Aquisphaera Insulae Sp. Nov., a New Member in Isosphaeraceae Isolated From the Floating Island of Loktak Lake and Emended Description of the Genus Aquisphaera

Gaurav Kumar  
University of Hyderabad

Lhingjakim Khongsai L  
J. N. T. University Hyderabad

Jagadeeshwari U  
University of Hyderabad

Shabbir A  
University of Hyderabad

Dhanesh Kumar  
University of Hyderabad

Gulam Mohammad Kashif  
J. N. T. University Hyderabad

Sasikala Ch  
University of Hyderabad

Ch. Venkata Ramana (✉ cvr449@gmail.com)  
University of Hyderabad  https://orcid.org/0000-0003-2362-9824

Short Report

Keywords: Planctomycetes, Isosphaeraceae, Aquisphaera, fruiting body, sporotan

DOI: https://doi.org/10.21203/rs.3.rs-313235/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Strain JC669\textsuperscript{T} was isolated from a floating island of Loktak lake, Manipur, India and shared highest 16S rRNA gene sequence identity with \textit{Aquisphaera giovannonii} OJF2\textsuperscript{T}. The strain is an aerobe, Gram-stain-negative, yellowish orange coloured, non-motile, NaCl intolerant, spherical to oval shaped, grows in single or aggregates and produce structures which appear like fruiting bodies. Strain JC669\textsuperscript{T} grows well up to pH 9.0, has MK6 as respiratory quinone, C\textsubscript{18:1}\textomega\textsubscript{9c}, C\textsubscript{16:0} and C\textsubscript{18:0} as major fatty acids and phosphatidylcholine, an unidentifed amino lipid, an unidentifed choline lipid (UCL) and six unidentifed lipids (UL1,2,3,4,5,6) as polar lipids. The genome size of strain JC669\textsuperscript{T} is 10.04 Mbp and genomic G+C content of 68.5 mol\%. Based on phylogenetic, polyphasic including genomic analyses support strain JC669\textsuperscript{T} as a novel species of the genus \textit{Aquisphaera}, for which we propose the name \textit{Aquisphaera insulae} sp. nov. Type strain is JC669\textsuperscript{T} (=KCTC 72672\textsuperscript{T} = NBRC 114306\textsuperscript{T}).

Introduction

The phylum Planctomycetes is one among the PVC superphylums (Wagner and Horn 2006; Wiegand et al. 2020) and members of this phylum are ubiquitously distributed (Rensink et al. 2020; Wiegand et al. 2020). It is difficult to get axenic cultures of these members and those which were obtained as axenic cultures are mostly from cultures which were isolated from brackish and marine sources (Bondoso et al. 2014, 2015; Yoon et al. 2014; Lage et al. 2017; Wiegand et al. 2018, 2020; Kumar et al. 2020a,b; Peeters et al. 2020). Planctomycetes are also presupposed to be untapped ‘adroit producers’ of small bioactive molecules that might comprise putative novel antimicrobial compounds with a potential therapeutic character such as novel antibiotics (Graca et al. 2016; Jeske et al. 2015). Planctomycetal genome analysis revealed that Planctomycetal genomes encode up to 15 biosynthetic gene clusters (BGCs).

There are six genera (\textit{Paludisphaera}, \textit{Aquisphaera}, \textit{Singulisphaera}, \textit{Tundrisphaera}, \textit{Isosphaera}, and \textit{Tautonia}) under the family \textit{Isosphaeraceae} and many of these are monospecific except for \textit{Singulisphaera} and \textit{Tautonia} (Kaushik et al. 2020). \textit{Isosphaeraceae} members are mesophilic, non-motile and their cells divide by polar budding. The Genus \textit{Aquisphaera} belongs to the family \textit{Isosphaeraceae} and it was first described by Bondoso et al. (2011). Genus \textit{Aquisphaera} contains only one species with valid name; \textit{Aquisphaera giovannonii} which was isolated from a fresh water aquarium’s sediments. The genus \textit{Aquisphaera} is characterized by diphosphatidylglycerol and phosphatidylcholine as major polar lipids with C\textsubscript{16:0} and C\textsubscript{18:1}\textomega\textsubscript{9c} as major fatty acids. \textit{A. giovannonii} is an obligate aerobe, slow-growing, chemoheterotroph, spherical and yellowish-orange/pink pigmented bacterium reproduce through budding.

Loktak lake is the largest freshwater lake of northeast India, having a spanning area of about 289 km\textsuperscript{2}. Loktak lake is famous for its floating islands (Phumdis; Fig. 1), which are heterogeneous biomass of vegetation, soil and organic matter at various decomposition stages (Reddy et al. 2005). Phumdis constitute a dense rhizosphere extending up to the lake sediment and hence, serve as an ecological
habitat for several groups of bacteria like Actinobacteria, Acidobacteria, Proteobacteria, Verrucomicrobia and Planctomycetes (Puranik et al. 2016). This lake is of ecological hotspot with remarkable flora and fauna diversity and declared as Ramsar site in 1990. Insight of the previous metagenome study (Puranik et al. 2016) and during our survey for planctomycetes from India (Kumar et al., 2020a,b,c; Kaushik et al., 2020; Kumar et al. 2021a,b), strain JC669T was isolated from the rhizosphere soil of a plant from the floating island of the Loktak lake, whose 16S rRNA gene sequence identity was highest (96.8%) with A. giovannonii OJF2T. We characterized strain JC669T using a polyphasic approach and genomic information and compared the characters with the type species of the genus Aquisphaera, A. giovannonii.

Materials And Methods

Isolation, cultivation and preservation

Rhizospheric soil of Zizania latifolia (commonly known as Manchurian wild rice) of the phumdis (floating island) was collected from Loktak lake situated in the northeast state of Manipur (GPS: 24°30’21” N 93°47’43”), India. The lake at the time of sample collection had a pH of 7.0 and temperature of 18°C. Soil samples were subjected to enrichment and cultivation in a medium as described by (Kaushik et al. 2020). The rhizospheric soil sample (100 mg) was mixed in a 20 ml transparent serum vials with 10 ml of media and sealed with butylated stopper and further incubated at 25°C for five months. After five months of incubation light yellowish orange colour colonies appeared at the bottom of the serum vial, further purified on the same solid media after subsequent sub-culturing. Light yellowish orange cultures were maintained on the agar plates by repeated streaking / preserved at 4ºC and this culture is designated as strain JC669T.

Isolation of DNA and 16S rRNA gene sequence analysis

The axenic culture was used to isolate genomic DNA using commercial DNA isolation it (Nucleo-pore gDNA Fungal Bacterial Mini Kit, from M/s. Genetix Biotech Asia Pvt. Ltd, India). Genomic DNA was used for genome sequencing and 16S rRNA gene amplification as well. Planctomycetes specific primers F40 (Köhler et al., 2008) combined with universal primer R1388 (Stackebrandt et al. 1993) are used to perform PCR for 16S rRNA gene amplification. Amplified PCR products were further purified and sequenced (16S rRNA gene) by Agri Genome Pvt. Ltd. (Kochi, India).

Genomic and in-silico analysis

Genome sequencing was outsourced to AgriGenome Pvt. Ltd, Kochi, India. Illumina HiseqX10 platform was used to generate whole genome sequencing (WGS) and sequence coverage of 100x was generated using the paired-end library. Unicycler assembler was used for De novo assembly (Wick et al. 2017) with default k-mer sizes and for further downstream analysis unicycler assembly was used. Contamination in the genome sequence was checked using ContEst service of EZBiocloud (https://www.ezbiocloud.net/tools/contest16s; Yoon et al. 2017) which showed single 16S rRNA
sequence of only one organism concluding the genome sequence as contamination free. Annotation the genome was done using NCBI–PGAP online freely available RAST server (http://rast.theseed.org/FIG/rast.cgi) (Aziz et al. 2008). EzBioCloud (https://www.ezbiocloud.net/tools/ani) (Yoon et al. 2017), Genome-to-Genome Distance Calculator (GGDC 2.1) (http://ggdc.dsmz.de/distcalc2.php) (Auch et al. 2010) and AAI calculator developed by the Konstantinidis lab (Rodriguez and Konstantinidis 2014) online services were used for estimating OrthoANI, dDDH and AAI scores, respectively between the strains JC669<sup>T</sup> and <i>A. giovannonii</i> OJF2<sup>T</sup>. Further, PATRIC 3.6.2 (https://www.patricbrc.org/) (Wattam et al. 2017), Interactive pathway explorer (iPath3) (https://pathways.embl.de/login.cgi) (Darzi et al. 2018) and antiSMASH5.1 (http://antismash.secondarymetabolites.org) (Blin et al. 2019) were used for carrying out the <i>In-silico</i> metabolic characterization including <i>In silico</i> identification of biosynthetic genetic clusters (BGCs) in strain JC669<sup>T</sup> and <i>A. giovannonii</i> OJF2<sup>T</sup>.

**Phylogenetic analysis**

ContEst16S service of EZBiocloud was used to extract the 16S rRNA gene sequence (1514 nt) of the strain JC669<sup>T</sup> and NCBI- BLAST (Johnson et al. 2008) was used for the analysis of its sequence identity. The full length 16S rRNA gene sequences of all type species of the family Isosphaeraceae, including strain JC669<sup>T</sup> were obtained from EZBioCloud. The sequences were aligned using MUSCLE implemented in MEGA7.0 (Kumar et al. 2016). In a pairwise deletion procedure, the distances were calculated by using the Kimura 2-parameter (Kimura 1980). To finally construct the phylogenetic tree based on 16S rRNA gene sequences, MEGA7 software was used using minimum evolution (ME), Neighbor-joining (NJ), and maximum likelihood (ML) methods having Bootstraps of 1000 replication (Felsenstein 1985). Ninety two core genes were used for the RAxML based phylogenomic tree (Na et al. 2018; Kumar et al. 2021) for which the genome sequences were obtained from publicly available genomes.

**Physiological analysis**

Ten ml of basal medium (Bondoso et al. 2011) in test tubes (25 x 250 mm) was used to test organic substrates and nitrogen sources utilization of strain JC669<sup>T</sup> and <i>A. giovannonii</i> DSM 22561<sup>T</sup> (= OJF2<sup>T</sup>) with slight modifications. Yeast extract (0.05% w/v) was supplemented to the basal medium and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.1% w/v) as nitrogen source. For the organic carbon substrate utilization, various organic carbon substrates (0.5% w/v) were tested for the growth of both strains. Similarly, for nitrogen source utilization, glucose was used as a carbon source and nitrogen sources (0.1% w/v) were tested for their utilization as described earlier by Kaushik et al. (2020). Vitamin B<sub>12</sub> requirement and nitrate reduction (Smibert et al. 1981) of strain JC669<sup>T</sup> and <i>A. giovannonii</i> DSM 22561<sup>T</sup> were tested in liquid media as described by Kaushik et al. (2020). Basal media containing glucose (0.5% w/v) and ammonium sulphate (0.1% w/v) as carbon and nitrogen sources, respectively was used to test NaCl and temperature tolerance of strain JC669<sup>T</sup> and <i>A. giovannonii</i> DSM 22561<sup>T</sup>. API ZYM kit (Biomerieux, France) was used to assay enzymatic activities of strain JC669<sup>T</sup> and <i>A. giovannonii</i> DSM 22561<sup>T</sup> using the manufacturer’s protocol.
Chemotaxonomic characterization

Analysis of fatty acids of strain JC669<sup>T</sup> and <i>A. giovannonii</i> DSM 22561<sup>T</sup> was done from cells which were harvested by centrifugation (8,000 g for 15 min at 4 °C) when cell density reached 70% of the maximum optical density (100% = 0.9 OD<sub>660</sub>). The methylated cellular fatty acids were analysed from the data generated by M/s Royal Research Labs, Secunderabad, India, who performed the analysis according to the Microbial Identification System's instructions (Microbial ID; MIDI 6.0 version; method, RTSBA6; Sasser, 1990). Polar lipids of strain JC669<sup>T</sup> and <i>A. giovannonii</i> DSM 22561<sup>T</sup> were extracted, separated and characterized as described previously by Kates (1972) and Oren et al. (1996). Quinones were extracted and analysed through HPLC as described earlier by Imhoff (1984). Polyamines for strain JC669<sup>T</sup> and <i>A. giovannonii</i> DSM 22561<sup>T</sup> were identified using HPLC after a process of extraction which was previously described (Kumar et al. 2020b).

“Sporotan” staining of fruiting bodies like structures

The protocol used by Senthilnathan et al. (2020) and Ali et al. (2020) was adapted in this study also. Late stationary phase liquid cultures (approximately 4 months old) of strain JC669<sup>T</sup> and <i>A. giovannonii</i> DSM 22561<sup>T</sup> were pellet (8,000 rpm at 4°C for 5 min), washed and re-suspended in PBS buffer (pH 7.8). 100 µl of the pellet was incubated for 20 min at room temperature (~ 26°C) with “sporotan” (6 µl of 4 mM “sporotan” solution in DMSO) and observed for fluorescence using confocal laser microscope (Carl Zeiss LSM900).

Microscopy

Carl Zeiss LSM880 microscope was used for observing cell shape, morphology, size and cell division, Philips XL3O was used for scanning electron microscopic studies and H-7500 Hitachi was used for transmission electron microscopic studies.

Results And Discussion

Accession numbers of nucleotide sequences and 16S rRNA gene BLAST search analysis

The 16S rRNA gene sequence and whole-genome sequence of strain JC669<sup>T</sup> with the accession number LR782133 and JAALJH000000000 are deposited with EMBL and NCBI, respectively. BLAST analysis of both amplicon-based 16S rRNA gene sequence and that extracted from the genome of strain JC669<sup>T</sup> showed the 96.8 % identity with the only species of genus <i>Aquisphaera; Aquisphaera giovannonii</i> (Fig. 2).

Phylogenetic inference

The high POCP and AAI values between strain JC669<sup>T</sup> and <i>A. giovannonii</i> OJF2<sup>T</sup> are 67.3% and 76.6%, respectively. This values (Fig. 2) are within the recommended standards (Luo et al. 2014; Qin et al. 2014) for genera delineation and thus support strain JC669<sup>T</sup> belong to the genus <i>Aquisphaera</i>. OrthoANI and
DDH value between JC669<sup>T</sup> and <i>A. giovannonii</i> OJF2<sup>T</sup> are 81.4% and 24.3 %, respectively which are below the recommended cut off values of 95–96% for ANI and 70% for DDH for species delineation (Chun et al. 2018; Meier et al. 2014) (Fig. 2). Thus, the strain JC669<sup>T</sup> represents a novel species of the genus <i>Aquisphaera</i>.

Further, phylogenetic tree based on 16S rRNA gene sequences (Fig. 3) and RAxML tree based on constructed ninety two core genes (Fig. 4) confirmed the distinct clustering of strain JC669<sup>T</sup> with <i>A. giovannonii</i> OJF2<sup>T</sup>.

**Genomic characteristics**

Based on the NCBI - PGAP, the genome size of strain JC669<sup>T</sup> is 10.04 Mb with N<sub>50</sub> value of 90,612; the genome size of <i>A. giovannonii</i> OJF2<sup>T</sup> is 10.37 Mb. The genome of strain JC669<sup>T</sup> has 7,421 genes of which 7,189 are protein coding genes, 3 rRNA operons, 72 tRNA coding, 154 genes are pseudogenes whereas genome of <i>A. giovannonii</i> OJF2<sup>T</sup> contains a total of 7,692 genes of which 7,415 are protein coding, 73 tRNA genes, 9 rRNA operons and 171 genes are pseudogenes. G + C content of genome of strain JC669<sup>T</sup> is 68.5 mol% which is about 3.2% lower than G + C content (70.8%) of <i>A. giovannonii</i> OJF2<sup>T</sup>. The difference in G + C between the strains indicate that these two are distinct species since a 3% difference in the G + C content was recommended for species delineation.

PATRIC software was used to align the genome sequences to identify the multiple maximal matches and local collinear block (LCBs; www.patricbrc.org) of strain JC669<sup>T</sup> and <i>A. giovannonii</i> OJF2<sup>T</sup>. The results showed significant difference in the alignments of the LCBs in both strains (Fig. S1) which could be due to the shuffling or inversion of genes due to DNA rearrangement or recombination of homologous regions. Majority of proteins of the strain JC669<sup>T</sup> share 60–80% similarity with those of <i>A. giovannonii</i> OJF2<sup>T</sup> (Fig. S2). Both strain JC669<sup>T</sup> and <i>A. giovannonii</i> OJF2<sup>T</sup> share 20–30% similarity with other Isosphaeraceae family members (Fig. S2).

**In-silico metabolic characterization**

<em>In-silico</em> metabolic characterization shows that both strain JC669<sup>T</sup> and <i>A. giovannonii</i> OJF2<sup>T</sup> are able to synthesize isopentenyl pyrophosphate (IPP) through 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP) pathway. These five carbon isoprene units is the precursor for quinones' and carotenoids biosynthesis (Eisenreich et al. 2004; Zhao et al. 2013). In the genomes of strain JC669<sup>T</sup> and <i>A. giovannonii</i> OJF2<sup>T</sup>, major genes encoding for carotenoid biosynthetic enzymes are present like lycopene β-cyclase (5.5.1.19), zeaxanthin epoxidase (1.14.15.21), β-carotene hydroxylase (1.14.13.129) and neoxanthin synthase (5.3.99.9) were present. Thus, both strains are putatively capable of synthesizing neoxanthin from lycopene via intermediates like γ-carotene, β-carotene, zeaxanthin and violaxanthin (Fig. S3). The annotated genome of the strain JC669<sup>T</sup> showed that it has capability for fermentation and aerobic respiration and is well supported by the presence of fermentative enzymes like pyruvate dehydrogenase (EC 1.2.4.1), aldehyde dehydrogenase (EC 1.2.1.3), lactate dehydrogenase (EC
1.1.1.27), and alcohol dehydrogenase (EC 1.1.1.1) and fermentative products could be acetic acid, lactic acid, butanol and acetone.

Annotated genome shows that both strain JC669\textsuperscript{T} and \textit{A. giovannonii} OJF2\textsuperscript{T} contains complete Embden-Meyerhof-Parnas pathway, TCA cycle and both oxidative and non-oxidative pentose phosphate pathways. Sugars are probably not the preferred carbon source for energy and biomass formation since genes encoding for enzymes in gluconeogenesis and glyoxylate shunt were not detected (Table S1). The genome mining also showed genes \textit{nifH}, \textit{nifD}, \textit{nifK} that encode structural components of molybdenum-dependent (Mo-dependent) nitrogenase that help in dinitrogen fixation by converting dinitrogen to ammonia were observed in strain JC669\textsuperscript{T} and absent in \textit{A. giovannonii} OJF2\textsuperscript{T}. Strain JC669\textsuperscript{T} and \textit{A. giovannonii} OJF2\textsuperscript{T} also showed incomplete pathway of nitrification involving conversion of ammonia to nitrate. The gene encoding for methane/ammonia monoxygenase which helps in conversion of ammonia to hydroxylamine an intermediary step in nitrification is absent in both strains. The genome also revealed the presence of alcohol dehydrogenase (NADP+) [EC:1.1.1.2], protocatechuate 3,4-dioxygenase beta subunit [EC:1.13.11.3], gluconolactonase [EC:3.1.1.17], carboxymethylenebutenolidase [EC:3.1.1.45] and muconate cycloisomerase [EC:5.5.1.1] etc which are helpful in degradation of aromatic compounds like caprolactam, chlorocyclohexane, toluene, nitrotoluene and chlorobenzene. Strain JC669\textsuperscript{T} showed beta-Lactam resistance, Bla system pathway and the same is not observed in \textit{A. giovannonii} OJF2\textsuperscript{T}. Strain JC669\textsuperscript{T} showed genes encoding for chitin degradation and showed no genes encoding for N-acetylglucosamine-6-sulfatase and N-sulfoglucosamine sulfohydrolase that helps in degradation of sulphated glycopolymers. This is in line with the experimental analysis showing that N-acetyl glucosamine is not required for growth. The dbCAN metaserver is used for CAZy (Carbohydrate active enzymes) annotation and it predicted that both the strains have a similar genes responsible for encoding carbohydrate-active enzymes (CAZymes). Strains JC669\textsuperscript{T} and \textit{A. giovannonii} OJF2\textsuperscript{T} have large number of carbohydrate binding molecules (CBMs) followed by glycosyl transferases (GTs) and glycoside hydrolases (GHs). The major families in both strains are GH0, GT4, CBM48, GT2, CBM32 and CBM35 and are likely to arranged in clusters (Fig. S4) and also most of these enzymes in both strains are predicted to involve in the extracellular hydrolysis of substrates as they contain N-terminal signal peptides.

Twenty two functional categories of proteins were identified using Clusters of Orthologys (COG) and proteins belonging to categories general functional prediction only (R) nuclear structure (Y) were not observed in both strains. Both strains showed similar pattern and number of COG categories with a few minor differences where majority of proteins belongs to category S (unknown function) followed by energy production and conversion (C) and transport and metabolism (Fig. S5). Both strains are predicted to show resistance against antibiotics like fluoroquinolone and tetracycline as they have genes encoding for the resistance-nodulation-cell division (RND) antibiotic efflux pump. Both strain JC669\textsuperscript{T} and \textit{A. giovannonii} OJF2\textsuperscript{T} have the genes to putatively produce staurosporine (anti-cancerous compound) from L-tryptophan. Planctomycetes, believe to produce bioactive secondary metabolites since they have complex life style (Wiegand et al., 2020). Strains JC669\textsuperscript{T} and \textit{A. giovannonii} OJF2\textsuperscript{T} have putative
biosynthetic genetic clusters (BGCs) for producing secondary metabolites like bacteriocin, Type I Polyketide Synthase (T1PKS), Non-Ribosomal Peptide Synthetase clusters (NRPS), heterocyst-glycolipid synthase-like PKS (hglE-KS), terpenes and indole. Putative genetic cluster of lassopeptide and lanthipeptide is exclusive for strain JC669<sub>T</sub> and <i>A. giovannonii</i> OJF2<sup>T</sup> respectively (Fig. S6).

**Morphological and physiological analysis**

TEM image of the strain JC669<sub>T</sub> shows cytoplasm, nucleoid region, invagination of enlarge periplasm, cytoplasm, nucleoid region with fimbriae in daughter cell only and cell divide by budding (Fig. 5a). In SEM images, cells of strains JC669<sub>T</sub> (Fig. 5b,c) and <i>A. giovannonii</i> DSM 22561<sub>T</sub> (Fig. 5d,e) are spherical to oval-shaped with crateriform structures distributed throughout their surfaces (Fig. 5b,c,d,e). Both strain JC669<sub>T</sub> (Fig. 5b,c) and <i>A. giovannonii</i> DSM 22561<sub>T</sub> (Fig. 5d,e) produce fruiting bodies like structures which are distinct from each other. Free spore-like bodies were also observed (Fig. 5c) which were also stained (Fig. 6a,b) with the spore specific fluorescent stain “sporotan” (Senthilnathan et al., 2020; Ali et al., 2020). Out of curiosity we have done the genome mining of strain JC669<sub>T</sub> and <i>A. giovannonii</i> for genes encoding for putative proteins responsible for forming fruiting bodies/spores. Amino acids sequences of strain JC669<sub>T</sub> and <i>A. giovannonii</i> extracted from the NCBI and P-Blast was performed with the amino acids sequences of various important proteins involved in the formation of endospore as in <i>Firmicutes</i> represented by <i>Bacillus subtilis</i> and exospore as in <i>Actinobacteria</i> represented by <i>Streptomyces coelicolor</i>. Blast analysis in the form of heat maps showed important sporulation proteins in strain JC669<sub>T</sub> and <i>A. giovannonii</i>. However, their percentage of identity is low and mainly in the range of 20–40% (Fig. 7a,b). These findings are similar to the peptidoglycan synthesis and cell division proteins discovered among Planctomycetes members, where the percentage of identity was also low (Jeske et al. 2015; Wiegand et al. 2020). We do not conclude from this study with regard to the fruiting bodies and spores among the two taxa since it requires a more in-depth systematic analysis, however leave with a question of such possibility.

NaCl tolerance (0–7% w/v) for strain JC669<sub>T</sub> and <i>A. giovannonii</i> DSM 22561<sub>T</sub> was tested at 25°C and pH 7. Strain JC669<sub>T</sub> and <i>A. giovannonii</i> DSM 22561<sub>T</sub> didn't grow in the presence of NaCl even at 0.5%. The optimum temperature (5°C-45°C, at 5°C interval) required for cell growth was tested at pH 7. The range of temperature tolerance of the strain JC669<sub>T</sub> is between 10°C and 30°C with maximum growth was observed between 22–25°C (optimum temperature). The optimum temperature for the cell growth of <i>A. giovannonii</i> DSM 22561<sub>T</sub> is 28–30°C and growth was observed between 15°C and 40°C. pH tolerance (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0) for growth of both the strains JC669<sub>T</sub> and <i>A. giovannonii</i> DSM 22561<sub>T</sub> was tested at 25°C in buffered broth, as earlier described by (Bondoso et al. 2015). The optimum pH for cell growth of both strain was pH 7.0 and up to pH 9.0 cells growth was observed in both the strains. Strain JC669<sub>T</sub> and <i>A. giovannonii</i> DSM 22561<sub>T</sub> were unable to reduce nitrate. Vitamin B<sub>12</sub> was not obligate for their cell growth. Cell growth observed with various organic substrates/nitrogen sources of strain JC669<sub>T</sub> and <i>A. giovannonii</i> DSM 22561<sub>T</sub> are given in Table. 1 or in the species description.
Enzymatic activity for strain JC669\textsuperscript{T} and \textit{A. giovannonii} DSM 22561\textsuperscript{T} was performed using API ZYM kit. Strain JC669\textsuperscript{T} gave positive results for alkaline phosphatase, esterase (C4), esterase lipase (C8) and napthol-AS-B1-BD-phosphohydrolase and negative for acid phosphatase, lipase (C8), leucine arylamidase, valine arylamidase, \(\alpha\)-chymotrypsin, cysteine arylamidase, \(\alpha\)-galactosidase, \(\beta\)-galactosidase, \(\beta\)-glucuronidase, \(\alpha\)-glucosidase, \(\beta\)-glucosidase, \(\alpha\)-mannosidase, trypsin, N-acetylmuramoyl-L-\(\alpha\)-glucosaminidase and \(\alpha\)-fucosidase. While strain \textit{A. giovannonii} DSM 22561\textsuperscript{T} shows positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, trypsin, \(\alpha\)-chymotrypsin, leucine arylamidase, acid phosphatase, \(\beta\)-glucosidase, \(\alpha\)-glucosidase, cystine arylamidase and \(\alpha\)-fucosidase and negative for napthol-AS-B1-BD-phosphohydrolase, lipase (C14), \(\beta\)-galactosidase, \(\alpha\)-mannosidase, \(\alpha\)-galactosidase, \(\beta\)-glucuronidase and N-acetylmuramoyl-L-\(\alpha\)-glucosaminidase.

**Chemotaxonomic characterization**

The major fatty acid profile of the strain JC669\textsuperscript{T} was found to be in congruence with the type species of the genus mainly contain C\(_{18:1}\)\(\omega\)9c, C\(_{16:0}\) and C\(_{18:0}\). Anteiso-C\(_{15:0}\), C\(_{14:0}\), C\(_{20:0}\), C\(_{17:1}\)\(\omega\)8c, C\(_{18:1}\)\(\omega\)7c summed in feature 3 are present in both strains. While, iso-C\(_{19:0}\), iso-C\(_{16:0}\), C\(_{19:0}\), C\(_{20:0}\), C\(_{17:1}\)-cyclo, and C\(_{20:1}\)\(\omega\)9c are exclusively present in the strain JC669\textsuperscript{T}. However, C\(_{15:0}\) and C\(_{17:0}\) are exclusive for strain \textit{A. giovannonii} DSM 22561\textsuperscript{T} (Table S2).

The polar lipid profile of strain JC669\textsuperscript{T} (Fig. S7a) was not following type species of \textit{Aquisphaera}, \textit{A. giovannonii} DSM 22561\textsuperscript{T} (both from this study [Fig. S7a, b] and from the literature (Bondoso et al., 2015) using \textit{A. giovannonii} OJF2\textsuperscript{T}). However, the major polar lipids of \textit{A. giovannonii} DSM 22561\textsuperscript{T} in this study (Fig. S7b) are similar to what was reported earlier except for the absence of unidentified phospholipid, unidentified glycolipids, diphasphatidylglycerol and presence of six unidentified lipids (Bondoso et al., 2015). Polar lipids of strain JC669\textsuperscript{T} are phosphatidylcholine (PC), an unidentified amino lipid (AL), unidentified choline lipid (UCL), and six unidentified lipids (UL1-6) (Fig. S7a). For strain \textit{A. giovannonii} DSM 22561\textsuperscript{T} we identified phosphatidylcholine (PC), a phosphatidylglycerol (PG) and six unidentified lipids (Fig. S7b). Strain JC669\textsuperscript{T} differ from \textit{A. giovannonii} DSM 22561\textsuperscript{T} in not having phosphatidylglycerol but additionally having an unidentified aminolipid and an unidentified choline lipid. MK6 is the predominant quinone of both strains. Both strains have two polyamines; sym-homospermidine and putrescine, while \textit{A. giovannonii} DSM 22561\textsuperscript{T} in addition has an unidentified polyamine (Fig. S8).

Proposal of \textit{Aquisphaera insulae} sp. nov.

Strain JC669\textsuperscript{T} resembles the type strain of its phylogenetically closest neighbour regarding having light yellowish orange colonies, major polar lipids and major fatty acids. However, strain JC669\textsuperscript{T} has about 3.2% less in the G + C mol% than \textit{A. giovannonii} OJF2\textsuperscript{T}. The phenotypic differences (Table 1) are supported by the molecular differences (16Ss rRNA phylogenomic analysis) and genomic relatedness.
(gANI, dDDH, AAI) for the description of strain JC669\textsuperscript{T} into a new species of the genus *Aquisphaera* for which we propose the name *Aquisphaera insulae* sp. nov.
| Characteristics                  | 1                     | 2                     |
|---------------------------------|-----------------------|-----------------------|
| Cell shape                      | Spherical to oval     | Spherical             |
| Cell size (diameter µm)         | 1.8–2.2               | 1.6-2.0               |
| Organic substrates used for growth |                       |                       |
| D-xylose                        | +                     | -                     |
| Inositol                        | +                     | -                     |
| Na-succinate                    | +                     | -                     |
| Sorbitol                        | +                     | -                     |
| D-fructose                      | -                     | +                     |
| D-mannitol                      | -                     | +                     |
| Acetate                         | -                     | +                     |
| Pyruvate                        | -                     | +                     |
| Sorbitol                        | -                     | +                     |
| Nitrogen sources used for growth |                       |                       |
| Na-nitrate                      | +                     | -                     |
| L-phenylalanine                 | +                     | -                     |
| L-serine                        | -                     | +                     |
| Aspartate                       | -                     | +                     |
| Cysteine                        | -                     | +                     |
| Serine                          | -                     | +                     |
| Vitamin $B_{12}$ requirement.   | -                     | +                     |
| Polar lipids                    |                       |                       |

Data from this study

Species: 1, strain JC669$^T$; 2, strain *A. giovannonii* OJF2$^T$.

Both strains have quinone as MK6; both strains grow from pH 7–9 with optima at 7.0. Phosphatidylcholine and six unidentified lipids are common for both strains; major fatty acids are $C_{18:1}\omega9c$, $C_{16:0}$ and $C_{18:0}$; glucose supports the growth; does not require vitamin $B_{12}$ for growth and N-acetylglucosamine is not obligate for their growth; PG, phosphatidylglycerol; AL, unidentified amino lipid; UCL, unidentified choline lipids. +, substrate utilized; -, substrate not utilized;
| Characteristics | 1 | 2 |
|-----------------|---|---|
| PG              | - | + |
| AL              | + | - |
| UCL             | + | - |
| Fatty acids     | + | - |
| iso-C₁₉:0       | + | - |
| iso-C₁₆:0       | - | + |
| C₁₅:0           | - | + |
| C₁₇:0           | + | - |
| C₂₀:0           | + | - |
| C₂₀:₁ω₉c        | 68.5 | 70.8 |
| C₁₇:₁-cyclo     |   |   |

Genomic DNA G + C content (mol %)

Data from this study

Species: 1, strain JC669ᵀ; 2, strain *A. giovannonii* OJF2ᵀ.

Both strains have quinone as MK6; both strains grow from pH 7–9 with optima at 7.0. Phosphatidylcholine and six unidentified lipids are common for both strains; major fatty acids are C₁₈:₁ω₉c, C₁₆:₀ and C₁₈:₀; glucose support the growth; does not require vitamin B₁₂ for growth and N-acetylglucosamine is not obligate for their growth; PG, phosphatidylglycerol; AL, unidentified amino lipid; UCL, unidentified choline lipids. +, substrate utilized; -, substrate not utilized;

Description of *Aquisphaera insulae* sp. nov.

(in'su.lae. L. n. *insula*, island; L. gen. n. *insulae*, from an island, referring to the isolation of type strain from floating island of Loktak lake, Manipur, India).

Colonies are light yellowish orange in colour. Cells are Gram-stain-negative, round to oval shaped, non-motile and multiply by budding. Stalks and flagellated cells are not observed. Cells are intolerant to NaCl. Obligate aerobe and mesophilic. Temperature optima at 25°C (range 10–30 °C). Vitamin B₁₂ is not obligate for growth. Utilizes α-D-glucose, lactose, sucrose, D-galactose, mannose, maltose, D-xylose, starch, Na-propionate, inositol, rhamnose, Na-succinate and sorbitol. Fumarate, fructose, ascorbate, acetate, pyruvate, mannitol, malate, inulin, benzoate and citrate do not support growth. D-alanine, L-arginine, L-glycine, L-histidine, L-leucine, L-isoleucine, L-glutamine, L-methionine, DL-ornithine, D-valine, L-proline, L-phenylalanine, DL-threonine, Peptone, NAG, yeast extract, casamino acid and sodium nitrate are
good nitrogen sources for growth. L-serine, L-lysine, L-glutamic acid, L-aspartic acid, L-tyrosine, cysteine, L-tryptophan, L-glycine and urea do not support growth as nitrogen source. API ZYM kit analysis gives positive for alkaline phosphatase, esterase (C4), esterase lipase (C8) and naphthol-AS-B1-BD-phosphohydrolase and negative for acid phosphatase, lipase, leucine arylamidase, valine arylamidase, cysteine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, trypsin and α-fucosidase. Nitrate is not reduced. Major fatty acids are C_{18:1}ω9c, C_{16:0} and C_{18:0}. MK6 is the only respiratory quinone. Phosphatidylcholine, an unidentified amino lipid, unidentified choline lipid and six unidentified lipids make the polar lipid composition. Sym-homospermidine and putrescine are the major polyamines. Genomic DNA G+C content of the type strain is 68.5 mol%. The type strain JC669^{T} (= KCTC 72672^{T} = NBRC 114306^{T}) was isolated from a rhizosphere soil of Zizania latifolia of the floating island from Loktak lake, Manipur, India.

16S rRNA gene sequence GenBank accession number is LR782133. Genome of strain JC669^{T} has been deposited in GenBank under the accession numbers JAALJH0000000000. The version described in this paper is version JAALJH010000000.

Proposal to emend the genus description of "Aquisphaera"

The genus "Aquisphaera" is less understood as it contains single species ("A. giovannonii OJF2^{T}") which is described so far. The original description of this genus was based mostly on the morphological, chemotaxonomic and genomic features (Bondoso et al., 2011). We propose to broaden the genus characters of "Aquisphaera" by emending the genus description.

Emended description of the genus "Aquisphaera"

Colonies are yellowish-orange in colour. Cells have well-distributed crateriform structures on the cell surface. The genomic size varies from 10.04–10.37 Mb. DNA G+C content varies from 68.5 mol% to 70.8 mol%. Sym-homospermidine and putrescine, are the major polyamines. The major polar lipids are diphosphatidylglycerol and phosphatidylcholine. Some strains contain unidentified choline lipids and unidentified amino lipids.

Declarations

Funding Information

This work received specific grant from Department of Biotechnology, Government of India, with sanction No. BT/HRD/35/01/02/2015. Financial support received from Council for Scientific and Industrial Research (CSIR), New Delhi is acknowledged. Infrastructure facilities funded by DST-FIST, UGC-SAP (DRS), TEQIP and AICTE are acknowledged.

Acknowledgements
Gaurav and Ramana thank DBT, New Delhi for the award of SRF and TATA innovation fellowship, respectively. Sasikala, Dhanesh, Shabbir and Jagadeeshwari thank UGC for Mid-career award, RA, SRF and TEQIP awards for JRF respectively. We also thanks Mr. Bernhard Schink for providing species name and etymology for strain JC669T. Prof. T. Radhakrishnan and Dr. Senthilnathan Department of Chemistry are thanked for providing “Sporotan”.

**Author contributions**

KL performed sample collection, KG performed media optimization and polar lipid analysis, KG and KL isolated the strain, performed the initial cultivation, strain deposition and strain characterization; KG and DK performed the electron microscopic analysis; JU performed the genomic and phylogenetic analysis; SA performed and analyzed the data for polyamines; GMK and KG performed genome mining for genes related to sporulation; KG and KL 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.

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

**Ethical statement:** Not applicable

**References**

1. Ali A, Gaurav K, Senthilnathan N, Radhakrishnan T P, Ch Sasikala and Ramana CV (2020) “Sporotan” a new fluorescent stain for identifying cryptic spores of *Rhodobacter johnii*. J Microbiol Methods 177: 106019. doi:10.1016/j.mimet.2020.106019

2. Auch AF, Klenk HP, and Göker M (2010) Standard operating procedure for calculating genome-to-genome distances based on high-scoring segment pairs. Stand Genomic Sc2: 142-148. doi:10.4056/sigs.541628

3. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T et al. (2008) The RAST server:rapid annotations using subsystems technology. BMC Genomics9:75. doi:10.1186/1471-2164-9-75

4. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, and Weber T (2019) AntiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res47(W1):W81-W87. doi: 10.1093/nar/gkz310

5. Bondoso J, Albuquerque L, Lobo-da-Cunha A, da Costa MS, Harder J, and Lage OM (2014) *Rhodopirellula lusitana* nov. and *Rhodopirellula rubra* sp. nov., isolated from the surface of macroalgae. Sys Appl Microbiol 37:157-164. doi:10.1016/j.syapm.2013.11.004

6. Bondoso J, Albuquerque L, Nobre MF, Lobo-da-Cunha A, da Costa, MS., and Lage, O.M. (2011). *Aquisphaera giovannonii* nov., sp. nov., a planctomycete isolated from a freshwater aquarium. Int J Syst Evol Microbiol61: 2844-2850. doi: 10.1099/ijs.0.027474-0
7. Bondoso J, Albuquerque L, Nobre MF, Lobo-da-Cunha A, da Costa M S, and Lage OM (2015) *Roseimaritima ulvae* nov., sp. nov. and *Rubripirellula obstinata* gen. nov., sp. nov. two novel planctomycetes isolated from the epiphytic community of macroalgae. Syst. Appl. Microbiol. 38: 8-15. doi:10.1016/j.syapm.2014.10.004

8. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, Meyer SD, and Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Sys Evol Microbiol 68: 461-466. doi:10.1099/ijsem.0.002516

9. Darzi Y, Letunic I, Bork P, and Yamada T (2018) iPath3.0: interactive pathways explorer v3. *Nucleic Acid. Res* 46: W510-W513. doi:10.1093/nar/gky299

10. Eisenreich W, Bacher A, Arigoni D, and Rohdich F (2004) Biosynthesis of isoprenoids via the non-mevalonate pathway. Cell Mo Life Sci 61:1401–1426. doi. 10.1007/s00018-004-3381-z

11. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791. doi:10.1111/j.1558-5646.1985.tb00420

12. Graca AP, Calisto R, Lage OM (2016) Planctomycetes as novel source of bioactive molecules. Front Microbiol 7:1241. doi.org/10.3389/fmicb.2016.01241

13. Imhoff JF (1984) Quinones of phototrophic purple bacteria. *Microbiol Lett* 25: 85- 89. doi:10.1111/j.1574-6968.1984.tb01381.x

14. Jeske O, Schüler M, Schumann P, Schneider A, Boedeker C, Jogler M, and Spring S (2015) Planctomycetes do possess a peptidoglycan cell wall. *Nat Commun* 6: 7116-7123. doi:10.1038/ncomms8116

15. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis, S, and Madden TL (2008) NCBI BLAST: a better web interface. *Nucleic Acids Res* 36:W5-W9. doi:10.1093/nar/gkn201

16. Kates M (1972) Isolation, analysis and identification of lipids. Techniques in Lipidology 268-618.

17. Kaushik R, Sharma M, Gaurav K, Jagadeeswari U, Shabbir A, Sasikala C, Ramana CV, and Pandit MK (2020) *Paludisphaera soli* nov., a new member of the family Isosphaeraceae isolated from high altitude soil in the Western Himalaya. Antonie van Leeuwenhoek 113: 1663-1674. doi:10.1007/s10482-020-01471

18. Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111-120.

19. Köhler T, Stingl U, Meuse K, and Brune A (2008) Novel lineages of Planctomycetes densely colonize the alkaline gut of soil feeding termites (*Cubitermes*). *Environ Microbiol* 10: 1260-1270. doi:10.1111/j.1462-2920.2007.01540

20. Kumar D, Gaurav K, Jagadeshwari U, Sasikala Ch, and Ramana ChV (2020a) *Roseimaritima sediminicola* nov., a new member of Planctomycetaceae isolated from Chilika lagoon. Int J Syst. Evol Microbiol.70: 2616-2623. doi:10.1099/ijsem.0.004076

21. Kumar D, Gaurav K, Sreya PK, Shabbir A, Jajadeshwari U, Sasikala Ch, and Ramana ChV (2020b) *Gimesia chilikensis* nov., a halotolerant planctomycete isolated from Chilika lagoon and
emended description of the genus Gimesia. Int J Syst Evol Microbiol 70: 3647-3655. doi:10.1099/ijsem.0.004211

22. Kumar D, Kumar G, Uppada J, Ahmed S, Sasikala C, and Ramana C V (2020c) Descriptions of *Roseiconus nitratireducens* nov. sp. nov. and *Roseiconus lacunae* sp. nov. Arch Microbiol 203: 741-754. doi:10.1007/s00203-020-02078-5

23. Kumar D, Kumar G, Jagadeeshwari U, Sasikala C, and Ramana C V (2021a) “Candidatus Laterigemmans baculatus” gen. nov. sp. nov., the first representative of rod shaped planctomycetes with lateral budding in the family Pirellulaceae. Syst Appl Microbiol 126188 doi:10.1016/j.syapm.2021.126188

24. Kumar G, Kumar D, Jagadeeshwari U, Sreya P K, Shabbir A, Sasikala C, and Ramana C V (2021b) *Crateriforma spongiae* nov., isolated from a marine sponge and emended description of the genus “*Crateriforma*”. Antonie van Leeuwenhoek. 1-13. doi:10.1007/s10482-020-01515-1

25. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870-1874. doi:10.1093/molbev/msw054

26. Lage OM, Albuquerque L, Lobo-da Cunha A, & da Costa M S (2017) *Mariniblastus fucicola* nov., sp. nov. a novel planctomycete associated with macroalgae. Int J Sys Evol. Microbiol 67: 1571-1576. doi:10.1099/ijsem.0.001760

27. Luo C, Rodriguez-R LM, and Konstantinidi KT (2014) MyTaxa: an advanced taxonomic classifier for genomic and metagenomic sequences. Nucl Acids Res42: e73. doi:10.1093/nar/gku169

28. Meier-Kolthoff JP, Klenk HP, and Göker M (2014) Taxonomic use of DNA G+ C content and DNA–DNA hybridization in the genomic age. Int J Syst Evol Microbiol 64: 352-356. doi:10.1099/ijs.0.056994-0

29. Na SI, Kim YO, Yoon SH, Ha SM, Baek I, and Chun J (2018) UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56:280-285. doi:10.1007/s12275-018-8014-6

30. Oren A, Duker S, and Ritter S (1996) The polar lipid composition of Walsby's square bacterium. FEMS Microbiol Lett 138:135-140. doi:10.1111/j.1574-6968.1996.tb08146

31. Peeters SH, Wiegand S, Kallscheuer N, Jogler M, Heuer A, Jetten MS, and Jogler C (2020) *Lignipirellula cremea* nov., sp. nov., a planctomycete isolated from wood particles in a brackish river estuary. Antonie van Leeuwenhoek 113:1863–1875 doi:10.1007/s10482-020-01407-4

32. Puranik S, Pal RR, More RP, and Purohit HJ (2016) Metagenomic approach to characterize soil microbial diversity of Phumdi at Loktak Lake. Water Sci Technol 74: 2075-2086. doi:10.2166/wst.2016.370

33. Qin Q L, Xie BB, Zhang XY, Chen XL, Zhou BC, Zhou J, and Zhang YZ (2014) A proposed genus boundary for the prokaryotes based on genomic insights. J Bacteriol 196: 2210-2215. doi: 10.1128/JB.01688-14

34. Reddy HR, Katti RJ, Raveesha KP, Vikas SJ, Babu RN, and Kumar KS (2005) Habitat heterogeneity of the Loktak lake, Manipur. Current Science 88:1027.
35. Rensink S, Wiegand S, Kallscheuer N, Rast P, Peeters S H, Heuer A, and Jogler C (2020) Description of the novel planctomycetal genus *Bremerella*, containing *Bremerellavolcania* nov., isolated from an active volcanic site, and reclassification of *Blastopirellula cremea* as *Bremerella cremea* comb. nov. Antonie van Leeuwenhoek 113: 1823–1837. doi:10.1007/s10482-019-01378-1

36. Rodriguez RLM, and Konstantinidis KT (2014) Bypassing cultivation to identify bacterial species. Microbe 9: 111-118.

37. Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids.

38. Senthilnathan N, Kumar G, Raman, CV, and Radhakrishnan TP, (2020) Zwitterionic small molecule-basedfluorophores for efficient and selective imaging of bacterial endospores. J Mater Chem B 8:4601–4608. doi:10.1039/d0tb00470g

39. Smibert RM, and Krieg NR (1981) General characterization. In: Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, et al. (eds) Manual of methods for general microbiology, vol 24. American Society for Microbiology Washington, pp 409–443

40. Stackebrandt E, Liesack W, and Goebel BM (1993) Bacterial diversity in a soil sample from a subtropical Australian environment as determined by 16S rDNA analysis. FASEB J doi:10.1096/fasebj.7.1.8422969

41. Wagner M, and Horn M (2006) The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr Opin Biotechnol 17: 241–249. doi:10.1016/j.copbio.2006.05.005

42. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, and Bun C (2017) Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. Nucleic Acids Res 45: 535-542. doi:10.1093/nar/gkw1017

43. Wick RR, Judd LM, Gorrie CL, and Holt KE (2017) Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS* computational biology. 13, e1005595. doi:10.1371/journal.pcbi.1005595

44. Wiegand S, Jogler M, Boedecker C, Pinto D, Vollmers J, Rivas-Marín E, and Oberbeckmann S et al. (2020) Cultivation and functional characterization of 79 Planctomycetes uncovers their unique biology. Nature Microbiol 5: 126-140. doi:10.1038/s41564-019-0588-1

45. Wiegand S, Jogler M, and Jogler C (2018) On the maverick Planctomycetes. FEMS Microbiol Rev 42: 739–760. doi:10.1093/femsre/fuy029

46. Yoon J, Matsuo Y, Kasai H, and Lee MK (2014) Phylogenetic and taxonomic analyses of *Rhodopirellula caenicola* nov., a new marine Planctomycetes species isolated from iron sand. J Phylogenetics Evol Biol 3: 143.

47. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H et al. (2017) Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613-1617. doi:10.1099%2Fijsem.0.001755

48. Zhao L, Chang WC, Xiao Y, Liu HW, and Liu P (2013) Methylerythritol phosphate pathway of isoprenoid biosynthesis. Annu Rev Biochem 82:497–530. doi: 10.1146/annurev-biochem-052010
Figures

Figure 1

Detail of the sampling site showing floating island.
Figure 2

Phylogenetic markers using various methods (16S rRNA gene identity, AAI, POCP, ANI and dDDH) to analyse the delineation of the novel isolate JC669T along with Aquisphaera giovannonii OJF2T.

Figure 3

Phylogenetic relationship of strain JC669T with the A. giovannonii OJF2T and other closely related species within the family Isosphaeraceae on the basis of 16S rRNA gene sequences. The phylogenetic tree was made with MEGA 7 software and Gimesia maris DSM8797T was used as out-group. In parentheses the GenBank accession numbers for 16S rRNA gene sequences are shown. Bootstrap values
are shown at the nodes corresponding to NJ/ML/ME analysis. Bar, 0.02 nucleotide substitution per position.

Figure 4

RAxML based phylogenomics tree of strain JC669T along with publicly available genome sequence of Isosphaeraceae families and out-group used is of Gimesia maris DSM8797T. In parentheses the GenBank accession numbers of genome sequences are shown. Bar, 0.05 nucleotide substitution per position.
Figure 5

Transmission (a) and scanning (b,c,d,e) electron micrographs of cell of Strains JC669T and A. giovannonii OJF2T. (a) Ultrathin section showing cell membrane (cm), cytoplasm (cy), enlarged periplasm (ep), nucleoid (nu), cell multiplication through budding (bd), fimbriae in daughter cell and crateriform like structure (cr) in the strain JC669T. Bars, 0.2μm. (b) Isolated round cells of the strain JC669T showing crateriform like structure (cr) and fruiting bodies like structures (fb) attached to mother cells (mc). Bars, 1μm. (c) Free spore like structures (sp) detach from mother cells (mc) of the strain JC669T. Bars, 2μm. (d,e) Round cells of the A. giovannonii DSM 22561T showing crateriform like structure (cr) and fruiting bodies like structures (fb) attached to mother cells (mc). Bars, 1μm.
Figure 6

LSCM images: Sporotan stained fruiting bodies like structures and unstained mother cells of *A. giovannoni* DSM 22561^T^ (a) and the strain JC669^T^ (b). Green arrows show fruiting bodies like structures; Red arrows show mother cells.

Figure 7
Heat map representing the Percentage identity of some sporulating proteins in the strains JC669T and OJF2T with the Bacillus subtilis (a) and Streptomyces coelicolor (b).