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ORIGINAL PAPER

Description of three bacterial strains belonging to the new genus *Novipirellula* gen. nov., reclassification of *Rhodopirellula rosea* and *Rhodopirellula caenicola* and readjustment of the genus threshold of the phylogenetic marker *rpoB* for *Planctomycetaceae*

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Abstract Access to axenic cultures of Planctomycetes is crucial for further investigating their complex lifestyle, uncommon cell biology and primary and secondary metabolism. As a contribution to achieve this goal in the future, we here describe three strains belonging to the novel genus *Novipirellula* gen. nov. The strains were isolated from biotic and abiotic surfaces in the Baltic Sea and from the island Heligoland in the North Sea. Colony colours range from white to light pink. Cells are acorn-shaped and grew optimally at neutral pH and temperatures between 27 and 30 °C. Phylogenetic analyses revealed that the isolated strains represent three novel species belonging to a new genus, *Novipirellula* gen. nov. Beyond that, our analysis suggests that *Rhodopirellula rosea* LHWP3^T, *Rhodopirellula caenicola* YM26-125^T and *Rhodopirellula maiorica* SM1 are also members of this novel genus. Splitting the current genus *Rhodopirellula* into a more strictly defined genus *Rhodopirellula* and *Novipirellula* also allowed readjusting the genus threshold value for the gene *rpoB*, encoding the RNA polymerase β-subunit, which is used as phylogenetic marker for *Planctomycetales*. A threshold range of 75.5–78% identity of the analysed partial *rpoB* sequence turned out to be reliable for differentiation of genera within the family *Planctomycetaceae*.

Keywords *Novipirellula aureliae* · *Novipirellula artificiosorum* · *Novipirellula galeiformis* · *Planctomycetaceae* · Baltic Sea · Rosettes · Heligoland · *Rhodopirellula* · *rpoB* gene

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Introduction

Planctomycetes are Gram-negative bacteria belonging to the PVC superphylum (Spring et al. 2016; Wagner and Horn 2006). In addition to Planctomycetes, the environmentally, medically and biotechnologically relevant PVC superphylum consists of the phyla Verrucomicrobia, Lentisphaerae, Kiritimatiellaeota,

Candidatus Omnitrophica and Chlamydiae. Planctomycetes are ubiquitous microorganisms often found in aquatic environments, in which they play major roles in global carbon and nitrogen cycles. One important example are Planctomycetes capable of performing anaerobic ammonia oxidation (anammox) (Strous et al. 1999). By converting large amounts of ammonium to dinitrogen gas, anammox Planctomycetes find industrial applications for *N*-elimination during wastewater treatment (Peeters and van Niftrik 2018).

Planctomycetes share some characteristic morphological traits (Fuerst and Sagulenko 2011; König et al. 1984; Lonhienne et al. 2010) and were initially believed to link bacteria and eukaryotes (Fuerst and Sagulenko 2011). This picture changed with the advent of novel (super resolution) microscopic techniques and detailed physiological analyses of Planctomycetes (Jeske et al. 2015; Jogler et al. 2011; Jogler and Jogler 2013; Rivas-Marin et al. 2016; Van Teeseling et al. 2015), based on which the cell envelope architecture of Planctomycetes was classified as Gram-negative (Boedeker et al. 2017; Devos 2014). Nevertheless, Planctomycetes remain exceptional. For example, they divide by budding, binary fission or even a combination of both and lack proteins of the canonical divisome (such as FtsZ) (Wiegand et al. 2019). It was also found that many strains are resistant to several antibiotics (Cayrou et al. 2010; Godinho et al. 2019), either because of their degradation, intrinsic resistance or lack of targets (Jogler et al. 2012; Pilhofer et al. 2008).

Planctomycetes are not only found in 'nutrient-rich' suspensions such as wastewater, but also dwell in oligotrophic environments, e.g. seawater (Bengtsson et al. 2012; Bondoso et al. 2015; Bondoso et al. 2014; Bondoso et al. 2017; Lage and Bondoso 2014; Vollmers et al. 2017). Since decades, planctomycetal research is driven by the question how those microorganisms manage to survive in such rather nutrient-poor environments. To enable biomass formation and propagation, several Planctomycetes, e.g. strains of the *Pirellula* clade, attach to biotic surfaces such as algae or kelp and consume dissolved organic carbon released from these surfaces (Bondoso et al. 2015; Wiegand et al. 2019). The first strain of the *Pirellula* clade was isolated in 1973 and named '*Pasteuria ramosa*' (Staley, 1973). Later, it was renamed first to '*Pirella staleyi*' and finally to the currently valid name *Pirellula staleyi* (Schlesner and Hirsch, 1984; Schlesner and Hirsch, 1987). Twelve years later, a second species, '*Pirella marina*' (now designated *Blastopirellula marina*), was isolated from brackish water of the Baltic Sea (Schlesner 1986). In a systematic analysis published in 2004, ninety-seven isolated budding strains and the above-mentioned species were classified in the already existing genus *Pirellula* and the novel genera *Rhodopirellula* and *Blastopirellula* based on DNA–DNA hybridization, physiological properties and chemotaxonomic tests (Schlesner et al. 2004). Morphological analyses took the mode of budding, bud shape, presence of fimbriae, crateriform structures and holdfast structure into account.

The overall analysis yielded important properties allowing to differentiate the type species of the three genera and comprise strain pigmentation (*Rhodopirellula baltica* is pink to red, *Pirellula staleyi* and *Blastopirellula marina* lack pigmentation), salt tolerance (*R. baltica* and *B. marina* tolerate high salinity conditions, *P. staleyi* shows only limited tolerance), substrate utilisation pattern and fatty acid composition (Schlesner et al. 2004). Another relevant criterion for differentiation is the relation to oxygen. Known *Rhodopirellula* and *Blastopirellula* species are strict aerobes, whereas some *Pirellula* species are also capable to grow under anoxic conditions (Ward et al. 2006).

In this study, we characterise the three strains Poly41^T, Q31b^T and Pla52o^T which were isolated from natural or artificial surfaces in the North Sea and the Baltic Sea and analysed their phylogeny as well as basic phenotypic and genotypic characteristics. Based on the performed classification and by including information on already described species we were able to redefine the proposed genus threshold of the phylogenetic marker *rpoB* for the family *Planctomycetaceae*.

Materials and methods

Isolation of the strains

Strain Q31b^T was sampled on the 5th of June 2013 from jellyfish (*Aurelia aurita*) on the north-western shore of Heligoland island in the North Sea (54.188 N, 7.875 E). Strain Poly41^T was isolated from polystyrene particles embedded in an incubator in the water of

the Baltic Sea for 14 days in 2 m depth at Heiligendamm Pier, Germany (54.146 N, 11.843 E). Collection date was the 8th of October 2015, when the incubator was removed from the water. Strain Pla52o^T was isolated as described for strain Poly41^T, but pieces of wood were embedded in the incubator instead of polystyrene particles. The incubation took place at exactly the same location (54.146 N, 11.843 E), but 1 year earlier (sampling date: 4. September 2014). Strains were isolated from the artificial (polystyrene) or natural (wood, jellyfish) sampling material as described in detail before (Wiegand et al. 2019). Initial amplification and sequencing of the 16S rRNA gene was performed as previously described (Rast et al. 2017). For subsequent cultivations M1 medium with HEPES as buffering agent and additionally supplemented with *N*-acetyl glucosamine (NAG) and artificial seawater (ASW) was used (designated M1H NAG ASW). Medium preparation was described previously (Kallscheuer et al. 2019a).

Light microscopy and scanning electron microscopy

Phase contrast microscopy and field emission scanning electron microscopy were performed according to protocols published earlier (Kallscheuer et al. 2019a).

Genome analysis

Genome information of the three isolated strains is available from GenBank under accession numbers SJPV00000000 (Poly41^T), SJPY00000000 (Q31b^T) and SJPT00000000 (Pla52o^T). The corresponding 16S rRNA gene sequences are also available from GenBank under accession numbers MK554551 (Poly41^T), MK554555 (Q31b^T) and MK554549 (Pla52o^T). Completeness and contamination of the genomes was determined using CheckM v1.0.131 (Parks et al. 2015). 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. The carbohydrate active enzymes were determined by employing dbCAN2 (Zhang et al. 2018), which automatically mines the CAZy database (Lombard et al. 2014).

Physiological analysis

For determination of the pH optimum 100 mM HEPES was used for cultivations at pH 7.0, 7.5 and 8.0. For cultivations at pH 5.0 and 6.0 HEPES was replaced by 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), whereas 100 mM *N*-cyclohexyl-2-aminoethane-sulfonic acid (CHES) served as a buffering agent at pH 9.0 and 10.0. Cultivations for determination of the pH optimum were performed at 28 °C in triplicates. For determination of the temperature optimum the strains were cultivated at temperatures ranging from 10 to 40 °C in standard M1H NAG ASW medium at pH 7.5 in triplicates.

Phylogenetic analysis

16S rRNA gene phylogeny was computed for strains Poly41^T, Q31b^T and Pla52o^T, the type strains of all described planctomycetal species (assessed in August 2019) and all isolates recently published (Wiegand et al. 2019), including the strains described recently (Boersma et al. 2019; Kallscheuer et al. 2019a, b, c; Kohn et al. 2019; Peeters et al. 2019). The 16S rRNA gene sequences were aligned with SINA (Pruesse et al. 2012). The phylogenetic analysis was done with RAxML (Stamatakis 2014) employing a maximum likelihood approach with 1000 bootstraps, the nucleotide substitution model GTR, gamma distributed rate variation and estimation of proportion of invariable sites (GTRGAMMAI option). Three 16S rRNA genes of bacterial strains from the PVC superphylum were used as outgroup. The *average nucleotide identity* (ANI) was calculated using OrthoANI (Lee et al. 2016) and the *average amino acid identity* (AAI) was calculated using the aai.rb script of the enveomics collection (Rodriguez-R and Konstantinidis 2016). The *percentage of conserved proteins* (POCP) was calculated as described (Qin et al. 2014). The *rpoB* nucleotide sequences were taken from the above mentioned as well as other publicly available genome annotations and the sequence identities were determined as described (Bondoso et al. 2013). Upon extracting only those parts of the sequence that would have been sequenced with the described primer set the alignment and matrix calculation was done with Clustal Omega (Sievers et al. 2011). For the multilocus sequence analysis (MLSA) the unique single-copy core genome of all analysed genomes was

determined with proteinortho5 (Lechner et al. 2011) with the 'selfblast' option enabled. The protein sequences of the resulting orthologous groups were aligned using MUSCLE v.3.8.31 (Edgar 2004). After clipping, partially aligned *C*- and *N*-terminal regions and poorly aligned internal regions were filtered using Gblocks (Castresana 2000). The final alignment was concatenated and clustered using the maximum likelihood method implemented by RAxML (Stamatakis 2014) with the 'rapid bootstrap' method and 500 bootstrap replicates.

Results and discussion

Phylogenetic analysis

The phylogenetic positions of the three strains Poly41^T, Q31b^T and Pla52o^T within the planctomycetal phylum were determined by 16S rRNA gene and MLSA analysis (Fig. 1). In the phylogenetic trees, the strains form a monophyletic cluster together with *Rhodopirellula rosea* LHWP3^T, *Rhodopirellula maiorica* SM1 and *Rhodopirellula caenicola* YM26-125^T (Richter et al. 2014; Roh et al. 2013; Yoon et al. 2014). Similarity of the 16S rRNA gene (Fig. 2, Table S1) shows that all strains of this cluster yield identity values of 94.5% or above, indicating that all strains belong to the same genus (Yarza et al. 2014). However, when including other described *Rhodopirellula* species (*Rhodopirellula baltica*, *Rhodopirellula lusitana*, *Rhodopirellula europaea*, *Rhodopirellula islandica*, *Rhodopirellula bahusiensis*, *Rhodopirellula sallentina* and *Rhodopirellula rubra*) into the analysis, minimal 16S rRNA gene identity values of 92.9% are observed. These values below the genus threshold are not only found when the novel strains described in this study are included, but also when analysing already published species. For example, *R. lusitana* and *R. rosea* share a 16S rRNA gene identity of 93.0%, indicating a relation on family-level rather than on genus-level. These findings suggest that the genus *Rhodopirellula* was defined too broadly.

Based on the 16S rRNA gene identity values, we propose that the novel strains Poly41^T, Q31b^T and Pla52o^T form the novel genus *Novipirellula* gen. nov. and that *R. rosea* LHWP3^T, *R. maiorica* SM1 and *R. caenicola* YM26-125^T should be transferred to this novel genus. Supported by a minimal 16S rRNA gene identity of 95.4%, *R. baltica*, *R. lusitana*, *R. europaea*, *R. islandica*, *R. bahusiensis*, *R. sallentina* and *R. rubra* should remain in the now more strictly defined *Rhodopirellula* genus.

When comparing two-way AAI values for all strains described as *Rhodopirellula* and the novel isolates (Table S2), the minimal obtained value is 50.5%. The same analysis for the newly defined *Rhodopirellula* and *Novipirellula* genera gives minimal AAI values of 57.8% and 58.4%, respectively. Although all of these values are below the proposed AAI genus threshold of 60–80%, only the latter are still well within the ranges found for different bacterial genera (Luo et al. 2014). A similar pattern is found for POCP (Table S3). The larger clade comprising all strains has a minimal POCP of 44.1%, a value distinctly below the proposed genus threshold of 50% (Qin et al. 2014). The now smaller *Rhodopirellula* taxon and the *Novipirellula* reach higher minimal POCP values of 54.1% and 47.5%, respectively, which are either above or at least closer to the proposed border. In brief, the separation of the current genus *Rhodopirellula* to a more strictly defined genus *Rhodopirellula* and the novel genus *Novipirellula* is supported on genomic level.

While strains *R. rosea* LHWP3^T, *R. caenicola* YM26-125^T and *R. maiorica* SM1 are members of the novel genus, the latter is not a validly described species and is therefore excluded from more detailed taxonomic analyses. For the two former and our three isolates Poly41^T, Q31b^T and Pla52o^T, the identities of the 16S rRNA gene sequences range between 94.5% and 98.1% (Fig. 2). All values are below the species threshold of 98.7% (Stackebrandt and Ebers 2006), indicating that all five strains, including the novel isolates, represent individual species belonging to the same genus. For the novel strains with sequenced genomes, this finding is supported by ANI values (Fig. 2) unambiguously below the species threshold of 95% (Kim et al. 2014).

New thresholds for *rpoB* as marker gene

Previously, the comparison of a partial sequence of the *rpoB* gene encoding the β-subunit of RNA polymerase was proposed as reliable molecular marker to infer phylogeny of species of the order *Planctomycetales* (Bondoso et al. 2013). A threshold value of 72%

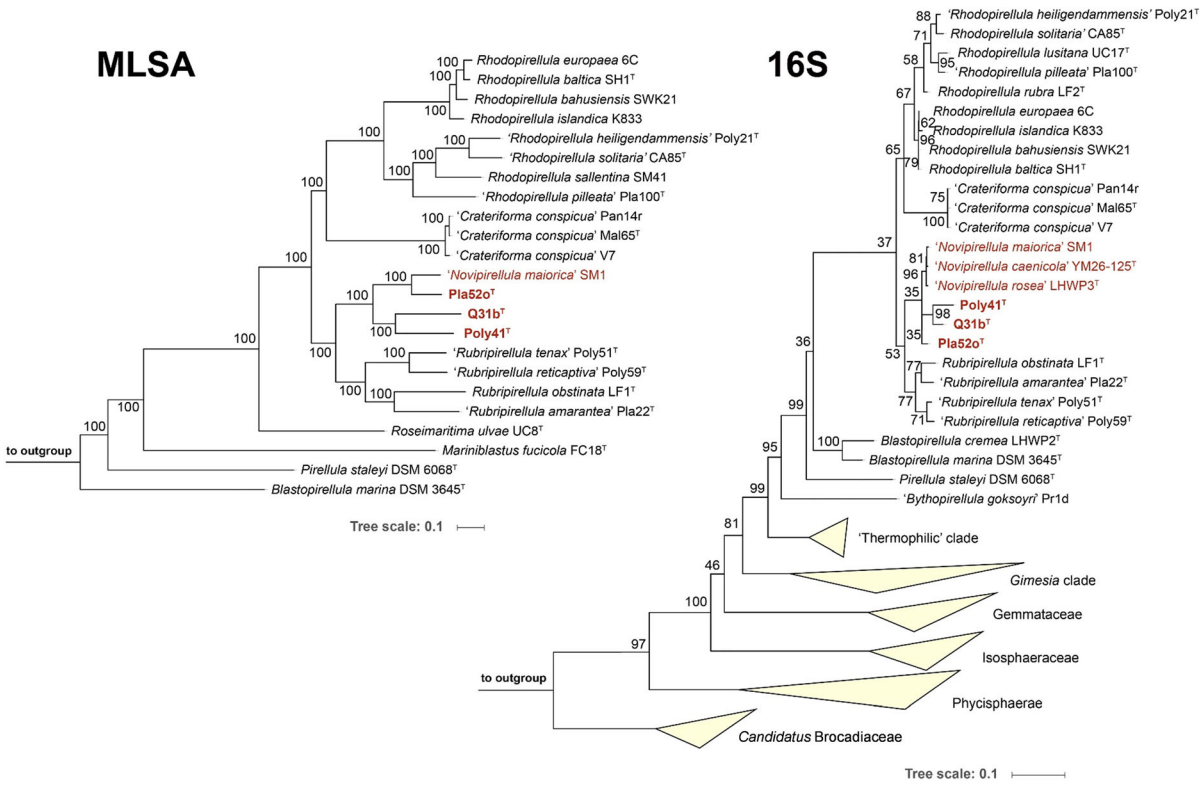

Fig. 1 MLSA- and 16S rRNA gene-based phylogeny. The phylogenetic trees show the position of the three here described strains in relation to their closest described relatives. Analysis was performed as described in the "Materials and methods" section. 16S rRNA gene phylogeny and MLSA were computed using the maximum likelihood method. Bootstrap values after 1000 re-samplings (16S rRNA gene)/500 re-samplings (MLSA) are given at the nodes (in %). The outgroup consists of three 16S rRNA genes from the PVC superphylum. In case of the MLSA tree '*Bythopirellula goksoyri*' Pr1d served as outgroup

identity was proposed for differentiating between genera of this order. To a certain extent, this analysis was built on strains of the now split genus *Rhodopirellula.* To adapt the threshold values to the reclassified taxa as well as to include all strains belonging to the family *Planctomycetaceae* recently published (Wiegand et al. 2019), we re-evaluated the partial *rpoB* sequence used earlier (Bondoso et al. 2013) (Table S4). Based on this analysis, we here propose a new genus threshold range of 75.5–78.0% partial *rpoB* sequence identity. The novel genus *Novipirellula* and the re-defined genus *Rhodopirellula* are also supported by this threshold. The *rpoB*-based species threshold found has not been affected by our analysis (Bondoso et al. 2013).

Morphological and physiological analysis

We employed both light microscopy (Fig. 3) and scanning electron microscopy (Fig. 4) to analyse the morphologies of Poly41^T, Q31b^T and Pla52o^T cells during the exponential growth phase. Detailed information on morphology and cell division is summarised in Table 1 and compared to closely related strains.

Cells of strain Poly41^T have a size of $2.0 \pm 0.4 \times 1.0 \pm 0.2$ µm (Fig. 3c) and show a characteristic acorn-shape with a polar 'fibre cap' covering up to 40% of the cell surface. Cells either live planktonic or form small rosettes of 3–8 cells (Fig. 4b). Division occurs by polar budding with the daughter cell having a round shape. Crateriform structures and flagella were observed. Colonies have a light pink colour. The preferred temperature and pH

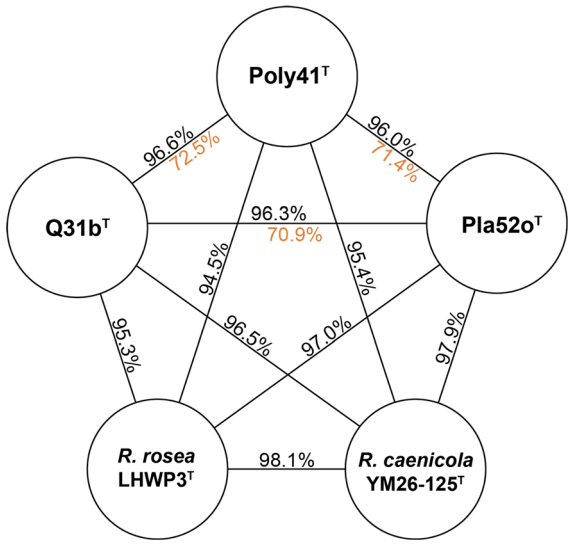

Fig. 2 Phylogeny of the novel genus comprising strains Q31b^T, Poly41^T and Pla52o^T as well as the former *Rhodopirellula* strains *R. rosea* LHWP3^T and *R. caenicola* YM26-125^T. The black font gives the 16S rRNA gene identity and the orange font gives the whole genome-based *average nucleotide identity* (ANI) values. Comparison of *rpoB* similarity values could not be performed for *R. rosea* LHWP3^T and *R. caenicola* YM26-125^T as their genome sequences are not available

are 27 °C and 7.5, respectively, while growth in a range of 15–27 °C and of pH 6.5–8.5 was observed (Fig. 5). Growth is thus mesophilic and neutrophilic. A maximal growth rate of 0.039 h^1 (generation time of 18 h) was observed during cultivation.

Q31b^T has a similar cell shape as Poly41^T but the cells are slightly smaller (1.6 ± 0.3 × 0.8 ± 0.1 µm) (Fig. 3f). Aggregate formation is more pronounced for Q31b^T as aggregates consisting of 10 to more than 50 individual cells were found (Fig. 4d, e). Mode of division and shape of the daughter cell are comparable to Poly41^T. The temperature optimum at 30 °C and the pH optimum at pH 7.5 are also similar to Poly41^T, but growth of strain Q31b^T was observed in a distinctly broader temperature (10–36 °C) and pH range (pH 5.5–9.0). The maximal growth rate of 0.018 h^{-1} (generation time of 39 h) was about half of the maximal growth rate of Poly41^T (Fig. 5).

Cells of strain Pla52o^T have the same colour, size and mode of division as Poly41^T. The cell shape also resembles an acorn, but cells appear elongated and more strongly deformed during SEM analysis compared to Poly41^T and Q31b^T (Fig. 4g). The number of cells per aggregate (typically 10–30) was more uniform compared to the other two strains. Pla52o^T showed the same temperature and pH profile as Q31b^T and, with μ_{max} = 0.078 h^{-1} (generation time of 9 h), the fastest growth of the three isolates (Fig. 5).

With regard to shape and temperature/pH preferences there are only slight differences when comparing the isolated strains with the related species '*Novipirellula maiorica*' SM1, '*Novipirellula rosea*' LHWP3^T and '*Novipirellula caenicola*' YM26-125^T (Table 1). The already published strains are slightly smaller and have more roundish shape than the strains described in this study. The degree of pigmentation within the genus *Novipirellula* varies from white to red.

Genomic characteristics

The genomic characteristics of the three isolated strains are summarised in Table 1. As '*N. rosea*' LHWP3^T and '*N. caenicola*' YM26-125^T have not been sequenced yet, obtained genome data could only be compared to '*N. maiorica*' SM1. All strains have a similar G + C content of 53–58%, but differ significantly in their genome sizes. While Q31b^T and Pla52o^T have a genome size of 7.3–7.4 Mb, about 20–25% larger genomes are present in Poly41^T and '*N. maiorica*' SM1 (9.2 and 8.9 Mb, respectively). These differences in genome size are not reflected in the strain relationships as Poly41^T clustered with Q31b^T in our phylogenetic analysis and both are more distantly related to Pla52o^T. Larger genomes correlated with higher numbers of open reading frames considering that all strains had similar coding densities (86–89%) and protein-coding genes per Mb (770–823). The number of tRNA genes is identical for Poly41^T and Q31b^T (78 tRNA genes), but is significantly lower in Pla52o^T (51 tRNA genes) and higher in '*N. maiorica* SM1' (95 tRNA genes). All sequenced strains harbour one copy of the 16S rRNA gene.

Genome-based analysis of the central carbon metabolism

Based on the genome sequences of the three novel isolates and of '*N. maiorica* SM1' for comparison we investigated the presence of pathways representing the microbial central carbon metabolism (Table 2). A complete set of genes coding for glycolytic enzymes

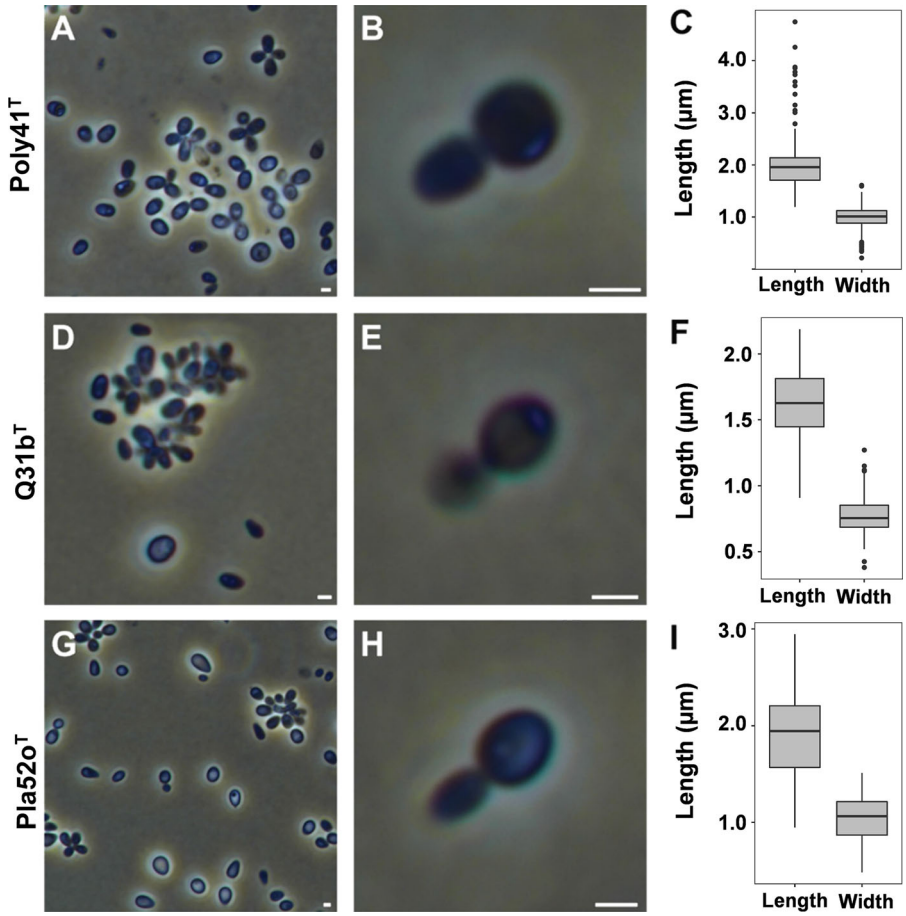

Fig. 3 Light microscopy images and cell size plots of strains Poly41^T, Q31b^T and Pla52o^T. The figure highlights the mode of cell division (**a**, **c**, **e**) and gives a general overview of cell morphology (**b**, **d**, **f**). The scale bar is 1 µm. Cell sizes (**g**, **h**, **i**) of at least 100 representative cells were counted manually or by using a semi-automated object count tool

of the common Embden–Meyerhof–Parnas pathway is present in all strains, while also hits for key enzymes of the Entner–Doudoroff pathway were found. Additionally, degradation of sugars via the pentose phosphate pathway appears to be possible in all four strains. This was somehow expected as important precursors for the biosynthesis of amino acids and nucleotides branch off from this pathway and an incomplete or inactive pentose phosphate pathway would be associated to multiple auxotrophies. Our analysis further suggests that all strains harbour a complete TCA cycle, except for Pla52o^T, in which we could not identify the gene encoding isocitrate dehydrogenase. This, however, might be explained by the non-closed genomes with several scaffolds. In contrast to many other bacteria, *Novipirellula* strains seem to lack the glyoxylate shunt, which is typically utilised as anaplerotic pathway during growth on acetate or for enabling biomass formation from acetyl-CoA obtained e.g. during β-oxidation of fatty acids. *Novipirellula* species might utilise alternative pathways for biomass formation from acetate or are unable to use acetate or fatty acids as sole carbon and energy source. The capability of de novo glucose biosynthesis should be given as genes required for a functional gluconeogenesis pathway are present.

Monomeric sugars as easily degradable carbon sources are typically not found in larger amounts in oligotrophic environments which Planctomycetes inhabit. For enabling breakdown of complex polysaccharides obtained from biotic surfaces, Planctomycetes harbour larger sets of glycolytic enzymes, which convert complex and decorated polysaccharides to monomeric units, which can then be channelled into

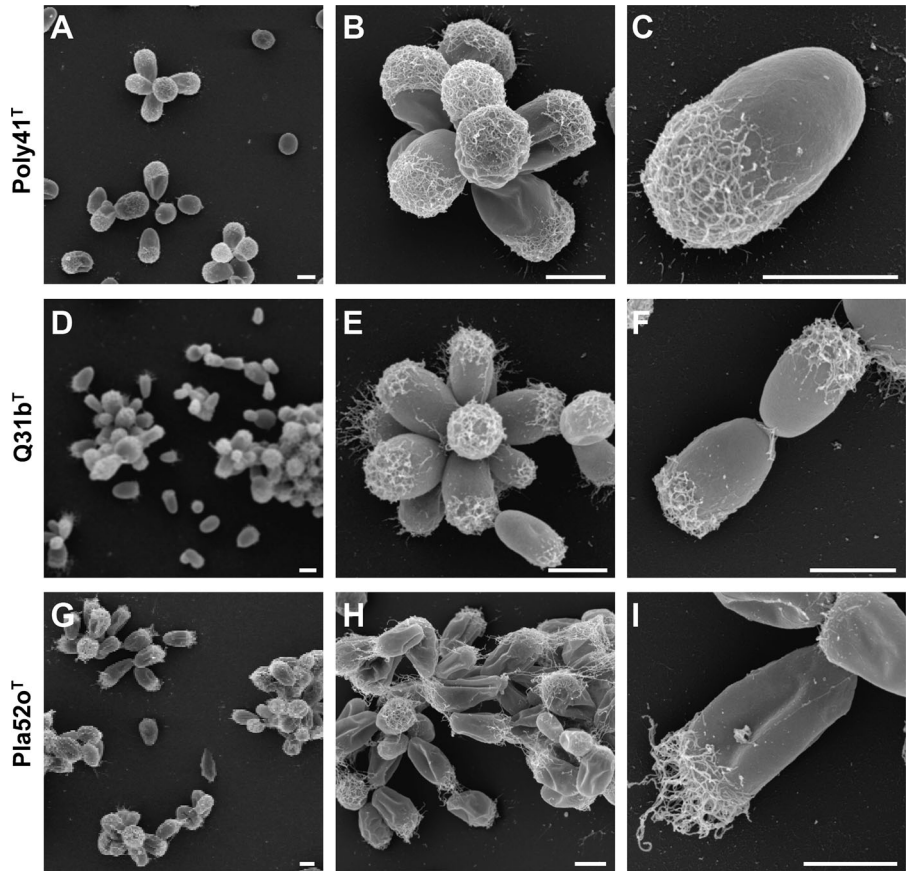

Fig. 4 Scanning electron microscopic pictures of strains Poly41^T, Q31b^T and Pla52o^T. The scale bar is 1 μm

the central carbon metabolism. For getting a first insight into the enzymatic set of *Novipirellula* strains, we analysed the most common classes of such glycolytic enzymes and compared the numbers of predicted enzymes in the four strains (Table 3). In our analysis, Poly41^T clearly stands out. With over 500 predicted glycolytic enzymes (of which 329 are putative glycoside hydrolases) it has by far the highest number compared to the other three strains, which have 230–330 putative glycolytic enzymes. These numbers also correlate with the genome size as Poly41^T also has the largest genome (9.20 Mb) of the investigated strains. '*N. maiorica*' SM1 (8.87 Mb) has the second largest genome and the total number of glycolytic enzymes is above 300, whereas it is below 300 for the other two strains. Taken together, a high number of glycolytic enzymes suggests large potential of *Novipirellula* strains for degradation of a broad range of different polymeric compounds with different sugar compositions and decorations of the sugar units.

Genes potentially involved in secondary metabolite production

For assessing the relevance of the three characterised strains as potential sources of novel secondary metabolites we performed AntiSMASH analyses based on the genome sequence of Poly41^T, Q31b^T and Pla52o^T and *N. maiorica* SM1 (Blin et al. 2019). In most cases, secondary metabolites are produced by dedicated enzymes of the families of polyketide synthases (PKS) or non-ribosomal peptide synthetases (NRPS). PKSs use coenzyme A (CoA)-activated carboxylic acids as substrates and often malonyl-CoA for carbon chain elongation while NRPSs condense proteinogenic or non-proteinogenic amino acids. We cannot exclude that Planctomycetes follow additional, yet unknown, strategies for secondary metabolite production, however relevant proteins most likely escaped the AntiSMASH analysis. According to our analysis, all four strains harbour

Table 1 Phenotypic and genotypic features of the three characterized strains in comparison to *N. maiorica* SM1 (Frank 2011; Winkelmann and Harder 2009), *N. rosea* LHWP3^T (Roh et al. 2013) and *N. caenicola* YM26-125^T (Yoon et al. 2014)

Characteristics	Poly41^T	Q31b^T	Pla52o^T	SM1	LHWP3^T	YM26-125^T
Phenotypic features						
Size	2.0 × 1.0 µm	1.6 × 0.8 µm	1.9 × 1.0 µm	1.2–1.6 × 1.0–1.4 µm	0.6–1.5 × 0.6–1.4 µm	1.0–1.1 µm (diameter)
Shape	Acorn-shaped	Acorn-shaped	Acorn-shaped	Almost round	Ovoid	Spherical
Colony colour	Light pink	Lucid white	Light pink	Light pink	Pink to red	Pink
Motility	Yes	Yes	Yes	Yes	Yes	Yes
Aggregates	Yes	Yes	Yes	Yes	n/a	n/a
Division	Budding	Budding	Budding	Budding	Budding	Budding
pH optimum	7.5	7.5	7.5	7.5–8.0	7.0	7.0
pH range	6.5–8.5	5.5–9.0	6.0–9.0	6.5–10.0	6.0–8.0	6.0–8.0
Temperature optimum	27 °C	30 °C	30 °C	28–32 °C	30 °C	28 °C
Temperature range	15–27 °C	10–36 °C	10–36 °C	16–37 °C	20–37 °C	20–30 °C
Genotypic features						
Genome size [bp]	9,199,719	7,267,653	7,403,604	8,874,084	n/a	n/a
Plasmids	n.d.	n.d.	n.d.	n.d.	n/a	n/a
G+C [%]	55.3 ± 1.6	52.9 ± 2.2	55.8 ± 2.2	54.7	53.0	57.5
Completeness [%]	98.28	98.28	97.41	97.41	n/a	n/a
Contamination [%]	2.87	1.72	1.72	2.04	n/a	n/a
Protein-coding genes	7088	5810	5751	7458	n/a	n/a
Hypothetical proteins	3039	2681	2562	4558	n/a	n/a
Protein-coding genes/Mb	770	799	777	823	n/a	n/a
Coding density [%]	88.6	85.8	84.8	86.4	n/a	n/a
16S rRNA genes	1	1	1	1	n/a	n/a
tRNA genes	78	78	51	95	n/a	n/a

n.d. not detected, *n/a* not available

two genes involved in terpenoid production (Table 3). None of the strains encodes an NRPS, however two putative mixed type I PKS-NRPS proteins are encoded in Poly41^T and Q31b^T. One or two putative type I PKS genes were identified in all strains except Pla52o^T, no type II PKSs are present, and a single putative type III PKS is encoded by all strains except '*N. maiorica*' SM1 (Table 3). Clusters involved in the production of bacteriocins, resorcinol or ectoine were not identified in the four genomes. Q31b^T and Poly41^T encode the same set of identified clusters involved in secondary metabolite production, which differs from the sets encoded in Pla52o^T and '*N. maiorica*' SM1. This observation also reflects the close relationship of Poly41^T and Q31b^T compared to Pla52o^T and '*N.*

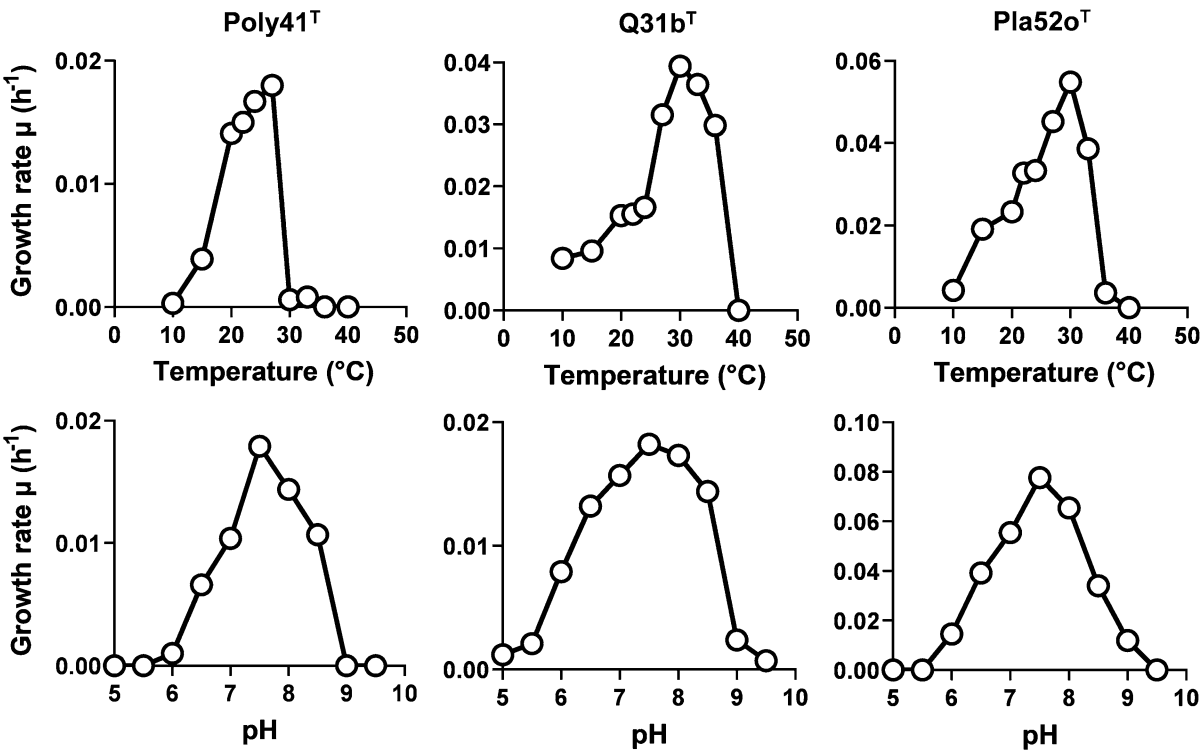

Fig. 5 Temperature and pH profiles of strains Poly41^T, Q31b^T and Pla52o^T. In the upper panel, the given data points show the average growth rates obtained after cultivation of the three isolated strains in M1H NAG ASW medium in biological triplicates at different temperatures and a constant pH of 7.5. In the bottom panel, the data points show the average growth rates for cultivation at different pH values at a constant temperature of 28 °C

maiorica' SM1, which is in line with the obtained results during the phylogenetic analysis.

Conclusion

Differentiation of the genera *Pirellula*, *Rhodopirellula*, *Blastopirellula* and *Novipirellula*

In addition to the genetic differentiation of the genera *Pirellula*, *Rhodopirellula*, *Blastopirellula* and *Novipirellula* based on different phylogenetic markers, attention should also be given to major differences in physiological features separating the respective type species of the genera from each other. Key physiological features are compared in Table 4. Major differences can be observed in cell size, strain pigmentation, relation to oxygen, salinity and polar lipid composition. Major fatty acids are very similar when comparing the type species of the four genera, but utilisation pattern of sugars could be used for strain differentiation, e.g. when investigating growth on D-mannitol/gluconate (*N. rosea*: +/−, *R. baltica*: −/+, *B. marina*: +/+, *P. staleyi*: −/−).

Based on our physiological and phylogenetic analysis of three novel isolates and of the transferred strains, we here introduce the novel genus *Novipirellula* gen. nov., which is separated from the genera *Rhodopirellula*, *Blastopirellula* and *Pirellula* by phylogenetic and physiological differences. We propose the names *Novipirellula artificiosorum* Poly41^T, *Novipirellula aureliae* Q31b^T and *Novipirellula galeiformis* Pla52o^T for the novel isolates. Our analyses suggest that the strains *Rhodopirellula maiorica* SM1, *Rhodopirellula rosea* LHWP3^T and *Rhodopirellula caenicola* YM26-125^T also belong to the novel genus *Novipirellula* gen. nov. and are thus reclassified.

Description of *Novipirellula* gen. nov.

Novipirellula (No.vi.pi.rel'lu.la. L. masc. adj. *novus* new; N.L. fem. n. *Pirellula* name of a bacterial genus;

Table 2 Genome-based primary metabolism of '*Novipirellula*' strains

Enzyme/reaction	EC number	Gene	Poly41^T	Pla52o^T	Q31b^T	SM1
Glycolysis						
Glucose-6-phosphate isomerase	5.3.1.9	*pgi*	Poly41_39540	Pla52o_37390	Q31b_23710	y
ATP-dependent 6-phosphofructokinase isozyme 1	2.7.1.11	*pfkA*	Poly41_33420	Pla52o_19050	Q31b_12530	y
			Poly41_08980		Q31b_45220	
			Poly41_33940		Q31b_14420	
Fructose-bisphosphate aldolase class 2	4.1.2.13	*fbaA*	Poly41_32850	Pla52o_08790	Q31b_09390	y
Triosephosphate isomerase	5.3.1.1	*tpiA*	Poly41_45600	Pla52o_44210	Q31b_00280	y
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*gapA*	Poly41_02070	Pla52o_28390	Q31b_04350	y
Phosphoglycerate kinase	2.7.2.3	*pgk*	Poly41_34820	Pla52o_20530	Q31b_06530	y
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	5.4.2.12	*gpmI*	Poly41_05930	Pla52o_07430	Q31b_56810	y
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	5.4.2.11	*gpmA*	n	Pla52o_36260	n	n
Enolase	4.2.1.11	*eno*	Poly41_10590	Pla52o_47060	Q31b_12580	y
Pyruvate kinase I	2.7.1.40	*pykF*	Poly41_03360	Pla52o_01890	Q31b_56760	y
			Poly41_53400			
Pyruvate dehydrogenase E1 component	1.2.4.1	*aceE*	Poly41_00990	Pla52o_37720	Q31b_56200	y
				Pla52o_30900	Q31b_34340	
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	2.3.1.12	*aceF*	Poly41_00980	Pla52o_37730	Q31b_56210	y
Gluconeogenesis						
Phosphoenolpyruvate carboxylase	4.1.1.31	*ppc*	Poly41_51550	n	Q31b_20640	y
Phosphoenolpyruvate synthase	2.7.9.2	*ppsA*	n	Pla52o_39950	Q31b_19450	n
Pyruvate, phosphate dikinase	2.7.9.1	*ppdK*	Poly41_47710	Pla52o_20240	Q31b_11930	n
Phosphoenolpyruvate carboxykinase (ATP)	4.1.1.49	*pckA*	n	Pla52o_54610		y
Phosphoenolpyruvate carboxykinase [GTP]	4.1.1.32	*pckG*	Poly41_43060	n	Q31b_45170	n
Fructose-1,6-bisphosphatase class 2	3.1.3.11	*glpX*	n	n	n	n
Fructose-1,6-bisphosphatase class 1	3.1.3.11	*fbp*	n	n	n	n
Pyrophosphate–fructose 6-phosphate 1-phosphotransferase	2.7.1.90	*pfp*	Poly41_53920	n	Q31b_04120	y
Pentosephosphate pathway						
Glucose-6-phosphate 1-dehydrogenase	1.1.1.49	*zwf*	Poly41_25190	Pla52o_19090	Q31b_46640	y
6-phosphogluconolactonase	3.1.1.31	*pgl*	Poly41_66660	Pla52o_45470	Q31b_40750	y
				Pla52o_32360		
				Pla52o_49640		
6-phosphogluconate dehydrogenase, decarboxylating	1.1.1.44	*gndA*	Poly41_59500	Pla52o_10120	Q31b_21120	y
Transketolase 2	2.2.1.1	*tktB*	Poly41_50790	Pla52o_09450	Q31b_37030	y
Transaldolase B	2.2.1.2	*talB*	candidates	Pla52o_33940	Q31b_21600	y
Entner-Doudoroff pathway						
KHG/KDPG aldolase	4.1.2.14	*eda*	Poly41_18820	Pla52o_18880	Q31b_04240	y
			Poly41_06450			
			Poly41_06820			
Phosphogluconate dehydratase	4.2.1.12	*edd*	Poly41_32860	Pla52o_57770	n	y
				Pla52o_08800		
TCA cycle						
Citrate synthase	2.3.3.16	*gltA*	Poly41_31360	Pla52o_47280	Q31b_30910	y
					Q31b_10950	

Table 2 continued

Enzyme/reaction	EC number	Gene	Poly41^T	Pla52o^T	Q31b^T	SM1
Aconitate hydratase A	4.2.1.3	*acnA*	Poly41_33530	Pla52o_07730	Q31b_11020	y
Isocitrate dehydrogenase [NADP]	1.1.1.42	*icd*	Poly41_65950	n	Q31b_03250	y
2-oxoglutarate dehydrogenase E1 component	1.2.4.2	*sucA*	Poly41_15470	Pla52o_45880	Q31b_12240	y
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	2.3.1.61	*sucB*	Poly41_38060	Pla52o_42890	Q31b_48870	y
Succinate–CoA ligase [ADP-forming] subunit alpha	6.2.1.5	*sucD*	Poly41_44000	Pla52o_25970	Q31b_06690	y
Succinate–CoA ligase [ADP-forming] subunit beta		*sucC*	Poly41_44010	Pla52o_25980	Q31b_06700	y
Succinate dehydrogenase flavoprotein subunit	1.3.5.1	*sdhA*	Poly41_52750	Pla52o_00540 Pla52o_14540	Q31b_46150	y
Succinate dehydrogenase iron-sulfur subunit		*sdhB*	Poly41_52760	Pla52o_14530	Q31b_46160	y
Succinate dehydrogenase cytochrome b556 subunit		*sdhC*	Poly41_52740	Pla52o_14550	Q31b_46140	y
Succinate dehydrogenase hydrophobic membrane anchor subunit		*sdhD*	n	n	n	n
Fumarate hydratase class I, an/aerobic	4.2.1.2	*fumA/B*	Poly41_44550	n	Q31b_28280	n
Fumarate hydratase class II	4.2.1.2	*fumC*	n	Pla52o_06710	Q31b_30360	y
Malate dehydrogenase	1.1.1.37	*mdh*	Poly41_31810	Pla52o_48950	Q31b_38290	y
			Poly41_17160	Pla52o_08390	Q31b_36540	
Glyoxylate shunt						
Isocitrate lyase	4.1.3.1	*aceA*	n	n	n	n
Malate synthase G	2.3.3.9	*glcB*	n	n	n	n

For the novel isolates the locus tag is given whenever a gene was identified. Presence of a gene in strain SM1 is indicated by 'y' and absence is indicated by 'n'. The analysis was based on the genomes with the following accession numbers: Poly41^T: SJPV00000000, Pla52o^T: SJPT00000000, Q31b^T: SJPY00000000, SM1: NZ_ANOG00000000

Table 3 Genome-based analysis of sugar catabolic enzymes and secondary metabolism

Numbers of putative enzymes involved in the degradation of sugars or of gene clusters involved in biosynthesis of secondary metabolites are shown. The analysis was based on the genomes with the following accession numbers: Poly41^T: SJPV00000000, Pla52o^T: SJPT00000000, Q31b^T: SJPY00000000, SM1: NZ_ANOG00000000

Enzyme class	Poly41^T	Pla52o^T	Q31b^T	SM1
Catabolism of sugars				
Carbohydrate esterases	41	43	25	45
Carbohydrate-binding	36	34	17	49
Glycoside hydrolases	329	66	157	119
Glycosyl transferases	84	76	76	106
Polysaccharide lyases	15	5	8	4
Auxiliary activities	4	3	2	2
	509	227	285	325
Secondary metabolite clusters				
Terpenoid	2	2	2	2
Type I PKS	1	0	1	2
Type II PKS	0	0	0	0
Type III PKS	1	1	1	0
NRPS	0	0	0	0
Type I PKS-NRPS	2	0	2	0
Total number of clusters	6	3	6	4

Table 4 Comparison of physiological characteristics of the type species of the genera *Novipirellula*, *Rhodopirellula*, *Blastopirellula* and *Pirellula*

Characteristic	*Novipirellula rosea* LHWP3^T	*Rhodopirellula baltica* SH1^T	*Blastopirellula marina* DSM 3645^T	*Pirellula staleyi* DSM 6068^T
Cell size	0.6–1.5 × 0.6–1.4	1.0–2.5 × 1.2–2.3	1.0–2.0 × 0.7–1.5	1.0–1.5 × 0.9–1.0
Pigmenation	Pink to red	Pink to red	Unpigmented	Unpigmented
Motility	Yes	Yes	Yes	Yes
Salinity	0–7% NaCl (w/v)	12–200% ASW	12–175% ASW	0–50% ASW
Relation to oxygen	Strictly aerobic	Strictly aerobic	Strictly aerobic	Aerobic*
Temperature range	20–37 °C	Up to 32 °C	Up to 35 °C	18–30 °C
Temperature optimum	30 °C	28–30 °C	27–33 °C	n/a
Polar lipid: phosphatidylcholine	Yes	Yes	No	No
Major fatty acids	16:0 (37.3%), 18:1 ω9c (22.2%), 18:0 (16.2%)	16:0 (39.2%), 18:1 ω9c (40.8%)	16:0 (27.5%), 18:1 ω9c (26.6%)	16:0 (33.8%), 18:1 ω9c (26.6%), 17:1ω9c (14.4%)
Carbon source utilization				
D-glucose	+	+	+	+
D-fructose	+	+	+	−
D-galactose	+	+	+	+
D-lyxose	+	+	+	−
Sucrose	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+
D-mannose	+	+	+	+
Rhamnose	+	+	+	−
Ribose	+	+	+	−
D-xylose	+	+	+	−
Glycerol	−	+	+	−
D-mannitol	+	−	+	−
Gluconate	–	+	+	−
N-acetyl glucosamine	+	+	+	+
16S rRNA gene similarity to *N. rosea* (%)	100	94.8	89.3	85.4

(+): utilized, (−): not utilized; n/a not available; * some *Pirellula* strains also showed growth under anoxic conditions

Information was taken from the respective species description manuscripts (Schlesner and Hirsch 1984; Schlesner 1986; Schlesner et al. 2004; Roh et al. 2013)

N.L. fem. n. *Novipirellula* a new type of *Pirellula*). Species of this genus are Gram-negative, aerobic, mesophilic, neutrophilic and heterotrophic. Cells are ovoid or acorn-shaped, motile and divide by budding. Colony colours range from white to red. The G + C content is between 51 and 59%. We introduce *Novipirellula rosea* as type species of the genus as it was the first validly described species of this genus (Roh et al. 2013).

Description of *Novipirellula artificiosorum* sp. nov.

Novipirellula artificiosorum (ar.ti.fi.ci.o.so'rum. L. masc. adj. *artificiosus* artificial, not natural; N.L. gen. pl. n. *artificiosorum* of artificial, not natural things, referring to the isolation of the strain from plastic). Cells are acorn-shaped (length: 2.0 ± 0.4 μm, width: 1.0 ± 0.2 μm), form aggregates and divide by polar budding. Colonies have a light pink colour. The type

strain grows at ranges of 15–27 °C (optimum 27 °C) and at pH 6.5–8.5 (optimum 7.5). The type strain genome has a size of 9,199,719 bp and a G + C content of 55.3 ± 1.6%. The genome (acc. no. SJPV00000000) and 16S rRNA gene sequences (acc. no. MK554551) of the type strain are available from GenBank.

The type strain is Poly41^T (DSM 103145^T = VKM B-3437^T) isolated from polystyrene particles incubated in the Baltic Sea close to a landing stage in Heiligendamm, Germany.

Description of *Novipirellula aureliae* sp. nov.

Novipirellula aureliae (au.re'li.ae. N.L. gen. n. *aureliae* of *Aurelia*; corresponding to the isolation of the strain from the common jellyfish *Aurelia aurita*). Cells are acorn-shaped (length: 1.6 ± 0.3, width: 0.8 ± 0.1 µm), form aggregates and divide by polar budding. Lucid white colonies are formed. The temperature optimum of the type strain is 30 °C (growth observed from 10–36 °C). The preferred pH is 7.5, but growth is also observed at pH 5.5–9.0. The genome of the type strain has a size of 7,267,653 bp and a G + C content of 52.9 ± 2.2%. The type strain genome (acc. no. SJPY00000000) and 16S rRNA gene sequence (acc. no. MK554555) are available from the GenBank database.

The type strain is Q31b^T (DSM 103929^T = LMG 29701^T) isolated from jellyfish *Aurelia aurita* on the shore of the island Heligoland.

Description of *Novipirellula galeiformis* sp. nov.

Novipirellula galeiformis (ga.le.i.for'mis. L. fem. n. *galea* a helmet; L. suff. adj. *formis* a form, a figure; N.L. fem. adj. *galeiformis* shaped like a helmet; corresponding to the helmet-shaped formation of the fibres). Cells are acorn-shaped (1.9 ± 0.4 µm x 1.0 ± 0.2 µm), form aggregates and divide by polar budding. Colonies have a light pink colour. The preferred temperature and pH of the type strain are 30 °C and 7.5, respectively, while growth is observed in the range of 10–36 °C and at pH 6.0–9.0. The type strain genome has a size of 7,403,604 bp and a G + C content of 55.8 ± 2.2%. The genome (acc. no. SJPT00000000) and 16S rRNA gene sequence (acc. no. MK554549) are available from GenBank.

The type strain is Pla52o^T (DSM 103357^T = LMG 29744^T; synonym Pla52Original) isolated from wood incubated in the Baltic Sea in 2 m depth near Heiligendamm, Germany.

Description of *Novipirellula rosea* comb. nov.

Basonym: *Rhodopirellula rosea* Roh et al. 2014

Strain characteristics as described before (Roh et al. 2013). The type strain is LHWP3^T (KACC 15560^T = JCM 17759^T).

Description of *Novipirellula caenicola* comb. nov.

Basonym: *Rhodopirellula caenicola* Yoon et al. 2015

Strain characteristics as described before (Yoon et al. 2014). The type strain is YM26-125^T (KCTC 32995^T = NBRC 110016^T).

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Author contributions NK and SW wrote the manuscript, analysed data and prepared figures, AH, PR and MJ isolated the strains and performed the initial cultivation and strain deposition, SHP and CB performed the light microscopic analysis, MSMJ contributed to text preparation and revised the manuscript, MR performed the electron microscopic analysis, CJ, PR and AH took the samples in the Baltic Sea and on Heligoland, and CJ supervised the study. 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.

Ethical statement This article does not contain any studies with animals performed by any of the authors.

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