



Maioricimonas rarisocia gen. nov., sp. nov., a novel planctomycete isolated from marine sediments close to Mallorca Island

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Abstract Planctomycetes are ubiquitous bacteria with environmental and biotechnological relevance. Axenic cultures of planctomycetal strains are the basis to analyse their unusual biology and largely uncharacterised metabolism in more detail. Here, we describe strain Mal4^T isolated from marine sediments close to Palma de Mallorca, Spain. Strain Mal4^T displays common planctomycetal features, such as division by polar budding and the presence of fimbriae and crateriform structures on the cell surface. Cell growth

was observed at ranges of 10–39 °C (optimum at 31 °C) and pH 6.5–9.0 (optimum at 7.5). The novel strain shows as pear-shaped cells of $2.0 \pm 0.2 \times 1.4 \pm 0.1$ µm and is one of the rare examples of orange colony-forming Planctomycetes. Its genome has a size of 7.7 Mb with a G+C content of 63.4%. Phylogenetically, we conclude that strain Mal4^T (= DSM 100296^T = LMG 29133^T) is the type strain representing the type species of a novel genus, for which we propose the name *Maioricimonas rarisocia* gen. nov., sp. nov.

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Introduction

Planctomycetes are bacteria that belong to the PVC superphylum (Wagner and Horn 2006), which includes the phyla *Planctomycetes*, *Verrucomicrobia*, *Chlamydiae*, *Lentisphaerae* and *Kirimatiellaeota* as well as some uncultured candidate phyla, such as *Candidatus* Omnitrophica. The PVC superphylum has environmental, medical and biotechnological relevance (Devos and Ward 2014).

Planctomycetes have been shown to be present in several environments, in which they play important roles in biogeochemical cycles, such as the carbon and nitrogen cycle (Wiegand et al. 2018). One example are

Planctomycetes of the class *Candidatus* Brocadiaae, which perform unique reactions during anaerobic ammonium oxidation (anammox) (Strous et al. 1999; Peeters and van Niftrik 2019). Members of the phylum *Planctomycetes*, in particular of the class *Planctomycetia*, colonise a variety of environments from terrestrial to aquatic, being able to dwell on various marine algal surfaces (Bengtsson et al. 2012; Bondoso et al. 2014, 2015, 2017; Lage and Bondoso 2014; Vollmers et al. 2017). They form biofilms on biotic surfaces (Bengtsson and Øvreås 2010), on which they metabolise complex carbon substrates (Lachnit et al. 2013; Jeske et al. 2013). Unique pili-forming crateriform structures and an enlarged periplasm are probably required for uptake and also cleavage of large polysaccharides obtained from the environment (Boedeker et al. 2017).

Planctomycetes possess large genomes with sizes of up to 12.4 Mb (Ravin et al. 2018), in which the presence of giant genes has been reported (Jeske et al. 2013; Guo et al. 2014; Kohn et al. 2016; Faria et al. 2018). These genome sizes are in line with their assumed capacity for secondary metabolite production (Graça et al. 2016; Jeske et al. 2016; Yadav et al. 2018). Furthermore, several members of the phylum *Planctomycetes* produce carotenoids, which could be associated with an increased tolerance against UV radiation or oxidative stress (Kallscheuer et al. 2019b).

Planctomycetes were considered exceptional due to several presumptively eukaryotic features, such as the lack of a peptidoglycan (König et al. 1984), a compartmentalised cell plan (Lindsay et al. 1997), a nucleus-like structure (Fuerst and Webb 1991) and the endocytosis-like uptake of macromolecules for an intracellular degradation (Lonhienne et al. 2010). However, with advances of microscopy techniques and the development of genetic tools (Jogler et al. 2011; Rivas-Marín et al. 2016b; Boedeker et al. 2017), many of these traits have been refuted or reinterpreted.

In recent years, the presence of peptidoglycan has been reported in several members of the *Planctomycetes* (Jeske et al. 2015; van Teeseling et al. 2015) and also in the sister phyla *Verrucomicrobia* (Rast et al. 2017) and *Chlamydiae* (Pilhofer et al. 2013; Liechti et al. 2014, 2016). With the exception of anammox-performing Planctomycetes (Jogler 2014; Neumann et al. 2014), the proposed cell plan has been found to feature large invaginations of the cytoplasmic membrane instead of closed compartments

(Santarella-Mellwig et al. 2013; Acehan et al. 2014; Boedeker et al. 2017). These discoveries contributed to the reinterpretation of Planctomycetes as bacteria with a cell envelope architecture resembling that of Gram-negative bacteria, but with some variations (Devos 2014a, b; Boedeker et al. 2017). Nevertheless, Planctomycetes remain exceptional in other ways, e.g. they lack the protein FtsZ normally essential for bacterial division as well as other division proteins (Pilhofer et al. 2008; Jogler et al. 2012; Rivas-Marín et al. 2016a). Beyond that, phylum members divide by binary fission, budding or intermediate mechanisms (Wiegand et al. 2018, 2020). Presence and essentiality of sterols in the membranes of one of its members was recently reported (Pearson et al. 2003; Rivas-Marín et al. 2019).

The unusual cell biology of Planctomycetes prompted us to explore the uncharacterised planctomycetal diversity. In the present study, we describe the novel strain Mal4^T isolated from marine sediments in Palma de Mallorca (Spain) in terms of physiological, microscopic as well as genomic properties. Supported by phylogenetic analyses, we conclude that strain Mal4^T represents a novel species of a novel genus within the family *Planctomycetaceae*.

Materials and methods

Cultivation conditions and isolation

Strain Mal4^T was isolated from marine sediments at the coast of S'Arenal close to Palma de Mallorca (Spain) on the 23th of September 2014 (sampling location: 39.5126 N 2.7470 E) as previously described (Wiegand et al. 2020). For strain isolation and cultivation M1H NAG ASW medium was used. Medium preparation was previously described (Kallscheuer et al. 2019a). Cultures were incubated in baffled flasks at 28 °C with constant agitation at 110 rpm. Plates were cultivated at 28 °C for 2–3 weeks and isolated colonies were then streaked on fresh M1H NAG ASW plates. Initial amplification and sequencing of the 16S rRNA gene, intended to check whether isolated strains are members of the phylum *Planctomycetes*, was performed as previously described (Rast et al. 2017).

Physiological analyses

Cultivations for physiological assays were performed in M1H NAG ASW medium. For pH optimum determination, 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) was used for cultivations at pH 5.0, 5.5, 6.0 and 6.5. For cultivations at pH values ranging from 7.0 to 8.0, MES was replaced by 100 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), whereas 100 mM 3-(4-(2-Hydroxyethyl)piperazin-1-yl)-propane-1-sulfonic acid (HEPPS) served as a buffering agent at pH 8.5 and 100 mM *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) was used for pH maintenance at pH 9.0 and 9.5. Cultivations for determination of the pH optimum were performed at 28 °C. For temperature optimum determination, strain Mal4^T was cultivated at pH 8.0 at different temperatures ranging from 10 to 39 °C. Cell growth and maximal growth rates were inferred from optical density measurements at 600 nm (OD₆₀₀) of triplicate cultures.

Genome analysis

The genome of strain Mal4^T was previously published (Wiegand et al. 2020). The genome (accession number CP036275) and 16S rRNA gene sequence (accession number MK559979) are available from the GenBank database. The primary metabolism was analysed by examining locally computed InterProScan (Mitchell et al. 2019) results cross-referenced with information from the UniProt database (UniProt 2019) and BlastP results of ‘typical’ protein sequences. Numbers of carbohydrate-active enzymes were determined by employing dbCAN2 (Zhang et al. 2018), which automatically mines the CAZy database (Lombard et al. 2014).

Light microscopy and scanning electron microscopy

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

Phylogenetic analyses

16S rRNA gene sequence-based phylogeny was computed for strain Mal4^T, the type strains of all

described planctomycetal species (assessed in January 2020) and all isolates recently published (Kohn et al. 2016, 2020a, b; Boersma et al. 2019; Kallscheuer et al. 2019a; Dedysh et al. 2020; Wiegand et al. 2020). An alignment of 16S rRNA gene sequences was performed with SINA (Pruesse et al. 2012). The phylogenetic analysis was conducted employing a maximum likelihood approach with 1000 bootstraps, the nucleotide substitution model GTR, gamma distribution and estimation of proportion of invariable sites (GTRGAMMAI option) (Stamatakis 2014). Three 16S rRNA genes of bacterial strains from the PVC superphylum, but outside of the phylum *Planctomycetes*, were used as outgroup. The *rpoB* nucleotide sequences (encoding the RNA polymerase β -subunit) were taken from publicly available genome annotations and the sequence identities were determined as described previously (Bondoso et al. 2013) using Clustal Omega (Sievers et al. 2011). Alignment and matrix calculation were done after extracting only those parts of the sequence that would have been sequenced with the described primer set. The average nucleotide identity (ANI) was calculated using OrthoANI (Lee et al. 2016). The average amino acid identity (AAI) was obtained with the aai.rb script of the enveomics collection (Rodriguez-R and Konstantinidis 2016). The percentage of conserved proteins (POCP) was calculated as described before (Qin et al. 2014). The unique single-copy core genome of all analysed genomes for the multi-locus sequence analysis (MLSA) was determined with proteinortho5 (Lechner et al. 2011) (‘selfblast’ option enabled). The sequences of the obtained 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 inference

In the phylogenetic trees obtained after analysis of 16S rRNA gene sequences, as well as MLSA (Fig. 1),

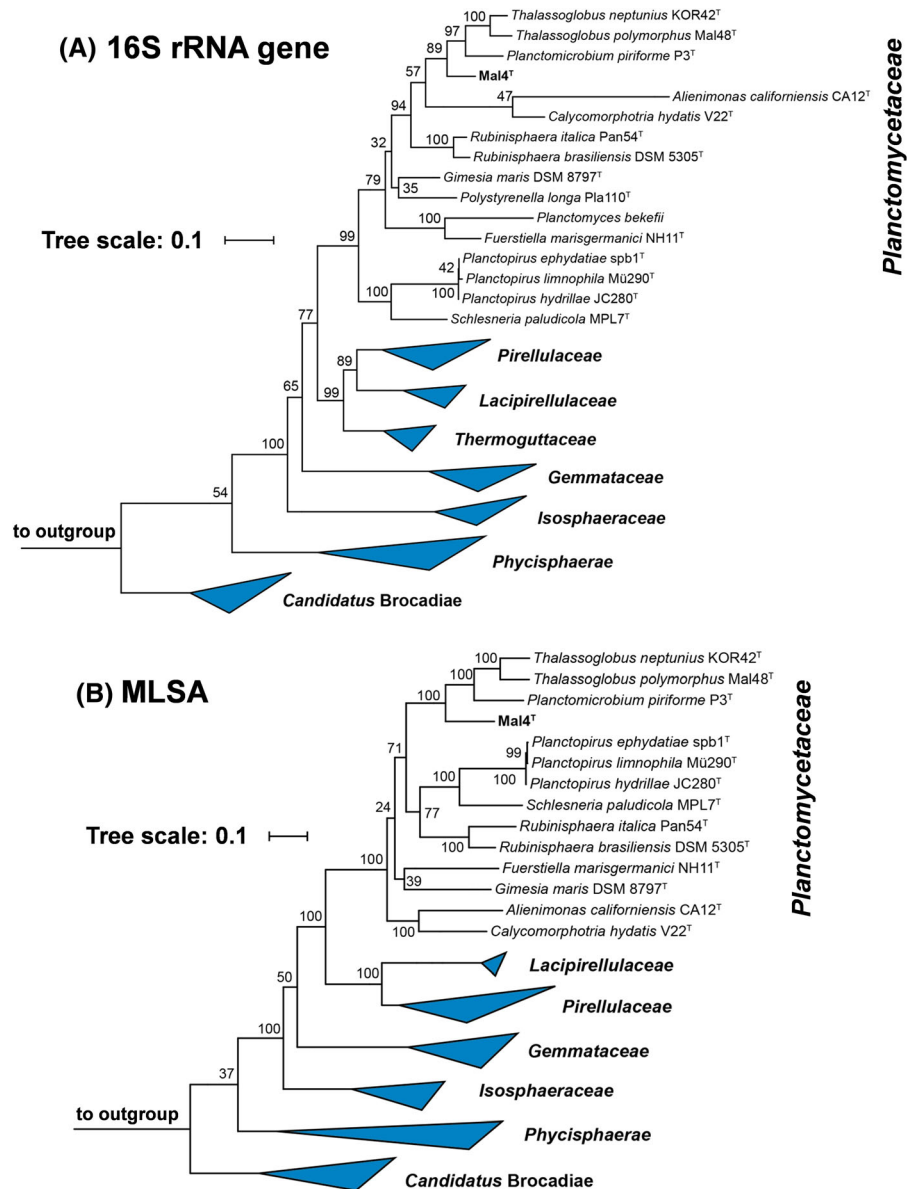


Fig. 1 Phylogenetic trees highlighting the position of strain *Mal4*^T. The outgroup consists of three 16S rRNA genes from the PVC superphylum outside of the phylum *Planctomycetes*.

strain *Mal4*^T clusters stably with members of two genera of the family *Planctomycetaceae*, namely *Planctomicrobium* and *Thalassoglobus*. 16S rRNA gene sequence identity between strain *Mal4*^T and the two genera is between 91.4% and 91.9% (Fig. 2). These values are below the proposed genus threshold of 94.5%, but above the threshold for separate families of 86.5% (Yarza et al. 2014), indicating that strain

Bootstrap values from 1000 re-samplings (500 re-samplings for MLSA) are given at the nodes (in %)

Mal4^T represents an distinct genus in the family *Planctomycetaceae*. Coherently, average nucleotide identities (ANI) below 95% confirm that strain *Mal4*^T is a distinct species. Phylogenetic assumptions on the genus level can also be obtained by analysing the *rpoB* gene sequence identities, AAI and POCP. For delineation of genera, the proposed threshold values for the above-mentioned markers are 75.5–78% (Kallscheuer

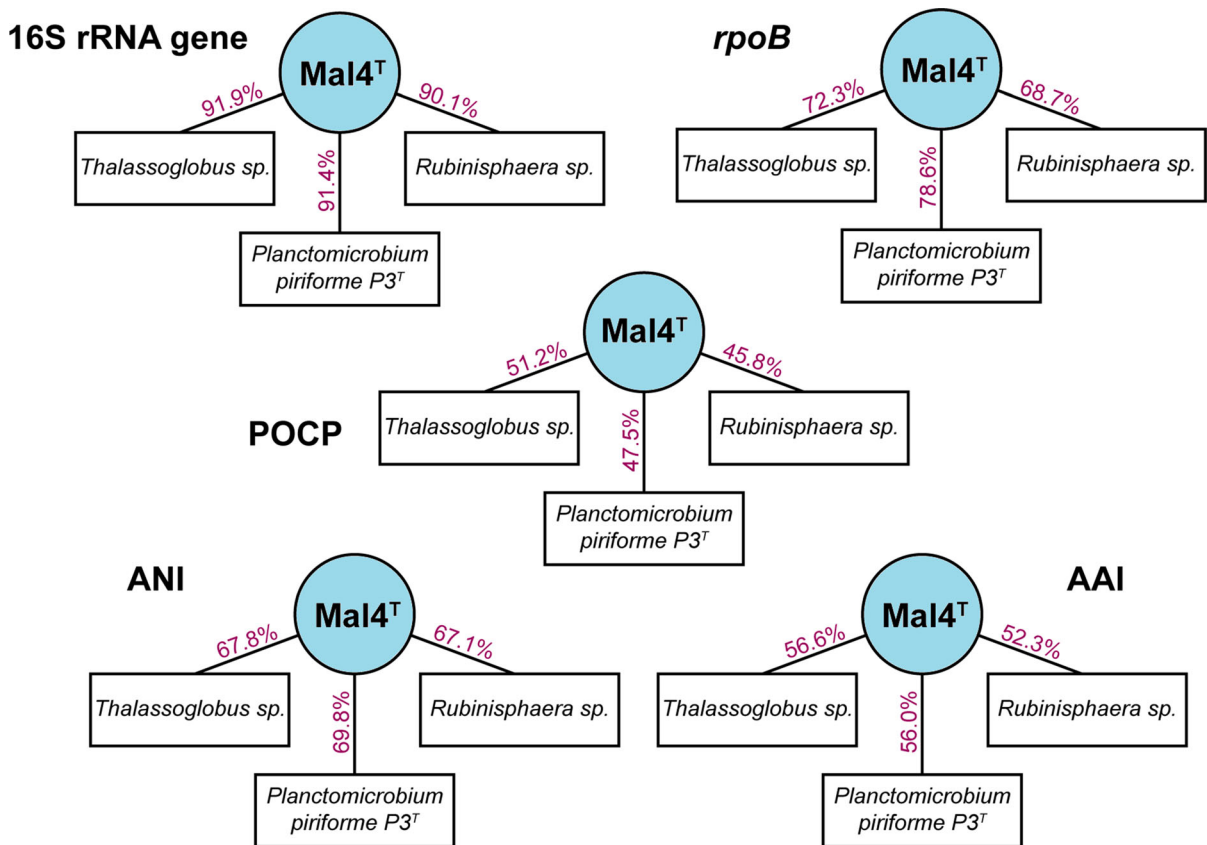


Fig. 2 Similarity values of the novel isolate Mal4^T in relation to species *P. piriforme P3^T*, *Thalassoglobus sp.* and *Rubinisphaera sp.* Methods used: 16S rRNA gene sequence identity, *rpoB* gene

identity, percentage of conserved proteins (POCP), average nucleotide identity (ANI) and average amino acid identity (AAI)

et al. 2019c), 45–65% (Konstantinidis et al. 2017) and 50% (Qin et al. 2014), respectively. The *rpoB* identity value and the AAI between strain Mal4^T and the members of the genus *Thalassoglobus*, which comprises *Thalassoglobus neptunius* KOR42^T (Kohn et al. 2020a) and *Thalassoglobus polymorphus* Mal48^T (Rivas-Marin et al. 2020), are below the given thresholds. POCP was found to be slightly above the threshold (51.2%), this, however, does not significantly influence the overall conclusion that strain Mal4^T belongs to a separate genus. Minimal comparative values of strain Mal4^T and the genus *Rubinisphaera*, another closely related genus; featuring *Rubinisphaera italica* (Kallscheuer et al. 2019a) and *Rubinisphaera brasiliensis* (Scheuner et al. 2014), are below these thresholds for all three phylogenetic markers (Fig. 2). Analogously, POCP between strain Mal4^T and *Planctomicrobium piriforme P3^T* (Kulichevskaya et al. 2015) was also found to fall below the

proposed threshold (Fig. 2), whilst the AAI value (56%) was in a ‘grey zone’ (45–65%), but well below the upper limit. Although the *rpoB* gene sequence identity of 78.6% is slightly above the proposed threshold, this sole deviance should not overrule the distinctiveness of the other values. In summary, the majority of analysed phylogenetic markers suggests that strain Mal4^T belongs to a novel genus.

Morphological and physiological analyses

Light microscopy and scanning electron microscopy (Fig. 3) were applied to analyse the morphological characteristic of Mal4^T cells harvested during the exponential growth phase. Detailed information on morphology and cell division is summarised in comparison to the current closest relatives (Table 1). Mal4^T cells are pear-shaped (2.0 ± 0.2 µm × 1.4 ± 0.1 µm) (Fig. 3a–c), occur as single cells and in rare

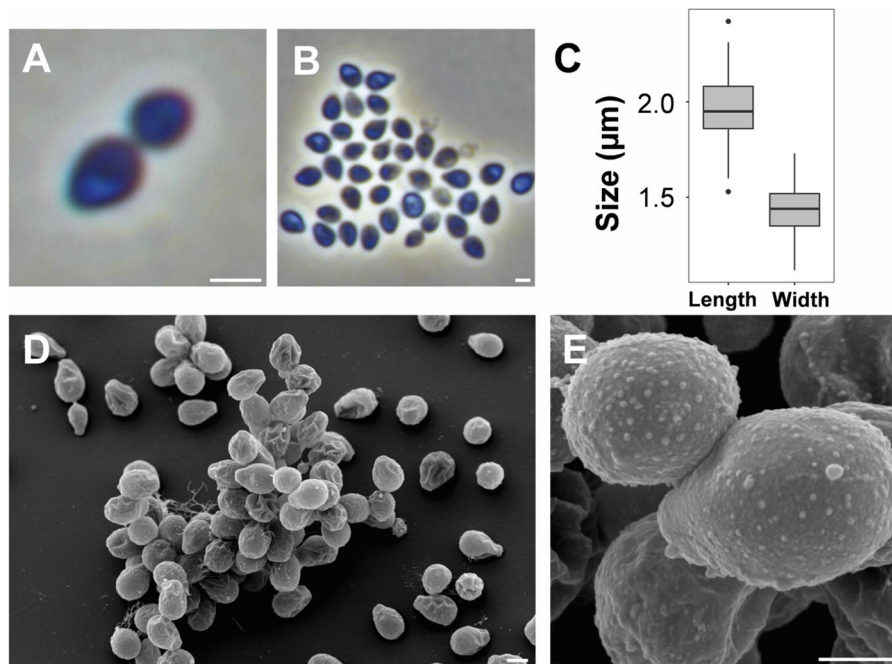


Fig. 3 Microscopy images and cell size plot of strain Mal4^T. Pictures from light microscopy (**a**, **b**) and scanning electron microscopy (**d**, **e**) are shown. The scale bars are 1 µm. For

determination of the cell size (**c**) at least 100 representative cells were counted manually or by using a semi-automated object count tool

cases form aggregates (Fig. 3d). The cell surface appears rough, evenly covered with crateriform structures and short fimbriae (Fig. 3d, e). A holdfast structure was not observed during electron microscopy. As shown for all described members of the family *Planctomycetaceae*, cell division takes place by polar budding with the daughter cell displaying a round shape. Optimal temperature and pH for growth were shown to be 31 °C and pH 7.5, respectively, however, Mal4^T cells are able to grow over a range of 10–39 °C and pH 6.5–9.0 (Fig. 4). These values are comparable to the two *Thalassoglobus* species, but differ from *P. piriforme* P3^T, which did not grow at temperatures exceeding 30 °C and favours moderate acidic conditions. The maximal observed growth rate of strain Mal4^T in M1H NAG ASW medium was determined to be 0.041 h⁻¹, corresponding to a generation time of approximately 17 h. Strain Mal4^T is amongst the rare examples of planctomycetal strains forming orange colonies and might thus be an interesting strain for further analysis of carotenoid

production and their function in Planctomycetes. Since most of the planctomycetal strains characterised so far are either pink to red or lack pigmentation (white), the pigmentation of the novel strain is an important phenotypic feature separating it from its current close phylogenetic neighbours. Strain Mal4^T is an aerobic heterotroph.

Genomic characteristics

The genomic characteristics of strain Mal4^T in comparison to *T. polymorphus* Mal48^T, *T. neptunius* KOR42^T and *P. piriforme* P3^T are summarised in Table 1. Its genome is 7.7 Mb in size, which is around 1 Mb larger compared to the other three strains. The G+C content is also the highest of the four strains. Automated gene prediction and annotation identified 5829 putative protein-encoding genes, of which 39% (2257 genes) are annotated as hypothetical proteins. These values correspond to 753 protein-coding genes per Mb and a coding density of 85.9%. Although the

Table 1 Phenotypic and genotypic features of strain Mal4^T in comparison to its current closest relatives

Characteristics	Mal4 ^T	<i>Thalassoglobus polymorphus</i> Mal48 ^T	<i>Thalassoglobus neptunius</i> KOR42 ^T	<i>Planctomicrobium piriforme</i> P3 ^T *
Phenotypic features				
Color	Orange	Beige	Cream	White
Size (µm)	2.0 × 1.4	1.6 × 0.9	1.7 (diameter)	1.7–2.8 × 0.9–1.3 µm
Shape	Pear-shaped	Pear-shaped	Spherical	Ellipsoidal to pear-shaped
Temperature range (optimum) (°C)	10–39 (31)	15–36 (30)	22–36 (33)	10–30 (20–28)
pH range (optimum)	6.5–9.0 (7.5)	6.5–8.0 (7.5)	5.5–8.5 (7.0–7.5)	4.2–7.1 (6.0–6.5)
Aggregates	Yes	Yes	Yes	Yes
Division	Budding	Budding	Budding	Budding
Dimorphic life cycle	n.o.	n.o.	n.o.	Yes
Flagella	n.o.	n.o.	n.o.	Yes
Crateriform structures	Yes, overall	n.o.	Yes	Yes, polar
Fimbriae	Yes, overall matrix or fibre	Yes, overall matrix or fibre	Few fibres	Yes
Capsule	n.o.	n.o.	n.o.	n.d.
Bud shape	Round	Like mother cell	Round	Like mother cell
Budding pole	Polar	Polar	n.o.	Polar
Stalk	Yes	Yes	n.o.	Yes
Holdfast structure	n.o.	n.o.	Yes	Yes
Genotypic features				
Genome size (bp)	7,744,989	6,357,355	6,734,412	6,317,004
Plasmids (bp)	No	No	n.d.	n.o.
G+C content (%)	63.4	50.3	52.8	58.8
Protein-coding genes	5829	4874	5508	5050
Protein-coding genes/ Mb	753	767	818	799
Hypothetical proteins	2257	1987	2516	2814
Coding density (%)	85.9	84.9	85.7	85.8
16S rRNA genes	2	2	1	1
tRNA genes	55	41	70	53

*Genomic data from GenBank acc. no. NZ_FOQD00000000

n.o. not observed, n.d. not determined

genome size of strain Mal4^T is larger, the coding density is in the same range in the other three species. Similar to its relatives, the strain lacks plasmids. Numbers of 41–55 tRNA genes are similar, except for *T. neptunius* KOR42^T, which has a slightly higher

number of 70 tRNA genes. As for *T. polymorphus* Mal48^T, strain Mal4^T harbours two copies of the 16S rRNA gene, whereas the gene occurs in single copy in the other two strains.

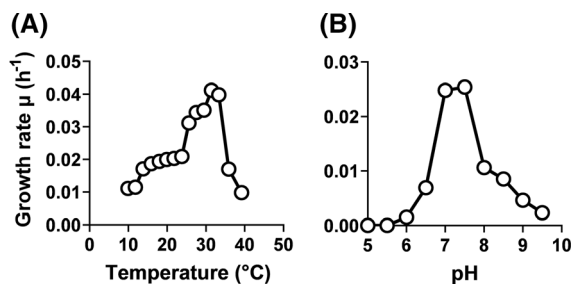


Fig. 4 Temperature and pH optima of strain Mal4^T. Data points show average growth rates obtained after cultivation in MIH NAG ASW medium in biological triplicates. Cultivations at different temperatures (a) were performed at pH 8. Cultivations at different pH values (b) were conducted at 28 °C

Genome-encoded features of the primary carbon metabolism

Based on the genome sequences, we analysed key metabolic capabilities in the primary metabolism of strain Mal4^T in comparison to the two *Thalassoglobus* species and *P. piriforme* P3^T (Table 2). Genes coding for enzymes participating in glycolytic pathways, gluconeogenesis, the tricarboxylic acid (TCA) cycle and anaplerotic reactions, such as pyruvate or phosphoenolpyruvate carboxylation and the glyoxylate shunt, were included. The resulting data suggest that strain Mal4^T is able to metabolise carbohydrates using at least two glycolytic pathways, the Embden-Meyerhof-Parnas pathway (the most common glycolytic pathway) and the pentose phosphate pathway. Additionally, its genome bears genes coding for putative 2-dehydro-3-deoxyphosphogluconate aldolase and phosphogluconate dehydratase, both involved in the alternative Entner-Doudoroff pathway. All four strains harbour a complete gene set required for a functional TCA cycle, which suggests that the central carbon metabolism of the strains is similar to canonical heterotrophic bacteria. With regard to gluconeogenesis, a minimal gene set required for this pathway has been identified, suggesting that the three strains are capable of *de novo* sugar biosynthesis. All four

strains lack the glyoxylate shunt, which is typically required during growth either with acetate or with compounds that are degraded to acetate or acetyl-CoA units. The lack of the glyoxylate shunt suggests that the strains are not able to use such compounds as the exclusive energy and carbon source. Alternatively, they may follow a different pathway with a similar function.

Based on the physiological, morphological and phylogenetic analyses of strain Mal4^T, we conclude that the characterised strain represents a novel species within the novel genus *Maioricimonas*. Thus, we propose the name *Maioricimonas rarisocia* gen. nov., sp. nov., represented by the type strain Mal4^T.

Maioricimonas gen. nov.

Maioricimonas (Ma.io.ri.ci'mo.nas. M.L. fem. n. *Maiorica* of Mallorca; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Maioricimonas* a monad from Mallorca, Spain).

Cells have a Gram-negative cell envelope architecture and divide by polar budding. Cells are mesophilic, neutrophilic, aerobic and heterotrophic and present crateriform structures and matrix or fimbriae. The genus is part of the family *Planctomycetaceae*, order *Planctomycetales*, class *Planctomycetia*, phylum *Planctomycetes*. The type species of the genus is *Maioricimonas rarisocia*.

Maioricimonas rarisocia sp. nov.

Maioricimonas rarisocia (ra.ri.so'ci.a. L. masc. adj. *rarus* few, infrequent; L. masc. adj. *socius* allied, united; N.L. fem. adj. *rarisocia*; corresponding to the characteristic of the cells to seldom form aggregates).

In addition to the features described for the genus, cells are pear-shaped (2.0 × 1.4 μ m), form orange colonies and mostly occur as single cells. Temperature and pH optimum of the type strain are 31 °C and 7.5, respectively, however growth is observed over a range

Table 2 Genome-based primary metabolism of strain Mal4^T compared to the close relatives *Thalassoglobus polymorphus* Mal48^T, *Thalassoglobus neptunius* KOR42^T and *Planctomicrobium piriforme* P3^T*

Enzyme	EC number	Gene	Mal4 ^T	Mal48 ^T	KOR42 ^T	P3 ^T *
Glycolysis (Embden–Meyerhof–Parnas pathway)						
Glucose-6-phosphate isomerase	5.3.1.9	<i>pgi</i>	Mal4_41440	Y	Y	Y
ATP-dependent 6-phosphofructokinase isozyme 1	2.7.1.11	<i>pfkA</i>	Mal4_28800	Y	Y	Y
Fructose-bisphosphate aldolase class 2	4.1.2.13	<i>fbaA</i>	Mal4_06980	Y	Y	Y
Triosephosphate isomerase	5.3.1.1	<i>tpiA</i>	Mal4_33520	Y	Y	Y
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	<i>gapA</i>	Mal4_10410	Y	Y	Y
Phosphoglycerate kinase	2.7.2.3	<i>pgk</i>	Mal4_43170	Y	Y	Y
2,3-Bisphosphoglycerate-independent phosphoglycerate mutase	5.4.2.12	<i>gpmI</i>	Mal4_57980	Y	Y	n.a.
2,3-Bisphosphoglycerate-dependent phosphoglycerate mutase	5.4.2.11	<i>gpmA</i>	N	N	N	Y
Enolase	4.2.1.11	<i>eno</i>	Mal4_26950	Y	Y	Y
Pyruvate kinase I	2.7.1.40	<i>pykF</i>	Mal4_16440	N	N	Y
Pyruvate dehydrogenase complex	1.2.4.1/ 2.3.1.12	<i>aceEF</i>	Mal4_36650/ Mal4_31770	Y	Y	Y
Gluconeogenesis						
Phosphoenolpyruvate synthase	2.7.9.2	<i>ppsA</i>	N	N	N	n.a.
Pyruvate, phosphate dikinase	2.7.9.1	<i>ppdK</i>	Mal4_37310	Y	Y	Y
Pyruvate carboxylase	6.4.1.1	<i>pyc</i>	Mal4_02890	Y	Y	Y
Phosphoenolpyruvate carboxykinase (ATP)	4.1.1.49	<i>pckA</i>	Mal4_29720	N	N	Y
Phosphoenolpyruvate carboxykinase (GTP)	4.1.1.32	<i>pckG</i>	N	N	N	N
Phosphoenolpyruvate carboxykinase (diphosphate)	4.1.1.38	<i>PEPCK</i>	Mal4_57980	Y	Y	n.a.
Fructose-1,6-bisphosphatase class 2	3.1.3.11	<i>glpX</i>	N	N	N	n.a.
Fructose-1,6-bisphosphatase class 1	3.1.3.11	<i>fbp</i>	Mal4_30740	N	N	n.a.
Pyrophosphate–fructose 6-phosphate 1-phosphotransferase	2.7.1.90	<i>pfp</i>	Mal4_26620	Y	Y	Y
Pentose phosphate pathway						
Glucose-6-phosphate 1-dehydrogenase	1.1.1.49	<i>zwf</i>	Mal4_45260	Y	Y	Y
6-Phosphogluconolactonase	3.1.1.31	<i>pgl</i>	Mal4_13550, Mal4_58700, Mal4_20230	Y	Y	Y
6-Phosphogluconate dehydrogenase, decarboxylating	1.1.1.44	<i>gndA</i>	Mal4_26580	Y	Y	Y
Transketolase 2	2.2.1.1	<i>tktB</i>	Mal4_57590/Mal4_57600	Y	Y	Y
Transaldolase B	2.2.1.2	<i>talB</i>	Mal4_01090	Y	Y	Y
KDPG (Entner–Doudoroff pathway)						
KDPG aldolase	4.1.2.14	<i>eda</i>	Mal4_43780	Y	Y	Y
Phosphogluconate dehydratase	4.2.1.12	<i>edd</i>	Mal4_05000	Y	Y	Y
TCA cycle						
Citrate synthase	2.3.3.16	<i>gltA</i>	Mal4_25960	Y	Y	Y
Aconitate hydratase A	4.2.1.3	<i>acnA</i>	Mal4_15300	Y	Y	Y
Isocitrate dehydrogenase [NADP]	1.1.1.42	<i>icd</i>	Mal4_26830	Y	Y	Y

Table 2 continued

Enzyme	EC number	Gene	Mal4 ^T	Mal48 ^T	KOR42 ^T	P3 ^{T*}
2-Oxoglutarate dehydrogenase complex	1.2.4.2/ 2.3.1.61	<i>sucAB</i>	Mal4_26900/Mal4_26910	Y	Y	Y
Succinate-CoA ligase complex	6.2.1.5	<i>sucCD</i>	Mal4_08670/Mal4_08660	Y	Y	Y
Succinate dehydrogenase complex	1.3.5.1	<i>sdhABC</i>	Mal4_42560/ Mal4_42550/ Mal4_42570	Y	Y	Y
Fumarate hydratase class I, an/aerobic	4.2.1.2	<i>fumAB</i>	N	N	N	n.a.
Fumarate hydratase class II	4.2.1.2	<i>fumC</i>	Mal4_09530	Y	Y	Y
Malate dehydrogenase	1.1.1.37	<i>mdh</i>	Mal4_25770	Y	Y	Y
Glyoxylate shunt						
Isocitrate lyase	4.1.3.1	<i>aceA</i>	N	N	N	n.a.
Malate synthase G	2.3.3.9	<i>glcB</i>	N	N	N	n.a.

*Genomic data from GenBank acc. no. NZ_FOQD00000000. Presence of a gene in strains used for comparison is indicated by ‘Y’ and absence is indicated by ‘N’

n.a. not available

of 10–39 °C and pH 6.5–9.0. The type strain genome (accession number CP036275) and 16S rRNA gene (accession number MK559979) are available from GenBank. The genome of the type strain has a G+C content of 63.4% and a size of 7.7 Mb.

The type strain is Mal4^T (= DSM 100296^T = LMG 29133^T, deposited as strain Malle4), isolated from marine sediments near the coast of S’Arenal in Palma de Mallorca, Mallorca Island, Spain.

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Author contributions E.R.M. wrote the manuscript, analysed the data and prepared the figures, S.W. and M.J. performed the genomic and phylogenetic analysis, A.H. isolated the strain and performed the initial strain cultivation and deposition, S.H.P. and C.B. performed the light microscopic analysis, N.K. and M.S.M.J. contributed to text preparation and revised the manuscript, M.R. performed the electron microscopic analysis, C.J. took the samples, supervised A.H. 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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