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ARTICLE

Planctopirus hydrillae sp. nov., an antibiotic producing *Planctomycete* isolated from the aquatic plant *Hydrilla* and its whole genome shotgun sequence analysis

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Abstract

An antibiotic producing novel *Planctomycete* strain, designated JC280^T, was isolated from the surface of the plant *Hydrilla verticillata* collected from an alkaline lake (Buffalo lake), University of Hyderabad, Hyderabad, India. The morphological and chemotaxonomic properties of strain JC280^T were in agreement with the characteristics of the genus *Planctopirus*. The cell shape was spherical to ovoid and some were tear drop shaped. The cells were Gram-stain-negative divided by budding presenting stalks and rosette formation and were non-sporulating. Crateriform structures with a sub-polar flagellum were observed. Characteristic polyamines were putrescine and spermidine. Diagnostic polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, an unidentified phospholipid (PL1), unidentified glycolipids (GL1-2), an unidentified aminophospholipid (APL), and an unidentified lipid (L3). Major (>10%) fatty acids were $C_{16:0}$, $C_{17:1}\omega8c$, $C_{18:1}\omega9c$, and summed feature-3. Major (88%) respiratory quinone was MK-6 with minor amount (12%) of MK-7. Strain JC280^T showed 99.7% 16S rRNA gene sequence similarity with *Planctopirus limnophila* DSM 3776^T. To resolve their full taxonomic position, the genome sequence was obtained and compared with the available *P. limnophila* DSM 3776^T genome. The genome sequence of strain JC280^T was 5,750,243 bp in size with a total of 4490 protein-coding genes, 66 RNA genes, and 2 CRISPR repeats. Based on whole-genome statistics, ANI value, in silico DDH, diversity of secondary metabolite biosynthetic gene clusters, distinct physiological, biochemical and chemotaxonomic differences, strain JC280^T represents a new species in the genus *Planctopirus*, for which the name *Planctopirus hydrillae* sp. nov. is proposed. The type strain is JC280^T (=KCTC 42880^T = LMG 29153^T).

Introduction

Planctomyces limnophilus belonging to the family *Planctomycetaceae* was described by Hirsch and Muller [1] and later reclassified as a novel genus, *Planctopirus limnophila*, by Scheuner et al. [2] in 2014. The genus *Planctopirus* encompasses aerobic, Gram-stain-negative, spherical to ovoid shaped non-sporulating cells with a sub-polar flagellum [1, 3]. They also have stalks forming rosette [1, 3]. Characteristic polyamines are putrescine and spermidine [2]. *Planctopirus* forms complex compartmentalization in the cell and have life cycle with two phases A and B. The sessile mother cells (phase A) at reproductive cell pole produce a small mirror image of the mother cell, those are swarmer (daughter buds) cells. These free-living daughter cell forms are transient while the adult cells are sessile [4]. At the time of manuscript preparation, there was only one

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species present in the genus *Planctopirus*: *Planctopirus limnophila* (http://www.bacterio.net/planctopirus.html).

Recently we have started exploring previously uncultivated and unexplored prokaryotes with the prospect that they might produce novel secondary metabolites with new scaffolds and for potential bacterial enzymes which will have pharmaceutical applications. Hence, we have selected one of the much unexplored phylum, *Planctomycetes* for the prospective of novel secondary metabolites. *Planctomycetes* become attractive to work because of their distinct structural and morphological features. Till date, there are no reports available on the nature of secondary metabolites produced by *Planctomycetes* to the best of our knowledge. Fortunately, we have partially achieved the goal by isolating a novel strain JC280^T, having prominent antimicrobial activity against pathogens and it belongs to the genus *Planctopirus*. Through this communication, we propose the novel taxonomic status of JC280^T based on the phenotypic characters and its genome analysis.

Materials and methods

Isolation of bacterial strain

Strain JC280^T was isolated from the surface of an aquatic plant, *Hydrilla verticillata*, collected from a slightly alkaline lake (pH 8.5) situated in the campus of the University of Hyderabad, India (17° 27′ 14″ N 78° 19′ 40.27″ E), during April, 2014. For enrichment of members of *Planctomycetes*, a few stems with leaves of the aquatic plant were introduced into a PYGV broth (ATCC medium 1521 [5]) containing a mixture of antibiotics (penicillin-G, ampicillin, and cycloheximide at a final concentration of 1 mg ml^{-1}) and incubated at 25 °C for 10 days. Enrichment was monitored daily by observing under phase contrast microscope for cells with typical oval to spherical cell shape with budding. Such cells were purified on the above media which was solidified using gellan gum (18 g l^{-1}; Himedia Lab, Mumbai, India) and the purified culture was designated as strain JC280^T.

Morphological observations

Morphological properties such as cell shape, cell size, and motility were observed by phase contrast light microscopy (Olympus BH-2). Morphology of cells was observed using a field emission scanning electron microscopy (FE-SEM, JSM-6500F, JEOL Co., Japan). Specimens were prepared as previously described [6]. The processed samples were mounted according to our previous study [7]. Transmission electron microscope (TEM, FEI Model Tecnai G2 S (200 kV) micrographs of JC280^T cells were taken after negative staining with aqueous 2% uranyl acetate (direct method [6]). Flagella and surface morphology was observed under TEM at various magnifications. Thin sections of strain JC280^T were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4 °C, and washed with phosphate buffer saline (PBS) two times each for 45 min, then post fixed in 1% aqueous Osmium Tetraoxide for 2 h, later washed with deionized distilled water four times, each for 45 min, dehydrated in series of graded alcohols, infiltrated and embedded in araldite 6005 resin [8]. For complete polymerization, the sections were incubated at 80 °C for 48 h. Ultra-thin (60 nm) sections were made with a glass knife on ultra-microtome (Leica Ultra cut UCT-A-D/E-1/00), mounted on copper grids, and stained with saturated aqueous uranyl acetate (UA) and counter stained with Reynolds lead citrate (LC) [8]. Ultra-thin sections were subsequently analyzed by using FEI Model Tecnai G2 S-200 kV TEM.

Cultural, physiological, biochemical, and chemotaxonomic analyses

Salinity 0–1.5% (w/v) and temperature (4, 10, 15, 20, 25, 30, 35, 40 45, 50 °C) ranges for growth were examined on PYGV medium [5] made up of gellan gum (18 g/l). pH tolerance range was examined by using buffered medium as described previously [9, 10]. Assimilation of carbon and nitrogen sources, oxidative and fermentative utilization of carbohydrates [11], and vitamin supplement requirement [5] were tested using PYGV medium as described by Hirsch et al. [1] and Staley [5]. Utilization of organic substrates (aspartate, pyruvate, fumarate, malate, glycerol, acetate, benzoate, ethanol, methanol, and succinate) in the presence of sulfate was tested as described previously [1, 5]. Various biochemical tests (starch/gelatin, indole production, oxidase and catalase activity) were carried out in the prescribed media to meet the requirements of the standard methods as described previously [9–11]. Fatty acids, polar lipids, carotenoids, quinones, hopanoids, and polyamines [12–14] were analyzed from cells grown till late exponential phase of growth as described previously [9, 10].

Phylogenetic analyses

A well isolated colony was used for 16S rRNA gene amplification and sequencing by using universal primers [9]. Sequencing was done by using 3730XL automated DNA sequencing system (Applied Biosystems) at Sci-Genom Labs, Cochin, India (http://www.scigenom.com). Calculation of pair wise 16S rRNA gene sequence similarity was done on the EzBioCloud [15] (http://www.ezbiocloud.net). Phylogenetic analysis was performed using 16S rRNA gene sequences of the closely related members of the family *Planctomycetaceae* using MEGA7 software [16]. For

Table 1 Morphological, physiological, and biochemical characters differentiating strain JC280^T from *Planctopirus limnophila* DSM 3776^T

Characteristics	Strain JC280^T	*P. limnophila* DSM 3776^T
Colony color	Dark pink	Pink to Red
Soluble pigment	Pink	Red
Reverse side pigment	Pale orange	Pink
Cell size (diameter; μm)	1.0–1.4	1.2–1.5
NaCl range (optimum)	0–1.5 (0–0.5)	0–0.5 (0)
pH range (optimum)	6.0–9.0 (6.5–7.5)	6.0–8.0 (6.5–7.0)
Temperature range (optimum) °C	10–45 (20–30)	4–50 (30)
Hydrolysis of		
Casein	+	–
Starch	+	–
Gelatin	–	+
Reduction of nitrate	–	+
Growth seen on c hitin medium (assimilation)	–	+
Carbon source utilized for growth (0.1% w/v)		
Cellobiose	–	+
Maltose	–	+
Sucrose	+	–
Glucuronic acid	+	–
D-Fructose	+	–
Mannitol	+	–
Glycerol (0.186%)	+	–
Nitrogen source utilized for growth (0.1% w/v)		
L-Arginine	+	–
L-Aspartate	+	–
DL-Alanine	+	–
DL-Phenylalanine	+	–
L-Serine	+	–
L-Typtophan	+	–
Aerobic acid formation from		
D-Fructose	+	–
D-Galactose	+	–
D-glucose	+	–
Anaerobic acid formation from		
D-fructose	+	–
Mannitol	+	–
Maltose	–	+
Galactose	–	+
Peroxidase	+	–
Lysine decarboxylase	+	–
Arginine decarboxylase	+	–
Formation of acetoin	–	+
Formation of H_2S	–	+
Vitamin supplements	+	–

Data presented is the comparative analysis done at authors' laboratory under identical growth/assay conditions. Characters common to both strains: adenine, chitin, and elastine are not hydrolyzed; growth occurs on D-glucose, D-galactose, and *N*-acetylglucosamine. Organic substrates which are not utilized for growth as carbon source include: D-ribose, starch, dextrin, inulin, salicin, pyruvate, α-oxoglutarate, succinate, fumarate, formate, foramide, methanol, ethanol, lactate, acetate, propionate, tartarate, glutamate, caproate, phthalate, citrate, and malate; Nitrogen sources not utilized: α-glutamate, L-glycine, L-histidine, L-leucine, L-proline, methylamine HCl, urea, nitrate, nitrite, and dinitrogen; Acid is not produced from saccharose, maltose, mannitol; Gas is not produced in Hugh–Leigson medium; indole is positive; Negative for urease, lysine deaminase, and phenylalanine deaminase; Positive for catalase and oxidase; Putrescine and spermidine are the polyamines

– Not utilized/negative/absent, + utilized/positive/present

constructing neighbor-joining [17] (NJ), maximum-likelihood [18] (ML), and minimum-evolution [19] (ME) phylogenetic trees, the following statistical methods were used. For NJ, Kimura two-parameter model [20], uniform rates, pair wise deletion was used. For ML and ME, the Kimura two-parameter model with uniform rates and the heuristic search algorithm nearest-neighbor-interchange (NNI) with complete deletion was used. The obtained tree topologies were evaluated using a bootstrap analysis [21]. Accession numbers for sequences used as references are indicated in the phylogenetic tree.

Genome analyses

Genomic DNA was isolated [22] and the mol% G+C content of the DNA was determined by reverse-phase HPLC [23]. In addition to the physiological, morphological, and chemotaxonomic analysis, genome level comparison was done by genome sequencing of strain JC280^T. Sequencing of the genome was performed at the SciGenom (Cochin, Kerala, India), using the Illumina HiSeq 2500 (Illumina Inc.) platform. Assembly of the raw sequencing data was performed using ABySS. Annotation of the assembled data was performed using the Rapid Annotation Using Subsystem Technology (RAST) server (http://rast.nmpdr.org/rast.cgi). Determination of average nucleotide identity [24] (ANI) and in silico DNA–DNA hybridization [25] (DDH) was performed by using the Kostas lab server (http://enve-omics.ce.gatech.edu/ani) and genome-to-genome distance calculator (GGDC) (http://ggdc.dsmz.de), respectively. Phylogenetic trees based on concatenated sequences of various housekeeping genes are known to be characterized by high robustness [26, 27]. Sequences of *recA*,

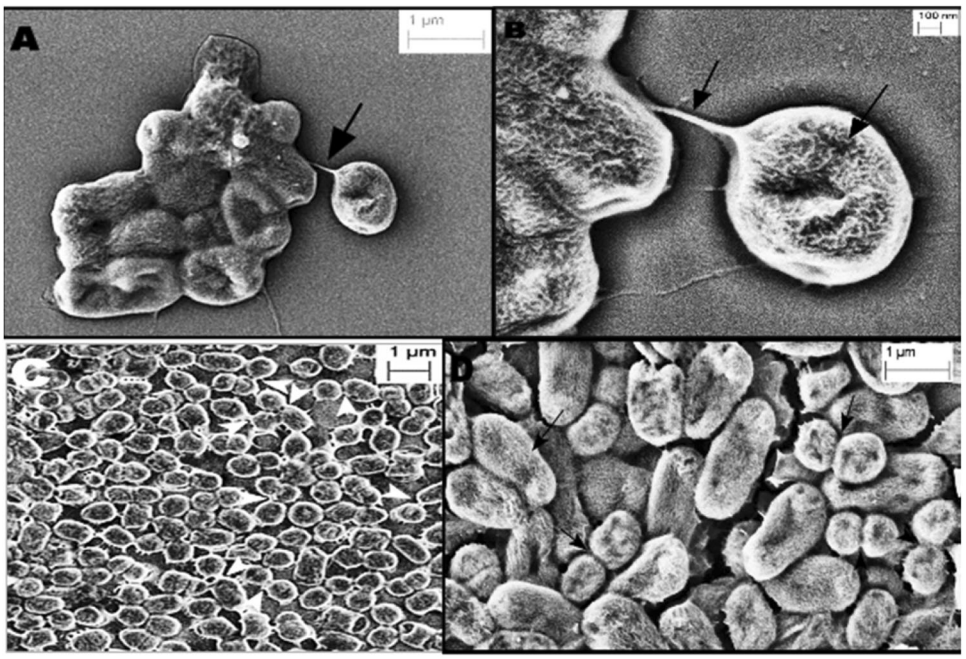

Fig. 1 Scanning electron microscopy photographs of strain JC280^T highlighting aggregate formation (**a**), tube-like stalk and crateriforms are on the cell surface (**b**), budding cells can be visualized in **c**, **d**

rpoA, *rpoB*, *fusA*, *gyrA*, and *gyrB* of most closely related members of *Planctomycetes* were extracted from the available genomes and the NJ tree was constructed using MEGA7 software (http://www.megasoftware.net/). Secondary metabolite biosynthetic gene clusters were analyzed by using antiSMASH v.3.5.0[28] and v.4.0.2[29] (https://antismash.secondarymetabolites.org/). Graphical circular map was prepared by using CGView server [30] (http://stothard.afns.ualberta.ca/cgview_server/index.html).

Results

Cultural, morphological, and physiological characteristics

Detailed cultural, morphological, and physiological characters of strain JC280^T and its closely related type strain *P. limnophila* DSM 3776^T are described in Table 1. Strain JC280^T formed dark pink-colored colonies. Cells were Gram-stain-negative, 1.0–1.4 µm in diameter, spherical to ovoid shaped forming aggregates divided by budding and crateriforms were seen on the cell surface (Fig. 1 a–d, Fig. 2b). Thin tube-like stalks were seen on aggregated cells. Cells of strain JC280^T have monotrichous and sub-polar flagella (fl) (Fig. 2a). Thin sections (60 nm) of strain JC280^T displayed a condensed nucleoid (Fig. 3a) and showed exceptional patterns of cytoplasmic invaginations (Fig. 3a–c). Cells of strain JC280^T divide through budding and contain holdfast (Fig. 4a, b), mother and daughter cells were connected by a tubular structure (Ts) (Fig. 4c, d).

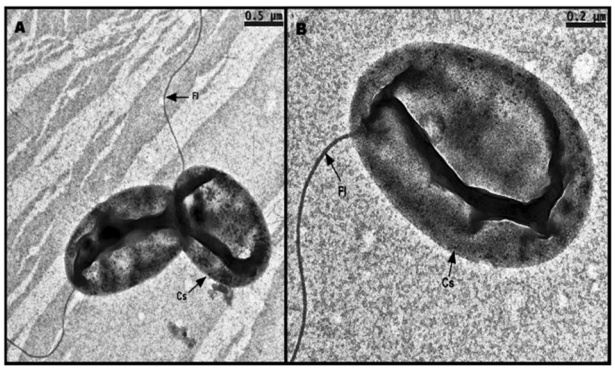

Fig.e 2 Transmission electron microscopic observation revealed monotrichous sub-polar flagella (fl) and crateriforms on the cell surface (**a**, **b**)

Visible colonies of strain JC280^T appeared after 3 days of incubation at optimal growth conditions while *P. limnophila* DSM 3776^T took 5 days. Growth of strain JC280^T occurred from pH 6.0–9.0 and optimum pH was 6.5–7.5. Strain JC280^T had a doubling time of 5 h when grown at 25 °C and at pH 7.0. Strain JC280^T grew faster than *P. limnophila* DSM 3776^T, which has a doubling time of 8 h. Maximum growth was recorded when *N*-acetylglucosamine was supplemented to the media.

Chemotaxonomic characteristics

Chemotaxonomic details are given in Table 2. MK-6 was the major (88%) respiratory quinone of the strain JC280^T with MK-7 as minor (12%) quinone. Strains JC280^T and *P. limnophila* DSM 3776^T contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, unidentified

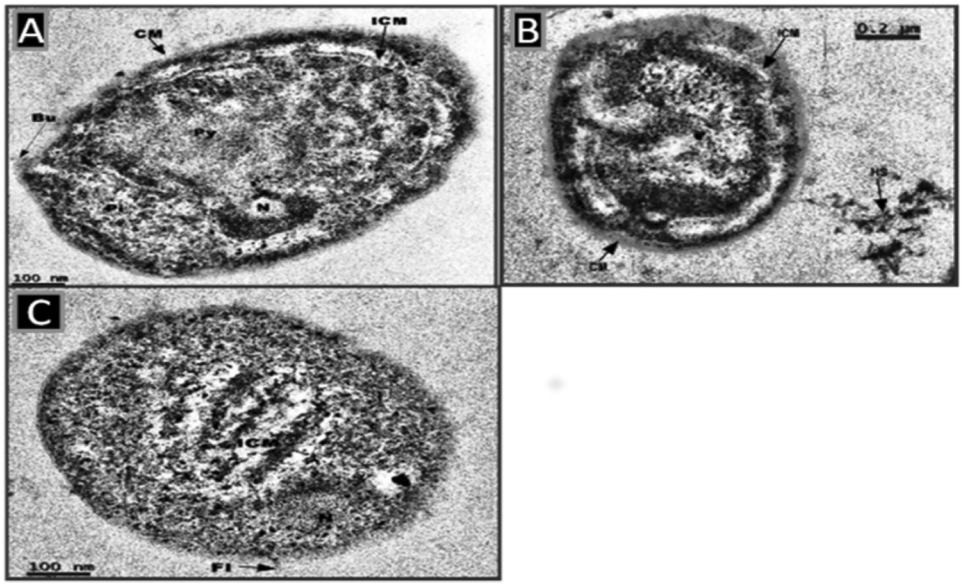

Fig. 3 Thin sections of strain JC280^T cells revealed different types of invaginations of the intracytoplasmic membrane (ICM) which divides the cytoplasm into a paryphoplasm (Py) and a pirellulosome (Pi), the nucleoid (N) is not covered by a further membrane but is always condensed while the size and organization of Py, Pi, and N differ between individual cells (**a**, **b**, **c**). CM cell membrane, Bu budding nick (just started), Fl flagella (broken), Hs holdfast substance

glycolipids (GL1-2), unidentified phospholipid (PL1), and unidentified lipids (L3). Strain JC280^T differs from *P. limnophila* DSM3776^T in polar lipids by the absence of unidentified phospholipid (PL2, 3) and unidentified lipids (L1, 2) (Figure S1). Both strains JC280^T and *P. limnophila* DSM 3776^T were pigmented and contained carotenoids. The absorption spectra and the retention time for the peaks observed in HPLC (Figure S2) does not match with the known carotenoids in the lipid bank (www.lipidbank.jp) and thus we were unable to identify the carotenoids in this study. Strain JC280^T differs from *P. limnophila* DSM3776^T in their hopanoids by the absence hop-22-ene, methylhop-21-ene, 2-methylhop-21-ene, and bacteriohopaneterol acetate. Unidentified hopanoids 5, 19, and 21 were present in both strains (Table 1). Cell wall polyamines putrescine and spermidine were present in both strains. Major (>10%) fatty acids of both strains included $C_{16:0}$, $C_{17:1}\omega8c$, $C_{18:1}\omega9c$, and summed feature-3. Minor (>1 but <10%) fatty acids included $C_{12:0}$, $C_{14:0}$, $C_{17:0}$, $C_{16:0}2OH$, $C_{15:1}\omega6c$, $C_{20:1}\omega9c$, summed feature-6, and summed feature-8. Strain JC280^T share major (>10%) fatty acids common with *P. limnophila* DSSM3776^T, however differ by the presence of $C_{12:0}$, $C_{14:0}$, $C_{16:0}2OH$ and absence of summed feature-7 (Table 2).

Phylogenetic analysis

EzBioCloud based BLAST search analysis of 16S rRNA gene sequence of strain JC280^T (1530 nt) showed 99.7% similarity with *P. limnophila* DSM 3776^T and <85.2% with other members of the family *Planctomycetaceae*. Phylogenetic trees based on NJ, ML, and ME confirm the clustering of strain JC280^T with *P. limnophila* DSM 3776^T and other members of the family *Planctomycetaceae* (Fig. 5). With the Gblock edited concatenated sequences (13 kb) of six housekeeping genes (*rpoA*, *rpoB, gyrA, gyrB, fusA*, and *recA*), strain JC280^T showed highest similarity with *P. limnophila* DSM 3776^T (80%) and other members of the family *Planctomycetaceae*. This indicated that strain JC280^T satisfied the threshold criterion (10% nucleotide substitution rate) of sequence diversity as distinct species and shares a common ancestor with members of the family *Planctomycetaceae* (Supplementary information, Figure S4).

Genome sequencing, assembly, and annotation

The calculated G+C mol% from the genome sequence of strain JC280^T was 53.8% which was very close to experimentally deduced value of 54.2% determined using HPLC. Finally 164 contigs were obtained and submitted to NCBI GenBank Ac. ID is LYDR00000000; genes were identified using Glimmer [31]. Genome properties were annotated by NCBI prokaryotic genome annotation pipeline. The genome consisted of a 5,75,243 bp, total G+C content 53.8%, total genes predicted were 4674, and total CDS were 4608, coding genes were 4490, and coding CDS were 4490 and 66 RNAs; rRNA were 3; tRNAs were 59; pseudo genes were 118 (https://www.ncbi.nlm.nih.gov/Traces/wgs/?val=LYDR01#contigs). Paralog gene clusters were 157, as identified by OrthoMCL (http://orthomcl.org/orthomcl/). Genes assigned to clusters of orthologous groups (COGs) were 3570, identified by using OrthoFinder [32] (http://www.stevekellylab.com/software/orthofinder). COG unassigned genes were 768. Pfam (https://xfam.wordpress.com/2017/03/08/pfam-31-0-is-released/) domain assigned were 2906 by using Pfam v.31.0. [33]. Identified signal peptides were 1239 by using SignalP 4.1 Server [34] (www.cbs.dtu.dk/services/SignalP/). Transmembrane helices

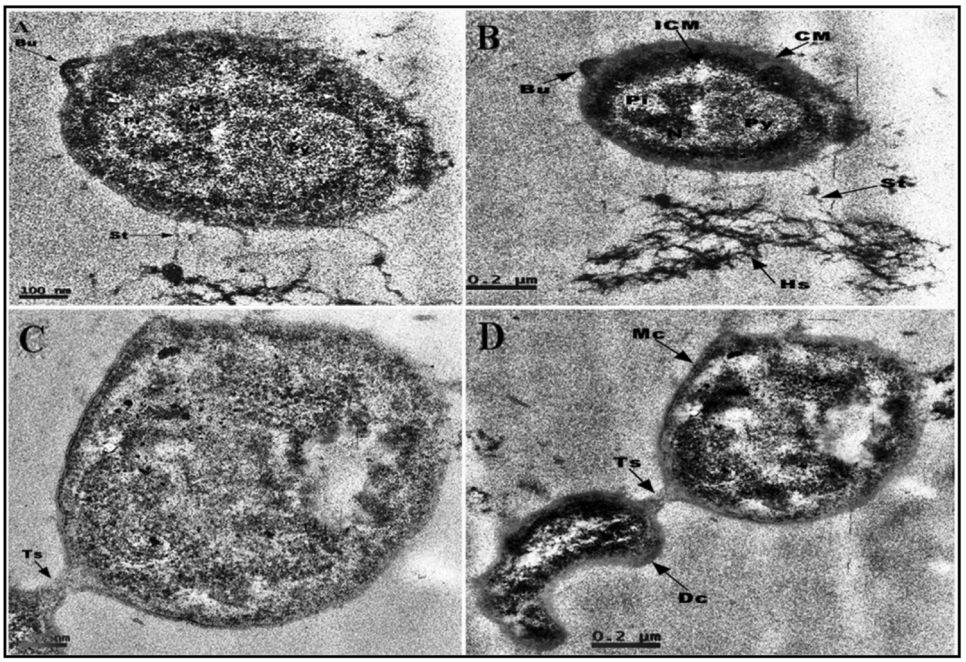

Fig. 4 Cell division through budding (Bu) (**a**, **b**) along with stalk (St) and holdfast substance (Hs) at opposite poles of the budding cells. Dividing cells are interconnected by a tubular structure (Ts) between mother and daughter cell (**c**, **d**)

(TMHMM) predicted were 1148 by using TMHMM Server v.2.0 [35]. Three CRISPR repeats were identified by CRISPR-finder [36] (http://crispr.i2bc.paris-saclay.fr/). Gene function prediction was done by using RAST server [37] and genome statistics are given as supplementary information (Table S2). COG classification was performed by using WebMGA [38] online server (http://weizhong-lab.ucsd.edu/metagenomic-analysis/server/cog/) details are given as supplementary information (Table S3). For whole-genome circular map generation, first all 164 contigs were merged into single chromosome by using EMBOSS [39] union program (http://emboss.sourceforge.net/). Circular genome map is shown in Fig. S5.

In silico DNA–DNA hybridization

ANI between strain JC280^T and *P. limnophila* DSM 3776^T was 91.2% which is far below the species cutoff (95–96%). Genome-to-genome distance between JC280^T and *P. limnophila* DSM 3776^T was 44.8% which is also well below the species cutoff (70%).

Secondary metabolite biosynthetic gene cluster analysis

The potential of strain JC280^T to produce secondary metabolites was evaluated using antiSMASH server. Six secondary metabolite biosynthetic gene clusters were (smBGC) predicted to be involved in secondary metabolism. They are other ketides (1), polyketides type I (1), polyketides type III (1), and terpenes (3). In *P. limnophila* DSM 3776^T, seven smBOC were predicted (Supplementary information, Table S1). Polyketide type III cluster was present in both species and smBOC flanking similarity was 92% and the predicted structure was nosiheptide. Nosiheptide_biosynthetic_gene_cluster similarity was 11% and the remaining clusters did not show any predicted structures. Polyketides cluster and Polyketides Type I cluster are having 81% similarities between the two species. Three terpene clusters were present in both the species; however, percentage similarities were only 55%, 32%, and 27%. *P. limnophila* DSM3776^T alone has bacteriocin cluster and the same was absent in strain JC280^T. We checked for antimicrobial property (using disk diffusion method) of both the strains from the culture supernatant of 15 days grown cultures. Strain JC280^T showed relatively more antimicrobial activity (zone of inhibition) than *P. limnophila* DSM 3776^T against *Staphylococcus* spp., *Streptococcus* spp., and *Escherichia coli* strains. Further work is in progress in identifying and characterizing the bioactive compounds. Based on the antiSMASH analysis, we are concluding that smBOC of both species are different. Hence, there is a greater probability to get novel compounds (graphical results given in Supplementary information, Figure S3) from the two *Planctopirus* strains.

Discussion

Morphological, phylogenetic, chemotaxonomic, and genome analyses indicated that the strain JC280^T belongs to the genus *Planctopirus*. Extensive study of strain JC280^T revealed differences and interesting properties between its closest relative *P. limnophila* DSM3776^T. Many differences

Table 2 Whole-cell fatty acids which differentiate strain JC280^T from *P. limnophila* DSM 3776^T

Characteristics	Strain JC280^T	*P. limnophila* DSM 3776^T
Fatty acids (%)		
Saturated		
$C_{12:0}$	2.8	–
$C_{14:0}$	2.3	–
$C_{16:0}$	30.4	25.0
$C_{17:0}$	5.5	7.2
Hydoxy		
$C_{16:0}$2OH	2.6	–
Unsaturated		
$C_{15:1}$ω6C	1.5	4.8
$C_{17:1}$ω8c	12.6	19.4
$C_{18:1}$ω9c	16.9	12.3
$C_{20:1}$ω9c	3.9	2.5
Summed feature-3	11.8	11.4
Summed feature-6	2.2	2.4
Summed feature-7	–	7.6
Summed feature-8	5.6	5.1
Hopanoids		
Hop-22-ene	–	+
Methylhop-21-ene	–	+
2-Methylhop-21-ene	–	–
Bacteriohopaneterol acetate	–	+
Unidentified hopanoid 5	+	+
Unidentified hopanoid 19	+	+
Unidentified hopanoid 21	+	+
Polar lipids		
Unidentified glycolipids (GL2)	+	–
Unidentifiedphospholipid (PL2)	–	+
Unidentified phospholipid (PL3)	–	+
Unidentified lipids (L1)	–	+
Unidentified lipids (L2)	–	+

Data presented is the comparative analysis done at author's laboratory under identical growth/assay conditions. Both strains have unidentified hopanoids 5, 19, and 21; Summed feature-3 was listed as $C_{16:1}$ω7c and/or $C_{16:1}$ω6c; summed feature-6 was listed as $C_{19:1}$ω11c and/or $C_{19:1}$ω9c; summed feature-7 was listed as $C_{19:1}$ω7c and/or $C_{19:1}$ω6c; summed feature-8 was listed as $C_{18:1}$ω7c and/or $C_{18:1}$ω6c

DPG diphosphatidylglycerol, *PC* phosphatidylcholine, *PG* phosphatidylglycerol, *GL1* unidentified glycolipids, *PL1* unidentified phospholipid, *L3* unidentified lipids, *APL* unidentified aminophospholipid

– Negative/absent, + Positive/present

found in physiological, biochemical, and chemotaxonomic properties are listed in Tables 1 and 2. ANI score and in silico DDH values confirmed that strain JC280^T represents a novel genomic species. At the genome level, variations between both the strains were many, a few are discussed here; the total genome size of strain JC280^T is 5,750,243 bp, while *P. limnophila* DSM 3776^T has 5,446,085 bp. Total genes in strain JC280^T were 4708, *P. limnophila* DSM 3776^T has 4370. Pseudo genes were 118 in strain JC280^T, while *P. limnophila* DSM 3776^T has only 46 (genome statistics taken from ref. [3]). Based on the genome size, all other assigned properties also showed variations such as ortholog and paralog clusters, no. of gene function prediction, Pfam domain assignments, signal peptides, and TMHMM predictions. An important difference between the two species was the CRISPR repeats, strain JC280^T contains two confirmed, one questionable CRISPR, while *P. limnophila* DSM 3776^T has one confirmed and two questionable CRISPR. In strain JC280^T, first CRISPR repeat was present at contig 69 with a length of 4310 bp and number of spacers were 58. Second CRISPR repeat was present at contig 77, of a length of 311 bp and number of spacers were 6. The third CRISPR repeat was questionable, present in contig 75, CRISPR length 101 bp and number of spacers were 1. *P. limnophila* DSM 3776^T has one confirmed CRISPR repeat present, length was 1415 bp and number of spacers were 19. The two questionable CRISPR repeats present have a length of 100 bp and 70 bp, both have 1 spacer, each. And also both species were differing in their antimicrobial activities (data not given). On the basis of the data presented in this study, strain JC280^T represents a novel species of the genus *Planctopirus*, for which the name *Planctopirus hydrillae* sp. nov. is proposed.

Description of *Planctopirus hydrillae* sp. nov

Hy.dril'lae. N.L. gen. n. *hydrillae* of *Hydrilla*, an aquatic plant from where the new species was isolated.

Colonies appear dark pink and smooth on gellan gum-solidified medium. Cells are spherical to ovoid, sub-polar monotrichous flagellated, crateriforms dispersed on the cell surface, and Gram-stain-negative. Mother cell diameter is 1.0–1.4 µm and multiplication occurs by budding on the distal cell pole. *N*-acetyl-D-glucosamine enhances growth yield and colony pigmentation. Obligate aerobe. Traces of yeast extract or added vitamins are required for growth. Growth occurs between pH 6.0 and 9.0 (optimum 6.5–7.5) under aerobic condition. No added NaCl is required for growth and tolerates up to 1.5% (w/v) NaCl. Optimum growth occurs at 20–30 °C (range 10–45 °C). Catalase and oxidase are positive. Indole is produced from L-tryptophan. Gelatin liquefaction is not observed. Starch and casein are hydrolyzed. Adenine, chitin, and elastine are not hydrolyzed. Growth occurs on D-glucose, D-galactose, and *N*-acetylglucosamine. Sucrose, glucoronic acid, D-fructose, mannitol, and glycerol are utilized. D-ribose, starch, dextrin, inulin, salicin, pyruvate, α-oxoglutarate, succinate, fumarate, formate, foramide, methanol, ethanol, lactate, acetate, propionate, tartarate, glutamate, caproate, phthalate, citrate,

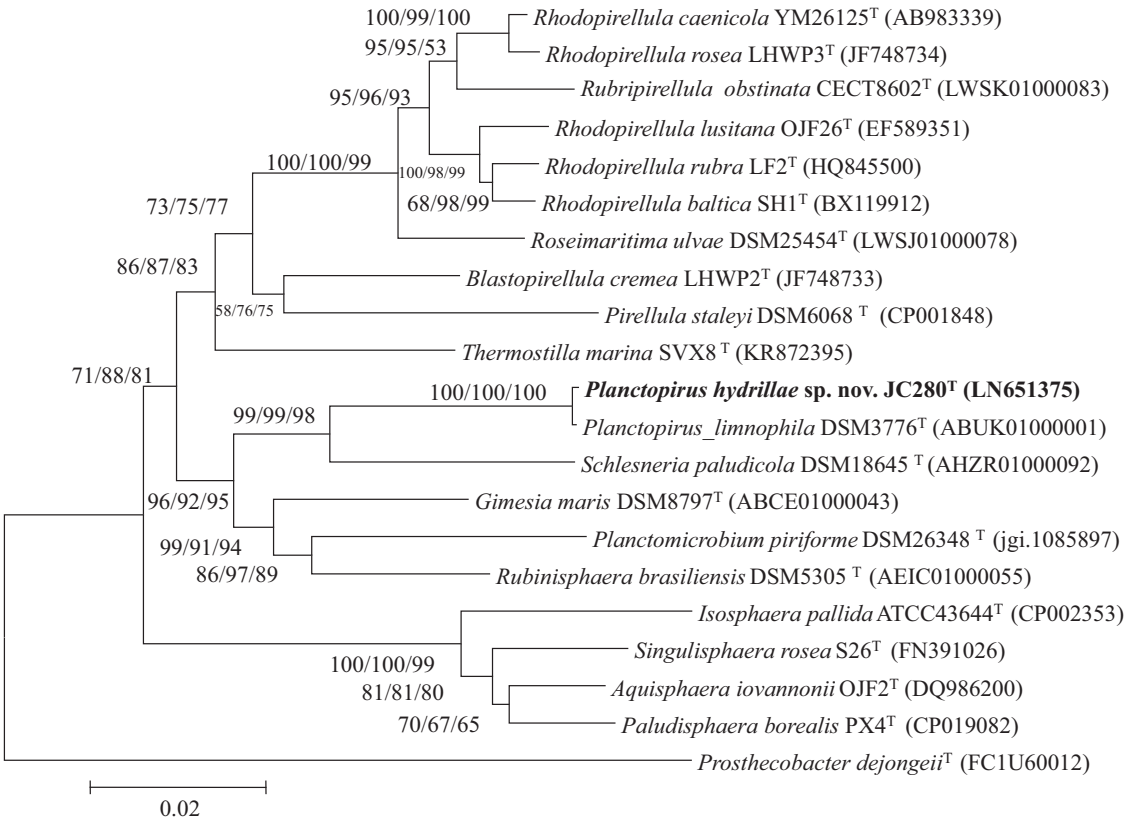

Fig. 5 Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain JC280^T along with other closely related members of the family *Planctomycetaceae*. The tree was constructed by the neighbor-joining method using the MEGA7 software and rooted by using *Prosthecobacter dejongeii*^{T} (FC1U60012) as the out-group. Numbers at nodes represent bootstrap values (based on 1000 resamples). Bootstrap percentages refer to NJ/ML/ME analysis. Bar 2 nucleotide substitutions per 100 nucleotides

and malate are not utilized. L-arginine, L-aspartate, DL-phenylalanine, L-serine, L-tryptophane are utilized as nitrogen sources. α-Glutamate, L-glycine, L-histidine, L-leucine, L-proline, methylamine HCl, urea, nitrate, nitrite, and dinitrogen are not utilized as nitrogen sources. Acid is produced from D-fructose, D-glucose, and D-galactose. Pigmentation of the culture is due to the presence of seven unidentified carotenoids. $C_{16:0}$, $C_{17:1}\omega8c$, $C_{18:1}\omega9c$, and summed feature-3 are present as major (>10%) fatty acids with minor (<10%) amounts of $C_{12:0}$, $C_{14:0}$, $C_{17:0}$, $C_{16:0}$-2OH, $C_{15:1}\omega6c$, $C_{20:1}\omega9c$. Sum in feature 6 and Sum in feature 8 are the fatty acid composition. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, two unidentified glycolipids (GL1-2), an unidentified phospholipid (PL1), an unidentified aminophospholipid (APL), and unidentified lipids (L1) and (L2) are the polar lipids present. Unidentified hopanoids 5, 19, and 21 are present. The calculated G+C content based on draft genome sequence is 53.8%.

The type strain JC280^T (=KCTC 42880^T = LMG 29153^T) was isolated from the plant *Hydrilla verticillata* collected from Hyderabad, India.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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