Alienimonas chondri sp. nov., a novel planctomycete isolated from the biofilm of the red alga *Chondrus crispus*

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ABSTRACT

The phylum *Planctomycetes* comprises bacteria with peculiar and very unique characteristics among prokaryotes. In marine environments, macroalgae biofilms are well known for harboring planctomycetal diversity. Here, we describe a novel isolate obtained from the biofilm of the red alga *Chondrus crispus* collected at a rocky beach in Porto, Portugal. The novel strain LzC2^T is motile, rosette-forming with spherical- to ovoid-shaped cells. LzC2^T forms magenta- to pinkish-colored colonies in M13 and M14 media. Transmission and scanning electron microscopy observations showed a division by polar and lateral budding. Mother cells are connected to the daughter cells by a tubular neck-like structure. The strain requires salt for growth. Vitamins are not required for growth. Optimal growth occurs from 15 to 30 °C and within a pH range from 5.5 to 10.0. Major fatty acids are *anteiso*-C15:0 (54.2%) and *iso*-C15:0 (19.5%). Phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid represent the main lipids and menaquinone 6 (MK-6) is the only quinone present. 16S rRNA gene-based phylogenetic analysis supports the affiliation to the phylum *Planctomycetes* and family *Planctomycetaceae*, with *Alienimonas* as the closest relative. Strain LzC2^T shares 97% 16S rRNA gene sequence similarity with *Alienimonas californiensis*. LzC2^T has a genome size of 5.3 Mb and a G+C content of 68.3%. Genotypic and phenotypic comparison with the closest relatives strongly suggest that LzC2^T (=CECT 30038^T=LMG XXXT) is a new species of the genus *Alienimonas*, for which we propose the name *Alienimonas chondri* sp. nov., represented by LzC2^T as type strain.

16S rRNA gene accession number: GenBank = MN757873.1.

Genome accession number: GenBank = WTPX00000000.

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Introduction

The phylum *Planctomycetes*, an underexplored group of bacteria, possess unique features which keep challenging the scientific community [52,53]. This phylum is classified within the *Planctomycetes-Chlamydiae-Verrucomicrobia* (PVC) superphylum

[51]. Although an extensive shift in planctomycetal research is currently taking place, these organisms still remain a mystery, mainly due to their peculiar cell division mechanism that does not rely on the otherwise universal bacterial division protein FtsZ [52,53]. While prokaryotes usually divide by binary fission, many of the cultured *Planctomycetes* are reported to reproduce by budding with the exception of members of the classes *Phycisphaerae* and *Cand. Brocadiae* (anammox-performing planctomycetes) [35,52]. Moreover, variations of the budding process were recently described, such as lateral budding [52], which unveiled novel models of cell division. *Planctomycetes* possess a complex system of cytoplas-

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matic membrane invaginations that were originally misinterpreted as structures resembling a eukaryotic nucleus. These membrane invaginations also account for an unusually enlarged periplasmic space [4,34].

Recent planctomycetal genome analyses revealed particular features. They display large genomes with an average genome size of 7.6 Mb, with members of the family *Gemmataceae* having the largest genome sizes of up to 12.4 Mb [35,52,53]. The G+C content of planctomycetal genomes differs significantly between species. Most planctomycetal genomes were also found to possess a large number of proteins with unknown function (40–50% of all annotated genes) [52,53]. Giant genes (with a size of up to 49 kb) have also been detected in planctomycetal genomes, but are most often of unknown function [20,27,28,52]. Planctomycetes are emerging as biotechnologically relevant bacteria, as already demonstrated in antimicrobial and anti-cancer studies [12,21,24]. The planctomycetes share common characteristics with known producers of bioactive metabolites, like Actinobacteria, namely large genomes and complex life cycles. Furthermore, they possess genes encoding multi-domain protein complexes, e.g. polyketide synthases (PKS) and non-ribosomal peptide synthases (NRPS), that are known to be involved in the production of two large classes of bioactive small molecules (also referred to as secondary metabolites) [18,21,23]. While found in most habitats on earth, only a low percentage of the diversity known from molecular environmental studies is covered by axenic cultures [35,53]. In marine environments, macroalgal biofilms harbor a great range of planctomycetal diversity [2]. In particular, five novel species and three genera were isolated from macroalgae of the Portuguese coast [8,10,32], all belonging to the '*Pirellula* clade' (now referred to as family *Pirellulaceae*) [17]. As the diversity of the described planctomycetes is still at an early stage, isolation and characterization of novel taxa is important to enlarge the knowledge on planctomycetal taxonomy, cell biology, ecology and evolution. This endeavor was brought forward by a recent study unveiling unique aspects of the biology of planctomycetes [52]. One of the many novel species was *Alienimonas californiensis*, recently described as a novel genus [5].

In this study, we focus on the biofilm of the red macroalga *Chondrus crispus* for the isolation of novel planctomycetes. One isolate, designated LzC2^T, was chosen for further characterization and its genome was sequenced. Here, we show that this isolate is a novel species belonging to the genus *Alienimonas* with the sole species *Alienimonas californiensis* as its closest relative. We propose the name *Alienimonas chondri* sp. nov., represented by LzC2^T as the type strain.

Material and methods

Isolation and ecology

Strain LzC2^T was isolated from the red macroalga *Chondrus crispus*. This alga was sampled on the rocky beach of Praia da Luz in Porto (Portugal) (41°09'12.38 N, 8°40'45.91 W). The alga was collected in October 2018 during a low tide and brought to the laboratory in a cold chamber. The seawater temperature was 10 °C. Pieces of the alga (about 1.5 × 1.5 cm) were washed in natural sterilized seawater to remove non-attached bacteria. To prevent fungal contamination, a subsequent washing step for 30 min was performed in seawater containing 1% (w/v) of the antifungal agent econazole nitrate (Pevaryl®). The biofilm on the algal surface was then scraped off and the obtained biological material was resuspended in sterile natural seawater. 50 µL of the mixture were inoculated in M13 medium plates [33], previously sprayed with Pevaryl® for one month. Isolated colonies were re-inoculated in new medium. Cultures of the isolated strain LzC2^T were then main-

tained in M13 medium or M14 medium [33] at 25 °C and preserved in medium M13 supplemented with 20% (v/v) glycerol at –80 °C.

Phylogenetic inference

Genomic DNA of strain LzC2^T was extracted using the E.Z.N.A. Bacterial DNA Isolation Kit (Omega). For the identification of the isolate, the 16S rRNA gene was amplified with the primers 27F and 1492R and the PCR was performed as previously described by Bondoso and collaborators [9]. PCR products obtained were purified using the GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences). Sequencing was performed at GATC Biotech™. Sequence analyses were carried out using Geneious R11 (Biomatters Ltd, Auckland, New Zealand). 16S rRNA gene-based phylogeny was computed for strain LzC2^T (GenBank accession number MN757873.1) and the type strains of related described planctomycetal species. 16S rRNA gene sequences were aligned with SINA [42]. The phylogenetic analysis was done employing a maximum likelihood approach with 1000 bootstraps, the nucleotide substitution model GTR, gamma substitution and estimation of proportion of invariable sites (GTRGAMMAI option) [49]. Three 16S rRNA genes of bacterial strains from the PVC superphylum served as outgroup. The average nucleotide identity (ANI) was calculated using OrthoANI [37] and the average amino acid identity (AAI) was obtained using the aai.rb script of the enveomics collection [44]. The percentage of conserved proteins (POCP) was calculated as described [43]. The *rpoB* gene sequences were taken from publicly available genome annotations and sequence identities were determined as previously described [11]. The alignment and matrix calculation were performed upon extracting only those parts of the sequence that would have been sequenced with the described primer set and the alignment and matrix calculation was done with Clustal Omega [48].

Genome sequencing and analysis

A paired-end sequencing library was prepared with the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, USA) according to the manufacturer's instructions and were sequenced on an Illumina MiSeq System employing 600 cycles with a MiSeq Reagent Kit v3 (Illumina). Adapter clipping and read trimming was done with Trimmomatic v0.35 [7]. After adapter removal, FastQ Screen v0.4.4 [54] was employed to filter the data set for contaminations and reads of low complexity were discarded with PRINSEQ lite v0.20.4 [46]. Subsequently, overlapping paired-end reads were merged using FLASH v1.2.11 [39]. The genome was assembled with SPAdes v3.7.0 [1]. Scaffolding of pre-assembled contigs based on the reuse of the paired-end library by using SSPACE [6] was performed. The genome assembly was examined by CheckM [41] and QUAST [22]. Finally, genome annotation was performed with Prokka v1.12 [47]. The genome was submitted to NCBI GenBank and is available under accession number WTPX00000000. The search for giant proteins was manually curated. The primary metabolism was analyzed by examining locally computed InterProScan [40] results cross-referenced with information from the UniProt database [50] and BLASTp results of 'typical' protein sequences. The program dbCAN2 [56], which automatically mines the CAZy [38] database, was used to determine the numbers of putative carbohydrate active enzymes. The genome of the strain was also analyzed for the presence of secondary metabolite biosynthetic gene clusters using the platform antiSMASH 5.0 [3]. Whole genome-based multilocus sequence analysis (MLSA) for complementary phylogenetic interference was determined with proteinortho5 [36] with the 'selfblast' option enabled. Alignment of protein sequences of the resulting orthologous groups was performed using MUSCLE v.3.8.31 [19]. After clipping, filtration

Fig. 1. Phylogenetic trees of the phylum Planctomycetes highlighting the position of strain LzC2^T. 16S rRNA gene phylogeny (A – top panel) and whole genome based MLSA (B – bottom panel) were computed using the maximum likelihood method. Bootstrap values after 1000 re-samplings (16S rRNA) and 500 re-samplings (MLSA) are given at the nodes. The outgroup consists of three non-planctomycetes PVC superphylum members.

of the partially aligned C- and N-terminal regions and poorly aligned internal regions was performed by Gblocks [13]. The final alignment was concatenated and clustered using the maximum likelihood method implemented by RaxML [49] with the “rapid bootstrap” method and 500 bootstrap replicates. Concatenated gene sets strains from the Phycisphaera and Candidatus Brocadiaceae served as outgroup.

Microscopy

Morphological characterization of strain LzC2^T was carried out by phase contrast, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) during the exponential phase of growth in M14 medium. Photographic records of colonies of the strain in M14 medium were also obtained. For phase contrast microscopy, cells were observed in a Nikon Eclipse Ci equipment under 1000x magnification. For SEM, cells were fixed in 2.5%

(v/v) glutaraldehyde in marine buffer, pH 7.0 for 2.5 h, dehydrated through a graded ethanol series, critical point-dried and observed with a HITACHI S-570 microscope. For TEM, cells were fixed in 2.5% (v/v) glutaraldehyde in marine buffer (pH 7.2) for 2 h and post-fixed in 1% (v/v) osmium tetroxide in the same buffer for 4 h followed by 1% (v/v) uranyl acetate for 1 h. Cells were dehydrated through a graded ethanol series, followed by propylene oxide treatment and were ultimately embedded in Epon resin. Ultrathin sections were stained for 10 min in 1% (v/v) uranyl acetate and for 10 min in Reynolds lead citrate. The sections were examined using a 100CXII transmission electron microscope (JEOL).

Physiology and chemotaxonomy

Unless otherwise stated, all physiological tests were performed at 25 °C using M13 medium. In liquid medium used for NaCl tolerance and pH range the results were recorded after seven days

Fig. 2. Phase contrast microscopy observation (a) and photographic record (b) of strain LzC2^T. Cells are ovoid to spherical shaped and cell division mode is budding (arrows) (a). Colonies of this strain appear circular and pink colored in M14 medium (b).

by measuring of the optical density at 600 nm (OD₆₀₀). The results of an analysis of vitamin requirements and growth temperature range were determined visually after cultivation for seven days on solid medium. NaCl tolerance was evaluated in duplicate in Falcon tubes by using artificial seawater (ASW) medium with added salt concentrations of 0%, 1%, 2%, 3%, 3.5%, 5%, 6%, 7% and 10% (each w/v). The pH range for growth was evaluated in 96-well plates in triplicate with M13 buffered with 10 mM of each of the following buffers: MES for pH 5.5, 6.0 and 6.5, Tris–HCl for pH 7.5 and CHES for pH 8.0, 8.5, 9.0 and 10.0. For analyzing vitamin requirements, M13 medium was prepared with and without a vitamin supplement. Temperature for growth was assessed in M13 medium at 5, 15, 20, 25, 30, 37 and 40 °C. Catalase test was performed by placing a drop of hydrogen peroxide on a microscope slide with a LzC2^T colony. Bubble formation indicated the presence of catalase. For chemotaxonomic analyses of respiratory quinones, polar lipids and fatty acids strain LzC2^T was cultured for 15 days; biomass was collected and lyophilized. The biomass was processed according to the protocols described previously [14–16]. Anaerobic growth was tested in anaerobic chambers (GENbox Anaer; BioMérieux) according to the manufactures and results were recorded after one week.

Results and discussion

Sequences obtained from the analysis of the 16S rRNA gene established the affiliation of strain LzC2^T to the family *Planctomycetaceae*. In the phylogenetic tree, LzC2^T clustered monophyletically with the recently described strain *Alienimonas californiensis* CA12^T (Fig. 1A). Strain LzC2^T shares a 16S rRNA gene sequence identity of 97.0% with *A. californiensis*. This value is below the proposed species threshold of 98.7% [55], but above the genus threshold of 94.5% [55], indicating that LzC2^T belongs to the genus *Alienimonas*, but is not a strain of the species *A. californiensis*. The computed MLSA phylogenetic tree supports this relation (Fig. 1B). A partial sequence of the RNA polymerase subunit beta gene (*rpoB*) is commonly applied as molecular phylogenetic marker in the order *Planctomycetales* [11]. Strain LzC2^T shares 92.9% *rpoB* similarity with *A. californiensis* CA12^T. This value is below the proposed species threshold of 96.3% [11], but above the genus threshold of 75.5–78% [25], thus confirming the results of the 16S rRNA gene comparison. Other phylogenetic markers, such as ANI [26], AAI [29] and POCP [39] were also applied to enhance the accuracy in the determination of phylogenetic affiliation. Percentages of similarity between LzC2^T and CA12^T were 79.5%, 78.7% and 79.7%, for ANI, AAI and POCP, respectively, which is in agreement with LzC2^T being placed as a novel species of the genus *Alienimonas*.

Genomic features (genome with 355 scaffolds) of LzC2^T in comparison to *A. californiensis* CA12^T genome are summarized in Table 1. LzC2^T has a genome size of 5.3 Mb and a G+C content of 68.3%. Other type strains of the family have a G+C content below 60.0% [52]. An interesting feature of most planctomycetal genomes

Table 1

Genomic features of LzC2^T compared with its closest relative *A. californiensis* CA12^T (retrieved from ¹Boersma et al. [5]).

Characteristics	LzC2 ^T	¹ <i>A. californiensis</i> CA12 ^T
Genome size (Mbp)	5.3	5.4
DNA G+C content (%)	68.3	70.7
16S rRNA gene sequence similarity to strain LzC2 ^T (%)	–	97.0
Completeness (%)	94.8	94.8
Contamination (%)	0	0
Number of predicted genes	4345	4382
Number of protein-coding genes	4232	4309
Coding density (%)	89.2	88.5
Number of hypothetical proteins	2310	1798
Hypothetical proteins (%)	54.6	41.7
Number of tRNA genes	57	65

is the considerably high number of genes with unknown function. The genome of LzC2^T is not an exception since more than 50% of the predicted proteins are annotated as hypothetical or uncharacterized proteins (Table 1). Extremely large genes (with a size of up to 49 kb) have been detected in some planctomycetal genomes [20], mostly encoding proteins with unknown function. The genome of our novel isolate harbors a single giant gene (with a size of 25 kb) related to a bifunctional hemolysin/adenylate cyclase-encoding gene. The presence of primary metabolic pathways involved in the catabolism of sugars (Embden–Meyerhof–Parnas pathway, gluconeogenesis, pentose phosphate pathway, tricarboxylic acid cycle and Entner–Doudoroff pathway) were also analyzed based on enzymes participating in these pathways encoded in the LzC2^T genome. Indeed, genes were found for all pathways, except for gluconeogenesis, for which only a partial set of genes coding for respective enzymes of this pathway could be identified. Genes coding for enzymes of the glyoxylate shunt, which is typically required for anaplerosis during growth with acetate or fatty acids as sole carbon and energy source, were not found. Data of *A. californiensis* CA12^T was used for comparison (Supplementary Table 1). Presence of genes encoding carbohydrate-degrading enzymes was also analyzed in the LzC2^T genome, as well as data of *A. californiensis* CA12^T for comparison (Table 2). The carbohydrate-degrading enzyme profile of LzC2^T was similar to the profile of strain CA12^T. As mentioned previously, planctomycetes are also emerging as biotechnologically important bacteria. Using the antiSMASH platform, the LzC2^T genome was analyzed for the presence of biosynthetic gene clusters and five putative clusters were detected: three of these appear to be related to the synthesis of terpenes (likely including the pigments carotenoids), one encodes a type III PKS and one cluster was unclassified.

A summary of some of the analyzed features of strain LzC2^T is shown in Table 3, as well as data of *A. californiensis* CA12^T for comparison. Morphologically, LzC2^T cells are ovoid to spherical, with

Fig. 3. Transmission electron and scanning electron microscopy observations of strain LzC2^T. Rosette organization (a,b); cell overview (c,d); polar budding with a tubular neck-like structure connecting mother and daughter cells (e,f); lateral budding (g,h). H – holdfast; CP – crateriform pits; F – fimbriae; CM – cytoplasmic membrane invaginations; TS – tubular shaped structure. Bars – 500 nm (a,h) and 200 nm (c–g).

sizes of $1.4 \pm 0.3 \times 1.0 \pm 0.2 \mu\text{m}$, while colonies appeared circular and pink-colored in both agar M13 and M14 media (Fig. 2). Motility of the cells was confirmed using phase contrast microscopy. SEM and TEM observations showed the characteristic rosette organization of planctomycetes, in which cells aggregate through their holdfast structures (Fig. 3a and b). Crateriform pits, commonly found in planctomycetes, are also easily observed to be distributed uniformly on the cell surface (Fig. 3c and d). Ultrathin sections of

LzC2^T showed an overall typical cell plan of planctomycetes with a large periplasm, invagination of the cytoplasmic membrane, cytoplasm with ribosomes, permanently condensed DNA and storage material (Fig. 3a, c, e and g). Crateriform pits were often associated with fimbriae (Fig. 3c). Reproduction by budding (a conserved feature of members of the class *Planctomycetia* [35,52,53]) occurs through an apical or a lateral tubular neck-like structure connecting the mother and daughter cells (Figs. 1a, b and 3e–h). A very sim-

Table 2
Carbohydrate-degrading enzymes of strain LzC2^T compared its closest relative *A. californiensis* CA12^T.

	LzC2 ^T	<i>Alienimonas californiensis</i> CA12 ^{Ta}
Carbohydrate esterases	28	26
Glycoside hydrolases	48	56
Glycosyltransferases	57	77
Polysaccharide lyases	5	5
Carbohydrate-Binding	20	19
Auxiliary activities	2	2
Unknown	4	3

^a Genomic data from GenBank acc. no. CP036265.

Table 3
Morphological and physiological features of strain LzC2^T in comparison with its closest relative. Data for *A. californiensis* was taken from the species descriptions [5].

Characteristics	LzC2 ^T	<i>A. californiensis</i> CA12 ^T
16S rRNA gene sequence similarity to strain LzC2 ^T (%)	100	97.0
Cell size (μm)	1.4 ± 0.3 × 1.0 ± 0.2	2.0 ± 0.2 × 1.5 ± 0.3
Cell shape	Spherical to ovoid	Spherical to ovoid
Rosette formation	Observed	Not observed
Reproduction	Budding	Budding
Distribution of crateriform structures	Uniform	Uniform, except for the pole at which the flagellum is located
Presence of fimbriae	Yes	Yes
Colony color	Pink	Light pink
Source (habitat)	<i>Chondrus crispus</i>	<i>Macrocystis pyrifera</i>
Temperature range (°C)	15–30	10–40
pH range	5.5–10	5.0–9.0

Table 4
Fatty acid composition of strain LzC2^T grown in M14 medium at 25 °C.

Fatty acids	ECL	LzC2 ^T
<i>iso</i> -C _{14:0}	13.619	3.7 ± 0.1
C _{14:0}	14.000	0.6 ± 0.1
Summed feature 1	14.478	1.8 ± 0.2
<i>iso</i> -C _{15:0}	14.623	19.5 ± 0.5
<i>anteiso</i> -C _{15:0}	14.713	54.2 ± 0.6
<i>iso</i> -C _{16:1} H	15.461	4.7 ± 0.2
<i>iso</i> -C _{16:0}	15.627	4.3 ± 0.2
Unknown 15.737	17.737	1.2 ± 0.1
Summed feature 3	15.822	1.5 ± 0.2
C _{16:0}	16.000	1.1 ± 0.1
Unknown 16.090	16.090	1.3 ± 0.1
Summed feature 9	16.416	0.8 ± 0.1
<i>anteiso</i> -C _{17:1} ω9c	15.524	2.4 ± 0.1
<i>anteiso</i> -C _{17:0}	16.723	1.2 ± 0.1
Summed feature 8	17.823	0.7 ± 0.1

Results are the percentage of the total fatty acids. ± SD, the results are the mean plus the standard deviation of two to four analyses; values for fatty acids present at less than 0.5% are not shown; ECL, equivalent chain length. A summed feature represents groups of two or three fatty acids that could not be separated by GLC with the MIDI System. Summed feature 1 comprises *iso*-C_{15:0} H and/or C_{13:0} 3-OH; Summed feature 3 comprises C_{16:1} w7c and/or C_{16:1} w6c and/or *iso*-C_{15:0} 2-OH; Summed feature 9 comprises *iso*-C_{17:1} w9c and/or C_{16:0} 10-methyl; Summed feature 8 comprises C_{18:1} w7c and/or C_{18:1} w6c.

ilar tubular neck-like structure has been reported before [27,31]. The closely related strain *A. californiensis* CA12^T was described to reproduce by unique consecutive polar budding, without completing a characteristic planctomycetal lifestyle switch [5]. However, this was not observed in our strain. The presence of a large amount of fibers as reported in *A. californiensis* was also not observed in LzC2^T. On the other hand, the previously referred tubular structure formed during the budding of our isolate was not detected in CA12^T. Although similar in size and shape, it is clear that its bud-

Table 5
Description of *Alienimonas chondri* sp. nov.

Genus name	<i>Alienimonas</i>
Species name	<i>Alienimonas chondri</i>
Specific epithet	<i>chondri</i>
Species status	sp. nov.
Species etymology	<i>chondri</i> (chon'dri. N.L. gen. n. <i>chondri</i> of the alga <i>Chondrus crispus</i>).
Description of the new taxon and diagnostic traits	Colonies on solid medium are pink-colored. Cells are 1.4 ± 0.3 × 1.0 ± 0.2 μm in size. Cells are motile. Aggregates in rosettes. Organism reproduces by apical and lateral budding and a tubular structure is formed between the mother and daughter cells. Crateriform structures are uniformly distributed, often associated with fimbriae. Catalase test is positive. Growth can occur between pH 5.5 and 10. At least 1% NaCl is needed for growth and it can tolerate up to 5% NaCl. Growth occurs between 25 and 30 °C. No need for vitamins. Major fatty acids are <i>anteiso</i> -C _{15:0} (54.2%) and <i>iso</i> -C _{15:0} (19.5%). Phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid are the main lipids. Menaquinone 6 (MK-6) is the only quinone present. Genome size of 5.3 Mb and a G+C content of 68.3%. The genes for the main primary biosynthetic pathways are present but only some genes are present for gluconeogenesis. No genes were found for enzymes of the glyoxylate cycle. Genes of different families of carbohydrate active enzymes were identified. Organism is obligate aerobic.
Country of origin	Portugal
Region of origin	Porto
Date of isolation (dd/mm/yyyy)	08/10/2019
Source of isolation	Macroalga <i>Chondrus crispus</i>
Sampling date (dd/mm/yyyy)	08/10/2019
Latitude (xx°xx'xx"N/S)	41°09'12.38 N
Longitude (xx°xx'xx"E/W)	8°40'45.91 W
Altitude (meters above sea level)	0
16S rRNA gene accession nr.	GenBank = MN757873.1
Genome accession number	GenBank = WTPX000000000
Genome status	Completeness of 94.8%
Genome size	5.3 Mb
G+C mol%	68.3
Number of strains in study	1
Information related to the Nagoya Protocol	Not applicable
Designation of the Type Strain	LzC2 ^T
Strain Collection Numbers	CECT 30038 ^T = LGMXXXX

ding process and morphology distinguish our strain from its peers [5,30,45].

Strain LzC2^T required salt to grow (at least 1% (w/v) NaCl) and could also tolerate concentrations of up to 5% NaCl. Growth occurred from 15 to 30 °C, in a pH range from 5.5 to 10 and did not require vitamins. LzC2^T is catalase positive. LzC2^T major fatty acids are *anteiso*-C_{15:0} (54.2%) and *iso*-C_{15:0} (19.5%) (Table 4). The major polar lipids of LzC2^T are phosphatidylglycerol, diphosphatidylglycerol one unidentified phospholipid and one unidentified glycolipid (Supplementary Fig. 1). The only respiratory quinone of strain LzC2^T is menaquinone 6 (MK-6). This strain is an obligate aerobic, as it did not grow on anaerobic conditions.

Conclusion

Both phenotypic and genotypic characteristics support that the novel isolate represents a novel species within the genus *Alienimonas*. Therefore, we propose the name *Alienimonas chondri* sp.

nov., with LzC2^T being the type strain. The formal description of the species is shown in Table 5.

Ethical statement

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

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Author contributions

The design of the experiments was done by IV and OML. The performing of the scientific work by IV, LA, SW, NK, ALC, and OML. Manuscript writing by IV, LA, SW, NK, MSC, ALC, CJ and OML.

Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <https://doi.org/10.1016/j.syapm.2020.126083>.

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