Alienimonas californiensis gen. nov. sp. nov., a novel Planctomycete isolated from the kelp forest in Monterey Bay

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Received: 23 June 2019 / Accepted: 26 November 2019 / Published online: 4 December 2019 © Springer Nature Switzerland AG 2019

Abstract Planctomycetes are environmentally important bacteria and are often found in association with nutrient-rich (marine) surfaces. To allow a more comprehensive understanding of planctomycetal lifestyle and physiology we aimed at expanding the collection of axenic cultures with new isolates. Here, we describe the isolation and genomic and physiological characterisation of strain CA12T obtained from giant bladder kelp (Macrocystis pyrifera) in Monterey Bay, California, USA. 16S rRNA gene sequence and whole genome-based phylogenetic analysis showed that strain CA12T clusters within the family Planctomycetaceae and that it has a high 16S rRNA sequence similarity (82.3%) to Planctomicrobium piriforme DSM 26348T. The genome of strain CA12T has a length of 5,475,215 bp and a G+C content of 70.1%. The highest growth rates were observed at 27°C and pH 7.5. Using different microscopic methods, we could show that CA12T is able to divide by consecutive polar budding, without completing a characteristic planctomycetal lifestyle switch. Based on our data, we suggest that the isolated strain represents a novel species within a novel genus. We thus propose the name Alienimonas gen. nov. with Alienimonas californiensis sp. nov. as type species of the novel genus and CA12T as type strain of the novel species.

Keywords Cell division · Marine bacteria · Planctomycetes · Primary metabolism · Carbohydrate active enzymes

Introduction

Planctomycetes are environmentally important bacteria that are found across all aquatic habitats, where they act as key players in global carbon and nitrogen cycles (Wiegand et al. 2018). Together with members of the phyla Verrucomicrobia, Chlamydiae and others, Planctomycetes belong to the medically and biotechnologically relevant PVC superphylum (Wagner and Horn 2006). The phylum Planctomycetes itself is divided into two classes: Planctomycetia and
Phycisphaerae. The order Planctomycetales is the only validly named order within the Planctomycetia and consists of three families: Planctomycetaceae, Isosphaeraceae, and Gemmataceae, with the vast majority of described planctomycetal species belonging to the family Planctomycetaceae (Scheuner et al. 2014; Kulichevskaya et al. 2016, 2017). The class Phycisphaerae consists of the orders Phycisphaerales, Sedimentisphaerales, and Tepidisphaerales, each consisting of only one family (Fukunaga et al. 2009; Kovaleva et al. 2015; Spring et al. 2018). Anammox Planctomycetes make up the Candidatus Brocadia clade, which holds this placeholder status due to the lack of axenic cultures (Peeters and van Niftrik 2019).

In the past, Planctomycetes were proposed to possess several eukaryotic traits, such as the lack of a peptidoglycan (PG) cell wall (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 (Lonhienne et al. 2010). Thus, Planctomycetes were thought to bridge the gap between bacteria and eukaryotes (Lonhienne et al. 2010; Fuerst and Sagulenko 2011; Devos et al. 2013). However, with the development of novel microscopic techniques and genetic tools for Planctomycetes (Jogler et al. 2011; Rivas-Marin et al. 2016; Boedeker et al. 2017), these traits were recently refuted. Planctomycetes were found to have a PG cell wall (Jeske et al. 2015; van Teeseling et al. 2015). This observation, together with the finding that their closest relatives, the Verrucomicrobia, also contain PG (Rast et al. 2017), now suggests that all known free-living bacteria have a PG cell wall (Rast et al. 2017). With the exception of anammox Planctomycetes (Jogler 2014, Neumann et al. 2014), the proposed cell compartments were found to rather be exceptionally large invaginations of the cytoplasmic membrane (Santarella-Mellwig et al. 2013; Acehan et al. 2014; Boedeker et al. 2017). Consequently, the cell plan of Planctomycetes was reinterpreted to more closely resemble that of Gram-negative bacteria than previously thought (Devos 2014a, b; Boedeker et al. 2017).

Despite this recent reinterpretation, Planctomycetes are still extraordinary. They are found in high abundance in biofilms on marine surfaces such as phototrophs (Bengtsson et al. 2012; Bondoso et al. 2014a, b, 2015, 2017; Lage and Bondoso 2014; Vollmers et al. 2017), where they can be the main players in the microbial community (Bengtsson and Øvrevås 2010; Kohn et al. 2019). Considering their slow growth compared to natural competitors on nutrient-rich algal surfaces, such as members of the Roseobacter clade (Frank et al. 2015; Wiegand et al. 2018), their dominance is remarkable. A possible explanation for this could be the production of yet for the most part uncharacterised small bioactive molecules that could e.g. be involved in mediating symbiotic interactions with algae or that could act as antibiotic agents (Graca et al. 2016; Jeske et al. 2016; Kallscheuer et al. 2019b). During the lifecycle of members of the Planctomycetaceae, a typical lifestyle switch can be observed (Jogler et al. 2011; Wiegand et al. 2018). It is hypothesised that in such cases a flagellated swimmer cell responds to an environmental clue, such as the secretion of dissolved organic carbon, and attaches to the phototroph by formation of a holdfast structure (Wiegand et al. 2018). A biofilm can then form, allowing the Planctomycete to degrade complex polysaccharides released by the host phototroph (Jeske et al. 2013; Lachnit et al. 2013).

To extend our knowledge on Planctomycetes, we here characterise the novel planctomycetal strain CA12T isolated from giant bladder kelp (Macrocystis pyrifera) in Monterey Bay, California, USA by using physiological, microscopic, genomic and phylogenetic methods. Based on these analyses, we propose that strain CA12T represents a novel species of a novel genus within the planctomycetal family Planctomycetaceae.

Materials and methods

Isolation and maintenance

*M. pyrifera* kelp material was collected in Monterey Bay, California, USA on the 28th of September 2014 (sampling location: 36.619 N 121.901 W) as described previously (Vollmers et al. 2017). For the isolation of strain CA12T kelp pieces were washed with 100 mg L⁻¹ cycloheximide solution dissolved in sterile filtrated natural seawater. Afterwards kelp pieces were swabbed over media plates solidified with gellan gum (8 g L⁻¹, autoclaved separately). To select for planctomycetal strains 1000 mg L⁻¹ streptomycin and 200 mg L⁻¹ ampicillin was added to the isolation medium. To prevent fungal growth
20 mg l⁻¹ cycloheximide was added. The medium for isolation consisted of 2.38 g l⁻¹ HEPES as buffer and 20 ml l⁻¹ mineral salt solution with 10 g l⁻¹ nitrotriacetic acid (NTA), 29.7 g l⁻¹ MgSO₄ × 7 H₂O, 3.34 g l⁻¹ CaCl₂ × 2 H₂O, 0.01267 g l⁻¹ Na₂MoO₄ × 2 H₂O, 0.099 g l⁻¹ FeSO₄ × 7 H₂O and 50 ml l⁻¹ metal salt sol. 44. NTA was dissolved in 700 ml distilled water by adjusting the pH to 7.2 with KOH. All further components were dissolved separately and added slowly. The solution was sterilised by filtration and stored at 4 °C. Metal salt solution 44 consisted of 250 mg l⁻¹ Na₂-EDTA, 1095 mg l⁻¹ ZnSO₄ × 7 H₂O, 500 mg l⁻¹ FeSO₄ × 7 H₂O, 154 mg l⁻¹ MnSO₄ × H₂O, 39.5 mg l⁻¹ CuSO₄ × 5 H₂O, 20.3 mg l⁻¹ CoCl₂ × 6 H₂O and 17.7 mg l⁻¹ Na₂B₄O₇ × 10 H₂O. In the first step, EDTA was dissolved and, if required, a few drops of concentrated H₂SO₄ were added to retard precipitation of the heavy metal ions. The solution was sterilised by filtration and stored at 4 °C. Furthermore, the medium was supplemented with 5 ml l⁻¹ vitamin solution consisting of 10 mg l⁻¹ p-aminobenzoic acid, 4 mg l⁻¹ biotin, 20 mg l⁻¹ pyridoxine hydrochloride, 10 mg l⁻¹ thiamine hydrochloride, 10 mg l⁻¹ Ca-pantothenate, 4 mg l⁻¹ folic acid, 10 mg l⁻¹ riboflavin, 10 mg l⁻¹ nicotinamide and 0.2 mg l⁻¹ vitamin B12. p-Aminobenzoic acid was dissolved first, the solution was sterilised by filtration and stored in the dark at 4 °C. In addition, 1 ml l⁻¹ trace element solution (1.5 g l⁻¹ Na-NTA, 500 mg l⁻¹ MnSO₄ × H₂O, 100 mg l⁻¹ FeSO₄ × 7 H₂O, 100 mg l⁻¹ Co(NO₃)₂ × 6 H₂O, 100 mg l⁻¹ ZnCl₂, 50 mg l⁻¹ NiCl₂ × 6 H₂O, 50 mg l⁻¹ H₂SeO₃, 10 mg l⁻¹ CuSO₄ × 5 H₂O, 10 mg l⁻¹ AlK(SO₄)₂ × 12 H₂O, 10 mg l⁻¹ H₃BO₃, 10 mg l⁻¹ NaMoO₄ × 2 H₂O and 10 mg l⁻¹ Na₃WO₄ × 2 H₂O) was added. The solution was sterilised by filtration and stored in the dark at 4 °C. HEPES and mineral salt solution were added before autoclaving, while vitamin and trace element solution were added after autoclaving. Furthermore, the medium was supplemented with 250 ml l⁻¹ concentrated artificial sea water (ASW) (46.94 g l⁻¹ NaCl, 7.84 g l⁻¹ Na₂SO₄, 21.28 g l⁻¹ MgCl₂ × 6 H₂O, 2.86 g l⁻¹ CaCl₂ × 2 H₂O, 0.384 g l⁻¹ NaHCO₃, 1.384 g l⁻¹ KCl, 0.192 g l⁻¹ KBr, 0.052 g l⁻¹ H₃BO₃, 0.08 g l⁻¹ SrCl₂ × 6 H₂O and 0.006 g l⁻¹ NaF) before autoclaving. During isolation N-acetyl glucosamine (NAG) (1 g l⁻¹) served as sole carbon and energy source and the pH was adjusted to 8.0. For maintenance of strain CA12ᵀ the medium was supplemented with 0.25 g l⁻¹ glucose, 0.25 g l⁻¹ peptone and 0.25 g l⁻¹ yeast extract in addition to NAG, while no antibiotics and antifungal reagents were used. The pH was adjusted to 7.5.

Morphological and physiological analysis

Physiological tests were performed in M1H NAG ASW medium composed of 250 ml l⁻¹ artificial sea water (ASW), 20 ml l⁻¹ Hutner’s basal salt solution, 0.25 g l⁻¹ peptone, 0.25 g l⁻¹ yeast extract, 0.25 g l⁻¹ glucose, 1 g l⁻¹ NAG, 5 ml l⁻¹ vitamin solution, 1 ml l⁻¹ trace element solution and buffered with either 100 mM MES (pH 5–6), HEPES (pH 7–8) or CHES (pH 9–10) depending on the desired pH. pH was adjusted by adding the appropriate amount of 5 M KOH. Glucose, NAG, vitamin solution and trace element solution were added after autoclaving.

The pH range and optimum were determined upon inoculating 4.5 ml M1H NAG ASW with 1.5 ml pre-culture and cultivation at 28 °C and 110 rpm. Growth was assessed by measuring the optical density at 600 nm (OD₆₀₀nm) for a period of 68 h employing a Varian Cary 50 UV-Vis Spectrophotometer (Agilent Technologies). Temperature range and optimum were determined upon inoculating 4.5 ml M1H NAG ASW of pH 8.0 with 0.5 ml pre-culture and cultivation at 28 °C and 110 rpm. Growth was assessed by measuring OD₆₀₀nm for a period of 148 h employing an Ultrospec II photometer (LKB Biochrom).

Microscopy techniques

Phase contrast microscopy was performed employing a Nikon Eclipse Ti inverted microscope with a Nikon N Plan Apochromat λ, 100x/1.45 oil objective and a Nikon DS-Ri2 camera objective. Cells were immobilised in a fixed focal plane in MalTek glass bottom dishes (35 mm, No. 1.5) using a 1% agarose cushion. The images were analysed using Nikon NIS-Elements software.

Field emission scanning electron microscopy was performed by fixing bacteria for 1 h on ice with 1% formaldehyde in HEPES buffer (3 mM HEPES, 0.3 mM CaCl₂, 0.3 mM MgCl₂, 2.7 mM sucrose, pH 6.9), after which they were washed with the same buffer. Cover slips with a diameter of 12 mm were coated with a poly-L-lysine solution (Sigma-Aldrich).
for 10 min, washed with distilled water and air-dried. 50 μl of fixed bacteria solution was placed on a coated cover slip and allowed to settle for 10 min. The cover slips containing the fixed bacteria were fixed in 1% glutaraldehyde in TE buffer (20 mM TRIS, 1 mM EDTA, pH 6.9) for 5 min at room temperature. After this, they were washed twice in TE buffer and dehydrated on ice in a graded series of acetone (10, 30, 50, 70, 90 and 100%) for 10 min in each concentration. After dehydration, the samples were brought to room temperature and placed in fresh 100% acetone. Samples were subsequently subjected to critical point drying with liquid CO2 (CPD 300, Leica), after which they were covered with an 80/20 gold/palladium film by sputter coating (SCD 500, Bal-Tec). The samples were examined with a field emission scanning electron microscope (Zeiss Merlin) employing the Everhart–Thornley HESE2 detector and the inlens SE detector with a 25:75 ratio and an acceleration voltage of 5 kV.

Transmission electron microscopy (TEM) was performed employing the floating grid method: carbon-coated copper grids were placed on 3 ml of CA12T culture in M1H NAG ASW medium in a 6-well plate and were incubated for 24 h or 48 h at 28 °C. After this, the grids were negative stained with 0.5% uranyl acetate (UA), by rinsing twice with 0.5% UA followed by 1 min incubation with 0.5% UA and subsequent blotting an air-drying. Micrographs were taken with a JEM 1400 (JEOL) employing an acceleration voltage of 120 kV. Images were captured with a Matataki Flash sCMOS camera (JEOL) using the Jeol TEM Center software (JEOL).

Time-lapse microscopy was performed employing a DMI8 inverted microscope (Leica) with a HC PL APO 100x/1.40 oil objective (Leica) and a DCF9000GT camera (Leica). Dividing CA12T cells were analysed using CellAsic Onix microfluidic plates, immobilising the cells in a fixed focal plane. Cells were loaded into the microfluidic plate by using the manufacturer’s loading protocol (CellASIC) three times consecutively. A constant flow of M1H NAG ASW medium was provided to the cells by applying 27 kPa of pressure. Cells were imaged under a constant flow of medium for up to 30 h and analysed using FIJI (Schindelin et al. 2012).

Phylogenetic analysis

16S rRNA gene phylogeny was computed for strain CA12T, the type strains of all described planctomycetal species (as of January 2019) and all isolates recently described (Kallscheuer et al. 2019a; Wiegand et al. 2019). An alignment of 16S rRNA gene sequences was made with SINA (Pruesse et al. 2012). Phylogenetic analysis was performed employing a maximum likelihood approach with 1000 bootstraps, the nucleotide substitution model GTR, gamma distribution and estimation of proportion of invariable sites (GTRGAMMAI option) (Stamatakis 2014). The out-group consisted of three 16S rRNA genes of bacterial strains from the PVC superphylum. The average amino acid identity (AAI) was determined with the aai.rb script of the enveomics collection (Rodriguez-R and Konstantinidis 2016) and the percentage of conserved proteins (POCP) was calculated as described before (Qin et al. 2014). The rpoB gene sequence was taken from the genome annotation and sequence identities were determined as described before (Bondoso et al. 2013) with clustal Omega (Sievers et al. 2011). The rpoB alignment and matrix calculation was performed upon extracting only those parts of the sequence that would have been sequenced with the described primer set. For the multi-locus 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 of 216 ubiquitous genes with a combined length of 103,564 conserved amino acid residues was concatenated and clustered using the maximum likelihood method implemented by RaxML (Stamatakis 2014) with the “rapid bootstrap” method and 500 bootstrap replicates. The outgroup consisted of concatenated gene sets from the Phycisphaera and Candidatus Brocadiaceae.

Genomic analysis

The genome of strain CA12T was published previously (Wiegand et al. 2019) and is available from NCBI under acc. no. CP036265. The GenBank acc.
no. of the 16S rRNA gene is MK554521. All other genomes were also gathered from NCBI and their accession numbers are given in the corresponding tables. Completeness and contamination of the genomes was determined using CheckM v1.0.131 (Parks et al. 2015). The primary metabolism was analysed by employing 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. The carbohydrate active enzymes were determined by employing dbCAN2 (Zhang et al. 2018), which automatically mines the CAZy database (Lombard et al. 2014).

**Results and discussion**

**Phylogenetic inference**

Based on 16S rRNA gene phylogeny and whole genome-based multilocus sequence analysis (MLSA), strain CA12T clusters within the planctomycetal family Planctomycetaceae (Fig. 1). However, the results about the current nearest neighbour are ambiguous, suggesting either *Planctomicrobium piriforme* P3T (Kulichevskaya et al. 2015) or *Fuerstella marisgermanici* NH11T (Kohn et al. 2016) in the 16S rRNA gene and MLSA trees, respectively. However, all phylogenetic markers employed below did not indicate *F. marisgermanici* NH11T, but instead either *P. piriforme* P3T or *Rubinisphaera brasiliensis* DSM 5305T as the current closest relative to strain CA12T. With 82.3%, strain CA12T has a high 16S rRNA gene sequence identity to *P. piriforme* P3T at 72%, while similarities < 72% indicate a novel genus (Bondoso et al. 2013). With a POCP value of 39.3%, *R. brasiliensis* DSM 5305T, rather than *P. piriforme*, has the current highest similarity to strain CA12T, but this still indicates the strain should be placed in a novel genus as the suggested cut-off value for delineation of genera is 50% (Qin et al. 2014). The AAI was highest to *R. brasiliensis* with both one-way AAs (46.39% and 45.45%) being below the suggested 60% cut-off value for delineation of genera (Luo et al. 2014), thereby again indicating a novel genus. ANI values < 70% ensure that strain CA12T and its relatives belong to separate species. Taken together, these different methods suggest that strain CA12T represents a novel species within a novel genus.

**Genomic characteristics**

The genome of strain CA12T is 5,475,215 bp in size and has a G+C content of 70.1%. It contains 4309 protein-coding genes, of which 1801 encode hypothetical proteins. These values correspond to 787 protein-coding genes per Mb and a coding density of 88.1%. Similar to its relatives, the strain lacks any plasmids. When compared to these close relatives (Table 1), it becomes evident that the genome of strain CA12T is relatively small while having a high G + C content. Due to its smaller genome, strain CA12T has a lower count of proteins and especially of those proteins annotated as hypothetical. In accordance, the strain’s coding density is higher than in its relatives and it carries no giant genes.

**Morphology and physiology**

Strain CA12T forms pink colonies on solid medium (Fig. S1A) and is light pink-coloured in liquid medium, where it has a strong tendency to form biofilms, even when shaken in a baffled flask (Fig. S1B). Such biofilms intensely adhere to the glass surface and are difficult to scrape off, indicating the production of an extracellular matrix or fibres that glue cells to each other and to the glass surface. This finding is not surprising as strain CA12T was obtained from a natural biofilm on the surface of *M. pyrifera*. The morphology of strain CA12T was determined employing a variety of microscopy techniques. Cells are spherical to ovoid-shaped and are 2.0 ± 0.2 μm x
1.5 ± 0.3 μm in size (Fig. 2a–c). The cell surface is evenly covered with large crateriform structures, with the exception of the pole at which the flagellum is located (Fig. 2g). Strain CA12 T produces a large amount of fibres, which often seem to originate from these crateriform structures. The quantity of fibres is most likely associated with the tendency to form biofilms and to grow in large aggregates (Fig. 2d).
Furthermore, cells seem to secrete an extracellular matrix, further stabilising the biofilm (Fig. 2d, e). No holdfast structure was observed during electron microscopic analysis.

As found for all members of the family Planctomycetaceae, strain CA12T divides through polar budding (Fig. 2a). Interestingly, the strain seems to form buds while the mother cell itself is still attached to its mother cell. This process of “consecutive budding” was found both in TEM (Fig. 2f) and in time-lapse microscopy experiments (Fig. 3; Movie S1) and has to our knowledge not been described before for members of Planctomycetaceae. It leads to the formation of short filaments, containing at least three cells (Fig. S2). Filament formation and multicellularity were described earlier for Isosphaera pallida (Giovannoni et al. 1987). In contrast to strain CA12T, I. pallida grows exclusively as filaments and intercalary bud formation always occurs on the chain axis (Giovannoni et al. 1987). The observed short filaments of strain CA12T always elongate via budding of the terminal cell. Whether strain CA12T is an intermediate form between filamentous and unicellular growth requires additional attention, but exceeds the scope of this study. In the light of the greater phylogenetic distance to I. pallida, a separate development appears more likely (Fig. 1). Nevertheless, future studies on strain CA12T should address this unusual cell division as a potential progenitor to planctomycetal filament formation.

Compared to its closest related strains, strain CA12T is of similar size and shape (Table 2). While the mode of division and the shape of the bud are very similar, strain CA12T is unique compared to its relatives in not having to fulfil a lifestyle switch in order to divide (Table 2). In addition, no stalk or holdfast structure was observed while both are present in P. piriforme, R. brasiliensis and Gimesia maris (Table 2). All four strains have a flagellum and produce fibres. No capsule could be observed for these strains.

Strain CA12T cells are capable of growth between 10 and 40 °C with optimal growth at 27 °C and between pH 5.0 and 9.0 with optimal growth at pH 7.5.
The strain reached a maximal growth rate of 0.065 h\(^{-1}\) corresponding to a generation time of approximately 11 h, which is within the range of 0.01–0.09 h\(^{-1}\) (generation time of 8–70 h) which we and others have observed for most of the Planctomycetes cultivated so far (Tekniepe et al. 1981; Giovannoni et al. 1987; Kulichevskaya et al. 2008; Bondoso et al. 2014a, b; Kohn et al. 2016). A generation time of 11 h is rather long compared to other bacteria that compete with strain CA12\(^T\) for its ecological niche in kelp biofilms.

The genome of strain CA12\(^T\) was previously found to harbour small molecule biosynthesis-related genes and gene clusters (Wiegand et al. 2019). Thus, the newly discovered strain might be a good candidate for further examination of the planctomycetal chemical interaction in marine biofilms. This might also be true with regard to the strain’s primary metabolism (Table 3). All close relatives are predicted to be able to convert glucose via the pentose phosphate pathway as well as by glycolysis via the Entner–Doudoroff (KDPG) and the Embden–Meyerhof–Parnas pathway. While strain

![Fig. 2 Morphology of strain CA12\(^T\). Morphology of strain CA12\(^T\) is shown by phase contrast (Phaco) microscopy (a, b), scanning electron microscopy (SEM) (d, e) and transmission electron microscopy (TEM) (f, g). Cells divide by polar budding (a) and mostly grow in dense aggregates (d). Large amounts of fibers are produced and seem to originate from large crateriform structures (g, white arrowhead), which evenly cover the cell surface except for the pole at which the flagellum (g, white arrow) is located. Cell size of the strain was determined from Phaco images (c). Scale bar 1 µm.](image)
CA12^T possesses the genes for the pentose phosphate pathway, it lacks the genes for the KDPG pathway. Interestingly, it also seems to lack *pgk*, which encodes phosphoglycerate kinase converting 1,3-bisphosphoglycerate to 3-phosphoglycerate. We identified some aldehyde dehydrogenases that might be able to bypass this reaction by converting glyceraldehyde-3-phosphate directly to 3-phosphoglycerate but it requires further analyses to investigate this hypothesis. During gluconeogenesis, strain CA12^T seems to depend on GTP (instead of ATP as in its relatives) to convert oxaloacetate to phosphoenolpyruvate. All compared strains appear to possess the expected enzymes for the citric acid cycle (TCA cycle), but all compared strains lack the glyoxylate shunt. In terms of its carbohydrate-degrading potential, CA12^T has slightly less enzymes than its close relatives, potentially due to the smaller genome (Table 4). However, the number of glycosyltransferases exceeds the number present in close relatives, pointing towards different nutritional sources of the different strains.

Description of *Alienimonas* gen. nov.

*Alienimonas* (A.li.e.ni.mon’as. L. masc. adj. alienus strange, foreign; Gr. fem. n. monas a unit, monad; N.L. fem. n. *Alienimonas* an alien bacterial unit).

Members of the genus are Gram-negative, aerobic, mesophilic, neutrophilic and heterotrophic. Cells are spherical to ovoid-shaped, divide by polar budding and produce fibres originating from large crateriform structures. The type species of the genus is *Alienimonas californiensis*.

Description of *Alienimonas californiensis* sp. nov.

*Alienimonas californiensis* (ca.li.for.ni.en’sis. N.L. fem. adj. *californiensis* of California; referring to the isolation of the type strain from California).

In addition to the general characteristics of the genus, cells are $2.0 \pm 0.2 \mu m \times 1.5 \pm 0.2 \mu m$ in size. They form pink colonies and have a strong tendency to form aggregates and biofilms. Cells divide...
by polar budding and have the ability to perform this consecutively, without fulfilling a lifestyle switch. The outer membrane is evenly covered with large crateriform structures, except near the pole where the flagellum is located. Fibres originating from crateriform structures are observed. Cells grow between pH 5.0 and 9.0 with optimal growth at pH 7.5 and between 10 and 40 °C with optimal growth at 27 °C. The G + C content of the type strain is 70.1%. The 5.5 Mb genome (acc. no. CP036265) and 16S rRNA gene (acc. no. MK554521) sequences of the type strain have been deposited at GenBank.

![Graph](image)

**Fig. 4** Temperature and pH optimum of strain CA12^T^ Cultivations were performed as described in the Materials and methods section

Table 2 Phenotypic features of strain CA12^T^ compared to the closest related genera

| Feature                | CA12^T^          | Planctomicrobium piriforme DSM 2634g^T^* | Rubinisphaera brasiliensis DSM 5305^T^** | Gimesia maris DSM 8797^T^*** | Fuerstella marisgermanici NH11^T^**** |
|------------------------|------------------|-----------------------------------------|------------------------------------------|----------------------------|--------------------------------------|
| Shape                  | Spherical to ovoid| Ellipsoidal to pear-shaped              | Spherical to ovoid                       | Ovoid, pear-shaped         |
| Cell size              | 2.0 ± 0.2 µm × 1.5 ± 0.3 µm | 1.7–2.8 µm × 0.9–1.3 µm | 0.7–1.8 µm                             | 0.4–1.5                     | 1.2–2.5 × 0.9–1.7                     |
| Aggregates             | Yes              | Rosettes                                | Yes and rosettes                        | n. d.                      | Yes                                  |
| Crateriform structures | Evenly covered, except for the pole at which the flagellum is located | Polar                                   | Whole cell surface                     | n. d.                      | Reproductive pole                     |
| Budding pole           | Polar            | Polar, opposite pole at which stalk is located | Polar, opposite pole at which stalk is located | Polar                     |
| Cell cycle             | No               | Yes                                     | Yes                                     | Yes                        | Yes                                  |
| Stalk                  | n.o.             | Yes                                     | Yes                                     | n.o.                      | n.o.                                 |
| Holdfast structure     | n.o.             | Yes                                     | Yes                                     | Yes                        | n.o.                                 |

*n.d. not determined; n.o. not observed

*Phenotypic data from (Kulichevskaya et al. 2015)

**Phenotypic data from (Schlesner 1989)

***Phenotypic data from (Bauld and Staley 1976)

****Phenotypic data from (Kohn et al. 2016)
Table 3: Genome-based primary metabolism of strain CA12T compared to the closest related genera

| Reaction/enzyme                           | EC number | Gene     | CA12T | Planctomicrobium piriforme DSM 26348T* | Rubinisphaera brasiliensis DSM 5305T** | Gimesia maris DSM 8797T*** | Fuerstiella marisgermanici NH11T***** |
|-------------------------------------------|-----------|----------|-------|----------------------------------------|----------------------------------------|---------------------------|--------------------------------------|
| Glycolysis (Embden–Meyerhof–Parnas pathway) |           |          |       |                                        |                                       |                           |                                      |
| Glucose-6-phosphate isomerase             | 5.3.1.9   | pgi      | CA12_32240 | y                                      | y                                      | y                         | y                                    |
| ATP-dependent 6-phosphofructokinase       | 2.7.1.11  | pfkA     | CA12_14770 | y                                      | y                                      | y                         | y                                    |
| Fructose-bisphosphate aldolase class 2    | 4.1.2.13  | fbaA     | CA12_43320 | y                                      | y                                      | y                         | y                                    |
| Trehalosephosphate isomerase              | 5.3.1.1   | tpiA     | CA12_39850 | y                                      | y                                      | y                         | y                                    |
| Glyceraldehyde-3-phosphate dehydrogenase  | 1.2.1.12  | gapA     | CA12_36350 | y                                      | y                                      | y                         | y                                    |
| Phosphoglycerate kinase                    | 2.7.2.3   | pgk      | n      | y                                      | y                                      | y                         | y                                    |
| NAD(P)-dependent glyceraldehyde-3-phosphate dehydrogenase | 1.2.1.90/1.2.1.9 | gapN | Candidates | Candidates | Candidates | Candidates | Candidates |
| 2,3-Bisphosphoglycerate-dependent phosphoglycerate mutase | 5.4.2.11 | gpmA | CA12_30720 | y                                      | y                                      | y                         | y                                    |
| Enolase                                   | 4.2.1.11  | eno      | CA12_00460 | y                                      | y                                      | y                         | y                                    |
| Pyruvate kinase I                         | 2.7.1.40  | pykF     | CA12_30440 | y                                      | y                                      | y                         | y                                    |
| Pyruvate dehydrogenase complex            | 1.2.4.1/2.3.1.12 | aceEF | CA12_20210/CA12_20200 | y                                      | y                                      | y                         | y                                    |
| Gluconeogenesis                           |           |          |       |                                        |                                       |                           |                                      |
| Pyruvate, phosphate dikinase              | 2.7.9.1   | ppdK     | CA12_36740 | y                                      | y                                      | y                         | y                                    |
| Phosphoenolpyruvate carboxykinase (ATP)   | 4.1.1.49  | pckA     | n      | y                                      | y                                      | y                         | y                                    |
| Phosphoenolpyruvate carboxykinase [GTP]    | 4.1.1.32  | pckG     | CA12_16520 | n                                      | n                                      | n                         | n                                    |
| Pyrophosphate–fructose 6-phosphate        | 2.7.1.90  | pfp      | CA12_41240 | y                                      | y                                      | y                         | y                                    |
| Pentose phosphate pathway                  |           |          |       |                                        |                                       |                           |                                      |
| Glucose-6-phosphate 1-dehydrogenase       | 1.1.1.49  | zwf      | CA12_01770 | y                                      | y                                      | y                         | y                                    |
| 6-Phosphogluconolactone                   | 3.1.1.31  | pgl      | CA12_42430 | y                                      | y                                      | y                         | y                                    |
| 6-Phosphogluconate dehydrogenase          | 1.1.1.44  | gndA     | CA12_41200 | y                                      | y                                      | y                         | y                                    |
| Transketolase                             | 2.2.1.1   | tkt      | CA12_17310 | y                                      | y                                      | y                         | y                                    |
| Transaldolase                             | 2.2.1.2   | tal      | CA12_24580 | y                                      | y                                      | y                         | y                                    |
| KDPG (Enner–Doudoroff) pathway             |           |          |       |                                        |                                       |                           |                                      |
| KDPG aldolase                             | 4.1.2.14  | eda      | n      | y                                      | y                                      | y                         | y                                    |
| Reaction/enzyme | EC number | Gene | CA12<sup>T</sup> | Planctomicrobium piriforme DSM 26348<sup>T</sup> | Rubinisphaera brasiliensis DSM 5305<sup>**</sup> | Gimesia maris DSM 8797<sup>***</sup> | Fuerstiella marisgermanici NH11<sup>****</sup> |
|----------------|-----------|------|-----------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Phosphogluconate dehydratase | 4.2.1.12 | edd | n               | y                                 | y                                 | y                                 | y                                 |
| TCA cycle      |           |      |                 |                                    |                                    |                                    |                                    |
| Citrate synthase | 2.3.3.16 | gltA | CA12_00670      | y                                 | y                                 | y                                 | y                                 |
| Aconitate hydratase A | 4.2.1.3 | acnA | CA12_34980      | y                                 | y                                 | y                                 | y                                 |
| Isocitrate dehydrogenase [NADP] | 1.1.1.42 | icd | CA12_15100      | y                                 | y                                 | y                                 | n                                 |
| Isocitrate–homoisocitrate dehydrogenase | 1.1.1.286 |      | CA12_34890      | y                                 | y                                 | y                                 | y                                 |
| 2-Oxoglutarate dehydrogenase complex | 1.2.4.2/ 2.3.1.61 | sucAB | CA12_20480/CA12_13510 | y                                 | y                                 | y                                 | y                                 |
| Succinate–CoA ligase complex | 6.2.1.5 | sucCD | CA12_41420/CA12_41410 | y                                 | y                                 | y                                 | y                                 |
| Succinate dehydrogenase complex | 1.3.5.1 | sdhABC | CA12_39470/CA12_39480/CA12_39460 | y                                 | y                                 | y                                 | y                                 |
| Fumarate hydratase | 4.2.1.2 | fumC | CA12_01380      | y                                 | y                                 | y                                 | y                                 |
| Malate dehydrogenase | 1.1.1.37 | mdh | CA12_04080      | y                                 | y                                 | y                                 | y                                 |

For strain CA12<sup>T</sup> the locus tag is given whenever a gene was identified. Presence of a gene in the other strains is indicated by ‘y’ and absence is indicated by ‘n’. When different enzymes are capable of performing a reaction, the enzymes are not separated by an underline. In case of EC 1.2.1.90 and EC 1.2.1.9 several aldehyde dehydrogenases were identified (‘candidates’).

* Genomic data from GenBank acc. no. NZ_FOQD00000000
** Genomic data from GenBank acc. no. CP002546
*** Genomic data from GenBank acc. no. CP042910
**** Genomic data from GenBank acc. no. CP017641
The type strain, CA12T (DSM 100707T = LMG 29076T), was isolated from *Macrocystis pyrifera* in Monterey Bay, California, USA.

**Acknowledgements** We thank Anne-Kristin Kaster (KIT Karlsruhe, Germany) and Alfred M. Spormann (Stanford, USA) as well as the Aquarius Dive Shop Monterey and the Hopkins Marine Station for sampling support. We further thank Ina Schleicher for excellent SEM sample preparation and skillful technical assistance and the General Instrumentation department at Radboud University for help during the TEM analyses. For their support during strain deposition we thank the BCCM/LMG bacteria collection and Brian Tindall and Regine Fähnrich from the DSMZ.

**Author contributions** ASB performed cultivations and wrote the manuscript; NK contributed to preparation of the text, analyzed the data and prepared the figures; SW and MJ performed the genomic and phylogenetic analysis; PR and AH isolated the strain and performed the initial strain cultivation and deposition; SHP and CB performed the light microscopic analysis; MR performed the electron microscopic analysis; CJ and MJ took the samples in Monterey Bay, CA; CJ supervised the study and all authors read and approved the final version of the manuscript.

**Funding** This work was funded by the Deutsche Forschungsgemeinschaft (DFG) grants KA 4967/1-1 and JO 893/4-1, and by the grant ALWOP.308 of the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO).

**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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**Table 4** Carbohydrate-degrading enzymes of strain CA12T compared to the closest related genera

| Enzyme Type                  | CA12T | *Planctomicrobium piriforme* DSM 26348T* | Rubinisphaera brasiliensis DSM 5305T*** | Gimesia maris DSM 8797T**** | Fuerstiella marisgermanici NH11T**** |
|------------------------------|-------|------------------------------------------|----------------------------------------|-----------------------------|-------------------------------------|
| Carbohydrate esterases       | 26    | 41                                       | 37                                     | 56                          | 60                                  |
| Glycoside hydrolases         | 56    | 61                                       | 55                                     | 78                          | 83                                  |
| Glycosyltransferases         | 77    | 56                                       | 75                                     | 60                          | 70                                  |
| Polysaccharide lyases        | 5     | 2                                        | 3                                      | 5                           | 5                                   |
| Carbohydrate-binding         | 19    | 27                                       | 34                                     | 50                          | 58                                  |
| Auxiliary activities         | 2     | 4                                        | 2                                      | 3                           | 4                                   |
| Unknown                      | 3     | 4                                        | 3                                      | 5                           | 6                                   |

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***Genomic data from GenBank acc. no. CP042910

****Genomic data from GenBank acc. no. CP017641

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