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ORIGINAL PAPER

Saccharopolyspora dendranthemae sp. nov. a halotolerant endophytic actinomycete isolated from a coastal salt marsh plant in Jiangsu, China

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Abstract A halotolerant actinomycete strain, designated strain KLBMP 1305^T, was isolated from a salt marsh plant *Dendranthema indicum* (Linn.) Des Moul collected from the coastal region of Nantong, Jiangsu Province, in east China and was studied in detail for its taxonomic position. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain KLBMP 1305^T is a member of the genus *Saccharopolyspora.* The 16S rRNA gene sequence similarity indicated that strain KLBMP 1305^T was most closely related to '*Saccharopolyspora pathumthaniensis*' S582^T (99.31 %), '*Saccharopolyspora endophytica*' YIM 61095^T (99.17 %) and *Saccharopolyspora tripterygii* YIM 65359^T (99.15 %); similarity to other type strains of the genus *Saccharopolyspora* was <97.2 %. The organism had chemical and morphological features consistent with its classification in the genus *Saccharopolyspora* such as *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell wall peptidoglycan and arabinose and galactose as the diagnostic sugars. The predominant menaquinone was MK-9(H_4). The polar lipids detected were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unknown glycolipid and an unknown lipid. The major fatty acids were iso-$C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and sum in feature 8 (18:1ω7*c*/18:1ω6*c*). The G+C content of the genomic DNA of the type strain was 68.7 mol%. DNA–DNA relatedness data, together with phenotypic differences, clearly distinguished the isolate from its closest relatives. On the basis of these phenotypic and genotypic data, the isolate represents a novel species, for which the name *Saccharopolyspora dendranthemae* sp. nov. is proposed. The type strain is KLBMP 1305^T (=KCTC 19889^T = NBRC 108675^T).

Keywords *Saccharopolyspora dendranthemae* sp. nov. · Coastal halophyte · Endophytic · Polyphasic taxonomy

Yue-Ji Zhang and Wen-Di Zhang contributed equally to this study.

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Introduction

The genus *Saccharopolyspora*, which belongs to the family *Pseudonocardiaceae*, was first described by Lacey and Goodfellow (1975). The genus encompasses aerobic, gram-positive, non-acid-fast organisms with substrate hyphae that either fragment into rod-shaped elements, do not fragment or are partially transformed into chains of spores and aerial hyphae that segment into bead-like chains of spores (Korn-Wendisch et al. 1989). Members of this genus are characterized chemotaxonomically by the presence of *meso*-diaminopimelic acid in the cell wall, arabinose and galactose as the characteristic sugars in the whole-cell hydrolysates with iso-branched and anteiso-branched-chain fatty acids, major amounts of phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylmethylethanolamine and MK-9(H_4) as the predominant menaquinone and lack mycolic acids (Embley et al. 1987; Goodfellow et al. 1989). To date, twenty-one species of *Saccharopolyspora* with validly published names have been described. The two species '*S. pathumthaniensis*' (Sinma et al. 2011) and '*S. endophytica*' (Qin et al. 2008a) were described recently but the names have not yet been validated. During a study on endophytic actinomycetes from coastal salt marsh plants in Jiangsu province (east China), strain KLBMP 1305^T was isolated and purified. The present study was carried out to determine the taxonomic status of this strain by using a polyphasic approach. Based on phenotypic and genotypic evidence, it is proposed that strain KLBMP 1305^T represents a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora dendranthemae* sp. nov. is proposed.

Materials and methods

Isolation and maintenance of organism

Strain KLBMP 1305^T was isolated from the healthy stems of a coastal salt marsh halophyte *Dendranthema indicum* (Linn.) Des Moul collected from the city of Nantong, Jiangsu Province, east of China. The samples were treated and surface-sterilized according to the five-step sterilization procedure (Qin et al. 2008b), and then aseptically crumbled into smaller fragments using a commercial blender (Joyoung, XC-001). The treated samples were then plated on the selective isolation medium agar (starch 5 g, glucose 5 g, casein 2 g, yeast 1 g, $CaCO_3$ 2 g, agar 15 g, 1 L distilled water, pH 7.0), incubated at 28 °C for 2–8 weeks. The strain was purified and maintained at 4 °C on yeast extract-malt extract agar (ISP 2 medium, Shirling and Gottlieb 1966) containing 3 % (w/v) NaCl.

Phenotypic characteristics

Cultural characteristics were determined after incubation for 2–4 weeks at 28 °C on media from the International *Streptomyces* Project (ISP) (Shirling and Gottlieb 1966), potato-dextrose (PDA), Czapek's and nutrient agars (Waksman 1967). All media were supplemented with 3 % (w/v) NaCl for growth. The colours of substrate and aerial mycelia and any soluble pigments produced were determined according to the ISCC-NBS centroid colour chart (Kelly 1964). The morphological characteristics of strain KLBMP 1305^T, including spore-chain morphology and spore surface ornamentation, were assessed by light microscopy (SA3300-PL) and scanning electron microscopy (Hitachi; S-3400N) using 3 weeks old ISP 2 agar medium culture (growth at 28 °C). Tests of growth at different temperatures (4, 10, 15, 20, 28, 32, 37, 45, 50 and 55 °C) and NaCl concentrations (0–20 %, w/v) (at intervals of 1 %, 28 °C) were examined by growing the novel strain on ISP 2 basal medium. Growth at various pH values (4.0–11.0) were examined as described by Xu et al. (2005) by growing the strain in ISP 2 broth basal medium. Media and procedures used for determination of physiological features, carbon and nitrogen source utilization were those described by Kurup and Schmitt (1973), Gordon et al. (1974), Williams et al. (1989).

Chemotaxonomic characterization

Biomass for chemotaxonomic and molecular studies was obtained after incubation at 28 °C for 4 days by growing in shake flasks of tryptic soy broth (Difco) containing 3 % NaCl. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomer type of diaminopimelic acid (DAP) in cell-wall peptidoglycan was determined by the method of Hasegawa et al. (1983). Analysis of whole-cell sugars was carried out using the methods of Lechevalier and Lechevalier (1970). Phospholipids were analyzed using the procedure of Minnikin et al. (1979). The

fatty acid profile was determined by the method of Sasser (1990), using the MIDI Sherlock Version 6. 1, MIDI database TSBA6. Menaquinones were extracted and purified as described by Collins et al. (1977) and analysed by HPLC (Groth et al. 1997). The G+C content of the DNA was determined by the method of Mesbah et al. (1989).

Determination of 16S rRNA gene sequence and phylogenetic analysis

Genomic DNA was extracted as described previously by Li et al. (2007) and PCR amplification of 16S rRNA gene was carried out according to the procedures described by Qin et al. (2009). The 16S rRNA sequence has been deposited in the GenBank data library and assigned the accession number JQ819260. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence identities were achieved using the EzTaxon-e database (Kim et al. 2012). The phylogenetic relationship between the isolate and closely related strains was investigated using the neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Kluge and Farris 1969) and maximum-likelihood (Felsenstein 1981) algorithms. Phylogenetic trees were generated using molecular evolutionary genetics analysis (MEGA) software version 5 (Tamura et al. 2011). The stability of the clades in the trees was appraised using a bootstrap value with 1,000 repeats (Felsenstein 1985). DNA–DNA hybridization was performed using the microplate hybridization method (Ezaki et al. 1989; He et al. 2005). DNA–DNA relatedness was calculated as the mean of triplicate measurements.

Results and discussion

Morphological and biochemical characteristics

Strain KLBMP 1305^T exhibited good growth on ISP 2, ISP 4, NA, PDA and Czapek's agar media, moderate on ISP 3 and ISP 5 agar. The extensively branched substrate mycelium was well developed, but not fragmented. The colour of the substrate mycelium was pale yellow to orange yellow. The aerial mycelium was sparse and formed flexuous chains of 10–20 spores per chain. The spores were non-motile, smooth-surfaced and oval-shaped (Fig. 1). No soluble pigments were produced. Strain KLBMP 1305^T growth occurred in the presence of 0–17 % (w/v) NaCl (optimum 3 %), at pH 6–10 (optimum pH 7–7.5) and at 15–37 °C (optimum 28 °C). The morphological and physiological characteristics described above are consistent with those of the genus *Saccharopolyspora*. It used the majority of sugars for its growth. The detailed physiological and biochemical properties are given in the species description and Table 1. Strain KLBMP 1305^T can be distinguished from its closest relatives by many phenotypic characteristics, including differences in utilization of sole carbon and nitrogen sources, degradation activity and different cultural characteristics on tested media. Unlike the three nearest recognized *Saccharopolyspora* species, strain KLBMP 1305^T exhibited positive for milk peptonization, grow at 17 % NaCl and on L-valine as sole nitrogen source, but negative for growth at 45 °C, hydrolysis of esculin, nor growth on D-galactose or sorbitol as sole carbon sources.

Phylogenetic analysis based on 16S rRNA gene sequence comparison and DNA–DNA relatedness

The almost-complete 16S rRNA gene sequence (1,445 nt) was determined from strain KLBMP 1305^T. Blast sequence analysis of the 16S rRNA gene sequence showed that the strain was affiliated to the genus *Saccharopolyspora*. The closest phylogenetic

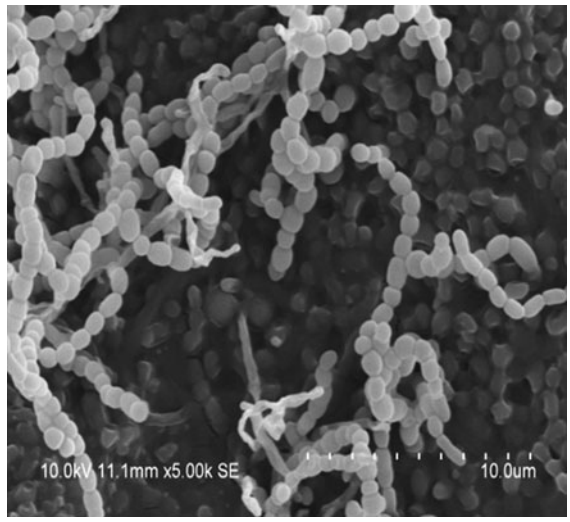

Fig. 1 Scanning electron micrograph of strain KLBMP 1305^T grown on ISP 2 medium for 21 days at 28 °C. *Bar* 10 μm

Table 1 Different characteristics of strain KLBMP 1305^T and its closely related *Saccharopolyspora* species

Characteristic	1	2	3	4
Colour of soluble pigment	None	None	Pink	None
Colour of colony on ISP 3 medium	Pale yellow	Pale yellow	White	White
Casein	−	+	−	−
Esculin	−	+	+	+
Starch	+	+	+	−
Tween 80	+	+	+	−
Milk peptonization	+	−	−	−
Production of soluble pigment	−	−	+	−
Reduction of nitrate	−	+	+	−
Growth on 17 % NaCl	+	−	−	−
Growth at 45 °C	−	+	+	+
Assimilation of sole carbon sources				
D-Arabinose	+	+	−	−
Dextrin	+	+	−	+
D-Galactose	−	+	+	+
D-Lactose	−	+	−	+
D-Mannose	−	−	+	−
D-Raffinose	−	−	+	−
D-Rhamnose	+	−	+	+
D-Ribose	+	+	−	−
Sorbitol	−	+	+	+
Xylitol	+	−	+	+
Acid produced from:				
D-Arabinose	+	w	−	−
D-Cellobiose	−	+	−	−
D-Fructose	−	+	−	−
D-Galactose	−	+	+	+
D-Maltose	−	+	+	w
D-Rhamnose	−	−	+	−
D-Ribose	+	−	−	−
Xylose	−	+	+	+
Assimilation of sole nitrogen sources				
L-Asparagine	−	+	+	−
L-Histidine	−	−	−	+
L-Lysine	−	+	−	+
L-Glutamic acid	−	−	−	+
L-Serine	−	−	+	+
L-Valine	−	−	−	−
DNA G+C content (mol%)	68.7	70.2*	66.2*	70.5*
Polar lipids*	DPG, PG,PE, PC, GL, L	PC, PE	DPG, PG, PC, PE, PIM, PI	DPG, PG, PE, PME, PC, PIM, PI, PL

Strains 1 KLBMP 1305^T, 2 '*S. pathumthaniensis*' S582^T, 3 '*S. endophytica*' YIM 61095^T, 4 *S. tripterygii* YIM 65359^T. All the data are from this study. + Positive or present, *w* weakly positive, − negative or absent

Polar lipids: *DPG* diphosphatidylglycerol, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannosides, *PE* phosphatidylethanolamine, *PC*, phosphatidylcholine *PME* phosphatidylmethylethanolamine, *GL* unknown glycolipid, *PL*, unknown phospholipid, *L* unknown lipid

* Data from Sinma et al. (2011), Li et al. (2009), Qin et al. (2008a)

relatives were '*Saccharopolyspora pathumthaniensis*' S582^T (99.31 %), '*Saccharopolyspora endophytica*' YIM 61095^T (99.17 %) and *Saccharopolyspora tripterygii* YIM 65359^T (99.15 %), lower sequence similarities (<97.2 %) were found with the type strains of all other members of the genus *Saccharopolyspora* with validly published names. It is evident from the phylogenetic tree (Fig. 2) that strain KLBMP 1305^T formed a distinct phyletic line with '*S. pathumthaniensis*' S582^T, '*S. endophytica*' YIM 61095^T and *S. tripterygii* YIM 65359^T and this phylogenetic relationship was supported by a high bootstrap value of 94 %. The phylogenetic relationship was also found and supported in trees constructed with other maximum-parsimony and maximum-likelihood tree-making algorithms. It has been shown that some *Saccharopolyspora* species have high 16S rRNA gene sequence similarities, but have low DNA–DNA relatedness values below the 70 % cut-off point. For example, the nearest neighbour '*S. pathumthaniensis*' S582^T showed 16S rRNA gene sequence similarities of 99.5 and 99.0 % respectively to the type strains '*S. endophytica*' YIM 61095^T and *S. tripterygii* YIM 65359^T, but shared relatively low DNA–DNA values of 53.3 and 44.8 % (Sinma et al. 2011). Recently, Stackebrandt and Ebers (2006) recommended an increase of about 2 % (from 97 to 98.7–99 %) to the 16S rRNA gene sequence similarity threshold used to determine the uniqueness of a new isolate provided that these data are supported by clear phenotypic difference. Although strain KLBMP 1305^T showed relatively high 16S rRNA gene similarities between the nearest relatives, the levels of DNA–DNA relatedness with these three strains were 51.7 ± 2.2, 53.4. ± 3.0 and 49 ± 1.8 %, respectively. These values were below the threshold value of 70 % recommended by Wayne et al. (1987) for assignment of strains to the same species, suggesting strongly that strain KLBMP 1305^T represents a novel species of the genus *Saccharopolyspora.*

Chemotaxonomic characteristics

The results of chemical analysis indicated that strain KLBMP 1305^T has chemotaxonomic markers characteristic of the genus *Saccharopolyspora.* The isolate contains *meso*-diaminopimelic acid as the wall diamino acid. Arabinose and galactose were detected as the major components of sugars in whole-cell hydrolysates. The predominant menaquinone was

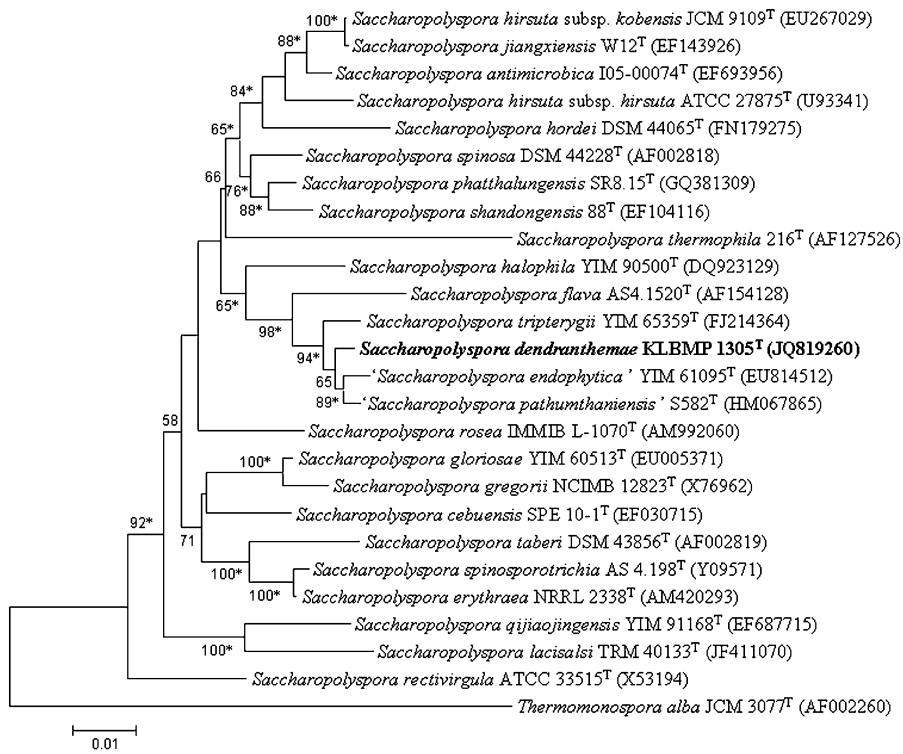

Fig. 2 A neighbour-joining phylogenetic dendrogram based on 16S rRNA gene sequences showing the position of strain KLBMP 1305^T among members of the genus *Saccharopolyspora* species. Numbers on branch nodes are percentage bootstrap values (1,000 resamplings). Outgroup sequence used for analysis was from *Thermomonospora alba* JCM 3077^T. *Asterisks* that the corresponding nodes were also recovered in the maximum-parsimony and maximum-likelihood trees. *Bar* 0.01 substitutions per nucleotide position

MK-9(H_4) (96.7 %) and MK-9(H_2) (3.3 %) was also detected as a minor component. The polar lipids were found to include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unknown glycolipid and an unknown lipid (see Online Supplementary Fig. S1). The major fatty acids were determined to be iso-$C_{16:0}$ (25.51 %), anteiso-$C_{17:0}$ (17.14 %), sum in feature 8 (18:1ω7*c*/18:1ω6*c*) (11.09 %), anteiso-$C_{15:0}$ (10.31 %) and iso-$C_{15:0}$ (9.17 %) (Table 2). This fatty acid profile was similar to those closest phylogenetic *Saccharopolyspora* species, although there were differences in the proportions of some components. The DNA G+C content was 68.7 mol%.

Taxonomic conclusion

The results of the morphological and chemotaxonomic investigations and phylogenetic analysis supported the affiliation of strain KLBMP 1305^T to the genus *Saccharopolyspora.* However, strain KLBMP 1305^T could be distinguished from its closest relatives by many phenotypic characteristics, such as differences in the hydrolysis of casein, esculin, starch and Tween 80, in milk peptonization and production of soluble pigment, in nitrate reduction, in the utilization of sole carbon and nitrogen sources and the acid production, in tolerance to NaCl, in the temperature ranges for growth and in the compositions of fatty acids and polar lipids. In conclusion, strain KLBMP 1305^T is considered to represent a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora dendranthemae* sp. nov. is proposed.

Description of *Saccharopolyspora dendranthemae* sp. nov

Saccharopolyspora dendranthemae (den.dran.the'-ma.e. N.L. n. *Dendranthema* a botanical genus name; N.L. gen. n. *dendranthemae* of *Dendranthema*, the plant genus from which this species was isolated).

Aerobic, gram-positive, non-acid–alcohol-fast, non-motile actinomycete that forms extensively branched pale yellow substrate mycelia. Smooth and non-motile spores (about 0.5–0.8 × 0.8–1.3 μm) are arranged in curved long spiral chains on aerial mycelia. Good growth occurs on ISP 2, ISP 4, NA, PDA and Czapek's agar and moderate growth on ISP 3 and ISP 5 media. Diffusible pigments are not produced. Tweens 20, 40, 80, L-tyrosine and starch are degraded, but adenine, casein, cellulose, chitin, esculin and gelatin are not. Growth occurs at 15–37 °C (optimum 28 °C) and pH 6.0–10.0 (optimum pH 7–7.5). The NaCl tolerance range is up to 17 % (w/v). Negative for the production of H_2S and for nitrate reduction. Positive for milk coagulation and peptonization. Uses D-arabinose, D-cellobiose, dextrin, erythritol, D-fructose,

Table 2 Fatty acid profiles (%) of strain KLBMP 1305^T and its closely related *Saccharopolyspora* species

Fatty acid	1	2	3	4
$C_{10:0}$	–	–	1.10	–
$C_{12:0}$	–	–	0.54	–
$C_{14:0}$	–	–	0.78	–
$C_{16:0}$	1.15	3.16	10.18	1.12
$C_{17:0}$	1.49	2.12	4.61	0.95
$C_{18:0}$	1.30	0.97	3.03	0.53
$C_{15:1}$ω6*c*	0.88	0.91	–	0.78
$C_{17:1}$ω8*c*	4.35	3.89	2.22	3.92
$C_{17:1}$ω6*c*	–	0.55	–	–
$C_{18:1}$ω9*c*	–	0.84	2.36	0.88
Iso-$C_{14:0}$	2.98	2.26	1.56	0.74
Iso-$C_{15:0}$	9.17	14.02	4.93	12.61
Anteiso-$C_{15:0}$	10.31	6.54	5.59	2.42
Iso-$C_{16:0}$	25.51	22.66	17.82	18.76
Iso-$C_{16:1}$ H	1.60	3.54	–	4.83
Iso-$C_{17:0}$	3.91	6.08	3.41	15.52
Anteiso-$C_{17:0}$	17.14	18.25	26.47	17.30
Anteiso-$C_{17:1}$ω9*c*	–	0.87	–	2.16
$C_{17:0}$ 10-methyl	1.01	2.87	1.50	3.97
$C_{17:0}$ 10-methyl TBSA	–	–	–	0.97
$C_{18:0}$ 10-methyl TBSA	–	–	0.59	
$C_{16:1}$ 2 OH	–	–	–	0.59
Sum in feature 1*	–	–	–	1.34
Sum in feature 3*	1.59	3.01	6.59	2.52
Sum in feature 5*	–	–	0.76	–
Sum in feature 8*	11.09	–	–	–
Sum in feature 9*	1.87	4.12	2.60	6.78

Strains 1 KLBMP 1305^T, 2 '*S. pathumthaniensis*' S582^T, 3 '*S. endophytica*' YIM 61095^T, 4 *S. tripterygii* YIM 65359^T. All the data are from this study. Values are percentages of total fatty acids; fatty acids amounting to <0.5 % in all species are not shown; – not detected

* Summed features represent groups of two or three fatty acids that cannot be separated by GC with the MIDI system. Summed features 1, 3, 5, 8, 9 comprised 15:1 iso H/13:0 3OH, 16:1ω7*c*/16:1ω6*c*, 18:2ω6, 9*c*/18:0 ante, 18:1ω7*c*/18:1ω6*c*, 17:1 iso ω9*c*/16:0 10-methyl, respectively

glucose, maltose, D-rhamnose, D-ribose, D-trehalose, xylitol and D-xylose as sole carbon sources for growth, but not D-galactose, inositol, D-lactose, D-mannose, D-raffinose and sorbitol. The diagnostic diamino acid of the peptidoglycan is *meso*-diaminopimelic acid. Whole-cell sugars are arabinose and galactose. The major menaquinone is MK-9(H_4). The major fatty acids are iso-$C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and sum in feature 8 (18:1ω7*c*/18:1ω6*c*). The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unknown glycolipid and an unknown lipid. The G+C content of the genomic DNA of the type strain is 68.7 mol%.

The type strain KLBMP 1305^T (=KCTC 19889^T = NBRC 108675^T) was isolated from surface-sterilized stems of a coastal salt marsh plant *Dendranthema indicum* (Linn.) Des Moul collected from the city of Nantong, Jiangsu Province, east of China.

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