaCl (w/v) and pH 7.0–8.0. In contrast to strains *O. fritillariae* JCM 12284^T, *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T, EGI 6500322^T tolerated wider temperature range (5–45 °C) and higher salt concentration (up to 7 % NaCl), which distinguished the novel strains from its closest related reference strains. The strain was oxidase-positive, which is the same as the type strain of *O. fritillariae* JCM 12284^T and significantly different to the type strains of *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T. The main characteristics that differentiate strain EGI 650022^T from species of the genera *Okibacterium* and *Plantibacter* are listed in Table 1.

Chemotaxonomic characteristics

The predominant MKs detected in strain EGI 650022^T were determined to be MK-12 (51.6 %), MK-11 (22.0 %), MK-12(H_4) (16.0 %) and MK-10 (10.4 %). The amino acids in the peptidoglycan layer of strain EGI 650022^T comprised glutamic acid, glycine, alanine, homoserine and lysine. The peptidoglycan type of EGI 650022^T was similar to those of the type strain of *O. fritillariae* JCM 12284^T, but differs significantly compared with *Plantibacter* species; L-2,4-diaminobutyric acid was not detected. Mycolic acids were absent. The polar lipids detected in strain EGI 650022^T were diphosphatidylglycerol, phosphatidylglycerol, two unidentified glycolipids and two unidentified phospholipids (Supplementary Fig. S1). The major fatty acid profile mainly consisted of (>10 % of the total fatty acids) anteiso-$C_{15:0}$ (37.5 %), anteiso-$C_{17:0}$ (36.7 %) and Sum in Feature 4 (iso-$C_{17:1}$ and/or anteiso-$C_{17:1}$; 10.4 %). Qualitative and quantitative differences were noted between the cellular fatty acid composition and proportion of strain EGI 650022^T and its closest phylogenetic neighbours (Supplementary Table S1). In comparison with strains *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T, strains EGI 650022^T and *O. fritillariae* JCM 12284^T contained larger amounts of anteiso-$C_{17:1}$, and also showed smaller amounts of anteiso-$C_{15:0}$. However, strain EGI 650022^T could also be distinguished from *O. fritillariae* JCM 12284^T by the presence of anteiso-$C_{17:1}$ A.

Phylogenetic analysis

The DNA G+C content of strain EGI 650022^T was determined to be 66.0 mol% which is similar to the range of the genus *Okibacterium* (66.6–67.2 %; Evtushenko et al. 2002), but a value lower than those

Table 1 Comparison of phenotypic characteristics of strain EGI 650022^T with the reference type strains of the related taxa

Characteristics	1	2	3	4
Colony colour	White	Yellow	Yellow	Yellow
Temperature range for growth (°C)	5–45	5–35	10–35	5–35
NaCl range for growth (%)	0–7	0–6	0–3	0–4
Optimal NaCl (%)	2–4	1–3	2	2
Oxidase	+	+	−	−
H_2S production	−	W	−	−
Hydrolysis of				
Starch	−	+	−	+
Tween 60	+	−	+	+
Tween 80	−	−	−	+
API 20 NE				
Reduction of nitrates to nitrites	+	−	−	−
N-Acetyl-glucosamine, gluconate	−	−	+	+
Enzyme activity (API ZYM)				
Alkaline phosphatase	−	−	−	+
Trypsin	−	+	−	+
Chymotrypsin	−	+	−	−
β-Galactosidase	−	+	+	+
Acid production from (API 50CH)				
Glycerol, D-galactose, D-mannitol, amygdalin	−	+	+	+
Gentiobiose, D-turanose				
Xylitol, D-arabinose	−	+	−	−
D-Tagatose, inositol, D-adonitol	−	−	+	−
Lactose, arbutin, methyl-β-D-xylopyranoside	−	−	+	+
Metezitose, D-lyxose				
Raffinose	−	−	−	+
D-Glucose, L-arabinose	+	+	W	+
D-Ribose	+	+	−	+
D-Sorbitol, L-sorbose, melibiose	+	−	−	−
L-Rhamnose	+	−	+	+
Utilization of (Biolog GN III MicroPlate)				
N-Acetyl neuraminic acid, fusidic acid				
D-Serine, lincomycin, myo-inositol	−	−	+	−
Minocycline, guanidine HCl, Niaproof 4				
Vancomycin, tetrazolium violet				
Tetrazolium blue, bromo-succinic acid				
Dextrin, D-lactose, *N*-acetyl-β-D-mannosamine	−	−	+	+
N-Acetyl-D-galactosamine, L-histidine				
D-Melibiose, troleandomycin, rifamycin sv	+	−	+	−
L-Galactonic acid lactone				
β-Hydroxy-D,L-butyric acid				
Gentiobiose, D-turanose, acetoacetic acid	−	+	+	+
Sodium bromate				
Stachyose, D-galacturonic acid, formic acid	+	−	−	−

Table 1 continued

Characteristics	1	2	3	4
D-Raffinose, potassium tellurite	+	−	−	+
p-Hydroxy-phenylacetic acid, lithium chloride	+	−	+	+
Glycyl-L-proline, α-keto-butyric acid	+	+	+	−
D-Lactic acid methyl ester	+	+	−	−
L-Pyroglutamic acid	−	+	+	−
γ-Amino-butyric acid	−	+	−	−
D-Arabitol	−	+	−	+
Predominant menaquinones (MK)	11/12/12(H4)	11/10[a]	9/10[a]	10/11[a]
Polar lipids	PG/DPG/GL	PG/DPG[a]	PG/DPG/PI/GL	PG/DPG
Peptidoglycan amino acid	Lys	Lys	DAB[a]	DAB[a]
DNA G+C content (mol%)	66.0	67.0[a]	68.0[a]	70.0[a]

All data were obtained from this study except where indicated. Both strains are positive for the following characteristics: presence of catalase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase and β-glucosidase; hydrolysis of Tween 20, Tween 40 and aesculin; assimilation of glucose, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, malic acid, D-trehalose, D-cellobiose, sucrose, β-methyl-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, D-fructose, D-galactose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, glycerol, D-glucose-6-phosphate, D-fructose-6-phosphate, gelatin, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-serine, pectin, D-gluconic acid, methyl pyruvate, L-lactic acid, α-keto-glutaric acid, D-malic acid, L-malic acid, α-hydroxy-butyric acid, propionic acid and acetic acid; sensitivity of 1 % sodium lactate, nalidixic acid, aztreonam and sodium butyrate; acid production from D-xylose, fructose, D-mannose, aesculin, salicin, cellobiose, maltose, sucrose and trehalose. Both strains are negative for the following characteristics: presence of lipase (C14), β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, β-fucosidase, arginine dihydrolase and urease; assimilation of potassium gluconate, capric acid, adipic acid, citrate, phenylacetic acid, 3-methyl glucose, D-aspartic acid, D-glucuronic acid, glucuronamide, mucic acid, quinic acid and D-saccharic acid; sensitivity of D-serine; acid production from erythritol, L-xylose, dulcitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, inulin, starch, glycogen, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate; hydrolysis of casein, gelatin; and in methyl red, glucose fermentation and indole production tests

Strains 1 EGI 650022^T (*Okibacterium endophyticum* sp. nov.), *2 O. fritillariae* DSM 12584^T, *3 Plantibacter auratus* DSM 19586^T, *4 P. flavus* DSM 14012^T. + Positive, − negative, *W* weakly positive, *PG* phosphatidylglycerol, *DPG* diphosphatidylglycerol, *PI* phosphatidylinositol, *GL* unidentified glycolipids

[a] Data were obtained from Evtushenko et al. (2002), Behrendt et al. (2002) and Lin and Yokota (2006)

reported for *Plantibacter* species (68.0–70.0 %; Behrendt et al. 2002; Lin and Yokota 2006). The almost complete 16S rRNA gene sequence of strain EGI 650022^T determined in this study (Gen Bank accession number KM114213) comprised 1,529 nucleotides. The results from the EzTaxon server (http://www.eztaxon.org/) indicated that the closest phylogenetic neighbours were *O. fritillariae* JCM 12284^T, *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T, which showed 98.0, 97.5 and 97.2 % of 16S rRNA gene sequence similarities to strain EGI 650022^T, respectively. Analysis based on the neighbor-joining method showed that strain EGI 650022^T grouped with the type species of the genus *Okibacterium*, and formed a cluster with *O. fritillariae* DSM 12584^T, *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T (Fig. 1). Furthermore, strains *O. fritillariae* JCM 12284^T, *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T were selected as the representative strains to perform DNA–DNA hybridization studies. The experiments showed that DNA–DNA relatedness values with *O. fritillariae* JCM 12284^T, *P. flavus* DSM 14012^T and *P. auratus* DSM 19586^T were 39.7, 19.7 and 22.0 %, respectively (Supplementary Table S2), which is significantly less than 70 % cut-off point according to the criterion recommended for the delineation of bacterial species by Stackebrandt and Goebel (1994).

Conclusion

The results obtained from the chemotaxonomic and phylogenetic analysis clearly indicate that strain EGI

650022^T is a member of the genus *Okibacterium* (Table 1; Fig. 1). Strain EGI 650022^T could be distinguished from the type strain of *O. fritillariae* by differences in phenotypic and chemotaxonomic features, including colony color, decomposition of starch and Tween 60, H_2S production, nitrate reduction, utilization and product acid of some substrates, activity of some enzymes and susceptibility to some chemical substrates, MKs composition (Table 1). These differences, in combination with the phylogenetic analysis and DNA–DNA hybridization data of strain EGI 650022^T, suggest that the novel strain EGI 650022^T is differentiated from the type strain of the *O. fritillariae*. Therefore, strain EGI 650022^T is considered to represent a new species of the genus *Okibacterium*, for which the name *Okibacterium endophyticum* sp. nov. is proposed.

Description of *O. endophyticum* sp. nov.

Okibacterium endophyticum (en.do.phy'ti.cum. Gr. pref. *endo*, within; Gr. n. *phyton*, plant; L. fem. suff. –icum, adjectival suffix used with the sense of belonging to; N.L. neut. adj. *endophyticum*, within plant, *endophytic*, pertaining to the original isolation from plant tissues).

Cells are Gram-stain positive, catalase-positive, oxidase-positive, aerobic and non-motile, irregular rods (length 0.78–1.25 μm, width 0.27–0.29 μm). Colonies grown on ISP 2 agar are white-colored, convex and round. Growth occurs at 5–45 °C on ISP 2 medium, with optimum growth occurring at 25–30 °C. Cells grow in the presence of 0–7 % (w/v) NaCl (optimum 2–4 %) and at pH 6.0–9.0 (optimum pH 7.0–8.0). Hydrolyses Tween 20, Tween 40 and Tween 60, but not starch, casein and Tween 80. H_2S production and methyl red test are negative. In the API 20NE test system, positive for aesculin hydrolysis, nitrate reduction, assimilation of arabinose, glucose, malic acid, maltose, mannitol and mannose, but negative for arginine dihydrolase, β-galactosidase, gelatin hydrolysis, glucose fermentation, indole production, urease and assimilation of *N*-acetyl-glucosamine, adipic acid, capric acid, citrate, phenylacetic acid and potassium gluconate. In the API ZYM test system, positive for acid phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), α-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase, but negative for *N*-acetyl-β-glucosaminidase, alkaline phosphatase, chymotrypsin, β-fucosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin. In the API 50CH test system, acid is produced only from aesculin, L-arabinose, cellobiose, fructose, D-glucose, maltose, D-mannose, melibiose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, sucrose, trehalose and D-xylose. According to the Microplates (Biolog GN III) assay, positive for acetic acid, *N*-acetyl-D-glucosamine, L-alanine, L-arginine, L-aspartic acid, aztreonam, D-cellobiose, formic acid, D-fructose, D-fructose-6-phosphate, D-fucose, L-fucose, D-galactose, L-galactonic acid lactone, D-galacturonic acid, gelatin, D-gluconic acid, D-glucose-6-phosphate, L-glutamic acid, glycerol, glycyl-L-proline, α-hydroxy-butyric acid, β-hydroxy-D,L-butyric acid, *p*-hydroxy-phenylacetic acid, inosine, α-keto-butyric acid, α-keto-glutaric acid, L-lactic acid, D-lactic acid methyl ester, lithium chloride, D-melibiose, methyl pyruvate, β-methyl-D-glucoside, nalidixic acid, pectin, potassium tellurite, propionic acid, D-raffinose, L-rhamnose, rifamycin sv, D-salicin, L-serine, sodium butyrate, 1 % sodium lactate, D-sorbitol, stachyose, sucrose,D-trehalose, troleandomycin and Tween 40; but negative for acetoacetic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl neuraminic acid, γ-amino-butyric acid, D-arabitol, D-aspartic acid, bromo-succinic acid, dextrin, fusidic acid, gentiobiose, glucuronamide, D-glucuronic acid, guanidine HCl, L-histidine, myo-inositol, α-D-lactose, lincomycin, 3-methyl glucose, minocycline, mucic acid, niaproof 4, L-pyroglutamic acid, quinic acid, D-saccharic acid, D-serine, sodium bromate, tetrazolium blue, tetrazolium violet, D-turanose and vancomycin. The major cellular fatty acids are anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The predominant MKs are MK-12, MK-11 and MK-12 (H4). The cell wall peptidoglycan contains the amino acids glutamic acid, glycine, alanine, homoserine and lysine. The polar lipid profile comprises diphosphatidylglycerol, phosphatidylglycerol, two unidentified glycolipids and two unidentified phospholipids. The G+C content of the genomic DNA of the type strain is 66.0 mol%.

The type strain, EGI 650022^T (=KCTC 29492^T = JCM 30086^T), was isolated from the roots of a healthy halophyte sample of *S. affinis* C. A. Mey, collected from suburban district of Urumqi, Xinjiang province, north-west China. The 16S rRNA gene

sequence of strain EGI 650022^T has been deposited in GenBank under the accession number KM114213.

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