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ORIGINAL PAPER

Paludisphaera soli sp. nov., a new member of the family *Isosphaeraceae* isolated from high altitude soil in the Western Himalaya

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Abstract A novel strain of Planctomycetes, designated JC670^T, was isolated from a high altitude (~ 2900 m above sea level) soil sample collected from Garhwal region in the Western Himalaya. Colonies of this strain were observed to be light pink coloured with spherical to oval shaped cells having crateriform structures distributed all over the cell surface. The cells divide by budding. Strain JC670^T was found to grow well at pH 7.0 and pH 8.0 and to tolerate up to 2% NaCl (w/v). MK6 was the only respiratory quinone identified. The major fatty acids of strain JC670^T were identified as $C_{18:1}\omega9c$, $C_{18:0}$ and $C_{16:0}$, and phosphatidylcholine, two unidentified phospholipids and six unidentified lipids are present as the polar lipids. The polyamines putrescine and sym-homospermidine were detected. Strain JC670^T shows high 16S rRNA gene sequence identity (95.4%) with *Paludisphaera borealis* PX4^T. The draft genome size of strain JC670^T is 7.97 Mb, with G + C content of 70.4 mol%. Based on phylogenetic analyses with the sequences of ninety-two core genes, low *d*DDH value (20.6%), low gANI (76.8%) and low AAI (69.1%) results, differential chemotaxonomic and physiological properties, strain JC670^T (= KCTC 72850^T = NBRC 114339^T) is recognised as the type strain of a new species of the genus *Paludisphaera,* for which we propose the name *Paludisphaera soli* sp. nov.

Keywords Soil bacteria · Western himalaya · Planctomycetes · Isosphaeraceae · *Paludisphaera* · High altitude

Abbreviations

NCBI	National Center for Biotechnology Information
ANI	Average Nucleotide Index
AAI	Average Nucleotide Index
*d*DDH	Digital DNA-DNA Hybridization
HPLC	High-Pressure Liquid Chromatography
KCTC	Korean Collection for Type Cultures

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Introduction

The phylum *Planctomycetes* is an important member of the *Planctomycetes/Verrucomicrobia/Chlamydiae* (PVC) superphylum, which is well-known for its biotechnological relevance (Wagner and Horn 2006). Though its members were initially considered as an intermediate between prokaryotes and eukaryotes (McInerney et al. 2011) they have now been recognised as bacteria due to the presence of peptidoglycan cell walls and their cell structure is similar to that of Gram-stain-negative bacteria (Wiegand et al. 2018). Members of *Planctomycetes* are ubiquitous in distribution and are found in diverse habitats with most species reported from aquatic environments (Wiegand et al. 2018). Though members of *Planctomycetes* can be the abundant phylum in soil microbial communities (Borneman et al. 1996; Borneman and Triplett 1997; Neef et al. 1998), relatively few have been cultivated so far (Buckley et al. 2006; Dedysh and Ivanova 2019; Dedysh et al. 2020; Ivanova and Dedysh 2012; Wang et al. 2002), although progress has recently been made in describing taxa cultivated by Wiegand et al. (2018) such as *Tautonia plasticadhaerens* (Jogler et al. 2020).

Taxonomically, the phylum *Planctomycetes* is subdivided into three classes: *Planctomycetia*, *Phycisphaerae* and *Candidatus* Brocadiae (Wiegand et al. 2018). At present, class *Planctomycetia* contains the majority of the species in the phylum and consists of four orders with validly published names viz., *Planctomycetales*, *Gemmatales*, *Pirellulales* and *Isosphaerales* (Dedysh et al. 2020). The families *Planctomycetaceae* and *Pirellulaceae* contain the majority of the species with validly published names. The family *Isosphaeraceae* is currently represented by six genera (*Paludisphaera*, *Tundrisphaera*, *Isosphaera*, *Aquisphaera, Singulisphaera* and *Tautonia*), which are generally monospecific taxa except the genera *Singulisphaera* and *Tautonia*. Recently, one more member has been added to the genus *Tautonia,* i.e. *T. plasticadhaerens* (Jogler et al. 2020). Members of the family *Isosphaeraceae* are stalk-free planctomycetes with spherical cells that divide by budding and are known to colonise a wide range of habitats such as hot springs, peat moss and aquatic sediments (Kulichevskaya et al. 2016). Genomic attributes of the strains belonging to the phylum *Planctomycetes* indicate genome sizes of 3–12 Mb and a DNA G + C content of 40–71% (Wiegand et al. 2018). Within the family *Isosophaeraceae*, the genome size ranges from 5.4–10.4 Mb while DNA G + C content ranges from 62.2–71.1% (Bondoso et al. 2011; Wiegand et al. 2018; Jogler et al. 2020).

The genus *Paludisphaera* was described by Kulichevskaya et al. (2016) to accommodate a new planctomycete of the family *Isosphaeraceae* isolated from a boreal *Sphagnum* peatbog. So far, the genus *Paludisphaera* has a single species, namely *Paludisphaera borealis*. The species is characterised by bright-pink colonies, small (1.5–2.5 µm) spherical cells that occur singly, in pairs or in short chains containing up to 10 cells and reproduce by budding (Kulichevskaya et al. 2016). Genome-based investigation of *P. borealis* PX4^T indicates high glycolytic properties and production of a diverse range of secondary metabolites (Ivanova et al. 2017).

In the present study, soil samples were collected from Gangotri, in Uttarkashi district of Uttarakhand state in India. Gangotri is located on the banks of the river Bhagirathi, the main tributary of the Ganga. Being part of the Great Himalayan range, the area exhibits a distinctive diversity of flora and fauna that are adapted to cold environment at high altitude. Several studies have reported the bacterial diversity from high altitude Gangotri soil (Baghel et al. 2005; Kumar et al. 2019) and the presence of taxonomically unclassified sequences indicates the untapped novel bacterial diversity of these ecosystems. During our survey of soil bacterial diversity, we isolated strain JC670^T from a soil sample collected nearly 30 km from the town of Gangotri. Combining a polyphasic taxonomic approach together with genomic information, we conclude strain JC670^T should be classified as the type strain of a new species of the genus *Paludisphaera*. To the best of our knowledge, this is the first report of planctomycetes from the Himalaya. This study not only extends the studies on collection of axenic strains of planctomycetes from the Indian subcontinent, their characterisation and description of novel taxa (Kumar et al. 2020a, b), but also represents one of the first attempts to isolate and perform polyphasic characterisation of a Planctomycetes strain of the family *Isosphaeraceae* from a soil sample.

Materials and methods

Home habitat and isolation of novel strain

Strain JC670^T was isolated from a soil sample collected from the bank of the river Bhagirathi (2900 m above sea level, 31° 00′ 3.2″ N 78° 54′ 32.7″ E) at a depth of 10 cm from soil surface. The pH and salinity of the soil were 7.2 and 0.00 ppt, respectively. Soil samples were subjected to enrichment and cultivation in a medium containing (g l^{-1} in distilled water; pH 7.0): *N*-acetylglucosamine, 2.0; KH_2PO_4, 0.1; peptone, 0.1; yeast extract, 0.1; vitamin solution, 10 ml l^{-1}; Hutner's basal salts, 20 ml l^{-1} prepared in distilled water. The antibiotics (g l^{-1}) streptomycin, 0.4, ampicillin, 0.2 and cycloheximide, 0.025 were added to the media. Vitamin solution contained (mg l^{-1}): vitamin B_{12}, 0.2; biotin, 4; thiamine-HCl.$2H_2O$, 10; Calcium pantothenate, 10; folic acid, 4.0; riboflavin, 10; nicotinamide, 10.0; p-aminobenzoic acid, 10; pyridoxine HCl, 20. Hunter's basal salts contain (g l^{-1}): nitrilotriacetic acid, 10; $MgSO_4.7H_2O$, 30; $CaCl_2.2H_2O$, 3.5; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.01; $FeSO_4.7H_2O$, 0.1; and metals stock solution 50 ml. Metal stock solution contain (g l^{-1}): Na-EDTA, 0.25; $ZnSO_4.7H_2O$, 1.1; $FeSO_4.7H_2O$, 0.5; $MnSO_4.H_2O$, 0.15; $CuSO_4.5H_2O$, 0.04; $Co(NO_3)_2.6H_2O$, 0.025; $Na_2B_4O_7.10H_2O$, 0.018. The soil sample (100 mg) was mixed with 10 ml medium in a serum vial of 20 ml capacity and the vial was sealed with butylated rubber stoppers. The serum vial was then incubated for two months at 25 °C to enrich planctomycetes. After two months of incubation, a light pink globular bacterial colony was observed at the bottom of the serum vial. The pink globular colony was further streaked on an agar plate containing the same medium. After three weeks of incubation, pink colonies appeared along with white colonies on the agar plates. The pink colonies were purified through repeated streaking. Pure cultures were maintained on agar plates by repeated sub-culturing and preserved at 4 °C. Purified cultures were grown in the above medium without antibiotics, unless otherwise mentioned. The pink coloured culture isolated from the soil sample was designated strain JC670^T.

DNA isolation, PCR, 16S rRNA gene sequencing and BLAST analysis

DNA was isolated from the pure cultures using a commercial DNA isolation kit (Nucleo-pore gDNA Fungal Bacterial Mini Kit, from M/s. Genetix Biotech Asia Pvt. Ltd, India) which was used for PCR amplification and genome sequencing. PCR was performed for 16S rRNA gene amplification using planctomycetes specific primers F40 (Kohler et al. 2008) and R1388 (Stackebrandt et al. 1993). Purified PCR products were sent to AgriGenomePvt. Ltd. (Kochi, India) for 16S rRNA gene sequencing.

Genomic information and *in-silico* metabolic characterisation

To further investigate the taxonomic position of strain JC670^T, whole genome sequencing was carried out using an Illumina HiseqX10 platform and a paired end library generated with sequence coverage of 100x. Genome sequencing was outsourced to AgriGenome Pvt. Ltd, Kochi, India. De novo assembly was performed using Unicycler (Wick et al. 2017) assembly software. The default k-mer sizes were used for Unicycler assembly. Unicycler assembly was used for all further downstream analyses. The genome sequence was checked for any possible contamination using the ContEst service of EZBiocloud (https://www.ezbiocloud.net/tools/contest16s; Yoon et al. 2017) which yielded 16S rRNA gene sequence of only one organism indicating the draft genome sequence was free from sequences of other organisms.The genome was further annotated using the NCBI Prokaryotic Genome Annotation Pipeline and also through the online freely available RAST server (https://rast.theseed.org/FIG/rast.cgi) (Aziz et al. 2008). In silico metabolic characterisation of strain JC670^T and *P. borealis* PX4^T was carried out on the basis of its genome information through the online tool PATRIC 3.6.2 (https://www.patricbrc.org/) (Wattam et al. 2017) and using Interactive pathway explorer (iPath3) (https://pathways.embl.de/login.cgi) (Darzi et al. 2018). In silico identification for the presence of gene clusters responsible for the biosynthesis of secondary metabolites in strain JC670^T and *P. borealis* PX4^T were carried out using the online freely available tool antiSMASH5.1 (https://antismash.secondarymetabolites.org) (Blin et al. 2019).

OrthoANI score was calculated from the genome sequences using the online service in the EzBioCloud (https://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017). *d*DDH estimate between strain JC670^T and *P. borealis* PX4^T was calculated based on the genome sequences using the Genome-to-Genome Distance Calculator (GGDC 2.1) online service (https://ggdc.dsmz.de/distcalc2.php) (Auch et al. 2010). Average amino acid identity (AAI) was calculated between the genomes of strains JC670^T and *P. borealis* PX4^T using the AAI calculator developed by the Konstantinidis lab (Rodriguez and Konstantinidis 2014).

Phylogenetic analysis

The 16S rRNA gene sequence of strain JC670^T extracted from the genome using ContEst16S has a sequence length of 1524 nt and analysis of identity was performed using NCBI BLAST (Johnson et al. 2008). The full length 16S rRNA gene sequences of strain JC670^T, *P. borealis* PX4^T, clone sequences related to *Paludisphaera* sp. (obtained from NCBI) along with all other closely related type strains of *Isosphaeraceae* (obtained from EZBioCloud) were aligned using MUSCLE implemented in MEGA7.0 (Kumar et al. 2016) and the distances were calculated by using the Kimura 2-parameter (Kimura 1980) in a pairwise deletion procedure. Neighbor-joining (NJ), minimum evolution (ME) and maximum likelihood (ML) methods in the MEGA7 software were used to construct phylogenetic trees having Bootstraps of 1000 replication (Felsenstein 1985).

The genome sequence of *P. borealis* PX4^T from the NCBI database (accession number: NZCP019082) was used. The phylogenomic tree was constructed using 92 core genes from publicly available genomes mainly representing the families *Pirellulaceae, Planctomycetaceae* and *Isosphaeraceae*. These 92 core genes were retrieved using the Up-to-date Bacterial Core Gene (UBCG) tool (Na et al. 2018). A concatenated sequence of 92 genes was used to construct the RAxML based phylogenomic tree. The tree was generated with random samples of 50% of the homology groups used for the main tree, in a process known as gene-wise jackknifing. Hundreds of these 50% gene-wise jackknife trees were made using RAxML, and the support values at the nodes indicate the number of times a particular branch was observed in the support tree.

Physiological analysis

For organic substrates and nitrogen source utilisation of strain JC670^T, liquid basal medium was used as described by Bondoso et al. (2011) with slight modifications. Medium was supplemented with 0.05% w/v yeast extract. $(NH_4)_2SO_4$ (0.1% w/v) and glucose (0.1% w/v) were used as nitrogen and organic carbon sources respectively, unless otherwise mentioned. For organic substrate utilisation, $(NH_4)_2SO_4$ (0.1% w/v) was used as a nitrogen source and cell growth was tested with different organic substrates at a concentration of 0.1% (w/v). For nitrogen source utilisation, glucose (0.1% w/v) was used as a carbon source and cell growth was tested with different nitrogen substrates at 0.1% (w/v). To examine the hydrolysis of phytagel, strain JC670^T was streaked onto the same medium solidified with 2% phytagel (Sigma-Aldrich) and incubated for 4 weeks (Kulichevskaya et al. 2016). The phytagel hydrolysing property was also checked on media supplemented with N-acetylglucosamine as a sole source of carbon and nitrogen. Both organic and nitrogen substrate utilisation were tested in test tubes (25 × 250 mm) containing 10 ml of liquid basal medium as described above. Requirement of vitamin B_{12} was tested by growing the cells in liquid media with and without vitamin B_{12} (0.02 mM). The requirement for vitamin B_{12} was confirmed by repeated transfer into media lacking vitamin B_{12}. NaCl tolerance (0–7% w/v; final salt concentration in the media) was tested at 25 °C and at pH 7. The optimum temperature (5, 10, 15, 20, 25, 30, 35, 40, 45 °C) required for cell growth was tested at pH 7. pH tolerance (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0) for cell growth of strain JC670^T was tested at 25 °C in buffered broth, as described earlier (Bondoso et al. 2015). Nitrate reduction was tested using conventional methods (MacFaddin et al. 1985). Enzymatic activities were assayed using the API ZYM kit (Biomerieux, France) following the manufacturer's protocol.

Chemotaxonomic characterisation

For fatty acid analysis, growing cells were harvested by centrifugation (10,000 g for 15 min at 4 °C) at a cell density of 70% of the maximum optical density (100% = 0.9 OD_{660}). Cellular fatty acids were methylated, separated and identified according to the

instructions of the Microbial Identification System (Microbial ID; MIDI 6.0 version; method, RTSBA6) (Sasser 1990), which was carried out by Royal Research Labs, Secunderabad, India. Polar lipids of strain JC670^T were extracted, separated and characterised as described previously (Kates 1972; Oren et al. 1996). Quinones were extracted with a chloroform/methanol (2:1, v/v) mixture, purified by TLC and analysed by HPLC (Imhoff et al. 1984). Polyamines of strain JC670^T were extracted and identified according to a recent method (Kumar et al. 2020b).

Microscopy

Cell morphology, cell size, cell shape and cell division were observed under transmission and scanning electron microscope (Carl Zeiss LSM880 /Philips XL3O). Transmission electron microscopy (H-7500 Hitachi) was used to observe cross sections of the cells.

Results and discussion

Phylogenetic inference

The 16S rRNA gene sequence of strain JC670^T extracted from the draft genome has a sequence length of 1524 nt and a sequence similarity of 95.4% with that of *P. borealis* PX4^T as determined by BLAST and ClustalW analyses. The 16S rRNA gene sequence based phylogenetic tree with combined bootstrap values obtained from NJ, ME, ML trees (Fig. 1) and 92 core genes based phylogenomic tree (Fig. 2) confirmed the clustering of strain JC670^T with the type species of the genus *Paludisphaera*, *P. borealis*.

POCP (Table S2) and AAI (Table S1) values between the strain JC670^T and *P. borealis* PX4^T were 67.3% and 69.1%, respectively. A POCP threshold below 50% was proposed to determine new genera (Qin et al. 2014) and the value of 67.3% observed between the two strains clearly indicates that they belong to the same genus. Further, AAI value of 69.1% between the strains fell close to the recommended cut-off of 80% used for the genus delineation (Rodriguez and Konstantinidis 2016). The OrthoANI value between JC670^T and *P. borealis* PX4^T was 76.8%. The *d*DDH value between strain JC670^T and *P. borealis* PX4^T was 20.6%. The ANI and *d*DDH values between the strain JC670^T and *P. borealis* PX4^T are far below the recommended cut off values (95–96% cut off for ANI and 70% cut off for *d*DDH) for prokaryotic species delineation (Meier-Kolthoff et al. 2014; Chun et al. 2018). Thus, strain JC670^T is concluded to represent a novel species of the genus *Paludisphaera.*

Genomic characteristics

Based on the NCBI Prokaryotic Genome Annotation Pipeline, the genome size of strain JC670^T is 7.97 Mb with N_{50} value of 217, 617, while the genome size of *P. borealis* PX4^T is 7.65 Mb. The genome of strain JC670^T has 6,453 predicted genes of which 6,213 are protein coding genes, 3 rRNA operons, 55 tRNA coding and179 genes are pseudogenes; and no CRISPR repeats were found. The genome of *P. borealis* PX4^T was predicted to contain a total of 5877 genes of which 5642 are protein coding, 150 pseudogenes, 73 tRNA genes, 9 rRNA operons, 3 genes coding for other RNAs and one CRISPR repeat.

The difference (5.8 mol%) in G + C content between strain JC670^T and *P. borealis* PX4^T (Table S3) further indicates that these two are distinct species since strains of a species will have less than 3% difference in the G + C content (Meier-Kolthoff et al. 2014). The genome sequences of strain JC670^T and *P. borealis* PX4^T were aligned using PATRIC software to identify the multiple maximal matches and local collinear blocks. The genome sequence of strain JC670^T was used as a reference against *P. borealis* PX4^T. The alignments of the local collinear blocks in both strains differ from each other significantly (Fig. S1). The alignment facilitates detection of homologous regions that are shuffled or inverted due to DNA rearrangement or recombination. Comparison of the genome map based on the protein sequence identity shows that majority of the proteins of strain JC670^T share 30–90% similarity with those of *P. borealis* PX4^T (Fig. S2). Both strain JC670^T and *P. borealis* PX4^T share 10–20% protein sequence similarity with other members of the family *Isosphaeraceae* (Fig. S2). These results suggest a clear dissimilarity between strain JC670^T with *P. borealis* PX4^T and between the two strains and other taxa of the family *Isosphaeraceae*.

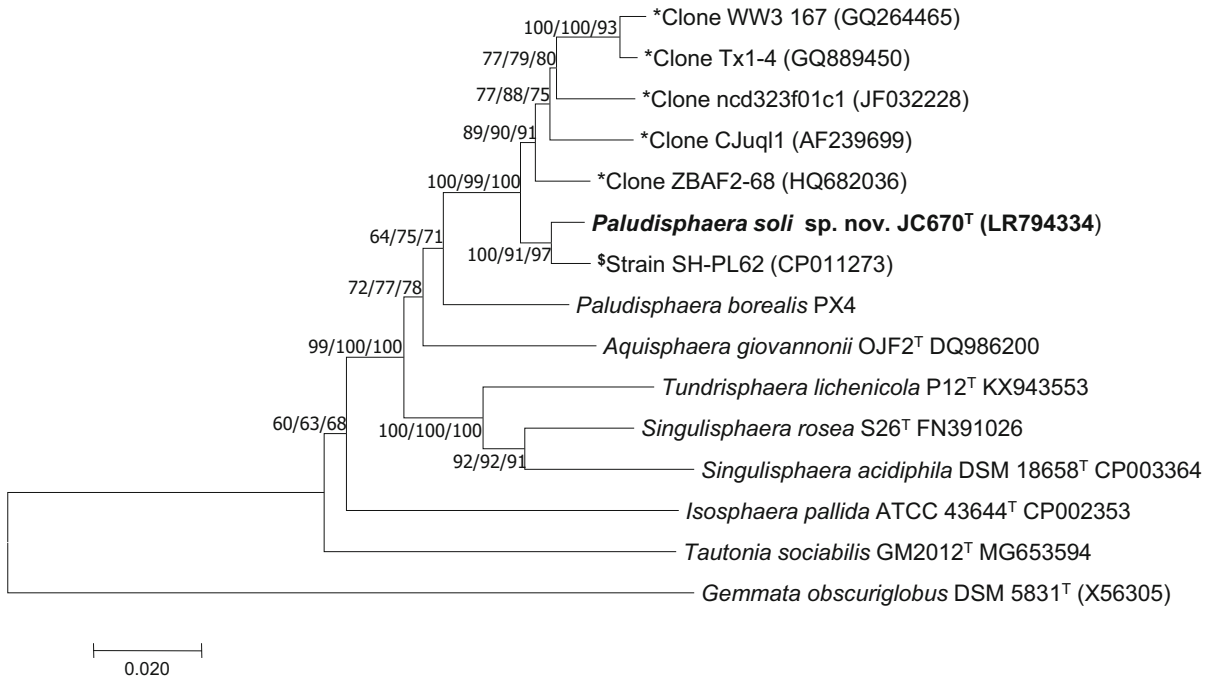

Fig. 1 Phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationship of strain JC670^T with *P. borealis* PX4^T and other closely related species. The tree was made with MEGA 7 software and *Gemmata obscuriglobus* DSM 5831 ^{T} was used as out-group. The GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses. Numbers at nodes indicate Bootstrap values from 1000 repetitions corresponding in the NJ/ME/ML analysis. Bar, 0.02 nucleotide substitution per position. *Clone sequences; $^$Axenic culture

In silico metabolic characterisation

In silico metabolic characterisation showed that strain JC670^T and *P. borealis* PX4^T have the 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway for the biosynthesis of five carbon isoprene units (isopentenyl pyrophosphate), which is eventually the precursor for the synthesis of carotenoids and quinones (Eisenreich et al. 2004; Zhao et al. 2013). Genes encoding for carotenoid biosynthetic enzymes such as lycopene β-cyclase (5.5.1.19), β-carotene hydroxylase (1.14.13.129), zeaxanthin epoxidase (1.14.15.21) and neoxanthin synthase (5.3.99.9) were found in both the strains. Hence, both strains are putatively capable of forming neoxanthin from lycopene via intermediates such as γ-carotene, β-carotene, zeaxanthin and violaxanthin. Gene clusters for the biosynthesis of neomycin, puromycin, kanamycin, tetracycline, novobiocin and gentamycin antibiotics are present in both of the strains. The streptomycin biosynthetic pathway gene cluster was exclusively predicted in *P. borealis* PX4^T. Both strains are capable of purine and pyrimidine metabolism. *P. borealis* PX4^T is putatively capable of degradation and metabolism of xenobiotic compounds such as xylene, atrazine, benzoate, toluene, ethyl benzene and caprolactam (Fig. S3). Due to the complex life cycle of planctomycetes, they are believed to produce bioactive secondary metabolites (Wiegand et al. 2020). There were six putative secondary metabolite gene clusters predicted in strain JC670^T and *P. borealis* PX4^T. Both strains have gene clusters for mixed heterocyst-glycolipid synthase-like and Type I polyketide synthases (Mixed hgIE-KS-Type I PKS) and terpenes biosynthesis. Type I and Type III polyketide synthase gene clusters were exclusively predicted in strain JC670^T.

Morphological and physiological analyses

SEM imaging showed that cells of strain JC670^T are present singly or in tissue-like aggregates (Fig. 3a).

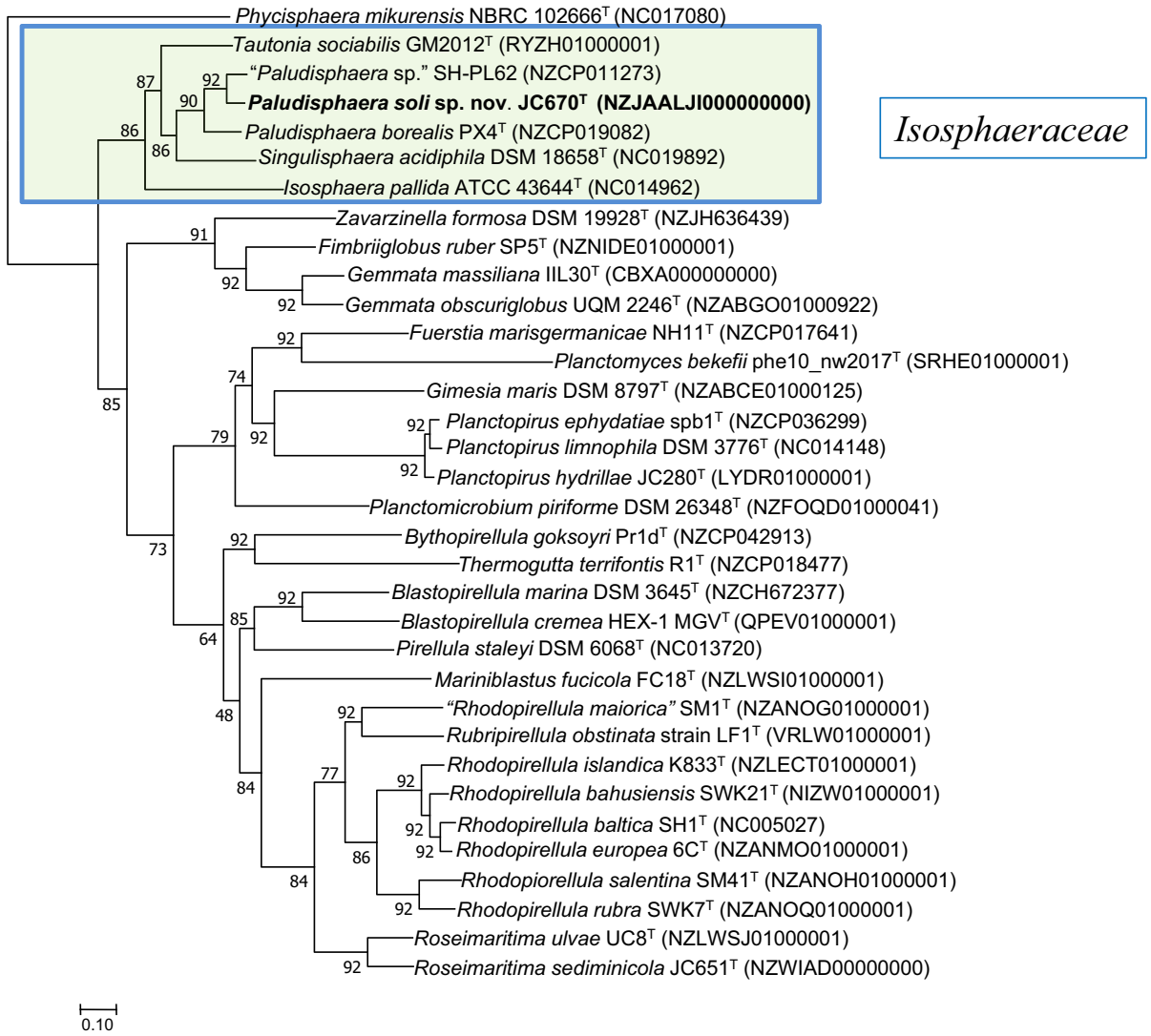

Fig. 2 RAxML based phylogenomic tree of strain JC670^T along with publicly available genome sequences of members of the families *Planctomycetaceae* and *Isosphaeraceae*. The GenBank accession numbers for genome sequences are shown in parentheses. Bar, 0.10 nucleotide substitution per position

Cells of strain JC670^T were observed to be spherical to oval shaped (1.6–1.7 × 1.3–1.5 µm; Fig. 3b), having crateriform structures distributed all over the surface. TEM images of JC670^T cells showed the presence of inner and outer membranes, invaginations of the cytoplasmic membrane, inclusion bodies, cytoplasm and nucleoid region (Fig. 3c). Cells of strain JC670^T multiply by budding and the daughter cells are smaller than the parent cells (Fig. 3d).

NaCl is not essential for growth of strain JC670^T, which tolerates up to 2% (w/v) NaCl with an optimum at 0%. The optimum temperature for the growth of strain JC670^T was determined to be 22–25 °C and no cell growth occurs above 30 °C or below 4 °C. The optimum pH for the growth of strain JC670^T was found to be 7.0 and cell growth was observed at pH 7.0 and pH 8.0. Strain JC670^T is unable to reduce nitrate and vitamin B_{12} is not required for its growth. Growth observations with different organic substrates/

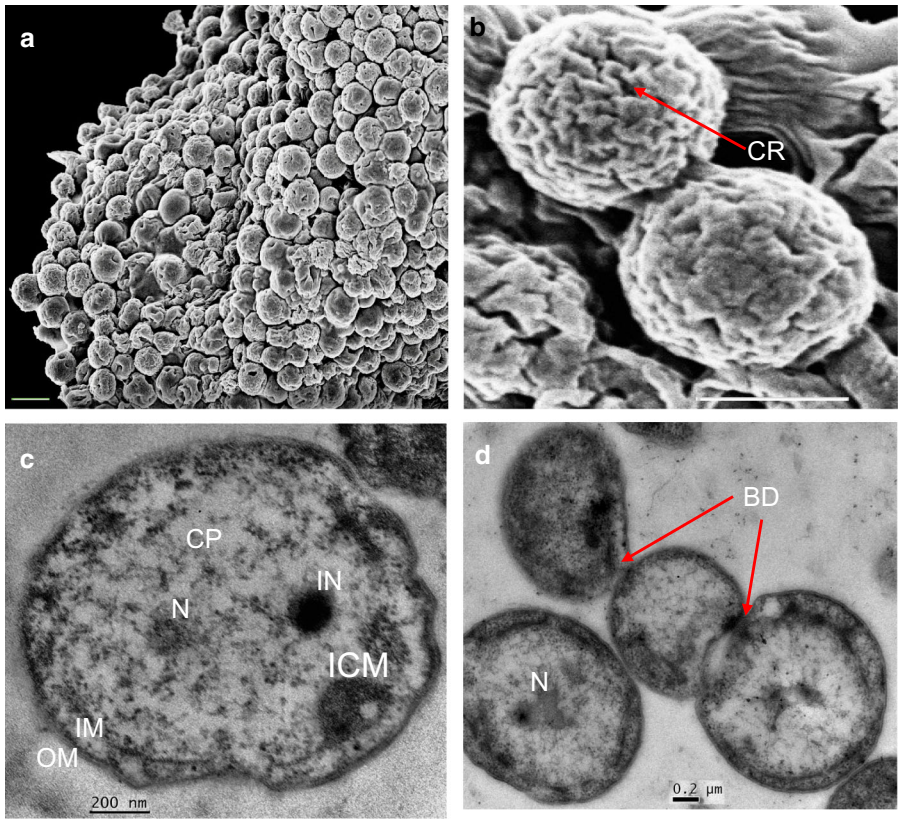

Fig. 3 Scanning (**a**, **b**) and transmission (**c**, **d**) electron micrographs of cell of strain JC670^T. **a** Spherical cells occur singly or in tissue-like aggregates. Bar, 2 µm. **b** Cells having crateriform structures (CR). Bar, 1 µm. **c** Ultrathin section showing invagination of cytoplasmic membrane (ICM), Inner membrane (IM), outer membrane (OM), inclusions (IN), cytoplasm (CP) and nucleoid region (N). **d** Cells of strain JC670^T multiply by budding (BD) wherein daughter cells are smaller than parent cells

nitrogen sources of strain JC670^T are given in the species description and Table 1.

Enzymatic activities determined for strain JC670^T using the API ZYM kit indicate positive results for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, with negative results for lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Strain JC670^T can hydrolyse phytagel (Fig. S4) only in the absence of *N*-acetylglucosamine in the medium, as also observed previously for *P. borealis* (Kulichevskaya et al. 2016).

Chemotaxonomic characterisation

The major fatty acids of strain JC670^T were identified as $C_{18:1}\omega9c$, $C_{16:0}$ and $C_{18:0}$ (Table S4). The polar lipids of strain JC670^T were found to include phosphatidylcholine, two unidentified phospholipids and six unidentified lipids (Fig. S5). In addition to phosphatidylcholine, trimethylornithine lipids were observed to be abundant in *P. borealis* (Kulichevskaya et al. 2016) but these were not detected in strain JC670^T. MK6 is the predominant quinone of strain JC670^T and polyamines are putrescine and sym-homospermidine (Fig. S6).

Table 1 Differences in the characteristics of strain JC670^T and *P. borealis* PX4^T

Characteristics	Strain JC670^T	*P. borealis* PX4^T*
Cell shape	Spherical to oval	Spherical
Cell size (L x W, in μm)	1.6–1.7 × 1.3–1.5	1.5 to 2.5
Arrangement of cell	Single or in tissue-like aggregates	Single, in pairs or short chains
pH range (optima)	7.0–8.0 (7.0)	pH 3.8–8.0 (5.0–5.5)
NaCl range (%w/v)	0–2	0–3
Temp range °C (optimum)	4–30 (22–25)	4–37 (15–25)
Nitrogen sources utilisation		
L-Phenylalanine	+	−
L-Lysine	+	−
DL-Threonine	+	−
Glycine	+	−
L-Isoleucine	+	−
L-Glutamine	+	−
L-Proline	+	−
Carbon sources utilisation		
Maltose	−	+
Succinate	−	+
Propionate	+	−
Activity of enzymes		
Alkaline phosphatase	+	−
β-Galactosidase	−	+
N-acetyl-β-glucosaminidase	−	+
G + C content (mol %)	70.4	66.3
Genome size (Mb)	7.9	7.6
RNAs	61	82
Coding sequences	6392	5792
CRISPR s	0	1
Fatty acids		
$C_{12:0}$	+	−
$C_{16:1}\omega7c$	−	+
$C_{16:1}\omega7c/C_{16:1}\omega6c$	+	−
$C_{17:0}$	+	−
anteiso-$C_{17:0}$	+	−
anteiso-$C_{17:1}$	+	−
$C_{18:0}$ 3-OH	−	+
$C_{18:1}\omega5c$	+	−
$C_{18:1}\omega7c$/ $C_{18:1}\omega6c$	+	−
$C_{18:3}\omega6c,9c,12c$	+	−
$C_{20:1}\omega9c$	+	−
$C_{20:0}$	+	−

Table 1 continued

Characteristics	Strain JC670^T	*P. borealis* PX4^T*
Long chain[a] hydroxy fatty acids	−	+

*Data taken fromKulichevskaya et al. (2016)

[a]Fatty acids with 25 or more carbon length

Both strains have MK6 as the only quinone; both strains have the major fatty acids $C_{18:1}\omega9c$, $C_{16:0}$ and $C_{18:0}$; grow well in the presence of glucose; does not require vitamin B_{12} for growth, can grow without N-acetylglucosamine and form depressions on phytagel

+ , Substrate utilised; − , Substrate not utilised

Proposal of Paludisphaera soli sp. nov.

Strain JC670^T shows similarity with *P. borealis* PX4^T with respect to cell structure, cell arrangement, quinone content, fatty acids and nitrate reduction. Additionally, vitamin B_{12} is not required for growth of either strain. However, strain JC670^T differs from *P. borealis* PX4^T in polar lipid composition, some enzyme activities and utilisation of some carbon and nitrogen sources for growth (Table 1). The phenotypic differences are supported by the molecular differences (16S rRNA gene sequence analysis) and genome relatedness (gANI/AAI, *d*DDH) values. Cumulatively these differences of strain JC670^T with respect to *P. borealis* PX4^T differentiate it into a new species of the genus *Paludisphaera*, for which we propose the name *Paludisphaera soli* sp. nov.

Description of *Paludisphaera soli* sp. nov.

Paludisphaera soli (so'li. L. neut. gen. n. *soli* of soil, referring to the isolation of the type strain from a soil sample).

Cultures are light pink in colour and strictly aerobic. Cells are spherical to oval shaped and multiply by budding. Non-motile. NaCl is not essential for growth and is tolerated up to 2% (w/v) with optimum at 0%. Vitamin B_{12} is not required for growth. Utilises α-D-glucose, sucrose, pyruvate, propionate, fructose, D-galactose, mannose, D-xylose and rhamnose as carbon source for growth while starch, ascorbate, acetate, fumarate, mannitol, lactose, inositol, maltose, malic acid, inulin, succinate, sorbitol, benzoic acid or citrate do not support growth. Ammonium sulphate, L-arginine, DL-alanine, L-glycine, L-phenylalanine, L-lysine, L-glutamine, L-proline, L-isoleucine, L-leucine-ornithine, DL-threonine, L-serine, L-tyrosine, Peptone, L-casamino acid, yeast extract, sodium nitrate are used as nitrogen sources for growth. Cysteine, L-methionine, L-aspartic acid, L-tryptophan, L-histidine, L-glutamic acid and urea do not support growth as nitrogen sources. Hydrolyses phytagel. $C_{18:1}\omega9c$, $C_{16:0}$ and$C_{18:0}$ are the major fatty acids. MK6 is the only respiratory quinone. Phosphatidylcholine, two unidentified phospholipids and six unidentified lipids are present in the polar lipids. Polyamines are putrescine and sym-homospermidine. The G + C content of the genomic DNA of the type strain is 70.4 mol%.

The type strain JC670^T (= KCTC 72850 ^{T} = NBRC 114339 ^{T}) was isolated from a soil sample from Gangotri region in Uttarakhand, India. The GenBank accession number for the 16S rRNA gene sequence of strain JC670^T is LR794334 and the genome (7.97 Mb) sequence has been deposited in GenBank under the accession number JAALJI000000000. The version described in this paper is version JAALJI010000000.

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Author contributions RK and MS performed sample collection. RK, MS and KG isolated the strain and performed the initial cultivation, strain deposition and strain characterisation, KG performed the electron microscopic analysis and media optimisation, JU performed the genomic and phylogenetic analysis, SA performed and analysed the data for polyamines, RK, MS and KG wrote the manuscript, Ramana, Sasikala and MKP supervised the study and contributed to text preparation and revised the manuscript. All authors read and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval Not applicable.

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