Rosistilla oblonga gen. nov., sp. nov. and Rosistilla carotiformis sp. nov., isolated from biotic or abiotic surfaces in Northern Germany, Mallorca, Spain and California, USA

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Abstract Planctomycetes are ubiquitous bacteria with fascinating cell biological features. Strains available as axenic cultures in most cases have been isolated from aquatic environments and serve as a basis to study planctomycetal cell biology and interactions in further detail. As a contribution to the current collection of axenic cultures, here we characterise three closely related strains, Poly24T, CA51T and Mal33, which were isolated from the Baltic Sea, the Pacific Ocean and the Mediterranean Sea, respectively. The strains display cell biological features typical for related Planctomycetes, such as division by polar budding, presence of crateriform structures and formation of rosettes. Optimal growth was observed at temperatures of 30–33 °C and at pH 7.5, which led to maximal growth rates of 0.065–0.079 h⁻¹, corresponding to generation times of 9–11 h. The genomes of the novel isolates have a size of 7.3–7.5 Mb and a G + C content of 57.7–58.2%. Phylogenetic analyses place the strains in the family Pirellulaceae and suggest that Roseimaritima ulvae and Roseimaritima sediminicola are the current closest relatives. Analysis of five different phylogenetic markers, however, supports the delineation of the strains from members of the genus Roseimaritima and other characterised genera in the family. Supported by morphological and physiological differences, we conclude that the strains belong to the novel genus Rosistilla gen. nov. and constitute two novel species, for which we propose the names Rosistilla carotiformis sp. nov. and Rosistilla oblonga sp. nov. (the type species). The two novel species are represented by the type strains Poly24T (= DSM 102938T = VKM B-3434T = LMG 26571T) and CA51T (= DSM 102939T = VKM B-3438T = LMG 26572T).
Introduction

For a long time since their discovery in 1924, the phylogeny of Planctomycetes was controversial. Initially identified as planktonic microorganisms in a Hungarian freshwater lake, Planctomycetes were primarily misinterpreted as eukaryotes, more specifically as fungi (Gimesi 1924), but later recognised as bacteria (Hirsch 1972). Planctomycetes are of ecological importance and play an important role in the global carbon and nitrogen cycle (Strous et al. 1999; Wiegand et al. 2018). Phylogenetically, the eponymous phylum Planctomycetes is part of the PVC superphylum along with Verrucomicrobia, Chlamydiae and other sister phyla (Wagner and Horn 2006). Currently, the phylum Planctomycetes is subdivided into the classes Planctomycetia, Phycisphaerae and Candidatus Brocadiae with members of the latter capable of performing anaerobic ammonium oxidation (anammox) (Peeters and van Niftrik 2019). Strains belonging to the classes Planctomycetia and Phycisphaera are typically found in aquatic habitats (Dedysh and Ivanova 2012; Spring et al. 2018), and also in soil environments (Buckley et al. 2006).

Research on Planctomycetes was driven by the observed eukaryotic-like traits (Fuerst and Sagulenko 2011), including apparent absence of a peptidoglycan cell wall (König et al. 1984), a compartmentalised cell plan (Lindsay et al. 1997) and a nucleus-like configuration (Fuerst and Webb 1991). Many of these features have been re-evaluated based on improved microscopic techniques and genetic methods developed for Planctomycetes (Jogler and Jogler 2013; Rivas-Marin et al. 2016). For instance, peptidoglycan has been found in Planctomycetes (Jogler et al. 2012; Pilhofer et al. 2008). In the context of the ecological significance of Planctomycetes, several studies showed high abundance of Planctomycetes in biofilms on marine biotic surfaces, such as macroscopic phototrophs (Bengtsson and Övreås 2010; Bondoso et al. 2014; Bondoso et al. 2017; Lage and Bondoso 2014; Vollmers et al. 2017; Wiegand et al. 2018). This points towards an important function in microbial (biofilm-forming) communities in such environments. A high abundance of Planctomycetes may appear counter-intuitive given that natural competitors in the same ecological niche grow much faster, e.g. Proteobacteria (Frank et al. 2014; Wiegand et al. 2018). In this context, small bioactive molecules secreted by Planctomycetes may take part in mediating symbiotic relationships with algae or serve as antibiotic agents (Grac ¸a et al. 2016; Jeske et al. 2016). The capability of Planctomycetes to degrade high molecular weight sugars secreted by primary photosynthetic producers may also provide a decisive growth advantage (Jeske et al. 2013; Lachnit et al. 2013). Pili originating from conspicuous crateriform structures and an enlarged periplasmic space are probably part of an uptake system for entire polysaccharide molecules (Boedeker et al. 2017). Metabolic versatility is further supported by high numbers of carbohydrate-active enzymes encoded in planctomycetal genomes, e.g. polysaccharide lyases or sulfatases (Dabin et al. 2008; Wegner et al. 2013).

During a detailed analysis of genome sequences, it was established that Planctomycetes is amongst the bacterial phyla with the highest numbers of hypothetical proteins (40–55% of the total number of annotated proteins).
proteins) (Overmann et al. 2017). Consequentially, much interesting cell biology and metabolic potential is expected to be found within this phylum.

As a contribution to the current collection of planctomycetal species, here we characterised three novel strains and analysed their phylogeny as well as basic phenotypic and genotypic characteristics.

Materials and methods

Isolation of the novel strains

The three novel strains were isolated from different locations. Strain Poly24T was sampled on 8 October 2015 from polyethylene particles stored in an incubator, which was placed at 2 m depth in the Baltic Sea below the pier of Heiligendamm, Germany (54.146 N, 11.843 E). Incubation time in the seawater was 14 days. Sterile-filtered natural seawater was added to the polyethylene particles and, after mixing, 50 μL of the seawater were streaked on an M1H NAG ASW plate containing 8 g/L gellan gum, 500 mg/L streptomycin, 200 mg/L ampicillin and 20 mg/L cycloheximide. Strain CA51T was isolated on 28 November 2014 from leaves of a giant bladder kelp (Macrocystis pyrifera) on the Californian coastline close to Monterey Bay, CA, USA (36.619 N 121.901 W). Initially, kelp pieces were washed with 100 mg/L cycloheximide dissolved in sterile-filtered natural seawater to prevent fungal growth, then swabbed over M1H NAG ASW plates containing 8 g/L gellan gum, 1000 mg/L streptomycin, 200 mg/L ampicillin and 20 mg/L cycloheximide and incubated at 20°C for 2 to 3 weeks. Strain Mal33 was sampled on 23 September 2014 from the surface of an alga found on the beach of S’Arenal, Mallorca, Spain (39.5126 N 2.7470 E). Isolation and initial cultivation was performed as described earlier (Wiegand et al. 2020). Single colonies were used to inoculate liquid M1H NAG ASW medium (Boersma et al. 2019). In order to check whether the isolated strains indeed represent members of the phylum Planctomycetes, their 16S rRNA genes were amplified by PCR and sequenced as previously described (Rast et al. 2017).

Genome information and analysis of genome-encoded features

Genome information of the three isolated strains is available from GenBank under accession numbers CP036348 (Poly24T), CP036292 (CA51T) and CP036318 (Mal33). 16S rRNA gene sequences are available from GenBank under accession numbers MK559990 (Poly24T), MK559969 (CA51T) and MK554528 (Mal33). DNA isolation and genome sequencing were performed as part of a previous study (Wiegand et al. 2020). Enzymes of the primary metabolism were 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.

Physiological analysis

Determination of the temperature and pH optimum was performed in M1H NAG ASW medium with 100 mM of the following buffers: 2-(N-morpholino)ethanesulfonic acid (MES) for pH 5.0 and 6.0, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic (HEPES) for pH 7.0, 7.5 and 8.0, 3-(4-(2-hydroxyethyl)piperazin-1-yl)propa-1-sulfonic acid (HEPPS) for pH 8.5 and N-cyclohexyl-2-aminoethanesulfonic acid (CHES) for pH 9.0 and 10.0. Cultivations were performed at 28°C. Cultivations for determination of the temperature optimum were performed at temperatures ranging from 10 to 40 °C in M1H NAG ASW medium at pH 8.0.

Light microscopy and scanning electron microscopy

Microscopic analyses were performed according to a previously published study (Boersma et al. 2019).

Phylogenetic analysis

16S rRNA gene sequence-based phylogeny was computed for strains Poly24T, CA51T and Mal33 and the type strains of all described planctomycetal species (as of May 2020), including recently published strains (Boersma et al. 2019; Dedysy et al. 2020a; Dedysy et al. 2020b; Kallscheuer et al. 2019a; Kallscheuer et al. 2020a; Kallscheuer et al. 2020b; Kallscheuer et al. 2019c; Kallscheuer et al. 2019d; Kohn et al. 2020;
Kumar et al. 2020; Peeters et al. 2020; Rensink et al. 2020). An alignment of 16S rRNA gene sequences was made with SINA (Pruesse et al. 2012). By employing a maximum likelihood approach with 1000 bootstraps, nucleotide substitution model GTR, gamma distributed rate variation and estimation of proportion of invariable sites (GTRGAMMAI option), the phylogenetic inference was calculated with RAxML (Stamatakis 2014). Three 16S rRNA genes of strains within the PVC superphylum outside of the phylum Planctomycetes were used as outgroup. For the multi-locus sequence analysis (MLSA), the unique single-copy core genome of the 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. OrthoANI was used to calculate the average nucleotide identity (ANI) (Lee et al. 2016), while average amino acid identities (AAI) were calculated using the aai.rb script of the enveomics collection (Rodriguez-R and Konstantinidis 2016). Percentage of conserved proteins (POCP) was calculated as described previously (Qin et al. 2014). The rpoB nucleotide sequences were taken from the above-mentioned genomes as well as other publicly available genome annotations and the sequence identities were determined as described before (Bondoso et al. 2013). The alignment and matrix calculation was performed with Clustal Omega extracting only those parts of the sequence that would have been sequenced with the described primer set (Sievers et al. 2011).

Results and discussion

Phylogenetic inference

In the phylogenetic trees obtained after analysis of 16S rRNA gene sequences and MLSA, the three novel isolates Poly24T, CA51T and Mal33 cluster monophyletically in the family Pirellulaceae (Fig. 1).

Analysis of five phylogenetic markers, namely 16S rRNA gene identity, AAI, ANI, rpoB similarity and POCP, suggests Roseimaritima ulvae (Bondoso et al. 2015) and Roseimaritima sediminicola (Kumar et al. 2020) as their current closest neighbours (Fig. 2, Table S1). An ANI of 57.5% far below the species threshold of 95% (Kim et al. 2014) indicates that the strains do not belong to the species R. ulvae or R. sediminicola. The maximum 16S rRNA gene similarity of the here described strains to members of the genus Roseimaritima was found to be 90.9% (Fig. 2), which is below the proposed genus threshold of 94.5% (Yarza et al. 2014). This finding is in line with values obtained during comparison of the novel isolates with Roseimaritima sp. for AAI and rpoB similarity, which were found to fall below the respective genus thresholds of 60% (Luo et al. 2014) and 75.5–78% (Kallscheuer et al. 2019d) (Fig. 2). Only the POCP of 54.2% is slightly above the suggested genus threshold of 50% (Qin et al. 2014). Taken together, it is reasonable to delineate the strains from the members of the genus Roseimaritima and all other genera of the family Pirellulaceae and to assign them to a novel genus instead.

In the next step, we compared the strains against each other in order to check for a relationship at the species level. The 16S rRNA genes of strain Mal33 and strain CA51T differ only at a single nucleotide position and thus have a similarity of 99.93%. This value is above the species threshold of 98.7% (Stackebrandt and Ebers 2006), suggesting that both strains are members of the same species. With an AAI of 97.9%, an ANI of 97.1% and an rpoB similarity of 98.9%, the results of the 16S rRNA gene comparison are substantiated when considering the species thresholds of 95% for AAI and ANI and of 96.3% for rpoB (Bondoso et al. 2013; Kim et al. 2014; Luo et al. 2014) (Fig. 2). Using the same phylogenetic markers, we also compared strain Poly24T to the other two strains. In all cases, the obtained values were found to be below the species thresholds, but above the genus thresholds (Fig. 2). Thus, Poly24T constitutes a separate species, but is part of the same genus as CA51T and Mal33.

Morphological and physiological analyses

Cell morphology of strains Poly24T, CA51T and Mal33 was analysed using light microscopy (Fig. 3)
and scanning electron microscopy (Fig. 4). To be able to investigate the division mode, cells were harvested during the exponential growth phase. Phenotypic features of the three strains are summarised in Table 1 and compared to *R. ulvae* and *R. sediminicola*. The cells of the novel strains have a similar size and a drop-like shape (Figs. 3, 4). However, strain Poly24T forms more elongated cells and cell poles appeared sharper during light microscopy, a shape which resembles carrots (Fig. 3a, b). The cell shapes significantly differ from the spherical to ovoid-shaped cells of *R. ulvae*, but are comparable to those of *R. sediminicola*. The three novel isolates all contain crateriform structures, but their distribution is different (Fig. 4). They cover the entire cell surface of Mal33 cells, but only occur on the pole(s) of the other two strains. All strains contain fimbriae or matrix originating from the budding pole. For strain Poly24T, we observed the presence of flagella and a holdfast
structure, while these features were not observed in case of the other two strains. This, however, does not necessarily mean that such structures are not present, as they may have been lost during sample preparation. The observed differences in the pigmentation of the strains were rather unexpected given their close relationship. Strain CA51T is white and thus apparently lacks pigmentation, while strain Mal33 is slightly pink and strain Poly24T shows a more intense pink pigmentation. Both *Roseimaritima* species show a similar pigmentation to strain Mal33. Since the exact metabolic pathway for carotenoid formation in Planctomycetes is not known (Kallscheuer et al. 2019b), we were not able to draw any conclusions regarding differences in pigmentation from the genome sequences.

The temperature and pH optima for growth were found to be highly comparable (Fig. 5), including *R. ulvae* UC8T. All four strains can grow up to temperatures of 35–36 °C with optimal growth at 30–33 °C, and tolerate a pH in the medium of 6.0–9.0. Only strain Mal33 showed a slightly narrower pH range of 6.5–8.5. In all cases, a pH of 7.5 was found to be optimal for the strains. *R. sediminicola* is able to grow up to temperatures of 40 °C although its temperature optimum is 5–8 °C lower compared to the strains used for comparison (Table 1). Maximal growth rates in M1H NAG ASW medium obtained for the three novel strains were 0.065 h⁻¹ (Poly24T), 0.069 h⁻¹ (CA51T) and 0.079 h⁻¹ (Mal33), which correspond to generation times of 11 h, 10 h and 9 h, respectively. All three strains are aerobic heterotrophs.
Genomic characteristics

The genomes of the novel isolates have a similar size of 7.3–7.5 Mb and are ~10% smaller than the genome of *R. ulvae* (8.2 Mb), but ~20% larger than the genome of *R. sediminicola* (6.3 Mb). The genomes have a G+C content of 58%, which is 1% lower than in *R. ulvae* and 5% lower compared to *R. sediminicola*. Given the similar genome size of the novel isolates, the numbers of protein-coding genes (5226–5515) and protein-coding genes per Mb (721–742) were also similar (Table 1). None of the strains (including *R. ulvae*) harbours plasmids (no information was available for *R. sediminicola* due to its non-closed genome). All five strains harbour a single 16S rRNA gene and 38–40% of the annotated proteins are of unknown function, except *R. sediminicola*, for which more than 62% of the annotated proteins are of unknown function. Values for the novel strains are thus in the lower range of up to 55% proteins of unknown function observed in most of the planctomycetal genomes sequenced so far. The number of tRNA genes is 1.7-fold higher in *R. ulvae* than in the other strains used for comparison (71 vs. 44–50 tRNA genes) (Table 1).

Genome-based analysis of encoded enzymes of the central carbon metabolism

A genome-based analysis was performed for the novel strains in order to gain a first insight into their central carbon metabolism. The analysis included glycolytic pathways (Embden-Meyerhof-Parnas pathway, pentose phosphate pathway, Entner-Doudoroff pathway), gluconeogenesis and the tricarboxylic acid (TCA) cycle including the glyoxylate shunt (Table 2). A
detailed description and discussion is not required in case of the three novel isolates since we were able to identify genes coding for all enzymes of the central carbon metabolism as found in typical heterotrophic bacteria. The above-mentioned pathways are thus likely fully functional, except for the glyoxylate shunt, which is absent in all planctomycetal genomes analysed so far.

### Conclusion

Based on the analysis of phenotypic and genomic characteristics and the phylogenetic position of the three novel isolates, it is concluded that the strains represent two novel species within a novel genus in the family Pirellulaceae. Thus, we propose the names *Rosistilla oblonga* gen. nov., sp. nov., represented by the type strain CA51<sup>T</sup>, and *Rosistilla carotiformis* sp. nov., represented by the type strain Poly24<sup>T</sup>.

### Table 1  Phenotypic and genotypic features of strains Poly24<sup>T</sup>, CA51<sup>T</sup> and Mal33 in comparison to closely related strains belonging to the genus *Roseimaritima*

| Characteristics | Poly24<sup>T</sup> | CA51<sup>T</sup> | Mal33 | *Roseimaritima ulvae* UC8<sup>T</sup> | *Roseimaritima sediminicola* JC651<sup>T</sup> |
|-----------------|-------------------|-----------------|-------|---------------------------------|----------------------------------|
| **Phenotypic features** | | | | | |
| Size (µm) | 2.2 ± 0.4 × 1.1 ± 0.2 | 2.5 ± 0.3 × 1.5 ± 0.2 | 2.3 ± 0.3 × 1.4 ± 0.2 | 1.1–1.8 × 0.9–1.5 | 1.0–2.0 × 0.8–1.5 |
| Shape | Elongated pear-shaped | Pear-shaped | Pear-shaped | Spherical to ovoid | Round to pear-shaped |
| Colour | Pink | White | Light pink | Light pink | Light pink |
| Temperature range (optimum) (°C) | 10–36 (30) | 10–36 (33) | 10–36 (33) | 15–35 (30) | 15–40 (25) |
| pH range (optimum) | 6.0–9.0 (7.5) | 5.0–9.0 (7.5) | 6.0–8.5 (7.5) | 6.5–10.0 (7.5) | 6.0–9.0 (7.5) |
| Aggregates | Yes, rosettes | Yes, rosettes | Yes, rosettes | Yes, rosettes | No |
| Division | Budding | Budding | Budding | Budding | Budding |
| Motility | Yes | n.o. | n.o. | Yes | No |
| Crateriform structures | At fibre pole | Polar | Overall | At reproductive pole | At one of the poles |
| Fimbriae | Matrix or fibre, at budding pole | Matrix or fibre, at budding pole | Matrix or fibre, at budding pole | Yes | Yes |
| Bud shape | Pill-shaped | Pill-shaped | Like mother cell | Like mother cell | Like mother cell |
| Stalk | n.o. | n.o. | n.o. | n.o. | n.o. |
| Holdfast structure | Yes, opposite fibre pole | n.o. | n.o. | Yes, opposite fibre pole | n.d. |
| **Genomic features** | | | | | |
| Genome size (bp) | 7,437,004 | 7,250,512 | 7,512,089 | 8,212,515 | 6,245,843 |
| Plasmids | No | No | No | No | n.d. |
| G + C content (%) | 57.7 | 58.2 | 58.1 | 59.1 | 62.4 ± 4.0 |
| Completeness (%) | 98.28 | 98.28 | 98.28 | 98.28 | 98.28 |
| Contamination (%) | 3.45 | 1.72 | 3.45 | 1.72 | 1.72 |
| Protein-coding genes | 5515 | 5226 | 5469 | 5919 | 4510 |
| Hypothetical proteins | 2199 | 1983 | 2189 | 2299 | 2816 |
| Protein-coding genes/Mb | 742 | 721 | 728 | 708 | 722 |
| Coding density (%) | 87.2 | 86.6 | 86.6 | 87.5 | 86.5 |
| 16S rRNA genes | 1 | 1 | 1 | 1 | 1 |
| tRNA genes | 47 | 44 | 44 | 71 | 50 |

Genomic analysis for *Roseimaritima ulvae* UC8<sup>T</sup> and *Roseimaritima sediminicola* JC651<sup>T</sup> is based on GenBank accession numbers CP042914 and GCA_009618275, respectively

n.o. not observed, n.d. not determined
Rosistilla gen. nov.

*Rosistilla* (Ro.si.stil’la. L. fem. n. *rosa* a rose; L. fem. n. *stilla* a drop; N.L. fem. n. *Rosistilla* a rosette-forming bacterium with drop-shaped cells).

Members of the genus have a cell envelope architecture similar to that of Gram-negative bacteria, are aerobic, neutrophilic, mesophilic and heterotrophic. Cells are elongated pear-shaped (drop-like shape), divide by budding and form rosettes. Cells are unpigmented or pink. Crateriform structures are present at the poles or cover the entire cell surface. The genomes have a G+C content of around 58%. The genus is part of the family *Pirellulaceae*, order *Pirellulales*, class *Planctomycetia*, phylum *Planctomycetes*. The type species of the genus is *Rosistilla oblonga*.

*Rosistilla oblonga* sp. nov.

*Rosistilla oblonga* (ob.lon’ga. L. fem. adj. oblonga oblong; corresponding to the elongated appearance of individual cells).

In addition to the characteristics given in the genus description, cells are pear-shaped and have a cell size of 2.3–2.5 × 1.4–1.5 μm. Colonies either lack pigmentation or are slightly pink. Cells contain fimbiae or matrix at the budding pole and polar crateriform structures. Stalk and holdfast structure may be present. Cells grow at temperatures of 10–36 °C with optimal medium in biological triplicates. Cultivation was performed as described in the Materials and methods section.

Fig. 5 Temperature and pH optima of the isolates strains Poly24<sup>T</sup>, CA51<sup>T</sup> and Mal33. The graphs show the average growth rates obtained from cultivation in M1H NAG ASW medium.
| Enzyme                                   | EC          | Gene          | CA51<sup>T</sup> | Mal33          | Poly24<sup>T</sup> |
|------------------------------------------|-------------|---------------|-------------------|----------------|-------------------|
| **Glycolysis**                           |             |               |                   |                |                   |
| Glucose-6-phosphate isomerase            | 5.3.1.9     | pgI           | CA51_28500        | Mal33_48960    | Poly24_26790      |
| ATP-dependent 6-phosphofructokinase isozyme 1 | 2.7.1.11    | pfkA          | CA51_07690        | Mal33_07120    | Poly24_11170      |
| Fructose-bisphosphate aldolase class 2   | 4.1.2.13    | frbA          | CA51_27320        | Mal33_50160    | Poly24_20430      |
| Triosephosphate isomerase                | 5.3.1.1     | tpiA          | CA51_20800        | Mal33_21820    | Poly24_21710      |
| Fructose-bisphosphate aldolase class 2   | 2.7.2.3     | fbaB          | CA51_35090        | Mal33_42070    | Poly24_38140      |
| Glyceraldehyde-3-phosphate dehydrogenase | 1.2.1.12    | gapA          | CA51_35090        | Mal33_42070    | Poly24_38140      |
| Phosphoglycerate kinase                  | 2.7.2.3     | pgk           | CA51_19370        | Mal33_39790    | Poly24_43390      |
| 2,3-Bisphosphoglycerate-independent phosphoglycerate mutase | 5.4.2.12 | gpmI          | CA51_00820        | Mal33_00850    | Poly24_51780      |
| Enolase                                  | 4.2.1.11    | eno           | CA51_02560        | Mal33_02740    | Poly24_02080      |
| Pyruvate kinase I                        | 2.7.1.40    | pykF          | CA51_08860        | Mal33_08330    | Poly24_08360      |
| Pyruvate dehydrogenase E1 component      | 1.2.4.1     | aceE          | CA51_39170        | Mal33_39790    | Poly24_43390      |
| Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex | 2.3.1.12 | aceF          | CA51_39160        | Mal33_39780    | Poly24_28990      |
| **Gluconeogenesis**                      |             |               |                   |                |                   |
| Phosphoenolpyruvate carboxylase          | 4.1.1.31    | ppc           | CA51_23330        | Mal33_54740    | Poly24_29970      |
| Pyruvate, phosphate dikinase             | 2.7.9.1     | ppdK          | CA51_24710        | Mal33_53210    | Poly24_24110      |
| Pyruvate carboxylase                     | 6.4.1.1     | pyc           | CA51_17250        | Mal33_20070    | Poly24_16690      |
| Phosphoenolpyruvate carboxykinase (ATP)  | 4.1.1.49    | pckA          | CA51_25590        | Mal33_52570    | Poly24_22680      |
| PP<sub>7</sub>-type phosphoenolpyruvate carboxykinase | 4.1.1.38 | PEPCK         | CA51_45950        | Mal33_31650    | Poly24_48970      |
| Pyrophosphate–fructose 6-phosphate 1-phosphotransferase | 2.7.1.90 | pfp            | CA51_28740        | Mal33_48680    | Poly24_25860      |
| **Pentose phosphate pathway**             |             |               |                   |                |                   |
| Glucose-6-phosphate 1-dehydrogenase      | 1.1.1.49    | zwf           | CA51_17800        | Mal33_17610    | Poly24_16800      |
| 6-Phosphogluconolactonase                | 3.1.1.31    | pgl           | CA51_26550        | Mal33_49970    | Poly24_29370      |
| 6-Phosphogluconate dehydrogenase, decarboxylating | 1.1.1.44 | gmdA          | CA51_27970        | Mal33_49500    | Poly24_21080      |
| Transketolase 2                          | 2.2.1.1     | tktB          | CA51_32040        | Mal33_45100    | Poly24_33010      |
| Transaldolase B                          | 2.2.1.2     | talB          | CA51_44840        | Mal33_32950    | Poly24_11860      |
| **Entner–Doudoroff pathway (KDPG)**       |             |               |                   |                |                   |
| KHG/KDPG aldolase                        | 4.1.2.14    | eda           | CA51_35880        | Mal33_41360    | Poly24_37690      |
| Phosphogluconate dehydratase             | 4.2.1.12    | edd           | CA51_17990        | Mal33_18570    | Poly24_12210      |
| **TCA cycle**                            |             |               |                   |                |                   |
| Citrate synthase                         | 2.3.3.16    | gha           | CA51_34870        | Mal33_42290    | Poly24_35470      |
| Aconitate hydratase A                    | 4.2.1.3     | acnA          | CA51_26170        | Mal33_51990    | Poly24_20280      |
| Isocitrate dehydrogenase [NADP]          | 1.1.1.42    | icd           | CA51_38400        | Mal33_38710    | Poly24_37940      |
| 2-Oxoglutarate dehydrogenase E1 component | 1.2.4.2 | sucA          | CA51_08330        | Mal33_06250    | Poly24_05710      |
| Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex | 2.3.1.61 | sucB          | CA51_18830        | Mal33_19360    | Poly24_28990      |
| Succinate–CoA ligase [ADP-forming] subunit alpha | 6.2.1.5 | sucD          | CA51_28020        | Mal33_49450    | Poly24_21030      |
| Succinate–CoA ligase [ADP-forming] subunit beta | 6.2.1.5 | sucC          | CA51_28010        | Mal33_49460    | Poly24_21040      |
| Succinate dehydrogenase flavoprotein subunit | 1.3.5.1 | sdhA          | CA51_43190        | Mal33_34540    | Poly24_44610      |
| Succinate dehydrogenase iron-sulfur subunit | 1.3.5.1 | sdhB          | CA51_43180        | Mal33_34550    | Poly24_44620      |
| Succinate dehydrogenase cytochrome b556 subunit | 1.3.5.1 | sdhC          | CA51_43200        | Mal33_34530    | Poly24_44600      |
| Fumarate hydratase class II              | 4.2.1.2     | fumC          | CA51_47870        | Mal33_29690    | Poly24_47530      |
growth at 33 °C. The pH optimum for growth is 7.5, and growth is observed at pH 5–9. The type strain genome has a size of 7.25 Mb.

The type strain is CA51T (= DSM 104080T = LMG 29702T), which was isolated from a giant bladder kelp (Macrocystis pyrifera) in Monterey Bay, CA, USA in November 2014. Strain Mal33 is an additional member of the species.

Rosistilla carotiformis sp. nov.

Rosistilla carotiformis (ca.ro.ti.for’mis. L. fem. n. carota a carrot; L. suff. adj. formis a form, a figure; N.L. fem. adj. carotiformis shaped like a carrot; corresponding to the carrot-shaped morphology of the cells).

In addition to the characteristics given in the genus description, cells form pink colonies. Cells have a size of 2.3 × 1.1 μm and are motile. Cells form fimbriae or matrix on one of the cell poles, on which also crateriform structures are present. A holdfast structure is present at the opposite pole. Cells of the type strain grow at temperatures ranging from 10 to 33 °C (optimum 30 °C) and over a pH range of 6.5 to 8.5 (optimum 7.5). The genome size of the type strain is 7.43 Mb.

The type strain is Poly24T (= DSM 102938T = VKM B-3434T = LMG 31347T = CECT 9848T), which was isolated from polyethylene particles incubated at 2 m depth in the Baltic Sea close to Heiligendamm, Germany in October 2015.

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Author contributions MW and MS wrote the manuscript, NK analysed the data, prepared the figures and revised the manuscript, SW and MJ performed the genomic and phylogenetic analyses, AH and PR 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 took the samples, supervised PR and AH and 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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