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Planococcus versutus sp. nov., Isolated from Antarctic soil

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Abstract:	A taxonomic study was performed on a novel Gram-staining-positive, cocci-shaped, orange-pigmented motile bacterium, designated strain L10.15T. The organism was isolated from a soil sample collected on Lagoon Island (close to Adelaide Island, western Antarctic Peninsula) using a quorum quenching enrichment medium. Growth occurred at 4-30 °C, pH 6-11, and at moderately high salinity (0-15 %), with optimal growth at 26 °C, at pH 7-8 and 6% NaCl. The 16S rRNA gene sequence analysis showed that strain L10.15T belonged to the genus Planococcus and was closely related to P. halocryophilus Or1T (99.3 %), P. donghaensis JH 1T (99.0 %), P. antarticus DSM 14505T (98.3 %), P. plakortidis AS/ASP6 (II)T (97.6 %), P. maritimus TF-9T (97.5 %), P. salinavum ISL-6 T (97.5 %), and P. kocurii NCIMB 629T (97.5 %). However, the ANI-MUMmer (ANIm) analysis showed low genomic relatedness values of 71.1-81.7% to the type strains of these closely related species of the genus Planococcus. The principal fatty acids were anteiso-C15 : 0, C16 : 1 ω7c, and anteiso-C17 : 0 and the major menaquinones of strain L10.15T were MK-5 (48 %), MK-6 (6 %) and MK-7 (44 %). Polar lipid analysis revealed presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and aminophospholipid. DNA G+C content was 39.4 mol%. The phenotypic and genotypic data indicate that strain L10.15T represents a novel species of the genus Planococcus, for which the name Planococcus versutus sp. nov. is proposed. The type strain is L10.15T (=DSM 101994T = KACC 18918T).

Planococcus versutus sp. nov., isolated from Antarctic soil

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA and complete genome sequence of the novel strain L10.15^T are KX516729 and CP016540-CP16542, respectively. The genome accession numbers for *Planococcus* species that are used in this study are: *P. donghaensis* DSM 22276^T (CP016543-CP016544), *P. plakortidis* DSM 23997^T (CP016539), *P. maritimus* DSM 17275^T (CP016538), *P. halocryphilus* DSM 24743^T (CP016537), *P.*

antarcticus DSM 14505^{T} (CP016534- CP016536), and *P. salinarum* DSM 23820^{T} (MBQG00000000).

A taxonomic study was performed on a novel Gram-staining-positive, cocci-shaped, orange-pigmented motile bacterium, designated strain L10.15^{T}. The organism was isolated from a soil sample collected on Lagoon Island (close to Adelaide Island, western Antarctic Peninsula) using a quorum quenching enrichment medium. Growth occurred at 4-30 °C, pH 6-11, and at moderately high salinity (0-15 %), with optimal growth at 26 °C, at pH 7-8 and 6% NaCl. The 16S rRNA gene sequence analysis showed that strain L10.15^{T} belonged to the genus *Planococcus* and was closely related to *P. halocryophilus* Or1^{T} (99.3 %), *P. donghaensis* JH 1^{T} (99.0 %), *P. antarticus* DSM 14505^{T} (98.3 %), *P. plakortidis* AS/ASP6 (II)^{T} (97.6 %), *P. maritimus* TF-9^{T} (97.5 %), *P. salinavum* ISL-6^{T} (97.5 %), and *P. kocurii* NCIMB 629^{T} (97.5 %). However, the ANI-MUMmer (ANIm) analysis showed low genomic relatedness values of 71.1-81.7% to the type strains of these closely related species of the genus *Planococcus*. The principal fatty acids were anteiso-$C_{15:0}$, $C_{16:1}\omega7c$, and anteiso-$C_{17:0}$ and the major menaquinones of strain L10.15^{T} were MK-5 (48 %), MK-6 (6 %) and MK-7 (44 %). Polar lipid analysis revealed presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and aminophospholipid. DNA G+C content was 39.4 mol%. The phenotypic and genotypic data indicate that strain L10.15^{T} represents a novel species of the genus *Planococcus,* for which the name *Planococcus versutus* sp. nov. is proposed. The type strain is L10.15^{T} (=DSM 101994^{T} = KACC 18918^{T}).

The genus *Planococcus* was proposed by Migula (1894) to accommodate aerobic, Gram-stain-positive, motile, cocci- or rod-shaped bacteria. In 2001, five *Planococcus* species were transferred to the newly proposed genus *Planomicrobium* to differentiate rod shaped, motile, non-sporogenous and low G+C content bacterial species within the original genus

Planococcus (Yoon *et al.*, 2001). These two genera can be differentiated through their 16S rRNA gene sequences, which were shown to have sequence signatures at positions 183 (T for *Planococcus* and C for *Planomicrobium*) and 190 (A for *Planococcus* and G for *Planomicrobium*), following the 16S rRNA gene sequence numbering of *E. coli*. To date, according to the *List of Prokaryotic with Standing in Nomenclature* (http://www.bacterio.net/planococcus.html), there are 12 species described in the genus *Planococcus*. Although 18 species are cited in the files of the genus *Planococcus* in LPSN, six of these have been reclassified to the genera *Planomicrobium* or *Marinococcus*.

Members of *Planococcaceae* are able to survive extreme environments having been isolated from a wide range of sources include deep sea sediments, marine solar salterns, glaciers, permafrost, Antarctic deserts, faeces, cyanobacterial mats and sea ice brine (Kim *et al.*, 2015; Margolles *et al.*, 2012; Pearson & Noller, 2011; Reddy *et al.*, 2002). All members of *Planococcus* are able to grow at moderately low temperatures (psychrotrophic) and are moderately halotolerent (halophilic). The type strain of *Planococcus halocryophilus*, which was isolated from Artic permafrost, was reported to grow and divide even at extremely low temperature (-15 °C) (Mykytczuk *et al.*, 2013). Members of *Planococcus* can be exploited in the field of biotechnological and industrial applications, for instance through their production of carotenoids, thermophilic and alkaline/salt-tolerant xylanases and biosynthesis of butanol (See-Too *et al.*, 2016; Huang *et al.*, 2015; Unverferth *et al.*, 2014; Kim *et al.*, 2015). Here, we provide a detailed taxonomic characterization of a novel species of the genus *Planococcus*, strain L10.15^{T}, which was recently isolated from Antarctic soil samples.

In this study, strain L10.15^{T} was isolated during an ecological survey of the quorum quenching (QQ) soil bacteria in Antarctic soil samples using QQ bacteria enrichment medium (Chan *et al.*, 2009). The soil sample was collected from an elephant seal wallow on

Lagoon Island, close to Adelaide Island, off the west coast of the Antarctic Peninsula (67° 35.689'S 068° 14.495"E). Briefly, around 1 g of soil sample and 5 ml sterile QQ bacteria enrichment medium with the sole carbon source of 100 µg synthetic C_6-HSL was added to a sterile 50 ml polypropylene conical tube and incubated at 4 °C with 150 rpm agitation. A total of 100 µL of the bacterial suspension was transferred into new QQ bacteria enrichment medium including C_6-HSL after 1 week of incubation. This step was repeated three times and, finally, 100 µl of bacterial suspension was plated onto Luria-Bertani (LB) agar. An orange-pigmented isolate, strain L10.15^T, was recovered. The cell suspensions were kept in 20 % w/v glycerol stock for long-term storage at -80 °C. Strain L10.15^T was then routinely cultured aerobically in LB broth or LB agar at 26 °C (optimum growth temperature). As this is the first reported *Planococcus* species with QQ activity, we sequenced its complete genome using Pacific Biosciences (PacBio) RSII to facilitate our investigation.

Colony morphology of strain L10.15^T was orange-pigmented, circular, entire, smooth, convex and 1-2 mm in size on LB agar after 48 h incubation at 26 °C. Gram-staining was performed using Difco Gram stain set and observed using a Leica DM 750 microscope (Leica Microsystems). Cells of strain L10.15^T were observed to be motile and Gram-positive with no spore formation. Electron micrographs were obtained using a table top scanning electron microscope (SEM, TM3030; Hitachi, Japan) and a scanning transmission electron microscope (STEM, LIBRA 120; Carl Zeiss AG, Germany). For SEM, a sample was prepared as described by Vali *et al.* (2004). For STEM, overnight suspension cells were stained using 1% phosphotungstic acid on a Formvar grid and observed at an operating voltage of 80 kV. Cells of strain L10.15^T were coccoid, typically 1.0-1.5 µm in diameter, mostly arranged as diplococci, but cells in single coccoid or tetrad were also observed (Fig. 1). A catalase test was conducted using 3 % (v/v) H_2O_2 and determined by observing the production of copious bubbles. Oxidase activity was determined using 1 % (w/v) *N*,*N*,*N*',*N*',-

tetramethyl 1,4-phenylenediamine (bioMérieux) as described by Smibert & Krieg (1994). API ZYM and Biolog GEN III Microplates were prepared according to the manufacturer's instructions. The activities of various enzymes were determined by using the API ZYM after incubation for 24 h. Antibiotic susceptibility was tested by using ATB PSE 5 strips (bioMérieux) and disc diffusion assay following the manufacturer's instructions. All tests were performed at 26 °C and in triplicate. The temperature range for growth was determined by plating on LBA and incubation at 4-37 °C with increments of 1 or 2 °C over 14 d. The pH range for growth of strain L10.15^T was determined on LBA plates adjusted to various pH values between 4 to 12 with 1 pH unit increments. Tolerance of salt was determined by growing on LBA media supplemented with 0-25 % (w/v) NaCl at increments of 1%. Both salt tolerance and pH range tests were conducted by incubating the LBA plates at 26 °C for up to 14 d. All results of physiological tests of strain L10.15^T, and comparison with closely related species, are presented in Table 1.

Genomic DNA of L10.15^T was extracted from an overnight cell suspension culture using the MasterPure™ Gram-positive DNA purification kit (Epicentre Technologies). A 20-kb SMRTbell template library was then constructed using the extracted genomic DNA. The whole genome sequencing was performed using Pacific Biosciences (PacBio) RSII sequencing platform with C4 chemistry in two single molecule real time (SMRT) cells. The complete genome of strain L10.15^T has been sequenced, enabling the discovery of the gene responsible for QQ activity (See-Too et al., unpublished data). To determine the identity of strain L10.15^T, the 16S rRNA partial gene sequence was amplified from the extracted DNA obtained as described above by using primers 27F and 1492R (Lane, 1991) and analyzed using the Ex-Taxon database (Kim *et al.*, 2012). Pairwise similarity analysis demonstrated that strain L10.15^T is a member of the genus *Planococcus*, with *P. halocryophilus* Or1^T (99.3 %), *P. donghaensis* JH 1^T (99.0 %), *P. antarticus* DSM 14505^T (98.3 %), *P. plakortidis*

AS/ASP6 (II)^T (97.6 %), *P. maritimus* TF-9^T (97.5 %), *P. salinavum* ISL-6^T (97.5 %) and *P. kocurii* NCIMB 629^T (97.5 %) as the closest relatives present in the database. Phylogenetic analyses of the 16S rRNA was carried out using the full 16S rRNA gene sequence (1538 bp) retrieved from complete genome sequence. MEGA 6.0 software (Tamura *et al.*, 2013) was used to performed the alignment using the MUSCLE algorithm (Edgar, 2004) and the phylogenies were constructed using default settings of neighbour-joining (NJ, Fig. 2), maximum likelihood (ML, Supplementary Fig. S1) and maximum parsimony (MP, Supplementary Fig. S2) algorithms. The 16S rRNA gene sequence of L10.15^T contained the signature nucleotides of *Planococcus*, T and A, respectively at positions 183 and 190 (*Escherichia coli* 16S rRNA gene sequence numbering) and thus clustered separately from the related genus *Planomicrobium* (Dai *et al*., 2005). All 16S rRNA phylogenies concordantly demonstrated that strain L10.15^T clustered within *Planococcus*, but formed a distinct branch separate from *P. halocryophilus* Or1^T, *P. donghaensis* JH1^T, *P. antarticus* DSM 14505^T, *P. plakortidis* AS/ASP6 (II)^T, *P. maritimus* TF-9^T, *P. salinavum* ISL-6^T, and *P. kocurii* NCIMB 629^T. The G+C content of strain L10.15^T was 39.4 mol% as determined from the complete genome sequence.

Average nucleotide identity (ANI) analysis was performed using JSpecies Web Service (JSpeciesWS; http://jspecies.ribohost.com/jspeciesws/) (Richter *et al*., 2015) in which strain L10.15^T demonstrated ANI-MUMmer (ANIm) values of between 71 % and 82 % similarity against all close relatives (*P. halocryophilus* Or1^T (81.2%), *P. donghaensis* JH 1^T (80.8 %), *P. antarticus* DSM 14505^T (79.6 %), *P. plakortidis* AS/ASP6 (II)^T (71.1 %), *P. maritimus* TF-9^T (72.0 %), *P. salinavum* ISL-6^T (73.0 %), and *P. kocurii* NCIMB 629^T (81.7 %)) (Supplementary Table S1). ANI-Blast (ANIb) values in comparison with all close relatives indicated 84 % to 88 % similarity (*P. halocryophilus* Or1^T (84.8 %), *P. donghaensis* JH1^T (84.8 %), *P. antarticus* DSM 14505^T (84.3 %), *P. plakortidis* AS/ASP6 (II)^T (85.0 %), *P.*

maritimus TF-9^T (84.6 %), *P. salinavum* ISL-6^T (88.2%), and *P. kocurii* NCIMB 629^T (86.1 %)) (Supplementary Table S2). The results were similar with OrthoANI analysis (Lee *et al*., 2016), which giving OrthoANI values ranging from 71.5 % to 82.2 % (*P. halocryophilus* Or1^T (81.4 %), *P. donghaensis* JH1^T (81.3 %), *P. antarticus* DSM 14505^T (79.9 %), *P. plakortidis* AS/ASP6 (II)^T (72.9 %)**,** *P. maritimus* TF-9^T (72.0 %), *P. salinavum* ISL-6^T (71.5%), and *P. kocurii* NCIMB 629^T (82.2 %)) (Supplementary Fig. S4). Richter *et al*. (2009) proposed a threshold of 94–96 % for species delimitation, with our analyses therefore indicating that strain L10.15^T does not belong to any of these related species.

The isoprenoid quinones were extracted using petroleum ether as described by Minnikin *et al.* (1984) and subsequently identified by HPLC (Shimadzu; Nexera-X2). The isoprenoid quinone profile of strain L10.15^T was characterized by the predominance of the menaquinones MK-5 (48 %), MK-6 (6 %) and MK-7 (44 %). The polar lipids of strain L10.15^T were extracted and analyzed by two-dimensional TLC following Embley & Wait (1994). Molybdophosphoric acid was used for the detection of total polar lipids, ninhydrin for amino lipids, molybdenum blue for phospholipids, Dragendorff reagent for choline-containing lipids and ***α***-naphthol/sulphuric acid reagent for glycolipids. Strain L10.15^T exhibited a complex polar lipid profile consisting of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminophospholipid, two unidentified lipids and four unidentified aminolipids (Supplementary Fig. S3). The predominant polar lipids of strain L10.15^T were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and aminophospholipid. This result is consistent with the description of *Planococcus plakortidis* (Kaur *et al*., 2012).

Cellular fatty acid profiles were determined following the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Pandey *et al*., 2002). Fatty acids

were extracted and fatty acid methyl esters were prepared and analyzed in the Microbial Identification System (MIDI). Briefly, overnight cultures of strain L10.15^T were harvested from LBA determined previously to be in the mid-exponential growth phase at 26°C. The fatty acids were separated using an Agilent GC (model 6890N) and were identified using Sherlock version 6.0 via the RTSBA6 database. The fatty acid profile of strain L10.15^T comprised (each constituting $\geqslant$0.5 % of the total): saturated fatty acids $C_{14:0}$ (0.6 %), $C_{15:0}$ (1.5 %), $C_{16:0}$ (4.0 %), $C_{17:0}$ (0.7 %) and $C_{18:0}$ (1.0 %), branched fatty acids anteiso-$C_{13:0}$ (0.6 %), anteiso-$C_{15:0}$ (46.2 %), anteiso-$C_{17:0}$ (10.7 %), iso-$C_{14:0}$ (3.4 %), iso-$C_{15:0}$ (1.9 %), iso-$C_{16:0}$ (5.5 %), iso-$C_{17:0}$ (1.9 %), Iso-$C_{17:1}$ ω10c (1.3 %) and iso-$C_{18:0}$ (0.7 %); unsaturated fatty acids $C_{16:1}$ ω*7c* alcohol (6.5 %), $C_{16:1}$ ω*11c* alcohol (5.6 %), $C_{17:1}$ ω*9c* alcohol (0. 8 %) and $C_{18:1}$ ω*9c* alcohol (0.7 %); summed feature 3 (iso-$C_{15:0}$ 2OH and/or anteiso- $C_{17:1}$; 0.6 %) and summed feature 4 (iso- $C_{17:1}$ and/or $C_{16:1}$ ω*7c*; 6.0 %). This profile is similar to those of recognized *Planococcus* species, although there were differences in the proportions of some fatty acids. Table 2 presents the fatty acids of strain L10.15^T and closely related species. The fatty acid profile of strain L10.15^T was similar to those of members of the genus *Planococcus* and contained anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ as the major fatty acids. The distinctive characteristic of L10.15^T compared to other member of the genus *Planococcus* lies in the menaquinone profile, in which the predominant menaquinones are MK-5, MK-6 and MK-7 instead of MK-6, MK-7 and MK-8. L10.15^T is also the only strain sensitive to fusidic acid of the reference strains tested.

Description of *Planococcus versutus* sp. nov.

versutus (ver.su'tus. L. masc. adj. *versutus* adroit, shrewd, ingenious)

The cells of L10.15^T are aerobic, Gram-stain-positive cocci, motile, and non-sporulating. Colonies on LB agar are orange-colored, circular, entire, smooth, convex and 1.0–2.0 mm in diameter. Strain L10.15^T grows at temperatures between 4 and 30 °C (optimum, 25 °C) and pH 6.0–11.0 (optimum, pH 7.0–8.0). Growth is observed between 0 and 14 % NaCl (optimum, 6 %). Tests positive for catalase, but negative for amylase. Strain L10.15^T is positive in assimilation of *N*-acetyl-D-glucosamine, *N*-acetyl neuraminic acid, *N*-acetyl neuraminic acid, α-D-glucose, inosine, D-mannitol, glycerol, D-fructose- 6-PO_4, glycyl-L-proline, L-alanine, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, L-serine, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, D-saccharic acid, D-lactic acid methyl ester, α-keto-glutaric acid, D-malic acid, L-malic acid, tween 40, β-hydroxy-D,L-butyric acid, acetoacetic acid, acetic acid and formic acid, dextrin, D-fructose, D-glucose- 6-PO_4, L-alanine, L-glutamic acid, pectin, D-galacturonic acid, glucuronamide, dextrin, D-fructose, D-glucose- 6-PO_4, L-alanine, L-glutamic acid, pectin, D-galacturonic acid, and glucuronamide L10.15^T. It is negative in assimilation of D-turanose, stachyose, D-mannose, 3-methyl glucose, D-sorbitol, citric acid, bromo-succinic acid, *N*-Acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, D-galactose, D-fucose, L-fucose, L-rhamnose, D-arabitol, myo-inositol, D-aspartic acid, D-serine, gelatin, L-arginine, L-histidine, quinic acid, *p*-hydroxy-phenylacetic acid, methyl pyruvate, L-lactic acid, γ-amino-butryric acid, α-hydroxy-butyric acid, α-keto-butyric acid and propionic acid. In the chemical sensitivity test, strain L10.15^T was resistant to D-serine, lincomycin, guanidine HCl, tetrazolium blue, potassium tellurite, 1 % sodium lactate, aztreonam and sodium butyrate, slightly resistant to tetrazolium violet and sodium bromate and sensitive to fusidic acid, nalidixic acid, lithium chloride, vancomycin, niaproof 4, troleandomycin, rifamycin SV and minocycline. The DNA G+C content of the type strain is 39.4 mol%. The respiratory menaquinones are MK-5, MK-6 and MK-7. Major fatty acids are anteiso-$C_{15:0}$, $C_{16:1}\,\omega$7c, and anteiso-$C_{17:0}$. The

predominant polar lipids are phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol, and aminophospholipids.

The type strain, strain L10.15^{T} (=DSM 101994^{T} = KACC 18918^{T}), was isolated from a soil sample collected from an elephant seal wallow on Lagoon Island (close to Adelaide Island, western Antarctic Peninsula).

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Fig. 1. Scanning (a) and scanning transmission (b) electron micrographs of cells of strain L10.15^T grown at 26 °C. Most of the cells are observed as diplococci and cell division septa at different stages were also observed. Scale bars: a, 5 μm; b, 0.5 μm.

Fig. 2. Phylogenetic tree constructed by neighbour-joining analysis based on 16S rDNA sequences, depicting the phylogenetic relationship of strain L10.15^T with related type species of the genus *Planococcus*. Scale bar represents evolutionary distance as 0.005 change per nucleotide position. Bootstrap values (%) > 50 % from 1,000 replicates are shown.

Table 1. Differential phenotypic characteristics of *P. versutus* L10.15^T and its phylogenetically closest related species. Strains: 1, L10.15^T; 2, *P. donghaensis* JH1^T; 3, *P. halocryphilus* Orl^T; 4, *P. antarcticus* DSM 14505^T; 5, *P. kocurii* DSM 20747^T; 6, *P. maritimus* JCM 11543^T; 7, *P. plakortidis* DSM 23997^T and 8, *P. salinarum* ISL-16 ^{T}. All strains are positive for the utilization of dextrin, D-fructose, D-glucose- 6-PO_4, L-alanine, L-glutamic acid, pectin, D-galacturonic acid, and glucuronamide. All strains are negative for utilization of D-turanose, stachyose, D-mannose, 3-methyl glucose, D-sorbitol, citric acid, and bromo-succinic acid. In chemical sensitivity assay, all strains are able to growth in 1 % sodium lactate, aztreonam and sodium butyrate, but not in vancomycin, niaproof 4, troleandomycin, rifamycin SV and minocycline. All data were obtained in this study.

Characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Growth:								
at pH	6.0-12	6.0-10	6.0-11	6.0-12	6.0-12	5.0-8	6-10	5.5-12
NaCl tolerance (%, w/v)	15	12	19	12	8	17	9	13
up to °C	30	37	37	28	37	41	37	38
From GenIII plate								
Assimilation of:								
D-Maltose	-	+	+	+	+	-	-	+
D-Trehalose	-	+	-	+	-	-	-	-
D-Cellobiose	-	-	-	+	-	-	-	-
Gentiobiose	-	+	-	-	-	-	-	-
Sucrose	-	+	-	-	-	-	-	-
D-Raffinose	-	-	-	+	-	-	-	-
α-D-Lactose	-	-	-	-	+	+	-	-
D-Melibiose	-	-	-	-	+	+	-	-
β-Methyl-D- Glucoside	-	+	+	+	-	-	-	-
D-Salicin	-	+	+	-	-	+	-	-
N-Acetyl-D- Glucosamine	+	+	+	-	+	+	-	-
N-Acetyl- *β*-D- Mannosamine	-	+	+	-	+	+	-	-
N-Acetyl-D- Galactosamine	-	-	-	+	-	+	-	-
N-Acetyl Neuraminic Acid	+	-	+	-	-	-	-	-
α-D-Glucose	+	+	+	-	+	-	-	-
D-Galactose	-	-	-	-	+	+	-	-
D-Fucose	-	+	-	-	-	-	-	-
L-Fucose	-	-	-	+	-	-	-	-

L-Rhamnose	-	-	-	-	-	+	-	-
Inosine	+	+	+	-	+	+	+	-
D-Mannitol	+	+	+	+	+	+	-	-
D-Arabitol	-	-	-	+	-	-	-	+
myo-Inositol	-	-	-	+	-	-	-	-
Glycerol	+	+	+	+	+	+	-	-
D-Fructose-6-PO_4	+	+	+	-	+	+	+	-
D-Aspartic Acid	-	+	+	+	-	+	-	-
D-Serine	-	-	+	+	-	-	-	+
Gelatin	-	+	+	+	+	+	-	-
Glycyl-L-Proline	+	+	+	+	+	+	-	-
L-Arginine	-	+	+	+	+	+	-	+
L-Aspartic Acid	+	+	-	+	+	+	-	+
L-Histidine	-	+	+	+	-	-	-	-
L-Pyroglutamic Acid	+	+	+	-	+	+	-	+
L-Serine	+	+	+	-	+	+	+	-
L-Galactonic Acid Lactone	+	+	+	-	+	+	+	+
D-Gluconic Acid	+	+	+	+	+	+	-	+
D-Glucuronic Acid	+	+	-	-	+	+	+	+
Mucic Acid	+	+	+	+	+	+	-	+
Quinic Acid	-	+	+	+	+	+	-	+
D-Saccharic Acid	+	+	+	-	+	+	-	-
p-Hydroxy- Phenylacetic Acid	-	-	-	+	-	-	-	-
Methyl Pyruvate	-	-	-	+	-	-	-	-
D-Lactic Acid Methyl Ester	+	+	+	-	+	-	-	+
L-Lactic Acid	-	+	+	+	-	+	-	-
α-Keto-Glutaric Acid	+	+	+	-	+	+	-	+
D-Malic Acid	+	+	+	+	+	+	-	+
L-Malic Acid	+	+	+	-	+	+	-	+
Tween 40	+	+	+	+	+	+	+	+
γ-Amino-Butryric Acid	-	-	-	+	-	-	-	-
α-Hydroxy- Butyric Acid	-	-	+	+	-	+	-	-
β-Hydroxy-D,L- Butyric Acid	+	+	+	-	+	+	+	+
α-Keto-Butyric Acid	-	-	+	-	-	+	+	-
Acetoacetic Acid	+	+	-	+	+	-	+	+
Propionic Acid	-	-	-	+	-	+	+	-
Acetic Acid	+	+	+	-	+	+	+	+
Formic Acid	+	+	+	-	+	-	-	+
Chemical Sensitivity:								
Fusidic Acid	+	-	-	-	-	-	-	-
D-Serine	-	-	+	-	-	-	-	-
Lincomycin	-	-	-	+	-	-	-	-
Guanidine HCl	-	-	-	+	-	-	+	-

Tetrazolium Violet	W	+	w	w	+	+	+	+
Tetrazolium Blue	-	-	-	w	-	-	-	-
Nalidixic Acid	+	+	-	w	+	-	+	+
Lithium Chloride	+	+	+	-	+	+	+	+
Potassium Tellurite	-	+	-	-	+	+	+	+
Sodium Bromate	W	w	-	+	-	-	+	-
API ZYM test:								
Alkaline phosphatase	-	+	-	-	+	+	+	+
Esterase	-	w	-	+	w	+	+	+
Leucine arylamidase	+	w	-	+	+	+	+	+
Valine arylamidase	-	+	-	w	+	+	w	+
Cystine arylamidase	+	-	-	+	+	+	+	+
α-chymotrypsin	+	+	-	w	-	-	-	+
β- galactosidase	-	w	-	+	+	+	-	-
β-glucosidase	-	+	+	-	-	-	-	-
Genome feature:								
Genome size (Mb)	3.37	3.32	3.42	3.83	3.49	3.29	3.28	NA
DNA G+C content (mol %)	39.4	40.1	40.1	43.2	40.9	47.2	50.0	NA
Number of genes #	4639	4417	4598	5040	4631	4609	4889	NA
Number of coding sequences #	4425	4196	4276	4811	4460	4365	4718	NA

Table 2. Cellular fatty acid profile of strain L10.15^{T} and close related species.

Strains: 1, *P. versutus* sp. nov. L10.15^{T}; 2, *P. donghaensis* JH1^{T}; 3, *P. halocryphilus* Orl^{T}; 4, *P. antarcticus* DSM 14505^{T}; 5, *P. kocurii* DSM 20747 ^{T}; 6, *P. maritimus* JCM 11543 ^{T}; 7, *P. plakortidis* DSM 23997^{T} and 8, *P. salinarum* ISL-16 ^{T}. Values are percentages of the total fatty acids and only fatty acids comprising 0.5 % are shown. 2, ND-Not detected. All data were obtained in this study.

Fatty acid	1	2	3	4	5	6	7	8
Straight chain								
$C_{14:0}$	0.6	1.2	0.5	0.7	0.6	-	0.6	1.2
$C_{15:0}$	1.2	0.9	0.6	1.6	4.1	1.1	1.9	-
$C_{16:0}$	4.0	**12.6**	6.8	4.1	2.6	1.5	4.4	3.5
$C_{17:0}$	0.7	1.9	0.5	1.2	2.9	1.9	-	0.8
$C_{18:0}$	1.0	4.8	0.9	1.4	0.6	1.2	1.9	1. 8
Branched chain								
anteiso-$C_{13:0}$	0.6	-	0.5	-	-	-	-	-
iso-$C_{14:0}$	3.4	2.4	2.2	1.5	2.1	3.4	2.4	3.2
iso-$C_{15:0}$	1.9	2.3	2.5	3.6	3.6	9.8	5.2	2.5
anteiso-$C_{15:0}$	**46.2**	**35.0**	**44.4**	**44.7**	**43.0**	**32.3**	**43.4**	**32.1**
iso-$C_{16:0}$	5.5	4.6	4.9	3.7	4.0	4.2	6.5	3.7
iso-$C_{17:0}$	1.9	3.2	3.6	7.5	5.3	5.5	-	2.9
iso-$C_{17:1}$ ω^{10c}	1.3	0.9	-	3.5	2.7	4.1	-	3.3
anteiso-$C_{17:0}$	**10.7**	**14.1**	**15.7**	**11.9**	9.6	5.9	-	9.3
iso-$C_{18:0}$	0.7	1.0	0.4	-	0.6	4.7	1.5	-
Unsaturated								
$C_{16:1}$ ω^{7c} alcohol	6.5	1.8	2.9	2.2	2.9	6.6	4.8	**10.1**
$C_{16:1}$ ω^{11c} alcohol	5.6	5.8	4.6	2.9	3.8	1.5	2.8	1.8
$C_{17:1}$ ω^{7}	0.8	1.1	0.3	0.7	3.0	4.1	-	-
$C_{18:1}$ ω^{9c}	0.7	2.1	0.8	0.9	1.0	1.6	1.8	0.8
Summed feature 3†	0.6	-	-	-	0.4	-	-	1.0
Summed feature 4††	6.0	3.3	6.1	5.3	6.0	5.4	2.9	8.6

†Summed feature 3 contains $C_{16:1}$ ω^{7c} and/or $C_{16:1}$, which could not be separated by GC with the MIDI system.

††Summed feature 4 contains iso-$C_{17:1}$ and/or anteiso-$C_{17:1}$, which could not be separated by GC with the MIDI system.

Fig. 1

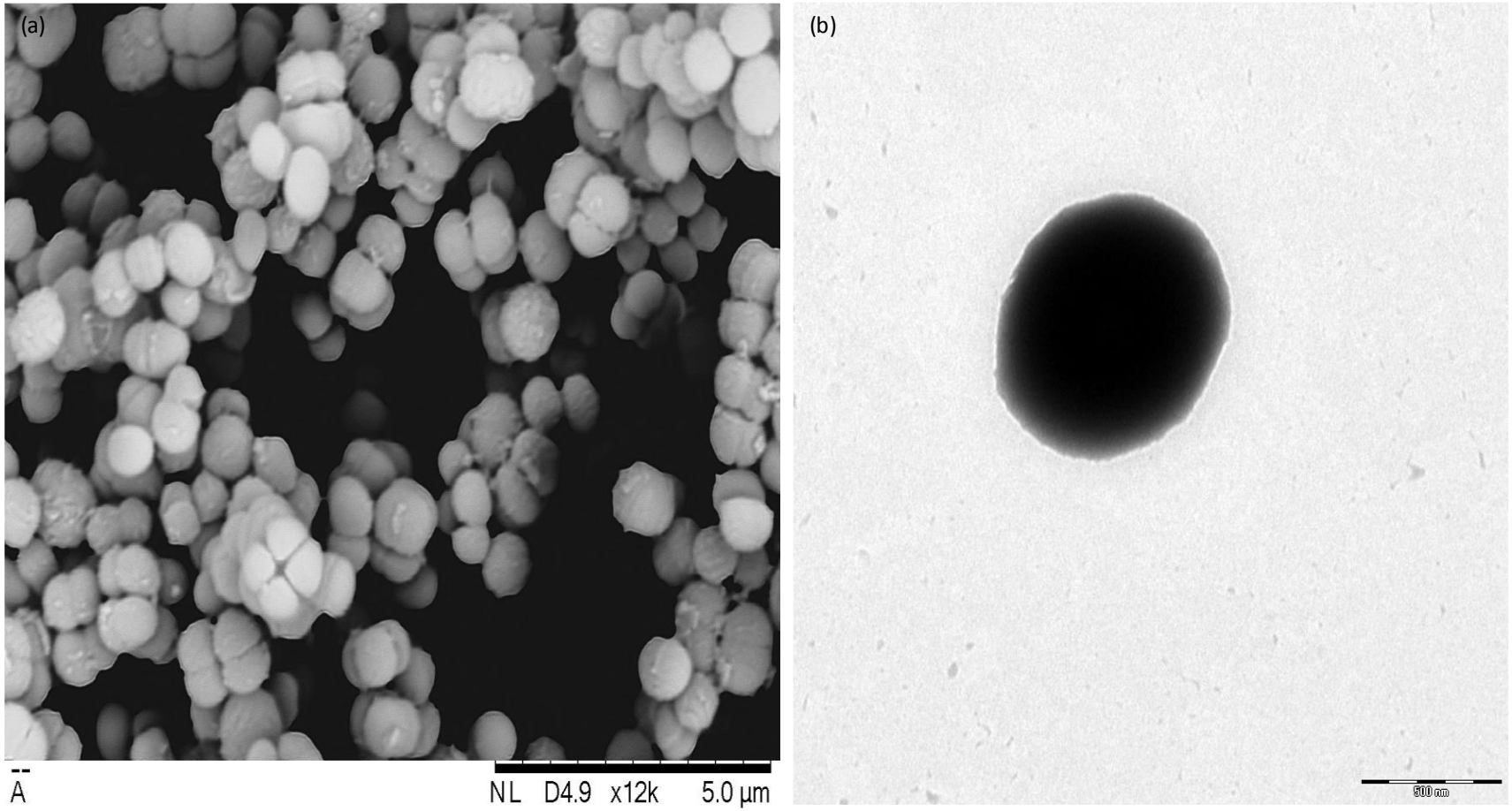
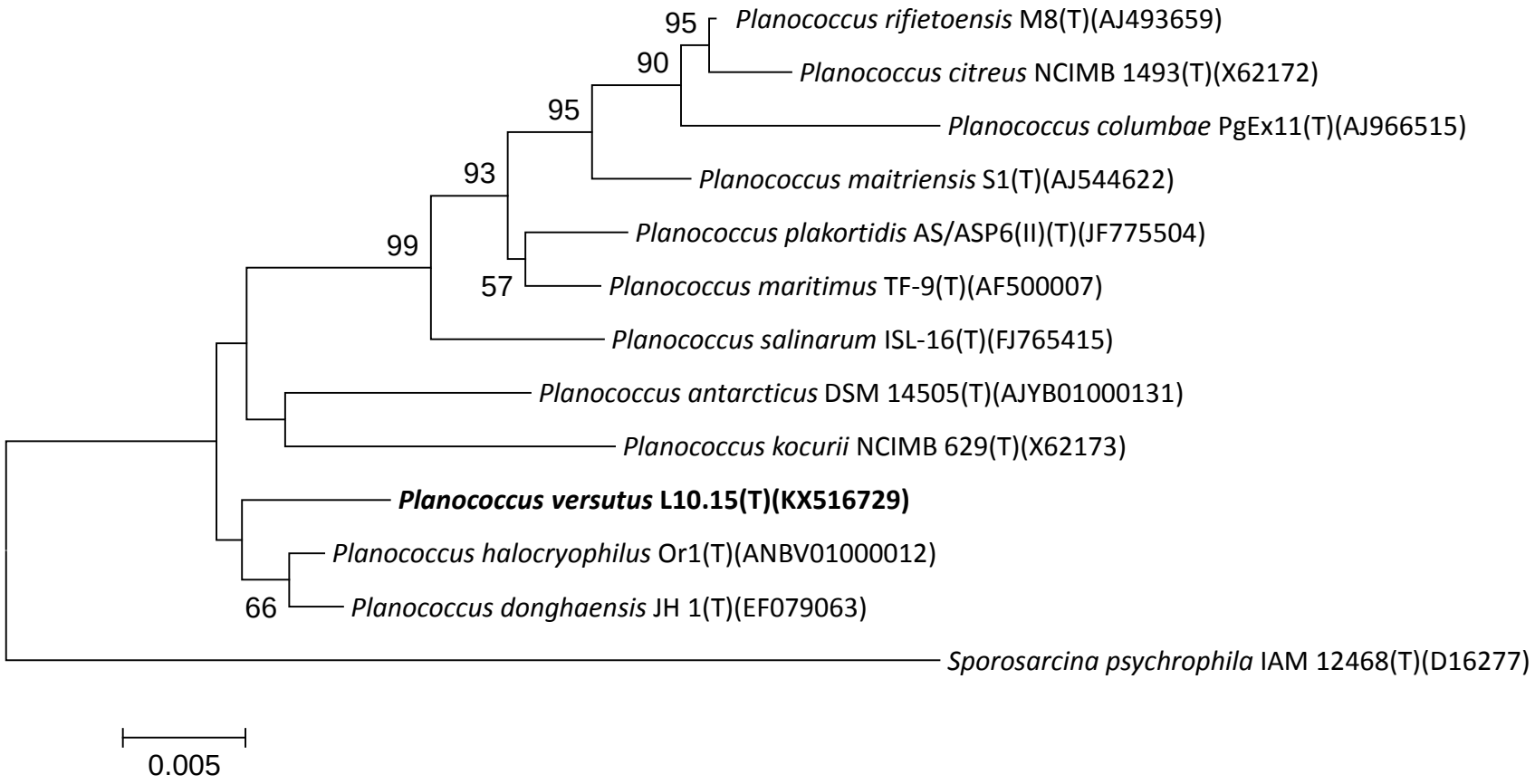


Fig.2


95
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Planococcus columbae PgEx11(T)(AJ966515)
Planococcus maitriensis S1(T)(AJ544622)
Planococcus plakortidis AS/ASP6(II)(T)(JF775504)
Planococcus maritimus TF-9(T)(AF500007)
Planococcus salinarum ISL-16(T)(FJ765415)
Planococcus antarcticus DSM 14505(T)(AJYB01000131)
Planococcus kocurii NCIMB 629(T)(X62173)
***Planococcus versutus* L10.15(T)(KX516729)**
Planococcus halocryophilus Or1(T)(ANBV01000012)
Planococcus donghaensis JH 1(T)(EF079063)
Sporosarcina psychrophila IAM 12468(T)(D16277)
0.005

Planococcus versutus sp. nov., isolated from Antarctic soil

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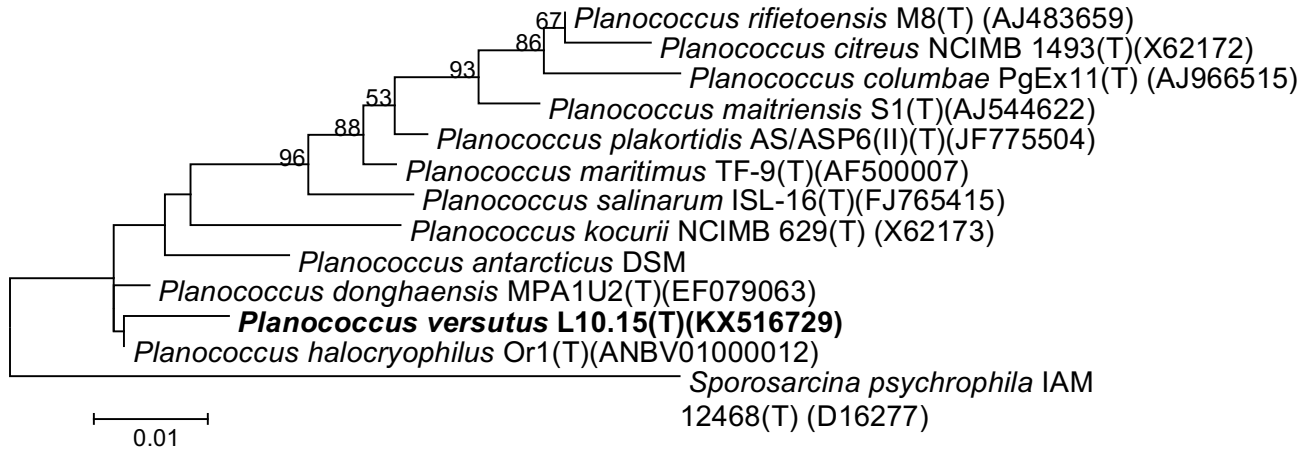
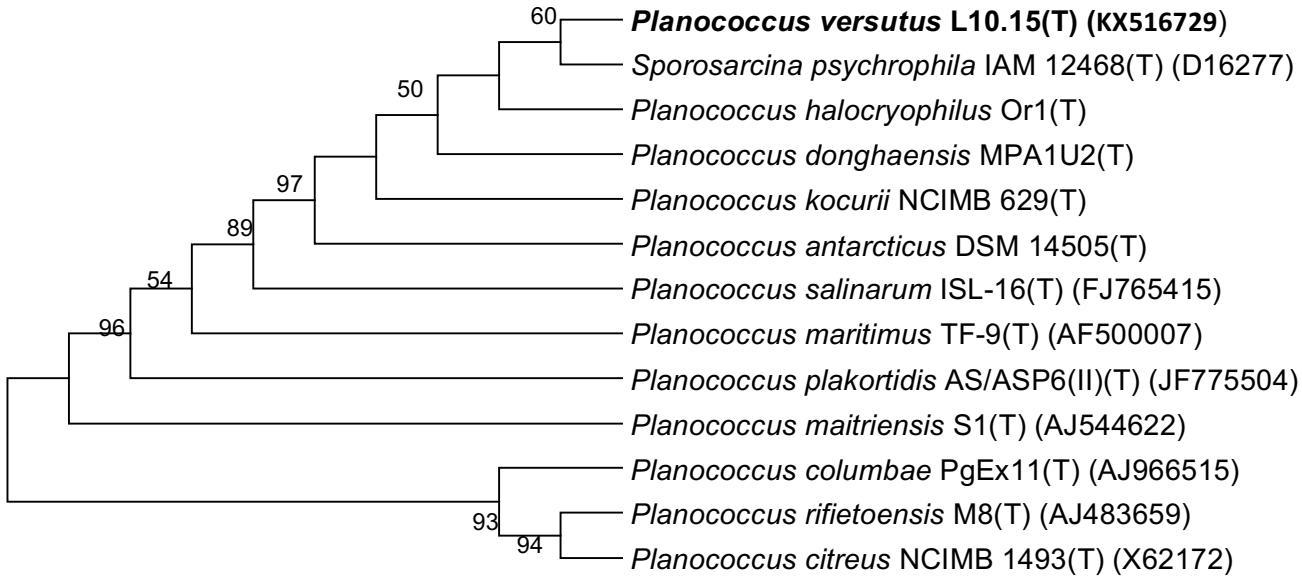
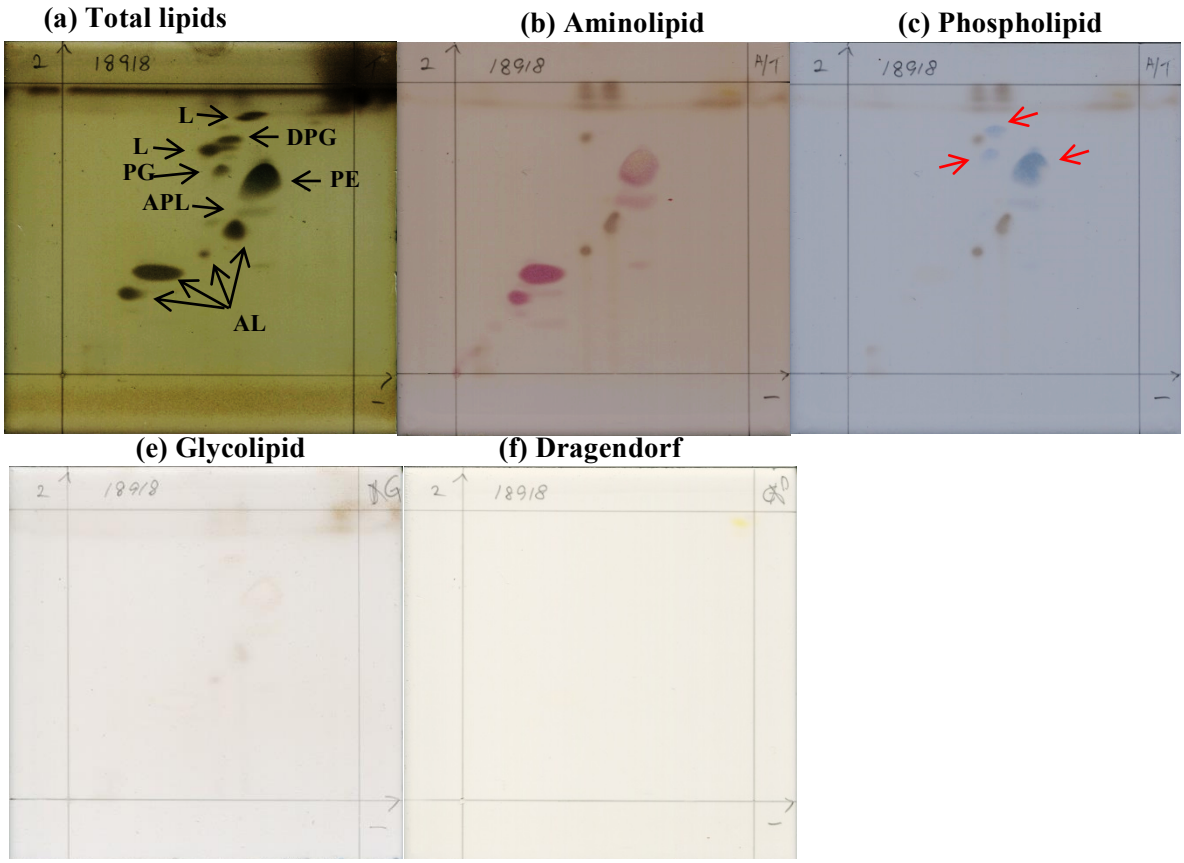


Supplementary Fig. S1. Phylogenetic tree constructed by maximum-likelihood analysis based on 16S rDNA sequences, depicting the phylogenetic relationship of strain L10.15^{T} with closely related type species of the genus *Planococcus*. Scale bar represents evolutionary distance as 0.01 change per nucleotide position. Bootstrap values (%) > 50 % from 1,000 replicates are shown.



Supplementary Figure S2. 16S rRNA phylogeny constructed using maximum parsimony phylogeny showing the taxonomic position of strain L10.15^{T} against 12 closest relatives retrieved from EzTaxon database. Bootstrap values (expressed as percentages of 100 replicates) greater than 50 % are shown at the branch points. *Sporoscarcina psychrophila* IAM 12468^{T} was used as an outgroup.



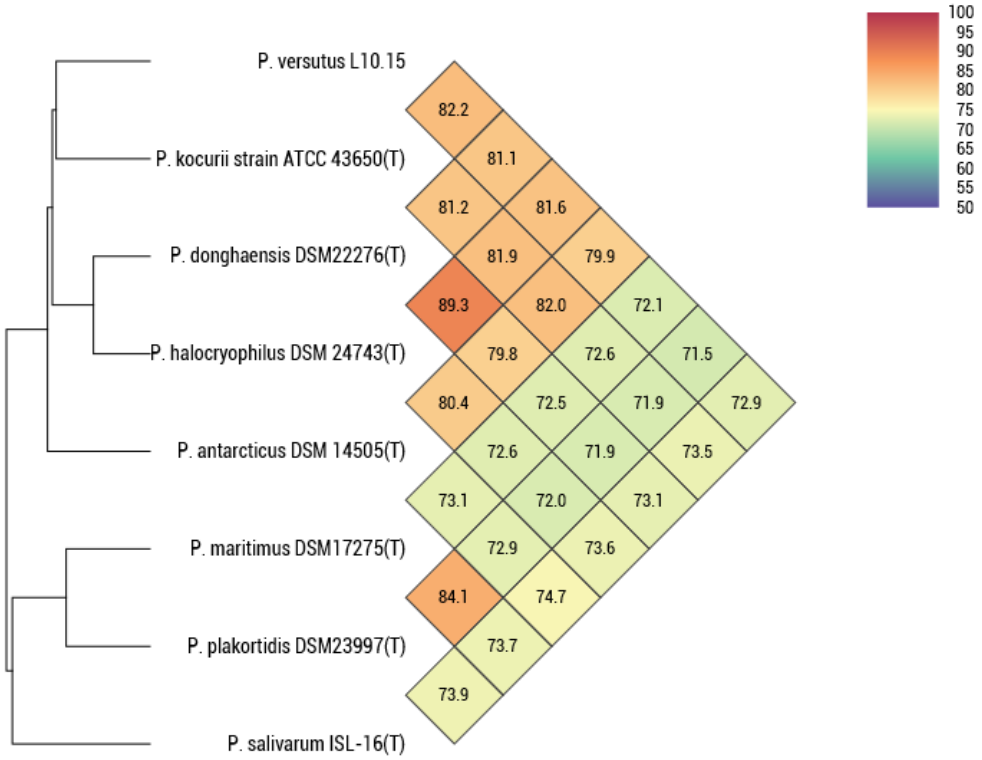
Supplementary Fig. S3. TLC chromatograms of polar lipid distribution of *P. versutus* strain L10.15^{T} visualized with 5 % ethanolic molybdatophosphoric acid for total lipids (a); ninhydrin (Sigma) for amino lipids (b); molybdenum blue (Sigma) for phospholipids (c); *α*-naphthol/sulphuric acid reagent for glycolipids (d) and Dragendorff reagent for choline-containing lipids (e). The polar lipid profile consisted of a mixture of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), an unidentified aminophospholipid (APL), two unidentified Lipids (L) and four unidentified aminolipids (AL).

Supplementary Table S1. ANI-MUMmer analysis and the aligned percentage in [#] of *P.versutus* L10.15^{T} and closest type species based on JSpeciesWS.

#ANIm and aligned percentage	*P. salivarum* ISL-16^{T}	*P. versutus* L10.15^{T}	*P. donghaensis* JH1^{T}	*P. halocryphilus* Or1^{T}	*P. antarcticus* CMS 26or^{T}	*P. plakortidis* AS/ASP6 (II)^{T}	*P. maritimus* TF-9^{T}	*P. kocurii* ATCC 43650^{T}
P. salivarum ISL-16^{T}	*	88.2 [7.0]	89.3 [7.8]	88.8 [8.0]	86.0 [11.3]	86.2 [8.8]	87.8 [7.7]	88.9 [7.7]
P. versutus L10.15^{T}	88.2 [2.4]	*	84.8 [41.5]	84.8 [46.6]	84.4 [39.5]	85.0 [4.9]	85.0 [5.5]	86.1 [46.6]
P. donghaensis JH1^{T}	88.6 [3.0]	84.8 [43.1]	*	89.6 [86.2]	84.0 [38.5]	85.5 [5.8]	85.5 [6.4]	85.2 [46.5]
P. halocryphilus Or1^{T}	88.4 [2.9]	84.8 [46.3]	89.6 [83.3]	*	84.1 [41.5]	85.1 [5.7]	84.4 [6.6]	85.6 [48.7]
P. antarcticus CMS 26or^{T}	86.0 [3.7]	84.3 [35.0]	84.0 [33.0]	84.2 [37.1]	*	84.1 [5.8]	84.7 [6.1]	84.9 [46.0]
P. plakortidis AS/ASP6 (II)^{T}	86.2 [3.1]	85.0 [5.0]	85.2 [5.5]	85.1 [5.8]	84.1 [6.7]	*	85.8 [69.4]	85.4 [5.5]
P. maritimus TF-9^{T}	87.4 [2.7]	84.6 [5.6]	85.5 [6.1]	84.4 [6.7]	84.7 [6.9]	85.8 [69.1]	*	84.8 [6.8]
P. kocurii ATCC 43650^{T}	88.9 [2.5]	86.1 [45.0]	85.2 [43.3]	85.6 [47.4]	84.9 [49.8]	85.4 [5.2]	84.8 [6.4]	*

Supplementary Table S2. ANI-Blast analysis and the aligned percentage in [#] of *P. versutus* L10.15^{T} and closest type species based on JSpeciesWS.

#ANIb and aligned percentage	*P. salivarum* ISL-16^{T}	*P. versutus* L1015^{T}	*P. donghaensis* JH1^{T}	*P. halocryphilus* Or1^{T}	*P. antarcticus* CMS 26or^{T}	*P. plakortidis* AS/ASP6 (II)^{T}	*P. maritimus* TF-9^{T}	*P. kocurii* ATCC 43650^{T}
P. salivarum ISL-16^{T}	*	73.2 [52.9]	73.4 [58.2]	73.4 [58.4]	74.7 [61.2]	74.2 [56.8]	73.8 [56.1]	73.6 [55.4]
P. versutus L1015^{T}	73.0 [16.7]	*	80.8 [68.9]	81.2 [71.4]	79.8 [70.2]	71.3 [52.8]	72.0 [53.7]	81.8 [72.2]
P. donghaensis JH1^{T}	73.4 [19.4]	80.8 [70.5]	*	89.0 [85.3]	79.6 [72.2]	71.7 [57.3]	72.3 [58.8]	81.2 [72.3]
P. halocryphilus Or1^{T}	73.6 [18.2]	81.2 [70.8]	88.9 [82.6]	*	80.2 [72.4]	71.6 [57.3]	72.2 [58.7]	81.7 [73.0]
P. antarcticus CMS 26or^{T}	74.8 [17.9]	79.6 [63.0]	79.5 [63.3]	80.1 [65.0]	*	72.6 [50.5]	72.7 [51.9]	81.5 [65.1]
P. plakortidis AS/ASP6 (II)^{T}	73.7 [19.3]	71.1 [54.7]	71.4 [58.8]	71.5 [59.6]	72.5 [58.8]	*	83.6 [80.6]	71.6 [57.0]
P. maritimus TF-9^{T}	73.7 [18.2]	72.0 [54.9]	72.2 [58.8]	72.3 [60.0]	72.8 [59.3]	83.7 [79.5]	*	72.5 [57.4]
P. kocurii ATCC 43650^{T}	73.3 [17.1]	81.7 [69.9]	80.9 [69.0]	81.6 [71.4]	81.5 [70.2]	71.6 [52.9]	72.3 [54.3]	*



Supplementary Fig. S4. OrthoANI analysis of *P. versutus* L10.15^{T} and closest type species.