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ORIGINAL PAPER

Three marine strains constitute the novel genus and species *Crateriforma conspicua* in the phylum *Planctomycetes*

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Abstract *Planctomycetes* is a ubiquitous phylum of mostly aquatic bacteria that have a complex lifestyle and an unusual cell biology. Here, we describe three strains of the same novel genus and species isolated from three different environments; from a red biofilm at a hydrothermal vent in the Mediterranean Sea, from sediment in a salt-water fish tank, and from the surface of algae at the coast of the Balearic island Mallorca. The three strains Mal65^T (DSM 100706^T = LMG 29792^T, Pan14r (DSM 29351 = LMG 29012), and V7 (DSM 29812 = CECT 9853 = VKM B-3427) show typical characteristics of the *Planctomycetaceae* family, such as cell division by budding, crateriform structures and growth in aggregates or rosettes. The strains are mesophilic, neutrophilic to alkaliphilic as well as chemoheterotrophic and exhibit doubling times between 12 and 35 h. Based on our phylogenetic analysis, the three strains represent a single novel species of a new genus, for which we propose the name *Crateriforma conspicua* gen. nov. sp. nov.

Keywords Marine bacteria · *Planctomycetaceae* · Biofilms · Algae · Fish tank · Mallorca · Red biofilm · Hydrothermal vent

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Introduction

Planctomycetes are Gram-negative bacteria belonging to the PVC superphylum, along with *Verrucomicrobia*, *Lentisphaerae*, *Kiritimatiellaeota*, *Candidatus* Omnitrophica and *Chlamydiae*. This phylum has medical and biotechnological relevance (Wagner and Horn 2006), and plays a major role in global biogeochemical cycles (Peeters and van Niftrik 2018; Strous et al. 1999; Wiegand et al. 2018).

Not only are Planctomycetes found in the eutrophic environment of wastewater, many Planctomycetes make their home on algal surfaces in larger water bodies (Bengtsson et al. 2012; Bondoso et al. 2014b, 2015, 2017; Lage and Bondoso 2014; Vollmers et al. 2017), where they can be highly abundant (Bengtsson and Øvreås 2010) and in which they can metabolize complex algal carbon substrates (Jeske et al. 2013; Lachnit et al. 2013). The abundance of

Planctomycetes in the algal surface biotope is surprising as Planctomycetes grow slowly in comparison to their natural competitors, such as members of the *Roseobacter* clade (Frank et al. 2014; Wiegand et al. 2018). In the past, Planctomycetes were thought to have a number of exceptional traits, such as, a compartmentalised cell plan (Lindsay et al. 1997), a nucleus-like structure (Fuerst and Webb 1991), endocytosis-like uptake (Lonhienne et al. 2010) and the lack of peptidoglycan (König et al. 1984). With the advent of novel high-resolution microscopic techniques and genetic tools for Planctomycetes (Jogler et al. 2011; Jogler and Jogler 2013; Rivas-Marin et al. 2016) this picture has changed. Planctomycetes do in fact possess peptidoglycan (Jeske et al. 2015; van Teeseling et al. 2015), as do the closely related Verrucomicrobia (Rast et al. 2017). Compartments of Planctomycetes were instead found to be invaginations of the cytoplasmic membranes (Acehan et al. 2013; Boedeker et al. 2017; Lage et al. 2013; Santarella-Mellwig et al. 2013), with the exception of the *Candidatus* Brocadiales clade (Jogler 2014; Neumann et al. 2014). The cell envelope architecture of the Planctomycetes was therefore re-interpreted as Gram-negative (Boedeker et al. 2017; Devos 2014a, b).

Even though Planctomycetes were removed from their special place on the evolutionary ladder, they are still exceptional in other ways. Members of the family *Planctomycetaceae* perform cell division via budding or binary fission while lacking canonical divisome proteins including the otherwise universal FtsZ (Jogler et al. 2012; Pilhofer et al. 2008; Wiegand et al. 2019). *Planctomycetaceae* can also perform a lifestyle switch between a planktonic swimmer- and sessile stalked mother-cell (Jogler et al. 2011). They possess unique cell surface alterations, so-called crateriform structures, that form pili which are potentially employed as an uptake mechanism for large polysaccharides from the environment (Boedeker et al. 2017). Their periplasm can be extremely enlarged, likely facilitating the digestion of said polysaccharides (Boedeker et al. 2017). They are also rich in giant genes (Kohn et al. 2016; Wiegand et al. 2019), which might be involved in small molecule biosynthesis or code for parts of unique structural components. They are potential producers of small molecules (Graça et al. 2016; Jeske et al. 2016; Wiegand et al. 2019), and represent the bacterial phylum with the most predicted genes of unknown function (Faria et al. 2018; Overmann et al. 2017). Taken together, Planctomycetes are among the most unusual of all bacterial phyla known thus far (Wiegand et al. 2018), which is the main motivation to explore Planctomycete diversity.

In this study, we describe the cultivation of three strains of the phylum *Planctomycetes* that are closely related to the *Pirellula* clade, a widespread marine clade of Planctomycetes, that contains the genera *Pirellula*, *Rhodopirellula*, *Rubripirellula*, *Blastopirellula*, *Roseimaritima*, *Mariniblastus* and *Novipirellula* (Bondoso et al. 2014a; Kallscheuer et al. 2019d; Wiegand et al. 2019). It is a typical *Planctomycetaceae* clade in most aspects, such as cell division and lifestyle. Despite the close relation, the three new isolates Mal65^T, Pan14r and V7 represent a new genus as well as a single new species, for which we propose the name *Crateriforma conspicua* gen. nov. sp. nov.

Materials and methods

Isolation and cultivation of planctomycetal strains

Strain Mal65^T was isolated from the surface of an algae sampled in the Bay of Palma on the coast of El Arenal, Mallorca, Spain (39.5126 N 2.7470 E). Pan14r was isolated from a red biofilm in a shallow hydrothermal vent located 5 km in southern eastern direction from Panarea island, Italy (38.5568 N 15.1097 E) (Maugeri et al. 2009). Strain V7 was isolated from sediment material in a salt water fish tank in Braunschweig, Germany (52.2689 N 10.5268 E). Isolation and initial cultivation was performed as described earlier (Wiegand et al. 2019). For further investigation, the strains were grown in M1H medium supplemented with *N*-acetylglucosamine (NAG) and artificial seawater (ASW) (designated M1H NAG ASW medium) as described before (Wiegand et al. 2019) and were incubated in baffled flasks at 28 °C under constant agitation at 110 rpm.

Light microscopy and scanning electron microscopy

Phase contrast images were taken with a Nikon Eclipse Ti inverted microscope with a Nikon DS-Ri2 camera. Specimens were immobilized in MatTek glass

bottom dishes (35 mm, No. 1.5) using a 1% (w/v) agarose cushion (Boedeker et al. 2017). Nikon NIS-Elements software (version 4.3) was used to examine cell size either manually or by using the object count tool (smooth: 4x, clean: 4x, fill holes: on, separate: 4x).

Field emission scanning electron microscopy was performed as described earlier (Boersma et al. 2019). Briefly, bacteria were fixed in formaldehyde, washed, and placed on cover slips coated with poly-L-lysine solution. Cover slips were then fixed in 1% (v/v) glutaraldehyde and washed twice before dehydrating in a graded series of acetone (10, 30, 50, 70, 90, 100%) on ice. Samples from the 100% acetone step were brought to room temperature before placing them in fresh 100% acetone. Samples were then subjected to critical-point drying with liquid CO_2 (CPD 300, Leica). Dried samples were covered with a gold/palladium (80/20) film by sputter coating (SCD 500, Bal-Tec) before examination in a field emission scanning electron microscope (Zeiss Merlin) using the Everhart–Thornley HESE2 detector and the inlens SE detector in a 25:75 ratio at an acceleration voltage of 5 kV.

Physiological and biochemical analyses

Determination of pH optima was performed at 28 °C, with buffering agent 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) at pH 5 and 6, 100 mM HEPES at pH 7, 7.5 and 8, or 100 mM *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) at pH 9 and 10. Temperature optima determination was performed at pH 7.5. Cell densities were inferred from optical density at 600 nm (OD_{600}).

Phylogenetic analysis

16S rRNA gene phylogeny was computed for the strains in question (GenBank acc. no. MK554558 (V7), MK554530 (Pan14r), and MK559980 (Mal65^T)), the type strains of all described planctomycetal species (as in August 2019) and all isolates recently published and/or described (Boersma et al. 2019; Kallscheuer et al. 2019a, b, c, d; Kohn et al. 2019; Wiegand et al. 2019) and with an outgroup of strains from outside the phylum *Planctomycetes* but part of the PVC superphylum. An alignment of 16S rRNA genes was made with SINA (Pruesse et al. 2012). Phylogenetic analysis was performed employing a maximum likelihood approach with 1000 bootstraps, the nucleotide substitution model GTR, gamma distribution, and estimation of proportion of invariable sites using GTRGAMMAI (Stamatakis 2014).

The genomes for the genome-based analyses were gathered from GenBank, including the sequences for strain Mal65^T (acc. no. CP036319), Pan14r (acc. no. SJPL00000000) and V7 (acc. no. SJPZ00000000) recently published (Wiegand et al. 2019). The primary metabolism was analysed by examining locally computed InterProScan (Mitchell et al. 2019) results cross-referenced with information from the UniProt database and BLASTp results of 'typical' protein sequences. Completeness and contamination of the genome was determined using CheckM v1.0.131 (Parks et al. 2015). The average nucleotide identity (ANI) was calculated using OrthoANI (Lee et al. 2016), the average amino acid identity (AAI) was computed with the aai.rb script from the enveomics collection (Rodriguez-R and Konstantinidis 2016) and the percentage of conserved proteins (POCP) was determined as previously described (Qin et al. 2014). The *rpoB* nucleotide sequences were taken from the genome annotations and the sequence identities were determined as described (Bondoso et al. 2013). Upon extracting only those parts of the sequences that would have been sequenced with the described primer set, the alignment and matrix calculation was done with Clustal Omega (Sievers et al. 2011).

Results and discussion

Morphological, physiological and biochemical analyses

Mal65^T, Pan14r and V7 all form pink colonies and cells are pear-shaped (Figs. 1a, 2) with slightly different average sizes of $2.1 \pm 0.3 \times 1.3 \pm 0.3$ µm (V7), $1.8 \pm 0.3 \times 1.0 \pm 0.2$ µm (Mal65^T) and $1.8 \pm 0.2 \times 0.9 \pm 0.1$ µm (Pan14r) (Fig. 1b). All strains produce fibres mainly from a single pole, which seem to originate from crateriform structures that cover about 30% of the surface (Fig. 2). The large number of fibres is likely what enables these strains to grow in dense biofilms and enables cells to attach to each other in aggregates of more than 10 cells or rosettes of between 3 and 5 cells (Fig. 2). Typical for the *Planctomycetaceae*, the three strains perform cell division by polar budding (Fig. 1a).

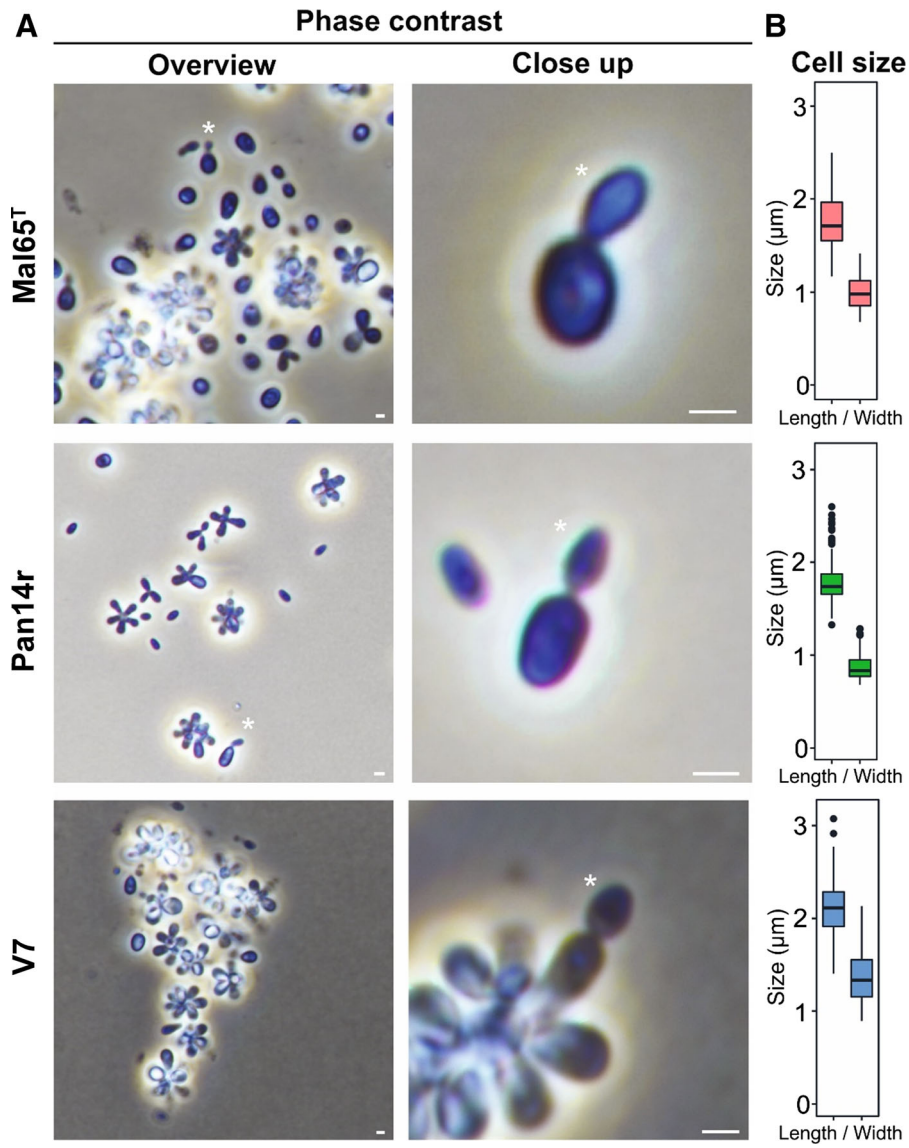

Fig. 1 Phase contrast micrographs of strains Mal65^T, Pan14r and V7 (**a**), and their cell size (**b**). Each of the strains grows in aggregates or rosettes and divides by polar budding, as can be observed in the overview and close up, respectively. Asterisks mark budding cells. Scale bar represents 1 µm

In batch experiments, all strains grew strictly aerobically and at temperatures ranging from 10 °C to 36 °C with optimal growth at 33–36 °C, indicating a mesophilic lifestyle (Fig. 3). pH values that permitted growth ranged from 5.0 to 9.5 with optimal growth at pH 7.0–7.5. The growth profile pointed to a slightly alkaliphilic lifestyle. Here, Pan14r stood out as it maintained 80–90% of the maximal growth rate (at pH 7.5) up to a pH of 9.5. Maximal growth rates of the three strains were calculated to 0.083 h^{-1} (Mal65^T), 0.028 h^{-1} (Pan14r) and 0.059 h^{-1} (V7). These values corresponds to doubling times of 8, 25 and 12 h, respectively.

Genomic characteristics and genome-encoded features of the metabolism

Mal65^T, Pan14r and V7 are all very similar in size, G + C content, number of protein-coding genes and hypothetical proteins (Table 1). The genotype of V7 is

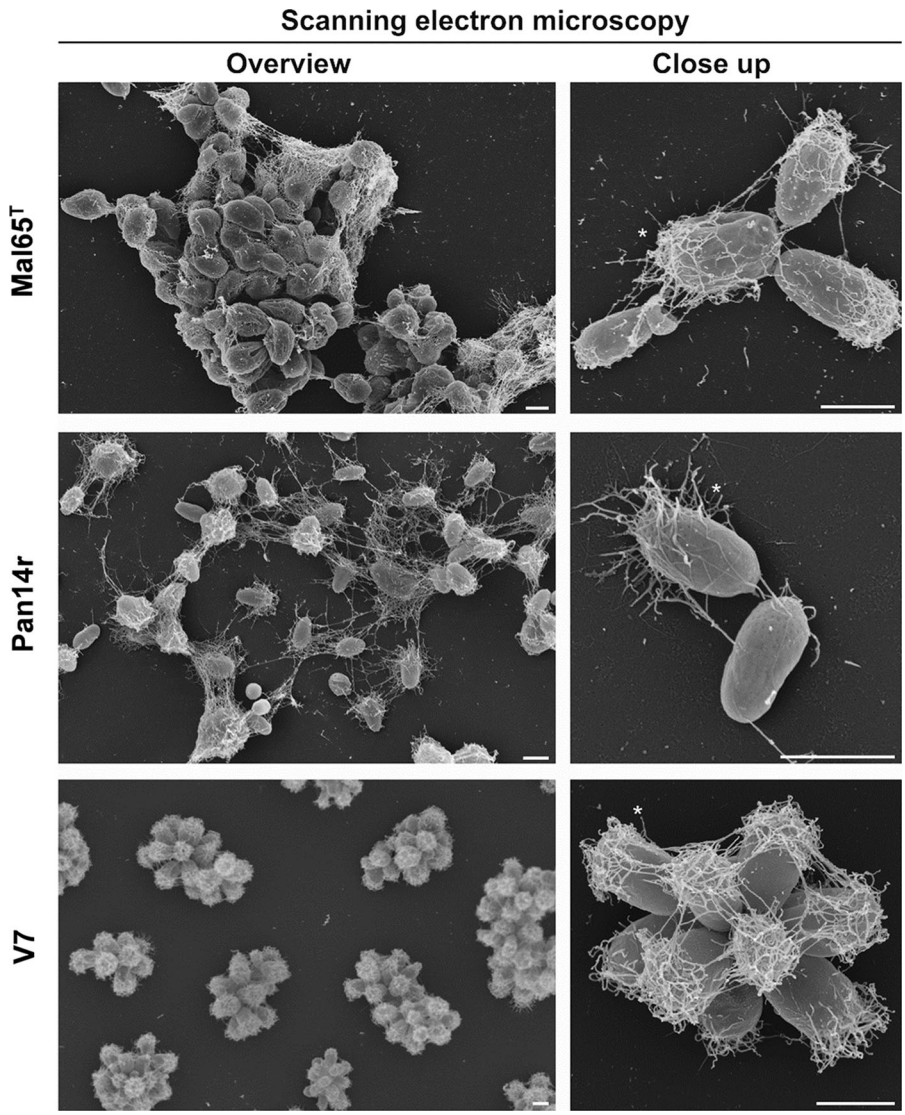

Fig. 2 Scanning electron microscopy micrographs of strains Mal65^T, Pan14r and V7. Asterisks indicate cell poles with fibers originating from crateriform structures. Scale bar represents 1 µm

more different from both, Mal65^T and Pan14r, which is also reflected in the phylogeny. These three strains differ from *Rhodopirellula baltica* mainly in a higher G + C content (57% vs. 55%), a slightly lower coding density and a lower proportion of hypothetical genes. Key genomic features of the strains in comparison to *R. baltica* are summarized in Table 1.

For getting a first insight into the central carbon metabolism of the three novel isolates, we searched for genes coding for enzymes participating in glycolytic pathways, the TCA cycle, gluconeogenesis and important anaplerotic reactions, such as pyruvate or phosphoenolpyruvate carboxylation and the glyoxylate shunt. *R. baltica* is closely related to the three here characterised strains and served for comparison. Our analysis suggests that all strains, including *R. baltica* SH1^T, harbor a complete Embden-Meyerhof-Parnas pathway (the most common glycolytic pathway), TCA cycle and pentose phosphate pathway as genes could be assigned to all enzymes participating in these pathways (Table 2). For the gluconeogenesis pathway, essential enzymes appear to be absent in all four strains, including *R. baltica* SH1^T. If this is indeed true, the strains would not be able to grow with TCA

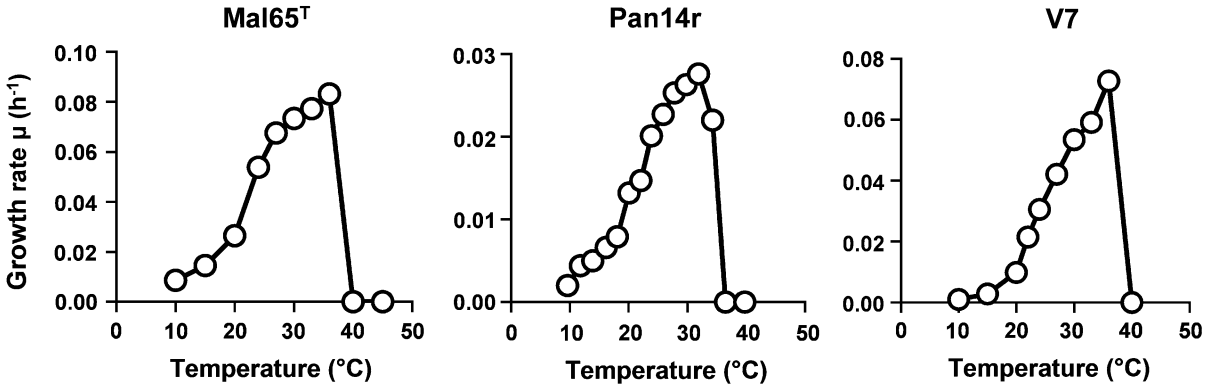

Fig. 3 Determination of the temperature optimum of the three novel isolates. Data shows growth rates calculated from three biological replicates cultivated in M1H NAG ASW medium at pH 7.5. Growth rates were assessed from the slope of the plot of $\ln(OD_{600})$ against the cultivation time

Table 1 Phenotypic and genotypic information of strains Pan14r, Mal65^T, V7 and *R. baltica* SH1^T

Characteristics	Pan14r	Mal65^T	V7	*R. baltica* SH1^T
Phenotypic features				
Shape	Pear-shaped	Pear-shaped	Pear-shaped	Pear-shaped
Cell size (μm)	1.8 ± 0.3 × 0.9 ± 0.1	1.8 ± 0.3 × 1.0 ± 0.2	2.1 ± 0.3 × 1.3 ± 0.3	1.0–2·5 × 1.2–2.3
Aggregates	Rosettes	Yes and rosettes	Yes and rosettes	Rosettes
Division	Polar budding	Polar budding	Polar budding	Polar budding
Colour	Pink	Pink	Pink	Pink
Doubling time (h)	25	8	12	12
Flagellum	Yes	Yes	Yes	Yes
Crateriform structures	Polar	Polar	Polar	Polar
Fimbriae	Fiber	Fiber	Fiber	Fiber
Capsule	n. o.	n. o.	n. o.	n.o.
Bud shape	Like mother cell	Like mother cell	Like mother cell	Like mother cell
Budding pole	Polar	Polar	Polar	Polar
Stalk	n. o.	n.o.	n.o.	n.o.
Holdfast structure	n. o.	n.o.	n.o.	n.o.
Genomic information				
Genome size (bp)	7,137,949	7,182,433	7,227,508	7,145,576
Plasmids (bp)	None	None	None	None
GC (%)	57.8 ± 2.5	57.8 ± 0	57.7 ± 2.7	55.4
Completeness (%)	94.83	95.69	94.83	98.28
Contamination (%)	0	0	0	1.72
Protein-coding genes	5400	5437	5531	5465
Hypothetical proteins	2081	2117	2173	3078
Protein-coding genes/Mb	757	757	765	765
Coding density (%)	88.2	88.5	88.5	88.7
Transposable elements	2	7	5	0

Phenotypic data of *R. baltica* SH1^T from Schlesner et al. (2004)

n. o. not observed

Table 2 Genome-based primary metabolism of the isolated strains compared to *Rhodopirellula baltica* SH1[T]

Enzyme/reaction	EC number	Gene	*R. baltica* SH1	V7	Pan14r	Mal65
Glycolysis						
Glucose-6-phosphate isomerase	5.3.1.9	*pgi*	Yes	V7x_11580	Pan14r_27110	Mal65_41760
ATP-dependent 6-phosphofructokinase isozyme 1	2.7.1.11	*pfkA*	Yes	V7x_30220	Pan14r_02160	Mal65_23940
Fructose-bisphosphate aldolase class 2	4.1.2.13	*fbaA*	Yes	V7x_12400	Pan14r_26380	Mal65_41030
Triosephosphate isomerase	5.3.1.1	*tpiA*	Yes	V7x_04530	Pan14r_34660	Mal65_49290
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*gapA*	Yes	V7x_41370	Pan14r_39950	Mal65_12800
Phosphoglycerate kinase	2.7.2.3	*pgk*	Yes	V7x_36030	Pan14r_08120	Mal65_17990
2,3-Bisphosphoglycerate-independent phosphoglycerate mutase	5.4.2.12	*gpmI*	Yes	V7x_11570	Pan14r_27120	Mal65_41770
Enolase	4.2.1.11	*eno*	Yes	V7x_31940	Pan14r_03750	Mal65_22390
Pyruvate kinase I	2.7.1.40	*pykF*	Yes	V7x_05710	Pan14r_33480	Mal65_48110
				V7x_06750	Pan14r_32360	Mal65_47080
Pyruvate dehydrogenase E1 component	1.2.4.1	*aceE*	Yes	V7x_28310	Pan14r_00630	Mal65_25450
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	2.3.1.12	*aceF*	Yes	V7x_28320	Pan14r_00640	Mal65_25440
Gluconeogenesis						
Phosphoenolpyruvate carboxylase	4.1.1.31	*ppc*	Yes	No	No	No
Pyruvate, phosphate dikinase	2.7.9.1	*ppdK*	Yes	V7x_14980	Pan14r_23880	Mal65_38540
Phosphoenolpyruvate carboxykinase (ATP)	4.1.1.49	*pckA*	No	No	No	No
Phosphoenolpyruvate carboxykinase (GTP)	4.1.1.32	*pckG*	No	No	No	No
Fructose-1,6-bisphosphatase class 2	3.1.3.11	*glpX*	No	No	No	No
Fructose-1,6-bisphosphatase class 1	3.1.3.11	*fbp*	No	No	No	No
Pyrophosphate–fructose 6-phosphate 1-phosphotransferase	2.7.1.90	*pfp*	No	No	No	No
Pentose phosphate pathway						
Glucose-6-phosphate 1-dehydrogenase	1.1.1.49	*zwf*	Yes	V7x_18810	Pan14r_19910	Mal65_34600
6-Phosphogluconolactonase	3.1.1.31	*pgl*	Yes	V7x_03560	Pan14r_35630	Mal65_50260
6-Phosphogluconate dehydrogenase, decarboxylating	1.1.1.44	*gndA*	Yes	V7x_13880	Pan14r_24880	Mal65_39540
Transketolase 2	2.2.1.1	*tktB*	Yes	V7x_12260	Pan14r_26520	Mal65_41170
Transaldolase B	2.2.1.2	*talB*	Yes	V7x_44140	Pan14r_41250	Mal65_10700
Entner–Doudoroff pathway						
KHG/KDPG aldolase	4.1.2.14	*eda*	No	V7x_14290	Pan14r_24470	Mal65_39130
Phosphogluconate dehydratase	4.2.1.12	*edd*	Yes	V7x_11490	Pan14r_27210	Mal65_41870
			Yes	V7x_30090	Pan14r_02030	Mal65_24060
			Yes	V7x_12410	Pan14r_26370	Mal65_41020
TCA cycle						
Citrate synthase	2.3.3.16	*gltA*	Yes	V7x_21170	Pan14r_18010	Mal65_32730
Aconitate hydratase A	4.2.1.3	*acnA*	Yes	V7x_03610	Pan14r_35580	Mal65_50210
Isocitrate dehydrogenase (NADP)	1.1.1.42	*icd*	Yes	V7x_52720	Pan14r_53880	Mal65_01240
2-Oxoglutarate dehydrogenase E1 component	1.2.4.2	*sucA*	Yes	V7x_06190	Pan14r_32990	Mal65_47630
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	2.3.1.61	*sucB*	Yes	V7x_33890	Pan14r_05900	Mal65_20190
Succinate–CoA ligase [ADP-forming] subunit alpha	6.2.1.5	*sucD*	Yes	V7x_15080	Pan14r_23780	Mal65_38440

Table 2 continued

Enzyme/reaction	EC number	Gene	*R. baltica* SH1	V7	Pan14r	Mal65
Succinate–CoA ligase [ADP-forming] subunit beta	6.2.1.5	*sucC*	Yes	V7x_15090	Pan14r_23770	Mal65_38430
Succinate dehydrogenase flavoprotein subunit	1.3.5.1	*sdhA*	Yes	V7x_19230	Pan14r_19500	Mal65_34190
Succinate dehydrogenase iron-sulfur subunit	1.3.5.1	*sdhB*	Yes	V7x_19220	Pan14r_19510	Mal65_34200
Succinate dehydrogenase cytochrome b556 subunit	1.3.5.1	*sdhC*	Yes	V7x_19240	Pan14r_19490	Mal65_34180
Succinate dehydrogenase hydrophobic membrane anchor subunit	1.3.5.1	*sdhD*	No	No	No	No
Fumarate hydratase class I, an/aerobic	4.2.1.2	*fumA/B*	No	No	No	No
Fumarate hydratase class II	4.2.1.2	*fumC*	Yes	V7x_18670	Pan14r_20050	Mal65_34740
Malate dehydrogenase	1.1.1.37	*mdh*	Yes	V7x_19900	Pan14r_18780	Mal65_33480
Glyoxylate shunt						
Isocitrate lyase	4.1.3.1	*aceA*	No	No	No	No
Malate synthase G	2.3.3.9	*glcB*	No	No	No	No

Presence of a gene in SH1^T is indicated by 'yes' and absence is indicated by 'no'. The analysis is based on genome sequences with the following accession numbers: NC_005027.1 (*R. baltica* SH1^T), CP036319 (Mal65^T), SJPL00000000 (Pan14r) and SJPZ00000000 (V7)

cycle intermediates or short-chain carboxylic acids, such as pyruvate or lactate, as sole carbon and energy source. The latter hypothesis is further substantiated by the lack of genes coding for enzymes of the glyoxylate shunt, which are typically required for replenishing the TCA cycle during growth on acetate. As acetyl-CoA units are formed as degradation product during β-oxidation of fatty acids the absence of the glyoxylate shunt would also prohibit growth on fatty acids as sole carbon and energy source. However, it cannot be excluded that the strains follow yet uncharacterized, non-canonical pathways for the utilization of acetate and other short- or long-chain carboxylic acids. Taken together, our analysis suggests that sugars are the preferred substrates for energy and biomass formation, which is in line with the postulated strategy of members of the '*Pirellula* clade' to obtain carbon and energy from degradation of complex algae-derived polysaccharides.

As the complex lifestyle of Planctomycetes is believed to be related to the production of bioactive secondary metabolites, we also checked for genetic clusters putatively involved in their biosynthesis. Genetic clusters known to be involved in the biosynthesis of such compounds code e.g. for polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) and thus we focused our analysis on these two classes (Table 3). The number of putative secondary metabolite-related clusters in the three strains is between 4 and 5. All three strains harbor one putative type I PKS and two putative NRPSs, but lack type III PKSs. Pan14r differs from Mal65^T and V7 in the number of putative mixed type I PKS-NRPS-encoding clusters. All three strains differ from *R. baltica* SH1^T, which harbors a putative type III PKS absent in the three here characterised strains. In turn, the three novel strains harbor a putative NRPS-related cluster, which was not identified in *R. baltica* SH1^T.

Phylogenetic inference

During comparison of 16S rRNA gene identity, strains Mal65^T and Pan14r showed identical sequences and V7 matched both other strains to 99.9% (Fig. 4). Being above the proposed species threshold of 98.7% (Stackebrandt and Ebers 2006), these identities indicate that all three strains belong to the same species. All cluster monophyletically within the family

Table 3 Numbers of genetic clusters putatively involved in production of polyketides and nonribosomal peptides

Strain	Type I PKS	Type II PKS	Type III PKS	Mixed type I PKS-NRPS	NRPS
R. baltica SH1^T	2	0	1	1	0
Pan14r	2	0	0	1	1
Mal65^T	2	0	0	2	1
V7	2	0	0	2	1

The analysis is based on genome sequences with the following accession numbers: NC_005027.1 (*R. baltica* SH1^T), CP036319 (Mal65^T), SJPL00000000 (Pan14r) and SJPZ00000000 (V7)

Planctomycetaceae (Fig. 4a) and their current closest relatives are *Rhodopirellula* species, with *Rhodopirellula rubra* LF2^T as closest relative of Mal65^T (94.0%), Pan14r (94.0%) as well as of V7 (94.1%). These values are lower than the suggested threshold for genera of 94.5% (Yarza et al. 2014), suggesting that the novel species also belongs to a novel genus.

For Planctomycetes, it is often observed that 16S rRNA gene sequence similarity as a sole basis for distinguishing separate genera is inconsistent (Kohn et al. 2019). Phylogenetic markers such as the RNA polymerase β-subunit gene *rpoB* (Bondoso et al. 2013), ANI (Lee et al. 2016), AAI (Konstantinidis and Tiedje 2005) and POCP (Qin et al. 2014) do provide additional accuracy (Fig. 4b).

Comparison of AAI values of the isolated strains to previously described *Rhodopirellula* species yielded a minimal similarity value of 52.8%. This value is below the genus threshold of 60%, affirming that the here described strains form a separate genus and do not belong to the genus *Rhodopirellula* (Luo et al. 2014). A minimal POCP value of 48.8% obtained during comparison of the novel strains with *Rhodopirellula* species further substantiates their assignment to a novel genus (criterion of $< 50\%$ for separate genera) (Qin et al. 2014).

Similarity of a 1200 base pair sequence fragment of the *rpoB* gene was originally introduced as a marker to infer phylogeny in genera belonging to the order *Planctomycetales*. Based on the large influx of novel Planctomycete genomes, the genus *Rhodopirellula* belonging to the *Planctomycetales* was split. This split has resulted in changed *rpoB* value thresholds compared to the original publication (Bondoso et al. 2013; Kallscheuer et al. 2019d).

For the novel strains in comparison to *Rhodopirellula* species, we observed a minimal similarity of the mentioned partial sequence of the *rpoB* gene of 77.9%, a value within the newly proposed genus threshold range of 75.5–78% (Kallscheuer et al. 2019d). In summary, AAI, POCP and *rpoB* identity confirm the 16S rRNA gene similarity-based assignment of a novel genus.

In addition to belonging to a novel genus, these three strains were also predicted to be the same species based on 16S rRNA similarity. A comparison of AAI values of the three strains ranges from 91.5 to 97.2% and is therefore in accordance with the species threshold of 80% (Fig. 4b) (Luo et al. 2014). The *rpoB* similarity of Mal65^T and Pan14r (99.6%) is above the species threshold, while V7 compared to the other two strains (95.7–95.8%) is below the species threshold (96.3%). The ANI of V7 is below the usual ANI threshold of 95–96%, but could still be in range when the proposed cutoff values of 93–96% are used instead (Rosselló-Móra and Amann 2015). Based on these results, we are confident that Mal65^T and Pan14r are the same species, but we could both argue that V7 is the same or a separate species using these results. As we cannot be sure, we opt to go for the more conservative option and put all three strains as one single species.

Conclusion

Based on the phylogenetic analyses and the morphological similarities, the three isolated strains belong to a single species within a new genus, for which we introduce the name *Crateriforma conspicua* gen. nov. sp. nov.

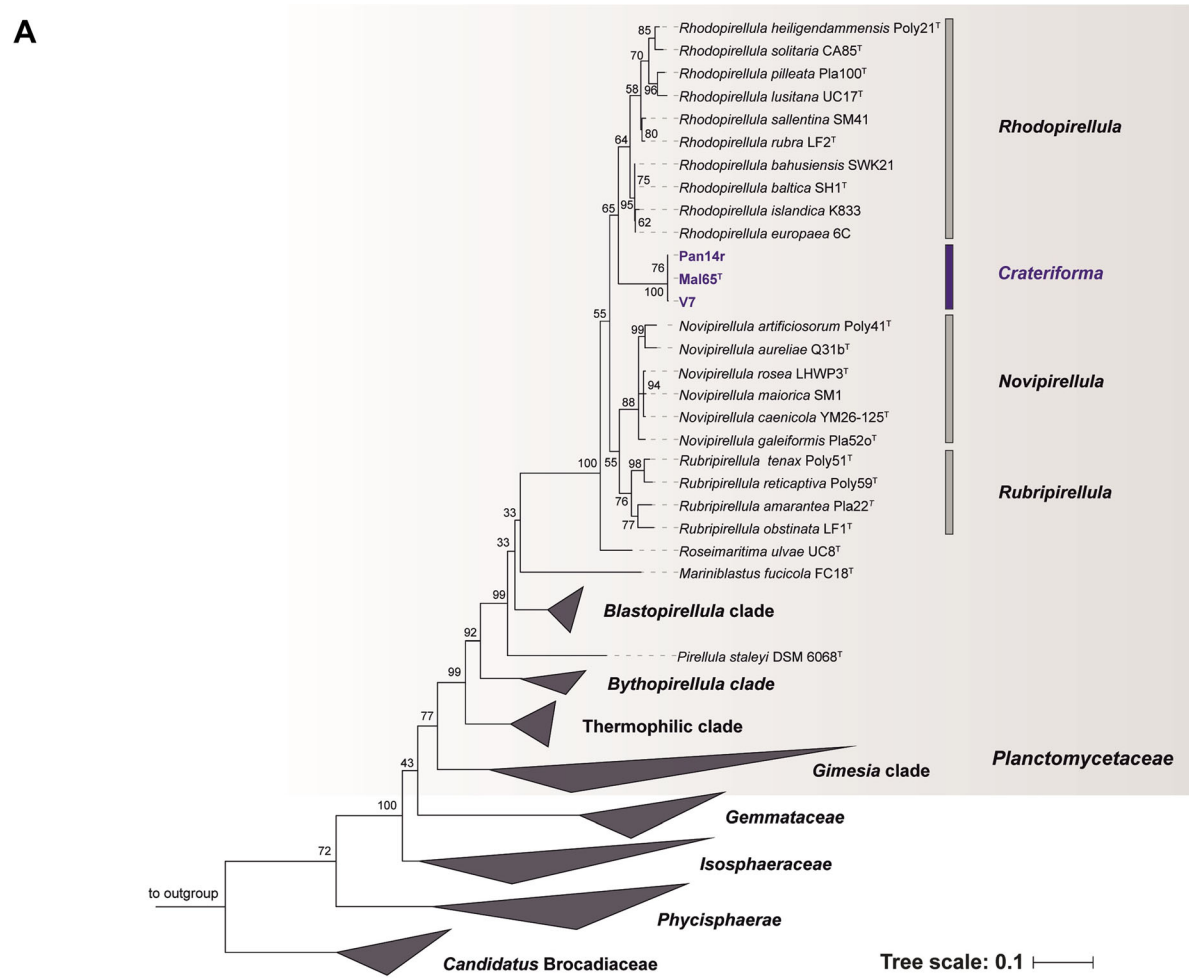

Fig. 4 a Phylogenetic tree of described planctomycetal species and of the novel isolates Mal65^T, Pan14r and V7. Bootstrap values indicated as a proportion of 1000 re-samplings (in %). The outgroup consists of three 16S rRNA genes from the PVC superphylum. **b** Comparison between methods to separate species and genera. Some methods are suitable for both genus and species differentiation, while others are only suitable for one. Methods used: 16S rRNA gene identity, average amino acid identity (AAI), percentage of conserved proteins (POCP), *rpoB* gene identity and average nucleic acid identity (ANI)

Description of *Crateriforma* gen. nov.

Crateriforma (*Cra.te.ri.for'ma.* L. masc. n. *crater* a crater; L. fem. n. *forma* a form, a figure; N.L. fem. n. *Crateriforma* a bacterium with crateriform structures).

Species of the genus are Gram-negative and pear-shaped. Cells divide by polar budding and produce fibres originating from conspicuous crateriform structures. The type species of this genus is *Crateriforma conspicua.*

Description of *Crateriforma conspicua* sp. nov.

Crateriforma conspicua (*con.spi'cu.a.* L. fem. adj. *conspicua* visible, clearly seen; corresponding to the clearly visible crateriform structures of the cells).

Colonies are pink and cells are pear-shaped (length: 1.8 ± 0.3 μm, width 1.0 ± 0.2 μm), form aggregates and divide by polar budding. Cells of the type strain grow at ranges of 10–36 °C (optimum 36 °C) and at pH 5.0–9.5 (optimum 7.5). The type strain is Mal65^T (DSM 100706^T = LMG 29792^T, synonym Malle65^T) and was isolated from the surface of an algae in the Bay of Palma of the coast of El Arenal, Mallorca, Spain. The genome of the type strain is 7,182,433 bp in length and features 57.8% G + C content. The genome (accession no. CP036319) and 16S rRNA gene sequence (accession no. MK559980) of the type strain are available from GenBank.

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Author's contributions SHP wrote the manuscript, analyzed the data and prepared the figures, SW performed the genomic and phylogenetic analysis, MJ, AH and PR isolated the strains and performed the initial cultivation and strain deposition, SHP and CB performed the light microscopic analysis, SW, NK and MSMJ contributed to text preparation and revised the manuscript, MR performed the electron microscopic analysis. CJ supervised the study and took the samples. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

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

Human and animal rights This article does not contain any studies with animals performed by any of the authors.

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