Paludisphaera rhizosphaerae sp. nov., a new member of the family Isosphaeraceae, isolated from the rhizosphere soil of Erianthus ravennae

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Abstract Two axenic cultures of Planctomycetota were isolated from distinct geographical locations of India. Strain JC665T was isolated from a rhizosphere soil of Loktak lake, Manipur, whereas strain JC747 was isolated from a soil sediment at Pallikkara village, Kerala, India. The two closely related strains shared the highest 16S rRNA gene sequence identity (94.6%) with Paludisphaera borealis PX4T, while the 16S rRNA gene sequence identity between both strains was 100%. Both strains grow aerobically, stain Gram negative, colonies are light pink-coloured, cells are non-motile, spherical to oval-shaped and tolerate NaCl up to 2% (w/v). While strain JC665T grows well up to pH 9.0, strain JC747 grows only up to pH 8.0. The respiratory quinone in both strains is MK-6. C16:0, C18:1ω9c and C18:0 are the major fatty acids. Phosphatidylcholine, two unidentified glycolipids, seven unidentified lipids and two unidentified phospholipids made up the polar lipid composition of both strains. Both strains have genome sizes of about 8.0 Mb and a DNA G + C content of 66.4 mol%. Both strains contain genes coding for enzymes putatively involved in the production of lycopene-related carotenoids. The phylogenetic position together with the results of the analysis of morphological, physiological and genomic features support the classification of strain JC665T as a new species of the genus Paludisphaera, for which we propose the name Paludisphaera rhizosphaerae

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sp. nov. Strain JC665^T (≡ KCTC 72671^T = NBRC 114305^T) and JC747 are the type and non-type strain of the new species, respectively.

**Keywords** Planctomycetota · Phumdis · Phylogenomics

**Abbreviations**
NCBI National centre for biotechnology information
gANI Genome average nucleotide identity
AAI Average amino acid identity
dDDH Digital DNA-DNA hybridization
HPLC High-pressure liquid chromatography
KCTC Korean collection for type cultures
NBRC Biological resource centre NITE

**Introduction**

The phylum *Planctomycetota* is a member of the PVC (Planctomycetota, Verrucomicrobiota and Chlamydiota) superphylum along with the phyla Verrucomicrobiota, Chlamydiota, Lentisphaerota, Kiritimatiellaeota and Candidatus Omnitrophica (names of the phyla used are as per the changes recommended by Oren and Garrity, 2021 and notified in the validation list published by Oren and Garrity, 2022). Members of the phylum *Planctomycetota* occur ubiquitously, play crucial roles in the global carbon and nitrogen cycle (Kuenen, 2008; Wiegand et al. 2018) and are recognised for their potential use in medical and in biotechnological issues (Rivas-Marín and Devos, 2018; Wagner and Horn, 2006). Phylum members are frequently isolated from various biotic and abiotic surfaces of marine and fresh water habitats (Lage et al. 2014; Wiegand et al. 2020; Gaurav et al. 2021; Kumar et al. 2021). Amongst other strategies for the enrichment or isolation of planctomycetes, the use of N-acetyl glucosamine as sole or additional carbon source or the exploitation of the natural resistance of strains to several antibiotics also led to the isolation novel phylum members from different terrestrial habitats like soil, peatlands and wetlands (Kaushik et al. 2020; Dedysh and Ivanova 2019).

Recent studies showed that also India harbours a rich diversity of *Planctomycetota* which is well supported by the isolation of several strains from different habitats including Loktak lake (Kumar et al. 2021; Gaurav et al. 2021; Kaushik et al. 2020). Loktak lake is an ecological hotspot with a remarkable diversity of flora and fauna and was declared as Ramsar site (a wetland site designated to be of international importance) in 1990. It has an area of about 289 km² and is the largest freshwater lake of Northeast India. Loktak lake is famous for its floating islands (Phumdis), which are heterogeneous masses of vegetation, soil and organic matter at various decomposition stages (Reddy et al. 2005). Phumdis constitute a dense rhizosphere extending down to the sediment of the lake and hence serve as an ecological habitat for several groups of bacteria including *Planctomycetota* (Puranik et al. 2016; Kumar et al. 2021).

The family *Isosphaeraceae*, to which the here characterised isolates belong, is currently composed of six genera, i.e. *Isosphaera*, Singulisphaera, Aquisphaera, *Paludisphaera*, Tundrisphaera and Tautonia. All of the currently characterised members of the family *Isosphaeraceae* are mesophilic, non-motile, spherical-shaped cells and divide by budding. The genome size ranges from 5.4 to 10.4 Mb while the DNA G+C content ranges from 62.2 to 71.1 mol% (Kulichevskaya et al. 2016, 2017; Kovaleva et al. 2019). The genus *Paludisphaera* was described by Kulichevskaya et al. (2016) as a new planctomycete of the family *Isosphaeraceae* isolated from a boreal Sphagnum peatbog. Recently, another species, “*Paludisphaera soli*”, was added to this genus (Kaushik et al. 2020), which was isolated from a high-altitude soil in the Western Himalaya.

In this study, we describe an additional member of *Paludisphaera*, isolated from two distinct and distantly located (≈ 4000 km) ecosystems, aquatic and wetland. Strain JC665^T was isolated from the rhizosphere soil of Plume grass (*Erianthus ravennae*) on a floating island of Loktak lake, Manipur (northeastern part of India), whereas strain JC747 was isolated from the wetland ecosystem of Pallikkara village, Kerala (southwestern part of India). Polyphasic taxonomic approach including genomic information, we define that strain JC665^T should be classified as the type strain of a new species of the genus *Paludisphaera* for which we propose the name *Paludisphaera rhizosphaerae* sp. nov. Strain JC747 is an additional member of the novel genus.
Materials and methods

Habitat and isolation

Rhizosphere soil of Erianthus ravennae (commonly known as “Plume grass”) of the phumdis (a floating island) was collected from Loktak lake located in the Northeastern part of India, Manipur (exact location: 24°30′21″ N 93°47′43″ E). In parallel, sediment samples were collected from the wetland (close to the village Pallikkara) located in the southwestern part of India, Kerala (12° 23′02″ N 75°02′33″ E). The samples had a pH of 7.0 and temperature of 22 °C. The rhizosphere soil and sediment sample were used for enrichment and cultivation in a medium prepared according to Kumar et al. (2021) containing (g l−1 in distilled water; pH 7.0): N-acetyl glucosamine, 2.0; KH2PO4, 0.1; peptone, 0.1; yeast extract, 0.1; vitamin solution, 10 ml l−l; Hutner’s basal salts, 20 ml l−l prepared in distilled water. The antibiotics (concentrations also in g l−1) streptomycin, 0.4, ampicillin, 0.2 and cycloheximide, 0.025 were added to the medium. The vitamin solution contained (in mg l−1): vitamin B12, 0.2; biotin, 4; thiamine-HCl ·2H2O, 10; Calcium pantothenate, 10; folic acid, 4.0; riboflavin, 10; p-aminobenzoic acid, 10; pyridoxine·HCl, 20. Hunter’s basal salts contained (in g l−1): nitrilotriacetic acid, 10; MgSO4 ·7H2O, 30; CaCl2 ·2H2O, 3.5; (NH4)6MoO7O24 ·4H2O, 0.01; FeSO4 ·7H2O, 0.1; and 50 ml metal stock solution. Metal stock solution contained (in g l−1): Na-EDTA, 0.25; ZnSO4 ·7H2O, 1.1; FeSO4 ·7H2O, 0.5; MnSO4 ·H2O, 0.15; CuSO4 ·5H2O, 0.04; Co(NO3)2 ·6H2O, 0.025; Na2B4O7 ·10H2O, 0.018. The samples (50 mg) were mixed with 10 ml medium in different serum vials of 50 ml capacity and the vial was sealed with butylated rubber stoppers. The serum vials were then incubated for five months at 25 °C to enrich planctomycetota. After five months of incubation, a light-pink globular bacterial colony was observed at the bottom of the serum vials of both samples. These colonies were streaked on agar (1.8% w/v) plates 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-mentioned medium without antibiotics, unless stated otherwise. The pink-coloured cultures isolated from the rhizosphere soil and sediment samples were designated strains JC665T and JC747, respectively.

DNA isolation, 16S rRNA gene sequencing and BLAST analysis

The Nucleo-pore gDNA Fungal Bacterial Mini Kit, from M/s. Genetix Biotech Asia Pvt. Ltd, India was used for DNA isolation, which was then used for 16S rRNA gene amplification and genome sequencing. For the PCR-based amplification of the 16S rRNA gene, the planctomycete-specific primers F40 (Kohler et al. 2008) and R1388 (Stackebrandt et al. 1993) and the TAKARA master mix (EmeraldAmp® GT PCR Master Mix) were used. Thermocycler conditions were as follows: an initial denaturation step (94 °C for 10 min) followed by 33 cycles of denaturation (94 °C for 1 min), annealing (52 °C for 54 s), and extension (72 °C for 1.4 min). Finally, the tubes were incubated at 72 °C for 15 min to ensure complete synthesis of the entire sequence. The amplified PCR products were sent to M/s. AgriGenome Pvt. Ltd. (Kochi, India) for purification and 16S rRNA gene sequencing. The closest relative of the isolated strains was identified based on the 16S rRNA gene sequence using BLAST search analysis in the EzBioCloud database (Yoon et al. 2017).

Genomic information and genome-based characterisation of central metabolism

Whole-genome sequencing (WGS) of strains JC665T and JC747 was outsourced to M/s. AgriGenome Pvt. Ltd, Kochi, India. WGS was carried out on an Illumina HiseqX10 platform. Paired-end libraries were generated with a sequence coverage of 100x. The Unicycler assembly software (Wick et al. 2017) was used for de novo assembly with default k-mer sizes and was also used for all further downstream analyses. The ContEst service (Yoon et al. 2017) was used for the detection of any possible contamination. The RAST server (http://rast.theseed.org/FIG/rast.cgi) (Aziz et al. 2008) was used for genome annotation. The average amino acid identity (AAI) was calculated using the AAI calculator developed by the
Konstantinidis lab (Rodriguez and Konstantinidis 2014). Orthologous gene clusters and distribution of proteins among the different strains were analysed by constructing a heat map and a Venn diagram with the help of the web server Orthovenn2 (https://orthovenn2.bioinfotoolkits.net/home). Carbohydrate active enzymes (CAZy) were determined on the dbCAN meta server (http://bcb.unl.edu/dbCAN2/) by choosing default parameters (Zhang et al. 2018).

In-silico characterization of the metabolism of strains JC665T and JC747 was carried out on the basis of the respective genome information by using KEGG mapper (Kanehisa and Sato 2020). An in-silico analysis of genetic clusters putatively responsible for the biosynthesis of secondary metabolites was carried out using antiSMASH 5.1 (http://antismash.secondarymetabolites.org) (Blin et al. 2019).

Phylogenetic analysis

The 16S rRNA gene sequences of strains JC665T and JC747 were extracted from the genome using ContEst16S (https://www.ezbiocloud.net/tools/contest16s) and the analysis of identity values was performed using NCBI BLAST (Johnson et al. 2008). The 16S rRNA gene sequences of strains JC665T and JC747 and other members of the family Isosphaeraceae were aligned using MUSCLE implemented in MEGA7.0 (Kumar et al. 2016) and the distances were calculated using Kimura 2-parameters (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, each having bootstraps of 1000 replication (Felsenstein 1985). The phylogenomic tree was constructed using 92 core genes (retrieved using the UBCG tool as described by Na et al. (2018)) from all publicly available genomes of Isosphaeraceae family members. A concatenated sequence of 92 genes was used to construct the RAxML-based phylogenomic tree as described by Kumar et al. (2021).

Physiological analyses

For carbon and nitrogen sources utilisation, basal medium was used as described previously (Bondoso et al. 2011) with slight modifications: The medium was additionally supplemented with trace amount of yeast extract (0.05%, w/v). For organic substrate utilisation, (NH4)2SO4 (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 organic carbon source and cell growth was tested with different nitrogen substrates at 0.1% (w/v). Both, organic and nitrogen substrate utilisation, was tested in test tubes (25×250 mm) containing 10 ml of basal medium as described above. 10 ml of broth in test tubes (25×250 mm) was used for determining the utilization of organic carbon/nitrogen substrates and vitamin B12 requirements as previously described (Kaushik et al. 2020). NaCl tolerance (1–10% w/v, at an interval of 1% w/v; final NaCl concentration was tested at 25 °C and pH 8.0. The optimal temperature (5, 10, 15, 20, 25, 30, 35, 40 °C) required for growth was tested at pH 8.0. The tolerated pH range (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0) of the strains was tested at 25 °C in buffered medium as described previously (Bondoso et al. 2015). To examine the hydrolysis of phytigel, the cultures were streaked onto media solidified with 2% (w/v) phytigel (Sigma-Aldrich) and incubated for 4 weeks (Kaushik et al. 2020; Kulichevskaya, et al. 2016). Phytigel-hydrolysing activities were also checked on media supplemented with N-acetyl glucosamine as a sole source of carbon and nitrogen. Enzyme activities were assayed using the API ZYM kit (Biomerieux, France) according to the manufacturer’s protocol. Anaerobic growth was tested using agar deeps in test tubes (25×250 mm) filled to the three quarters of the final volume with molten medium, sealed with subseal and the head space was replaced with ultra-pure argon gas.

Chemotaxonomic characterization

For fatty acid analysis, exponentially 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% =OD660 of 0.9). Cellular fatty acids were then methylated, separated and identified according to instructions for 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 were extracted, separated and
characterized as described previously (Kates 1972; Oren et al. 1996). Quinones were extracted with a chloroform/methanol (2:1, v/v) mixture, purified by Thin Layer Chromatography (TLC) and analysed by High Pressure Liquid Chromatography (HPLC; Imhoff 1984). Polyamines were extracted and identified according to a recently described method (Kumar et al. 2021).

Microscopy

Cell morphological features, including cell size, shape and cell division were observed under field emission scanning electron microscopy (FESEM) or transmission electron microscopy (TEM). For FESEM, one ml of log-phase culture was centrifuged at 7000 g for 10 min at 4 °C. The cell pellet was washed by resuspension in sterile MilliQ and centrifuged at 7000 g for 10 min at 4 °C. The pellet or cells were fixed in 2.5% (v/v) glutaraldehyde solution and kept for six hours incubation at 4 °C (Kumar et al. 2021). Cells were dehydrated sequentially with an increased ethanol concentration from 10–100% (v/v) (10% intervals). At last, the cells were resuspended in 100% ethanol. 10 μl of samples were kept on a small size glass slide, which was placed on the stab with adhesive tape (Kumar et al. 2021). Finally, stabs were kept for gold sputtering for six minutes and then cell morphology and division were examined under the FESEM (Philips XL3O) at the facility of the School of Physics, UOH. For TEM, ultrathin sectioning of the log phase cells was outsourced to RUSKA Diagnostic, Hyderabad. The sections of the cells were mounted on copper grids and observed under TEM (H-7500 Hitachi) at the facility of CCMB, Hyderabad.

Results and discussion

Genomic characteristics, BLAST analysis and phylogenetic inference

The genome sizes of the strains JC665T and JC747 are 8.05 Mb and 8.04 Mb with an obtained N50 value of 238,467 bp and 226,135 bp, respectively. The genome of strain JC665T harbours 6,444 genes of which 6,297 are protein-coding genes, 80 genes code for RNAs (3 genes code for rRNAs, 74 genes for tRNAs and 3 for other RNAs) and 67 genes are putative pseudogenes (Table S1). The genome of strain JC747 harbours 6,420 genes of which 6,299 are protein-coding genes, 80 genes code for RNAs (3 genes for encoding rRNAs, 74 genes for tRNAs and 3 for other RNAs) and 41 genes are putative pseudogenes (Table S1). The organization of orthologous clusters among the strains will provide a better understanding of the genome structure and gene/protein function. The automated genome annotation yielded 7106, 7102, 7373 and 6643 protein-coding genes for strains JC665T, JC747, “P. soli” JC670T and P. borealis PX4T, respectively. The predicted protein-coding gene of strains JC665 and JC747 led to 6973 and 6969 orthologous clusters and 103 single tons for which no orthologs could be found in other species (Fig. S1A). “P. soli” JC670T showed 4612 clusters and 2464 singletons. P. borealis PX4T showed 4268 clusters and 2160 singletons (Fig S1A). The comparative analysis of orthologous gene clusters shows that these species formed 7499 clusters, 4032 orthologous clusters (at least contains two species) and 3467 single-copy gene clusters (Fig. S1B). A total of 14,738 proteins were present in orthologous clusters found in all strains within the genus whereas strain JC670 and PX4 showed 314 and 190 proteins, respectively in clusters specific to them (Fig. S1C). Further comparison of shared orthologous gene clusters showed that 3556 clusters were observed in all the strains, no unique clusters were observed in the strains JC665T and JC747 whereas “P. soli” JC670T and P. borealis PX4T showed 127 and 73 unique clusters, respectively (Fig. S1D).

The genomic DNA G+C content of strains JC665T and JC747 is 66.4 mol% (Table S1). The 16S rRNA gene sequences extracted from the genomes have a sequence length of 1521 nt. BLAST analysis of 16S rRNA gene sequence of strains JC665T and strain JC747 on the EzBioCloud server gave an identity of 94.6% and 96.7% with P. borealis PX4T and “P. soli” JC670T, respectively (Fig. 1). Comparison of dDDH, gANI, and AAI values of strains JC665T and JC747 with Paludisphaera spp. yielded similarities of 19.4–20.3%, 62.4–68.6%, and (75.1–77.9%, respectively. dDDH, OrthoANI and AA1 values fell well below the recommended cut-off of 70, 95–96% and 80%, respectively for prokaryotic species delineation (Rodriguez and Konstantinidis 2014; Meier-Kolthoff et al. 2014; Chun et al. 2018). These values suggest that the isolated strains belong to a single
novel species of the genus Paludisphaera. However, high values of 16S rRNA gene sequence identity (100%), dDDH (100%), gANI (100%), and AAI (99.9%) between strains JC665^T and JC747 suggest these strains to belong to the same species. The 16S rRNA gene sequence-based phylogenetic tree with combined bootstrap values obtained from NJ, ME, ML trees (Fig. 2) and the 92 core gene-based phylogenomic tree (Fig. 3) confirmed the distinct monophyletic clustering of strains JC665^T and JC747 with Paludisphaera members within the family Isosphaeraceae and are in agreement with the suggestion of a novel species in the genus Paludisphaera.

In-silico metabolic characterisation

In order to have a better understanding on possible metabolic functions, the COGs annotation was performed. The results showed that both strains, JC665^T and JC747, showed similar results compared to the other members of the genus Paludisphaera. Most (about 1872 genes) of the predicted genes were of unknown function, followed by 690 genes involved in energy production and conversion (Fig. S2). The CAZy annotation of the genomes shows that the strains JC665^T and JC747 contain more genes encoding putative glycoside hydrolases and glycosyltransferases compared to other members of the genus Paludisphaera. The analysis also shows that 80–90% of the found enzymes belonged to families of glycoside hydrolases and glycosyl transferases (Fig. S3). The presence of higher number of carbohydrate-active enzymes with respect to bacterial metabolism needs further studies. The in silico metabolic characterisation showed that strains JC665^T and JC747 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), that act as precursors for the synthesis of carotenoids and quinones. The genes coding for a putative 15-cis-phytoene synthase, and phytoene desaturase are involved in the formation of lycopene were found in the genomes of both strains. The genes required for assimilatory nitrate reduction were observed only in P. borealis PX4^T. All strains belonging to genus Paludisphaera including JC665^T and JC747 showed putative gene clusters for synthesis of polyketide-derived compounds (type I and type III polyketide synthase gene-containing clusters) and terpenoid biosynthesis. In-silico genome analysis of all the four strains in the genus Paludisphaera also suggest the presence of putative hopanoid biosynthesis pathway genes like squalene synthase (hpnC), squalene/phytoene desaturase (hopC), squalene hopene cyclase (Shc; codes for the key enzyme of hopanoid biosynthesis), radical S-adenosyl-L-methionine (SAM) required for addition of adenosyl group to the hopane skeleton (hpnH), acetylornithine aminotransferase/amino-bacteriohopanetriol synthase (hpnO), hopanoid associated sugar epimerase (hpnA) and sterol desaturase family protein (erg32). However, genes putatively involved in the production of indole were predicted exclusively in JC665^T and JC747 (Fig. S4).

Morphological and physiological analysis

SEM images showed that cells of strains JC665^T and JC747 are spherical to oval shaped (1.7–1.8 × 1.3–1.5 μm; Fig. 4A). A uniform distribution of crateriform structures (CR) over the cell surface was observed. TEM images of the cells showed the presence of cytoplasmic membrane (CM), outer membrane (OM), invaginations of the cytoplasmic membrane (ICM), cytoplasm (CP), nucleoid region (N), ribosomes (RB) and cell reproduction by
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Fig. 2 Phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationship of strain JC665T, JC747 and other closely related members of the family *Isosphaeraceae*. The tree was constructed using MEGA7 software and *Gimesia maris* DSM 8797T was used as the outgroup. The GenBank accession numbers of 16S rRNA gene sequences are shown in parentheses. Numbers at nodes indicate Bootstrap values from 1000 repetitions in the NJ/ME/ML analysis. Bar, 0.02 nucleotide substitution per position.

Fig. 3 RAxML-based phylogenomic tree of strains JC665T and JC747 along with publicly available genome sequences of members in the family *Isosphaeraceae*. The GenBank accession numbers of genome sequences are shown in parentheses. The tree was computed with MEGA 7 software and rooted with *Alienimonas californiensis* CA12T as the outgroup. Bootstrap percentages refer to ML/ME/NJ analysis. Bar, 0.1 nucleotide substitution per position.
budding (BD), where a daughter cell is protruding from the mother cell (Fig. 4B).

NAG is not obligate for the growth of strains JC665T, JC747, “P. soli” JC670T and P. borealis DSM 28747T. All four strains utilize the following organic carbon sources: α-D-glucose, sucrose, Na-pyruvate, D-galactose, mannose, rhamnose, and trehalose. Neither of the strains was able to utilize the following organic carbon sources: starch, ascorbate, acetate, mannitol, malate, inulin, benzoate, Na-succinate and citrate. Lactose and maltose are only utilized by the strains JC665T, JC747, and P. borealis DSM 28747T. Fructose and D-xylose are utilised by the strains “P. soli” JC670T and P. borealis DSM 28747T. Cellobiose and ribose are exclusively utilised by the strain P. borealis DSM 28747T. Fumarate and propionate utilization are exclusive for the strains JC665T (including JC747) and “P. soli” JC670T, respectively. All the strains utilise the following nitrogen sources for biomass formation: ammonium sulphate, peptone, yeast extract, DL-alanine, L-arginine, casamino acids and sodium nitrate. Neither of the strains utilizes the following nitrogen sources: L-aspartic acid, urea and valine. The following nitrogen sources are exclusively utilized by the strains JC665T, JC747 and “P. soli” JC670T: glycine, L-phenylalanine, L-lysine, L-glutamine, L-proline, L-isoleucine, L-leucine, DL-ornithine and DL-threonine. However, L-methionine and cysteine are exclusively utilised by the strains JC665T and JC747. L-serine and L-tyrosine utilization turned out to be exclusive for strain “P. soli” JC670T (Table 1). Strain JC665T can hydrolyse phytage (Fig. S5) only in the absence of N-acetyl glucosamine in the medium, as also observed previously for P. borealis PX4 T and “P. soli” JC670T (Kaushik et al. 2020; Kulichevskaya et al. 2016).

All four strains turned out to be positive for esterase (C4), leucine arylamidase, and valine arylamidase. However, all strains lacked activity for lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. Alkaline phosphatase, esterase lipase (C8), and acid phosphatase were exclusively positive for the strains “P. soli” JC670T and P. borealis DSM 28747T. Naphthol-AS-BI-phosphohydrolase led to a positive signal for the strains JC665T, JC747 and “P. soli” JC670T. β-Galactosidase and N-acetyl-β-glucosaminidase signals were exclusively positive for P. borealis DSM 28747T.

Chemotaxonomic characterisation

The major fatty acids in strains JC665T, JC747, “P. soli” JC670T and P. borealis DSM 28747T are C_{18:1}ω9c, C_{18:0} and C_{16:0}. In terms of fatty acid composition, significant differences were found among all the tested strains (Table. S2). The polar lipids of
### Table 1  Differences in the characteristics of strains JC665<sup>T</sup>, JC747, “P. soli” JC670<sup>T</sup> and P. borealis DSM 28747<sup>T</sup>

| Characteristics                          | JC665<sup>T</sup> | JC747 | “P. soli” JC670<sup>T</sup> | P. borealis DSM 28747<sup>T</sup> |
|------------------------------------------|-------------------|-------|-----------------------------|-----------------------------------|
| shape                                    | Spherical to oval | Spherical to oval | Spherical to oval | Spherical                       |
| Cell size (LxW,in µm)                    | 1.8–1.7 x 1.5–1.3 | 1.8–1.7 x 1.5–1.3 | 1.6–1.7 x 1.3–15 | 1.5 to 2.5                      |
| Arrangement of cell                     | Single or in tissue-like aggregates | Single or in tissue-like aggregates | Single or in tissue-like aggregates | Single, in pairs or short chains |
| pH range(optima)                        | 6–9 (7.0)         | 6–8 (7.0)       | 7.0–8.0 (7.0)    | 3.8–8.0 (5.0–5.5)               |
| NaCl range (% w/v)(Optimum)             | 0–2 (0)           | 0–2 (0)         | 0–2 (0)          | 0–3 (0)                         |
| Temperature range (optimum)             | 4–34 (26–28)      | 8–30 (26–28)    | 4–30 (22–25)     | 4–37 (15–25)                    |
| Nitrogen sources utilization            |                   |                   |                 |                                  |
| L-Phenylalanine                          | +                 | +                | +               | −                                |
| L-Lysine                                 | +                 | +                | +               | −                                |
| DL-Threonine                             | +                 | +                | +               | −                                |
| Glycine                                  | +                 | +                | +               | −                                |
| L-Isoleucine                             | +                 | +                | +               | −                                |
| L-Glutamine                              | −                 | −                | +               | −                                |
| L-Proline                                | +                 | +                | +               | −                                |
| Carbon sources utilization              |                   |                   |                 |                                  |
| Maltose                                  | +                 | +                | −               | +                                |
| Succinate                                | −                 | +                | −               | +                                |
| Propionate                               | −                 | +                | −               | +                                |
| Activity of enzymes                      | −                 | +                | +               | −                                |
| Alkaline phosphatase                     | −                 | +                | +               | −                                |
| β-Galactosidase                          | −                 | −                | −               | +                                |
| N-acetyl-β-glucosaminidase               | −                 | +                | −               | +                                |
| Fatty acids composition                  |                   |                   |                 |                                  |
| C<sub>12:0</sub> anteiso                 | +                 | −                | −               | −                                |
| C<sub>13:0</sub>                         | +                 | −                | −               | −                                |
| C<sub>16:0</sub> N alcohol               | +                 | −                | −               | −                                |
| C<sub>16:1</sub>ω9c                      | −                 | −                | +               | +                                |
| C<sub>17:0</sub>                         | +                 | −                | +               | −                                |
| aLong chain hydroxy fatty acid           | −                 | −                | −               | +                                |
| Major Polar lipids                       |                   |                   |                 |                                  |
| Phosphatidylcholine                      | −                 | −                | +               | +                                |
| Phosphatidylethanolamine                 | −                 | −                | −               | +                                |
| Major Polyamines                         |                   |                   |                 |                                  |
| Spermidine                               | −                 | +                | −               | +                                |
| Sym homospermidine                       | +                 | −                | +               | −                                |
| Putrescine                               | +                 | −                | +               | −                                |
| Genomic features                         |                   |                   |                 |                                  |
| G + C content (mol%)                     | 66.4              | 66.4             | 70.4            | 66.3                             |
| Genome size (Mb)                         | 8.05              | 8.04             | 7.97            | 7.65                             |
| Coding sequences                         | 6364              | 6340             | 6389            | 5785                             |
| RNAs                                     | 80                | 80               | 61              | 85                               |
| CRISPRs                                  | 1                 | 1                | 0               | 1                                |

Data, from this study

* Fatty acid with 25 or more carbon atoms
strains JC665T and JC747 comprise phosphatidylcholine (PC), two unidentified glycolipids (GL1, 2), six unidentified lipids (UL1-7) and two unidentified phospholipid (PL1, 2) (Fig. S6A). The polar lipids of strain P. borealis DSM 28747T include phosphatidylcholine (PC), phosphatidylethanolamine (PE), one unidentified choline lipid (CL1), two unidentified glycolipids (GL1, 2), two unidentified lipids (UL1, 2), two unidentified amino lipids (AL1, 2) and four unidentified phospholipids (PL3-6) (Fig. S6B) and is not found in congruence of earlier study, as different methods were used for the identification of polar lipids (Kulichevskaya et al. 2016). The polar lipids of strain “P. soli” JC670T include phosphatidylcholine, two unidentified phospholipids and six unidentified lipids (Fig. S6C) and is in agreement with a previously published study (Kaushik et al. 2020). Polyamines of the strains JC665T and “P. soli” JC670T include sym-homospermidine and putrescine. Polyamines present in strain JC747 include spermidine and two unidentified polyamines (1, 3). Polyamines of P. borealis DSM 28747T include spermidine and two unidentified polyamines (2, 3) (Fig. S7). MK-6 is the predominant quinone for all strains.

Proposal of strain JC665T as the type strain of a new species in the genus Paludisphaera

Strains JC665T and JC747 showed clear phylogenomic differences with “P. soli” JC670T and P. borealis DSM 28747T but behave similar when compared against each other (Figs. 1,2,3; Fig. S1, Table S1). The phylogenomic differences are well supported by chemotaxonomic and phenotypic differences (Table 1), which together support to classify strain JC665T as a novel species of the genus Paludisphaera. For this reason, we propose the name Paludisphaera rhizosphaerae with the type strain JC665T and strain JC747 as an additional member of the species.

Description of Paludisphaera rhizosphaerae sp. nov.

Paludisphaera rhizosphaerae (rhi.zo.sphae’rae. Gr. n. rhiza, root; L. n. sphaera, sphere; N.L. gen. n. rhizosphaerae, from the rhizosphere).

The strain is pale pink pigmented. Cells are spherical to oval shaped and strictly aerobic. Cells divide by budding. NaCl is not obligate for growth and a concentration of up to 2% (w/v) can be tolerated. Optimum pH and temperature for growth are 7.0 (range 6.0–9.0) and 25 °C (range 4–34 °C) respectively. Growth occurs also in the absence of added N-acetylglucosamine. D-glucose, sucrose, pyruvate, D-galactose, Mannose, rhamnose, inositol, fumarate, lactate, maltose, sorbitol, and trehalose are utilized as carbon and energy source. Fructose, N-propionate, D-xylene, starch, ascorbate, acetate, manitol, malic acid, inulin, succinate, benzoic acid and citrate are not utilized. Ammonium sulphate, peptone, casamino acids, yeast extract, sodium nitrate, L-cysteine, L-methionine, L-histidine, L-glutamic acid, L-arginine, DL-alanine, glycine, L-glutamine, L-proline, L-iso-leucine, L-ornithine and DL-threonine are utilized as nitrogen source. L-serine, L-tyrosine, L-aspatic acid, L-tryptophan, urea and L-valine are not utilized as nitrogen source. Hydrolysates phytageal. Major fatty acids are C18:1ω9c, C16:0 and C18:0. Minor fatty acids include anteiso-C11:0, anteiso-C12:0, C13:0, anteiso-C15:0, C15:2:OH, C15:0:0c, C17:0, C17:1ω08c, anteiso-C17:0 and C18:3ω6c:9c,12c. Putrescine and sym-homospermidine are the major polyamines. The polar lipid profile comprises phosphatidylcholine, two unidentified glycolipids (GL1, 2), seven unidentified lipids (UL1-7) and two unidentified phospholipid (PL1, 2). MK-6 is the only quinone. Nitrate is not reduced. API ZYM shows positive signals for esterase (C4), leucine arylamidase, and valine arylamidase and naphthol-AS-BI-phosphohydrolase. Tested negative for lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-gluconidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase. Alkaline phosphatase, esterase lipase (C8), acid phosphatase, β-galactosidase and N-acetyl-β-glucosaminidase. The type strain JC665T (=NBRC 114,305 = KCTC 72671 T) was isolated from the rhizosphere soil of Erianthus ravenae (commonly known as “Plume grass”) collected from Loktak lake located in the Northeast part of India, Manipur (exact location: 24°30’21” N 93°47’43” E). JC747 is an additional strain isolated from a wetland located (village: Pallikkara) in the southwest part of India, Kerala (12° 23’ 02’’ N 75° 02’ 33’’ E). The genome size of the type strain is 8.05 Mb and the G+C content of the genomic DNA is 66.4 mol%. The GenBank accession numbers of the 16S rRNA gene sequence and genome sequence of strain JC65T and JC747 are LR746340.
OU374731 and JAALCR0000000000 and JAH-PZK0000000000, respectively.

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Author contributions  Khongsai L performed sample collection from Loktak lake, KG, Ramana, and Sasikala performed sample collection from Kerala, Khongsai L and KG isolated the strain, performed the initial cultivation, strain deposition and strain characterisation, KG performed media optimisation and polar lipid analysis and the electron microscopic analysis, JU performed the genomic and phylogenetic analysis, Smita N and Khongsai L performed the endometabolite and exometabolite analysis. SA and Khongsai L performed and analysed the data for polyamines, KG and Khongsai L wrote the manuscript. Ramana and Sasikala 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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Declaration

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

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