Antonie van Leeuwenhoek (2020) 113:1811–1822
https://doi.org/10.1007/s10482-019-01377-2

ORIGINAL PAPER

Blastopirellula retiformator sp. nov. isolated from the shallow-sea hydrothermal vent system close to Panarea Island

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Received: 2 October 2019 / Accepted: 11 December 2019 / Published online: 1 January 2020

Abstract Aquatic bacteria belonging to the deep-branching phylum *Planctomycetes* play a major role in global carbon and nitrogen cycles. However, their uncommon morphology and physiology, and their roles and survival on biotic surfaces in marine environments, are only partially understood. Access to axenic cultures of different planctomycetal genera is key to study their complex lifestyles, uncommon cell biology and primary and secondary metabolism in more detail. Here, we describe the characterisation of strain Enr8^T isolated from a marine biotic surface in the seawater close to the shallow-sea hydrothermal vent system off Panarea Island, an area with high temperature and pH gradients, and high availability of different sulphur and nitrogen sources resulting in a great microbial diversity. Strain Enr8^T showed typical planctomycetal traits such as division by polar budding, aggregate formation and presence of fimbriae and crateriform structures. Growth was observed at ranges of 15–33 °C (optimum 30 °C), pH 6.0–8.0 (optimum 7.0) and at NaCl concentrations from 100 to 1200 mM (optimum 350–700 mM). Strain Enr8^T forms white colonies on solid medium and white flakes in liquid culture. Its genome has a size of 6.20 Mb and a G + C content of 59.2%. Phylogenetically, the strain belongs to the genus *Blastopirellula*. We propose the name *Blastopirellula retiformator* sp. nov. for the novel species, represented by the type strain Enr8^T (DSM 100415^T = LMG 29081^T).

Keywords Marine bacteria · Planctomycetes · Hydrothermal area · Panarea · *Blastopirellula marina*

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Introduction

Members of the family *Planctomycetaceae,* which belong to the environmentally, medically and biotechnologically relevant PVC superphylum (*Planctomycetes-Verrucomicrobia-Chlamydiae*, and others) (Spring et al. 2016; Wagner and Horn 2006), are ubiquitous microorganisms dwelling mostly in aquatic environments, in which they play key roles in cycling of carbon and nitrogen. However, the mechanisms as to how these microorganisms gain nutrients from

biotic material in the oceans (in particular carbon and nitrogen for biomass and energy formation) are only partially understood. Species belonging to the family *Planctomycetaceae* are typically found to be attached to marine biotic surfaces, such as algae or kelp, and probably form biomass by using algal compounds as carbon and energy sources (Bondoso et al. 2015; Wiegand et al. 2019). Such nutrient-rich environments—in contrast to the oligotrophic seawater—attract also faster-growing competing microorganisms, e.g. *Roseobacter* sp. (Frank et al. 2014; Wiegand et al. 2018), but the latter fail to outcompete the slower-growing species of the family *Planctomycetaceae*. Two theories provide plausible explanations for this counter-intuitive observation: (I) *Planctomycetaceae* harbour a specialised machinery for uptake and degradation of complex polysaccharides released by algae or (II) they produce small molecules as a metabolic defence strategy. Both theories are supported by large planctomycetal genomes and the high number of predicted enzymes involved in the catabolism of complex sugars (Naumoff et al. 2014; Reisky et al. 2018; Wegner et al. 2013) (supporting theory I) or for production of secondary metabolites (supporting theory II) (Graça et al. 2016; Jeske et al. 2016; Wiegand et al. 2018, 2019). Uptake and catabolism of polymeric sugars might be facilitated by the unique pili-forming crateriform structures and an enlarged periplasm (Boedeker et al. 2017), while the mostly unexplored secondary metabolism could be a promising source for novel compounds, including those with health-promoting activities in humans (Graça et al. 2016).

Not only from a metabolic perspective, but also structurally, the phylum *Planctomycetes* is an interesting research topic. Eukaryotic-like morphological traits of Planctomycetes led to the conclusion that they might be beyond the bacterial cell plan (Fuerst and Sagulenko 2011; König et al. 1984; Lonhienne et al. 2010). In recent years, this picture changed with the advent of novel 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). The cell envelope of Planctomycetes was ultimately found to resemble that of Gram-negative bacteria (Boedeker et al. 2017; Devos 2014). But still, Planctomycetes remain exceptional. They divide by budding, binary fission or even a combination of both and lack proteins of the canonical divisome (Wiegand et al. 2019). Many strains have been shown to be resistant to several antibiotics (Cayrou et al. 2010; Godinho et al. 2019), either because of their degradation, or intrinsic resistance due to the lack of targets (Jogler et al. 2012; Pilhofer et al. 2008).

In conclusion, Planctomycetes have some characteristic traits (Wiegand et al. 2018, 2019), which encourages more detailed research and motivates us to steadily expand the collection of axenic cultures. In this study, we took samples in the surroundings of Panarea Island in the Tyrrhenian Sea off the southwestern coast of Italy. This region includes a shallow-sea hydrothermal vent system and consists of areas with increased temperatures, steep temperature gradients and high levels of different nitrogen and sulphur sources (Maugeri et al. 2010). Due to the expected microbial diversity (Manini et al. 2008), we considered this location a valuable source of so far unknown species of the family *Planctomycetaceae* and here describe the characterization of the novel strain Enr8^T isolated from a hydrothermal area close to Panarea Island.

Materials and methods

Isolation and cultivation conditions

Strain Enr8^T was isolated on the 10th of September 2013 from a marine biotic surface (Fig. 1) in a hydrothermal area in the Tyrrhenian Sea (sampling site 38.6387 N 15.1068 E) 2.5 km east of the port of

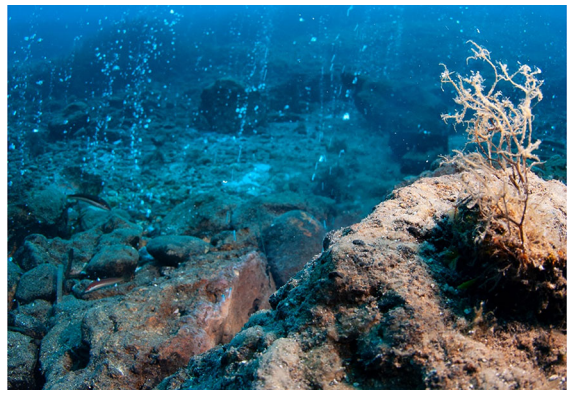


Fig. 1 Representative picture of the sampling location. Strain Enr8^T was isolated from the surface of the marine plant shown on the right side of the photograph

Panarea Island. The strain was isolated as described previously (Wiegand et al. 2019) and subsequently cultivated in M1 medium with HEPES as buffering agent and supplemented with *N*-acetyl glucosamine (NAG) and artificial seawater (ASW) (medium designation M1H NAG ASW) (Kallscheuer et al. 2019a).

Physiological analyses

The temperature optimum of strain Enr8^T was determined in M1H NAG ASW medium by cultivation at temperatures of 10, 15, 20, 22, 24, 27, 30, 33, 36 and 40 °C in a shaking incubator at 110 rpm with an initial pH of 8.0. For the determination of the pH optimum 100 mM HEPES was used as buffering agent 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) and at pH 9.0 and 10.0 100 mM *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) was used as buffering agent. Cultivations for determination of the pH optimum were performed at 28 °C in a shaking incubator at 110 rpm. For analysis of the salt tolerance, strain Enr8^T was cultivated in M1H NAG ASW medium with different concentrations of NaCl. The tested concentrations were 0, 100, 200 (standard NaCl concentration in ASW), 350, 500, 700, 900, 1200 and 1600 mM NaCl. Due to formation of white flakes by aggregation of cells in all experiments, growth was assessed by visual inspection of the culture taking the number and size of white flakes into account. Measurement of the optical density at 600 nm (OD_{600}) was not possible.

Light microscopy and electron microscopy

Microscopic analyses were performed according to a previously published protocol (Kallscheuer et al. 2019a).

Genome information and analysis of genome-encoded features

The genome (accession no. SJPF00000000) and 16S rRNA gene sequence (accession no. MK554541) of strain Enr8^T are available from GenBank (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.

Phylogenetic analysis

16S rRNA gene phylogeny was computed for strain Enr8^T, the type strains of all described planctomycetal species (as available in May 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, d; Kohn et al. 2019). The 16S rRNA gene sequences were aligned with SINA (Pruesse et al. 2012). The phylogenetic analysis was performed 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).

Results and discussion

Phylogenetic analysis

According to maximum likelihood-based 16S rRNA gene sequence analysis shown in Fig. 2, the current closest relative of strain Enr8^T is *Blastopirellula marina*, which is also the type species of the genus (Schlesner et al. 2004). The type strain of *B. marina*, DSM 3645^T, was originally isolated from brackish water in the Baltic Sea (Schlesner 1986) and together with *Blastopirellula cremea* (Lee et al. 2013) forms the current genus *Blastopirellula*. *B. marina* DSM 3645^T and strain Enr8^T share a 16S rRNA similarity of 98.9%. This value is slightly above the proposed species threshold of 98.7% (Stackebrandt and Ebers 2006), which could indicate that strain Enr8^T belongs to the species *B. marina*. However, it was shown before that 16S rRNA genes are not in all cases a reliable marker for Planctomycetes as some strains

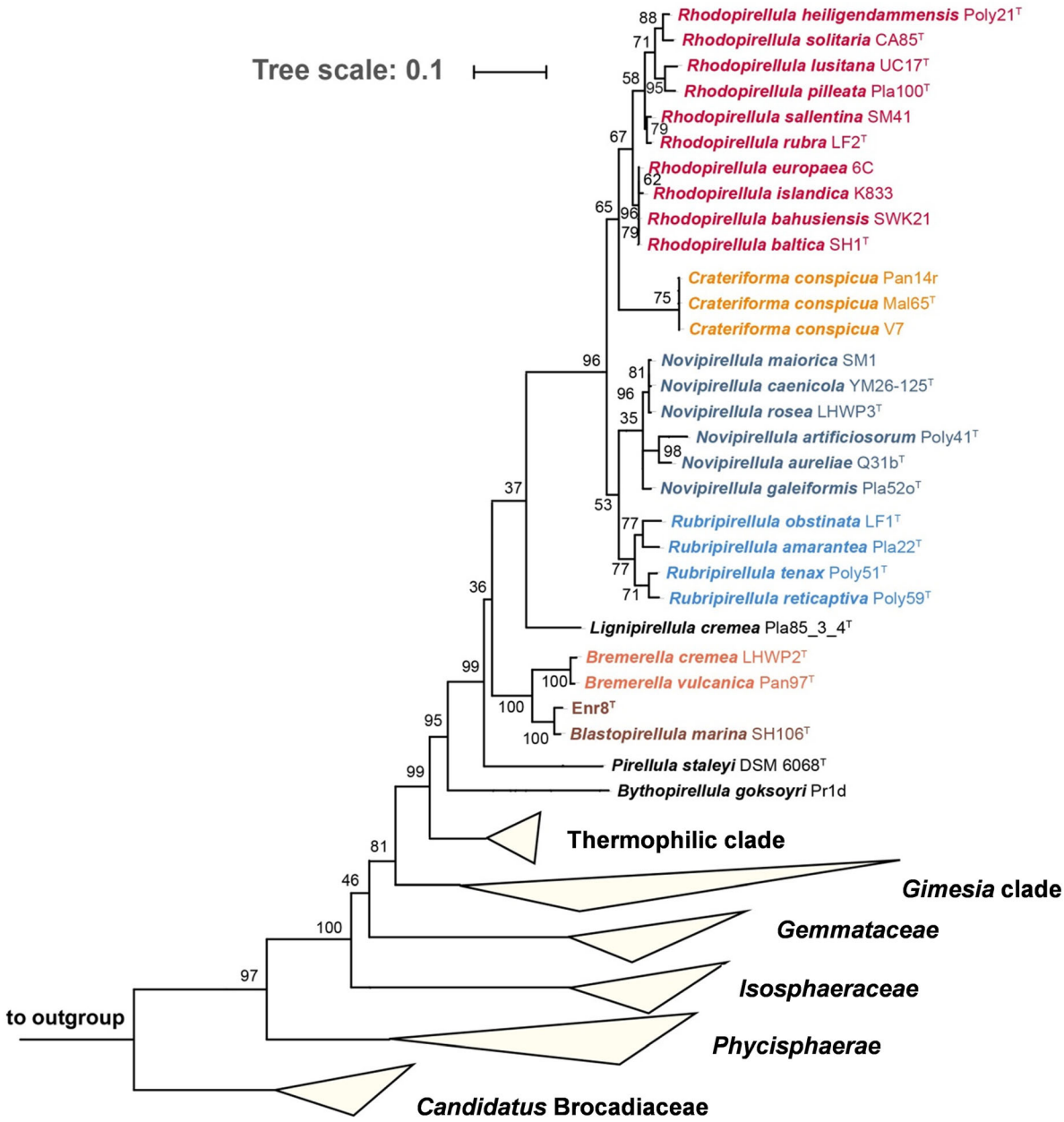


Fig. 2 Maximum likelihood analysis of 16S rRNA gene sequences. Phylogenetic tree showing the position of strain Enr8^T. 16S rRNA gene phylogeny was computed as described in the Materials and methods section. Bootstrap values after 1000 re-samplings are given in % at the nodes. The outgroup consists of three 16S rRNA genes from the PVC superphylum. The *Gimesia* clade includes species of the genera *Gimesia*, *Planctopirus*, *Fuerstiella*, *Schlesneria*, *Rubinisphaera* and *Planctomicrobium*, while the thermophilic clade includes species of the genera *Thermostilla*, *Thermogutta* and *Thermopirellula*

have high 16S rRNA gene sequence identities, but nevertheless belong to different species (Bondoso et al. 2013; Kohn et al. 2019). Thus, other phylogenetic markers need to be considered in order to achieve a reliable classification of strain Enr8^T. For example, the similarity of the analysed partial *rpoB* sequence is 88.4%, which is clearly below the proposed threshold of 95.5% for strains belonging to the same species

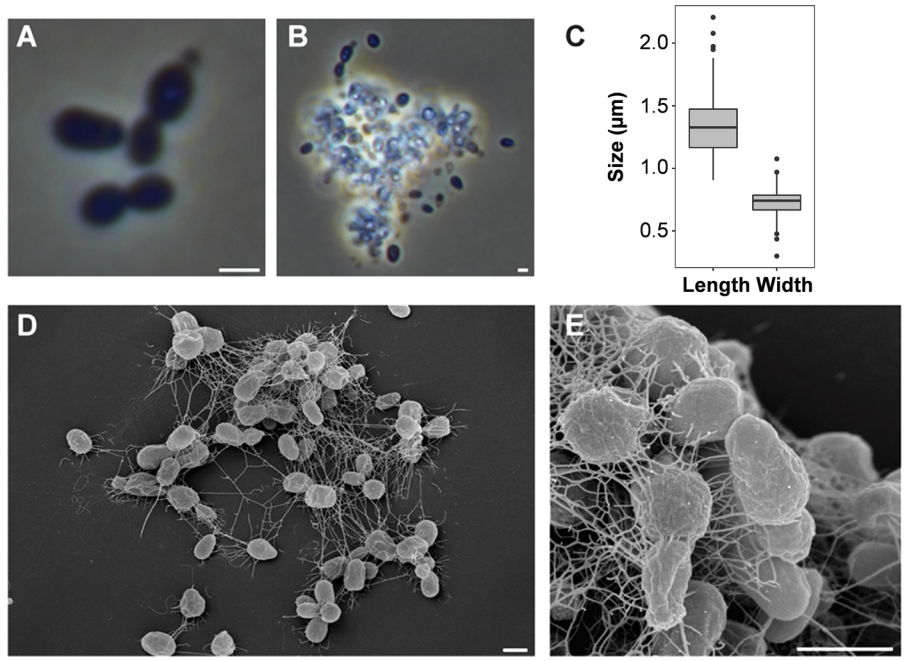


Fig. 3 Phase contrast and scanning electron microscopy images and cell size plot of strain Enr8^T. The figure shows the mode of cell division (**a**) and gives an overview on the cell morphology of strain Enr8^T (**b**, **d**, **e**). The scale bar is 1 µm. For determination of the cell size (**c**) at least 100 representative cells were counted manually or by using a semi-automated object count tool

(Bondoso et al. 2013). The same is true for ANI values of 78.3% when applying the species threshold of 95–96% (Kim et al. 2014). Taken together, *rpoB* similarity and ANI values clearly reinforce the conclusion that strain Enr8^T belongs to a separate species and does not represent a strain of the species *B. marina*. Thus, it appears likely that 16S rRNA gene identity comparisons in this clade do not follow the proposed species threshold of 98.7% (Stackebrandt and Ebers 2006). Accordingly, strain Enr8^T and *B. marina* have an AAI of 75.8% and a POCP of 77.6%. Both values are above the respective genus threshold ranges (AAI: 60–80% and POCP: 50%) (Luo et at. 2014; Qin et al. 2014), confirming that both strains belong to the same genus. In order to show that strain Enr8^T and *B. marina* DSM 3645^T do not belong to the recently proposed genus '*Bremerella*' (Rensink et al. 2019), minimal 16S rRNA gene sequence identities, AAI values, *rpoB* similarities and POCP values of strain Enr8^T and '*Bremerella vulcanica*' Pan97^T were compared. Comparison yields a 16S rRNA gene identity of 94.3%, an AAI value of 54.7% and a partial *rpoB* sequence of 74.7%, which are below the genus thresholds of 94.5%, 60–80% and 75.5–78%, respectively (Kallscheuer et al. 2019d; Luo et al. 2014; Yarza et al. 2014). The POCP value of 60.4% is only slightly above the genus threshold of 50% (Qin et al. 2014).

Morphological and physiological analyses

Strain Enr8^T was cultivated in M1H NAG ASW medium and exponentially growing cells were used for morphological characterisation using phase contrast microscopy and scanning electron microscopy (Fig. 3). Detailed information on morphology, locomotion and cell division in comparison to the closely related species *B. marina* is summarised in Table 1. Cells of strain Enr8^T appear ovoid to pear-shaped (length: 1.3 ± 0.2 µm, width: 0.7 ± 0.1 µm) (Fig. 3a), form strong aggregates and contain craterliform structures at the cell poles (Fig. 3b–e). Polar budding was observed as mode of cell division with the shape of the bud being similar to that of the mother cell (Fig. 3a). Fibres together with an extracellular matrix facilitate attachment to other cells or surfaces, thereby causing white flake formation visible with the naked eye in broth cultures. Colonies of strain Enr8^T

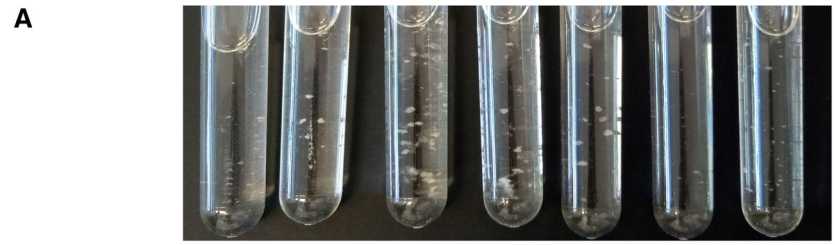
Table 1 Phenotypic and genotypic features of strain Enr8^T (Genbank acc. no. SJPF00000000) in comparison to *B. marina* DSM 3645^T (GenBank acc. no. AANZ00000000) (Schlesner 1986; Schlesner et al. 2004)

Characteristics	Enr8^T	*B. marina* DSM 3645^T
Phenotypic features		
Size (length × width)	1.3 × 0.7 µm	1.0–2.0 × 0.7–1.5 µm
Shape	Ovoid to pear-shaped	Ovoid, ellipsoidal or pear-shaped
Aggregates	Yes	Yes
Colony colour	White	Off-white to light brown
Division	Budding	Budding
Dimorphic life cycle	n.o.	n.o.
Flagella	Yes	n.o.
Crateriform structures	Yes	Yes
Fimbriae	Matrix or fibre	At reproductive pole
Capsule	n.o.	n.o.
Bud shape	Like mother cell	Bean-shaped
Budding pole	Polar	Polar
Stalk	n.o.	n.o.
Holdfast structure	n.o.	n.o.
Genotypic features		
Genome size (bp)	6,196,617	6,663,851
Plasmids (bp)	n.o.	n.a.
GC (%)	59.2 ± 2.6	57.4
Completeness (%)	98.28	96.55
Contamination (%)	1.72	1.72
Protein-coding genes	5033	5406
Hypothetical proteins	2107	3023
Protein-coding genes/Mb	812	811
Coding density (%)	86.0	86.8
16S rRNA genes	2	1
tRNA genes	72	56

n.o. not observed, *n.a.* not available

are white indicating the lack of carotenoid formation. Size, cell morphology and colour are similar to *B. marina* (Schlesner et al. 2004) (Table 1).

In cultivation experiments, strain Enr8^T was found to grow at a pH range of 6.0–8.0 and at temperatures from 15 to 33 °C (Fig. 4a, b). The optimal conditions were determined to be pH 7.0 and 30 °C. During cultivation, formation of strong aggregates was observed, which became visible as white flakes settling down in the cultivation tubes, for example as shown for the determination of the pH optimum (Fig. 4a). This rendered measurement of cell densities using OD_{600} impossible. Instead, optimal conditions (pH, temperature and NaCl concentration) were determined by visual inspection taking the observed differences in the number and size of flakes into account. The temperature optimum at 30 °C is the same as for *B. marina* and both failed to grow at 36–38 °C (Schlesner 1986). Due to aggregate formation, a maximal growth rate could not be calculated for strain Enr8^T. In the salt tolerance experiments, the strain showed growth in the presence of 0.6–7.0% (w/v) NaCl (100–1200 mM), but failed to grow without NaCl or with 9.3% (w/v) NaCl (1600 mM) (Fig. 4c). Optimal growth was observed in the range of 2.1–4.1% (w/v) NaCl (350–700 mM), which is in the range of natural seawater in the Mediterranean Sea with a total salt concentration of around 3.8% (w/v). The high salt tolerance of strain Enr8^T is a distinctive difference compared to *B. marina*. While concentrations of 1% NaCl were lethal for *B. marina* (Schlesner 1986), Enr8^T showed optimal growth at 2–4% NaCl and could even grow in the presence of 7% NaCl.



A

pH	5.0	6.0	7.0	7.5	8.0	9.0	10.0
Growth	-	+	+++	++	+	-	-

B

Temp.	5	10	15	20	24	27	30	33	36	40
Growth	-	-	+	+	++	++	+++	++	-	-

C

NaCl	0	100	200	350	500	700	900	1200	1600
Growth	-	+	+	+++	+++	+++	++	+	-

Fig. 4 Optimum of pH, temperature and NaCl concentration for strain Enr8^T. Cultivations in M1H NAG ASW medium were performed at different pH values (**a** constant temperature of 28 °C), different temperatures (**b** at pH 7.5, temperatures given in °C) and different NaCl concentrations (**c** given in mM) in biological triplicates. Due to strong formation of aggregates during cultivation it was not possible to measure cell density as OD_{600}. Instead, exemplary photographs of the cultures of the pH optimum determination experiment after a cultivation time of 170 h are shown (**a**). Growth was classified in the range from "−" (no growth) to "+++" (very good growth)

Genomic characteristics

The relevant genome characteristics in comparison to the closely related species *B. marina* are summarised in Table 1. The genome size of strain Enr8^T is 6.20 Mb. The genome is slightly smaller compared to *B. marina* DSM 3645^T (6.66 Mb), but both strains have a similar G + C content (Enr8^T: 59.2%, DSM 3645^T: 57.4%). Automated annotation yielded 5033 putative protein-encoding genes for strain Enr8^T, of which 42% (2107 genes) are annotated as hypothetical proteins. The calculated values, corresponding to 812 protein-coding genes per Mb and a coding density of 86%, are nearly identical to *B. marina*. Two copies of the 16S rRNA gene and 72 tRNA-encoding genes were detected in strain Enr8^T, while *B. marina* harbours only one 16S rRNA gene and 56 tRNA-encoding genes.

Genome-based analysis of the central carbon metabolism

Based on the genome sequences of Enr8^T and *B. marina* DSM 3645^T, we analysed the presence of key metabolic enzymes of the central carbon metabolism. The analysis included glycolytic pathways, gluconeogenesis, the tricarboxylic acid (TCA) cycle and anaplerotic reactions (Table 2). Both strains harbour the genes coding for enzymes involved in glycolytic reactions either using the Embden-Meyerhof-Parnas pathway (referred to as glycolysis) or the alternative Entner-Doudoroff pathway. However, we failed to identify the gene coding for phosphoglycerate mutase (Pgm), which catalyses the reversible isomerisation of 3-phosphoglycerate to 2-phosphoglycerate and is required for the activity of both pathways. As the degradation of glucose by both strains suggests that the glycolysis is active, the two strains either bypass the reaction, e.g. by using 2,3-bisphosphoglycerate as intermediate or the protein sequence of Pgm differs from canonical sequences and escaped our analysis. The pentose phosphate pathway and the TCA cycle appear to be fully functional in both strains since we were able to assign genes to all participating enzymes (Table 2). Both strains also encode phosphoenolpyruvate carboxykinase (ATP) and fructose-1,6-bisphosphatase, the key enzymes required for de novo

Table 2 Genome based-analysis of the central carbon metabolism of Enr8^T and *B. marina* DSM 3645^T

Enzyme	EC number	Gene	Enr8^T	*B. marina* DSM 3645^T
Glycolysis				
Glucose-6-phosphate isomerase	5.3.1.9	*pgi*	Enr8_27980	GCF_000153105_52400
ATP-dependent 6-phosphofructokinase isozyme 1	2.7.1.11	*pfkA*	Enr8_35550	GCF_000153105_47750
Fructose-bisphosphate aldolase class 2	4.1.2.13	*fbaA*	Enr8_24470	GCF_000153105_01670
Triosephosphate isomerase	5.3.1.1	*tpiA*	Enr8_45880	GCF_000153105_41410
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*gapA*	Enr8_00350	GCF_000153105_02490
Phosphoglycerate kinase	2.7.2.3	*pgk*	Enr8_07460	GCF_000153105_10610
2,3-Bisphosphoglycerate-independent phosphoglycerate mutase	5.4.2.12	*gpmI*	n	n
2,3-Bisphosphoglycerate-dependent phosphoglycerate mutase	5.4.2.11	*gpmA*	n	n
Enolase	4.2.1.11	*eno*	Enr8_34480	GCF_000153105_25320
Pyruvate kinase I	2.7.1.40	*pykF*	Enr8_14540	GCF_000153105_18700
Pyruvate dehydrogenase E1 component	1.2.4.1	*aceE*	Enr8_43650	GCF_000153105_45170
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	2.3.1.12	*aceF*	Enr8_43660	GCF_000153105_45160
Gluconeogenesis				
Phosphoenolpyruvate carboxylase	4.1.1.31	*ppc*	n	n
Phosphoenolpyruvate synthase	2.7.9.2	*ppsA*	n	n
Pyruvate, phosphate dikinase	2.7.9.1	*ppdK*	Enr8_43210	GCF_000153105_45620
Phosphoenolpyruvate carboxykinase (ATP)	4.1.1.49	*pckA*	Enr8_45450	GCF_000153105_41130
Fructose-1,6-bisphosphatase class 1	3.1.3.11	*glpX*	Enr8_23750	GCF_000153105_40330
Pyrophosphate–fructose 6-phosphate 1-phosphotransferase	2.7.1.90	*pfp*	n	n
Pentose phosphate pathway				
Glucose-6-phosphate 1-dehydrogenase	1.1.1.49	*zwf*	Enr8_32830	GCF_000153105_27210
6-Phosphogluconolactonase	3.1.1.31	*pgl*	Enr8_38350	GCF_000153105_20950
6-Phosphogluconate dehydrogenase, decarboxylating	1.1.1.44	*gndA*	Enr8_32810	GCF_000153105_27230
Transketolase 2	2.2.1.1	*tktB*	Enr8_02650	GCF_000153105_04910
Transaldolase B	2.2.1.2	*tal*	Enr8_28950	GCF_000153105_51220
Entner–Doudoroff pathway				
KHG/KDPG aldolase	4.1.3.16	*kdgA*	Enr8_37080	GCF_000153105_22230
Phosphogluconate dehydratase	4.2.1.12	*edd*	Enr8_11390	GCF_000153105_14920
TCA cycle				
Citrate synthase	2.3.3.16	*gltA*	Enr8_32230	GCF_000153105_47880
Aconitate hydratase A	4.2.1.3	*acnA*	Enr8_16410	GCF_000153105_26150
Isocitrate dehydrogenase (NADP)	1.1.1.42	*icd*	Enr8_11000	GCF_000153105_14510
2-Oxoglutarate dehydrogenase E1 component	1.2.4.2	*sucA*	Enr8_50880	GCF_000153105_37900
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	2.3.1.61	*sucB*	Enr8_50870	GCF_000153105_37910
Succinate–CoA ligase [ADP-forming] subunit alpha	6.2.1.5	*sucD*	Enr8_03400	GCF_000153105_05740
Succinate–CoA ligase [ADP-forming] subunit beta	6.2.1.5	*sucC*	Enr8_03390	GCF_000153105_05730
Succinate dehydrogenase flavoprotein subunit	1.3.5.1	*sdhA*	Enr8_25660	GCF_000153105_00080
Succinate dehydrogenase iron-sulfur subunit	1.3.5.1	*sdhB*	Enr8_25670	GCF_000153105_00070
Succinate dehydrogenase cytochrome b556 subunit	1.3.5.1	*sdhC*	Enr8_25650	GCF_000153105_00090
Fumarate hydratase class II	4.2.1.2	*fumC*	Enr8_30140	GCF_000153105_50000
Malate dehydrogenase	1.1.1.37	*mdh*	Enr8_10980	GCF_000153105_14490

Table 2 continued

Enzyme	EC number	Gene	Enr8^T	*B. marina* DSM 3645^T
Glyoxylate shunt				
Isocitrate lyase	4.1.3.1	*aceA*	n	n
Malate synthase	2.3.3.9	*glcB*	n	n

synthesis of sugar phosphates from the TCA cycle intermediate oxaloacetate. The gluconeogenesis pathway, however, also requires the phosphoglycerate mutase, which we could not identify in the two strains (as discussed above). Bacteria typically perform anaplerosis (replenishing of TCA cycle intermediates) by the glyoxylate shunt or by carboxylation of pyruvate or phosphoenolpyruvate (PEP). The carboxylation reactions rely on additional pathways providing pyruvate or PEP (typically glycolysis), while the glyoxylate shunt converts the TCA cycle intermediate isocitrate and the TCA cycle substrate acetyl-CoA to one molecule of succinate and malate. Both strains apparently lack the genes required for a functional glyoxylate shunt (Table 2), which is required e.g. during growth with acetate or fatty acids as sole carbon and energy source. This would imply that both strains are unable to use acetate or fatty acids as sole carbon and energy source, which may support the hypothesis that species of the family *Planctomycetaceae* consume complex sugars derived from phototrophs rather than short- or long-chain carboxylic acids.

Putative gene clusters involved in secondary metabolite production

To gain a first insight into the potential of strain Enr8^T as a source of secondary metabolites an AntiSMASH analysis based on its genome sequence was conducted (Blin et al. 2019). It appears reasonable to assume that the complex lifestyle and growth in competitive environments such as marine surfaces is related to the production of secondary metabolites allowing strain Enr8^T to cope with abiotic and biotic stresses. Of major interest in this case are multi-domain protein complexes of the families of polyketide synthases (PKSs) or non-ribosomal peptide synthetases (NRPSs) as these serve as molecular assembly lines for production of secondary metabolites starting from metabolites of the primary carbon metabolism (e.g. malonyl-CoA or amino acids) (Park et al. 2019). Strain Enr8^T harbours three genes or clusters involved in the production of terpenoids. These are probably not involved in the production of carotenoids (a major class of terpenoids) as the strain is white and thus lacks pigmentation. In addition, Enr8^T harbours genes coding for a putative type I PKS, a putative NRPS, a putative mixed NRPS-type I PKS and a protein related to bacteriocin biosynthesis. The same set of clusters is also encoded in the genome of *B. marina* DSM 3645^T except for the putative NRPS gene, which was not identified in *B. marina*. The exact products formed by the identified PKS and NRPS enzymes should be identified in future studies to gain insights into their biological significance.

Taken together, our phylogenetic analysis and the results of the morphological and physiological characterisation support the conclusion that strain Enr8^T represents a novel species of the genus *Blastopirellula*, for which we propose the name *Blastopirellula retiformator* sp. nov.

Description of *Blastopirellula retiformator* sp. nov

Blastopirellula retiformator (re.ti.for.ma'tor. L. neut. n. *rete* a net; L. masc. n. *formator* a shaper, creator; N.L. masc. n. *retiformator* corresponding to the characteristic fibre- and extracellular matrix-mediated formation of visible flake-like structures).

Colonies are white. Cells are pear-shaped (length: 1.3 ± 0.2 µm, width: 0.7 ± 0.1 µm) and form large aggregates which become visible as white flakes in liquid culture. Cells divide by polar budding and grow

at ranges of 15–33 °C (optimum 30 °C), pH 6.0–8.0 (optimum 7.0) and NaCl concentrations from 100 to 1200 mM (optimum 350–700 mM). The genome (accession no. SJPF00000000) and 16S rRNA gene sequence (accession no. MK554541) of the type strain are available from GenBank. The type strain genome has a size of 6.20 Mb and a G + C content of 59.2%. The type strain is Enr8^T (DSM 100415^T = LMG 29081^T, also designated Enrichment 8), isolated from a hydrothermal area close to the island Panarea, Italy.

Acknowledgements Part of this research was funded by the Deutsche Forschungsgemeinschaft Grants KA 4967/1-1 and JO 893/4-1, Grant ALWOP.308 of the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), SIAM (Soehngen Institute for Anaerobic Microbiology) Grant No. 024002002 and the Radboud Excellence fellowship. We thank Ina Schleicher for skilful technical assistance. Brian Tindall and Regine Fähnrich from the DSMZ as well as the BCCM/LMG Bacteria collection we thank for support during strain deposition. We thank the Scientific Diving Center of the Bergakademie Freiberg, Germany, Thomas Pohl, Peter Hornburger and all participants of the 2013 Panarea Expedition for sampling support.

Author's contributions NK wrote the manuscript, analysed the data and prepared the figures, SW performed the genomic and phylogenetic analysis, MJ, AH and PR isolated the strains and performed cultivations and strain deposition, SR and ASB performed the cultivation for determination of the pH optimum, SHP and CB performed the light microscopic analysis, MSMJ contributed to text preparation and revised the manuscript, MR performed the electron microscopic analysis, CJ and MJ took the samples 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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