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Antimicrobial Resistance Profile of *Planctomycetota* Isolated from Oyster Shell Biofilm: Ecological Relevance within the One Health Concept

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Abstract: Background: *Planctomycetota* isolation in pure culture is still challenging with most of the reported data coming from molecular-based methods. Here, we intended to isolate *Planctomycetota* from the filter-feeder Pacific oyster *Magallana gigas*, extending the search to a not yet explored natural reservoir and to characterize their antimicrobial resistance phenotype. Methods: Oyster samples from different supermarkets and from a farm producer were subject to isolation in selective medium. Inoculation was performed from the shell biofilm and after an enrichment of the edible content. Results: *Planctomycetota* isolates (n = 65) were only obtained from the shell biofilm with four different species identified: *Rhodopirellula baltica* (n = 62), *Rhodopirellula rubra* (n = 1), *Rhodopirellula heiligendammensis* (n = 1) and *Gimesia chilikensis* (n = 1). This study reports the first association of *Planctomycetota* members with oysters and the first description of *R. heiligendammensis* in Portugal. Moreover, *R. rubra*, originally identified in Portugal, was isolated from oysters of French origin. Antibiotic susceptibility testing, conducted in strains belonging to two species never assayed before revealed multidrug resistance phenotypes with bacteria showing resistance to several classes of clinically relevant antibiotics (e.g., β-lactams and aminoglycosides). Conclusion: The ecological role and impact of *Planctomycetota* on oyster holobiont and, ultimately, in public health, under the One Health concept, is discussed.

Keywords: *Magallana gigas*; culture-based method; antimicrobial resistance; One Health; microbiota

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1. Introduction

Members of the bacterial phylum *Planctomycetota* are fascinating Gram-negative bacteria, one of the groups in the superphylum *Planctomycetota–Verrucomicrobiota–Chlamydiota* (PVC) [1]. They possess unique distinguishing features, setting them apart from other bacteria. These include budding or binary fission cell division of different members, absence of the universal division protein FtsZ, highly condensed DNA, multiple invaginations of the cytoplasmic membrane, a complex life cycle in which a dimorphic lifestyle is characterized by a surface-attached mother cell that yields a planktonic daughter cell, presenting, in many members, motility. Furthermore, they present rare carotenoids, large genomes with a great percentage of genes with unknown function, membrane coat proteins (MC) that have a high

structural similarity to eukaryotic MC proteins such as clathrin, and unique cysteine-rich proteins with YTV-domain repeats [2–4]. The known *Planctomycetota* form colonies that range in colour from white and beige to pink and red or even orange, and the cells can be spherical, ovoid or pear-shaped and can possess crater-like pits in the cell wall associated with fimbria-like structures. Cells are gathered through a holdfast. *Planctomycetota* have relatively slow growth rates, making it very challenging to isolate them on standard media since they are quickly exceeded by bacteria with faster growth rates [5].

Some *Planctomycetota* have been found to be resistant to a vast range of antibiotic classes, such as aminoglycosides, β-lactams, and glycopeptides [6], although the main mechanisms behind these phenotypes still remains unclear. Due to this particular feature of *Planctomycetota*, studies on antimicrobial susceptibility are crucial to understand the role of their resistome within the antimicrobial resistance phenomenon. Inhabiting environmental ecological niches, these antibiotic resistant organisms might contribute to the spread of antibiotic resistance genes to other bacteria namely relevant pathogenic ones, and/or being accumulate throughout the trophic chain and ultimately reaching humans. In fact, antimicrobial resistance, a phenomenon mainly enhanced by the overuse and misuse of antibiotics within different areas, such as animal production systems, agricultural and aquacultural industries and human therapy, represents an actual threat of great concern worldwide involving cross-sectorial areas and demanding a coordinated and integrated global action following the One Health approach. This aims at providing optimized health for people, animals and environment, as established by the quadripartite collaboration of the FAO, United Nations Environment Programme (UNEP), World Health Organization (WHO), and the World Organization for Animal Health (WOAH).

Planctomycetota are empowered with a variable metabolic diversity that enables them to inhabit a vast range of environments [3]. Most of their members are aerobic, mesophilic, heterotrophic, and dwell in neutral pH, but they can also anaerobically oxidize ammonia in the case of the anammox *Planctomycetota* [2,7,8]. Bacteria belonging to this phylum have been found in terrestrial habitats such as soils [9] and peat bogs [10], and also in aquatic habitats including marine environments [5], freshwater [11], sediments [5] and deep-sea deposits [12]. They are also present in extreme habitats such as hot springs [13], desert soils [14], and also polluted environments [15,16], which displays their ability to adapt and survive in different surroundings. In addition, there are numerous examples of *Planctomycetota* living in association with other organisms such as sponges [17], macroalgae [18], the human skin and the digestive track [19–22]. The existence of an association between *Planctomycetota* and oysters was also reported before through molecular data analysis [23–29]. In fact, oysters are widespread in aquatic marine environments, where they coexist with a diverse range of microorganisms, including *Planctomycetota*, in important holobiontic relationships.

Oysters are marine invertebrates of the phylum Mollusca that represent an important component of the ecosystem. Being benthic, sessile filter-feeding bivalves, they eat algae and other food particles in suspension in the water column, and, by filtering the water, they remove organic and inorganic particles, including sequestration of excess nitrogen, that results in cleaner water, positively impacting on other species. They concentrate microbiota within their nutrient-rich mucosa and digestive organs, which perform several functions like the supply of vitamins, enzymes, and essential fatty acids, and the influence on the immune response and disease resistance [30]. Their shells are a hard substrate colonized by macroorganisms such as barnacles, mussels, and anemones, and also by a microbial biofilm, providing, thus, an essential habitat for many living beings.

Oysters are economically important organisms because they are nutritionally adequate and their consumption is more and more appreciated. As such, it is very important to assess their adequacy for consumption. In a previous study, we evaluated the microbiological quality of Pacific oysters (*Magallana gigas*) using, as parameters, various indicator microorganisms and hygiene bioindicators, and also assessing the oyster content in antibiotic-resistant bacteria [31]. The aim of the present study was to assess the associa-

tion of *Planctomycetota* members with Pacific Oysters *Magallana gigas* (formerly *Crassostrea gigas*) through culture-dependent methods, as this association has only previously been revealed by molecular-dependent approaches. Furthermore, and having in mind the extensive resistome of this phylum, the evaluation of antimicrobial susceptibility patterns of newly isolated *Planctomycetota* was conducted.

2. Material and Methods

2.1. Sampling

Three Pacific oyster (*Magallana gigas*) samples were purchased at different supermarkets located in Porto, between July 2021 and January 2022. One oyster sample (sample A) was from a French aquaculture and two samples (samples B and C) were from two distinct Portuguese aquaculture production systems. A fourth sample (sample D) was obtained directly from a Portuguese oyster farm. Samples were transported in their original packaging to the laboratory under refrigerated conditions for further processing.

2.2. Isolation of Planctomycetota from Oysters

Since *Planctomycetota* are known to be attached to surfaces [18], attempts of isolation were based on the analysis of the oyster shell surface. Furthermore, as they can be found in the water column, and oysters are filter-feeding organisms, isolation was also tried after an enrichment step of the oysters' edible content. For the isolation of *Planctomycetota*, medium M607 + NAG was used. This medium is composed of 0.025% yeast extract, 0.025% peptone, 0.025% glucose, 0.05% N-acetylglucosamine (NAG), 0.1 M Tris-HCI, pH 7.5, Hutner's basal salts, a vitamin solution [32], and 90% natural seawater.

2.2.1. Isolation of *Planctomycetota* from Surface Biofilm

Using sterile swabs, biological material was scraped off from the shell biofilm of different oysters in each sample (a pool of 4 to 5 individuals) and suspended in 0.9 mL of M607 + NAG broth supplemented with ampicillin (200 µg/mL), streptomycin (1000 µg/mL) and cycloheximide (20 µg/mL). Decimal serial dilutions were prepared and, subsequently, 100 µL of the initial suspension and further dilutions were spread, with glass beads, onto M607 + NAG agar medium supplemented with the antibiotics and the antifungal agent mentioned previously. Cultures were incubated at 25 °C, in the dark, until characteristic *Planctomycetota* colonies became noticeable, which were inoculated in new medium. All characteristic colonies were inspected by cell observation under optical microscopy. Isolated *Planctomycetota* were stored in M607 + NAG broth medium with 20% (*w*/*v*) glycerol at −80 °C.

2.2.2. Isolation of *Planctomycetota* from the Enrichment of the Edible Content

Twenty-five grams of the oysters' edible content obtained from pooled organism from each sample were placed in 225 mL M607 + NAG broth, supplemented with ampicillin (200 µg/mL), streptomycin (1000 µg/mL) and cycloheximide (20 µg/mL). After an incubation period of 2 months at room temperature and 80 r.p.m, this enrichment broth was decimally and serially diluted in M607 + NAG broth, and 100 µL of each dilution was spread on M607 + NAG agar medium supplemented with the same above-mentioned antimicrobial agents. All plates were incubated and processed as described above.

2.3. Amplification and Sequencing Analysis of 16S rRNA Gene and Bacterial Phylogenetic Analysis

Total genomic DNA was extracted using an E.Z.N.A. Bacterial DNA Isolation Kit from OMEGA, following the protocol with all optional procedures recommended by the manufacturer. The isolates were identified based on the analysis of the 16S rRNA gene sequence after amplification with the universal bacterial primers 27F and 1492R [33]. The PCR mixture contained 12.5 µL NZYTaq II 2× Green Master Mix, 0.1 µM of each primer, 2 µL of extracted genomic DNA, and 10 µL of MiliQ water to complete a final volume of 25 µL. The PCR program was performed in a thermocycler MyCycler™ Thermal Cycler (BIO-RAD®,

Hercules, CA, USA) and consisted of an initial denaturing step of 5 min at 95 °C, thirty cycles of 30 s at 94 °C, 30 s at 58 °C and 45 s at 72 °C, followed by a final extension of 10 min at 72 °C. PCR amplicons were analyzed by electrophoresis in Green Safe Premium (NZYTech, Lisboa, Portugal) stained 1.0% (*w*/*v*) agarose gel in 1× TAE buffer at 100 V for 30 min, using a GeneRuler 1 kb Plus DNA Ladder (Thermo Scientific™ Waltham, MA, USA). Image acquisition of the gels was performed using the GenoPlex (VWR® Radnor, PA, USA) gel documentation system with GenoCapture (VWR®) version 7.01.06 (Synoptics Ltd., Cambridge, UK). Amplicons were purified using the Illustra™ GFX™ PCR DNA and Gel Band Purification Kit (VWR®, Radnor, PA, USA) following the specifications of the manufacturer. Purified samples were visualized by electrophoresis as previously described. Purified PCR products were subsequently sequenced at Eurofins Genomics. Sequencing results were analyzed, and nucleotide alignment consensus sequences were generated utilizing software Geneious Prime 2021 (https://www.geneious.com; accessed on 7 July 2022). Consensus sequences of the 16S rRNA gene were subjected to standard nucleotide Basic Local Alignment Search Tool (BLAST) at the National Center of Biotechnology Information (NCBI) and EzBioCloud 16S rRNA gene database, to identify the differences between the isolates and validly described species [34,35]. Then, they were further analyzed by performing a multiple sequence alignment in the software Molecular Evolutionary Genetics Analysis (MEGA) (version 7.0) using the Clustal W algorithm [36,37]. The alignment was used to generate a phylogenetic tree applying a Maximum-likelihood algorithm in MEGA 7, and the following parameters: bootstrap method (1000 replicates), General Time Reversible model, Gamma distributed with Invariant sites (G + I) and complete deletion.

The *Planctomycetota* 16S rRNA gene sequences are deposited at the National Center for Biotechnology Information—NCBI, with the following GenBank accession numbers: OR888823–OR888887.

2.4. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility profile of the *Planctomycetota* isolates was determined by a modified Kirby–Bauer disk diffusion susceptibility test [6] for a total of 26 antibiotics representing several classes. Since *Planctomycetota* members are slow-growing bacteria and require specific growth conditions, isolates were tested on M607 + NAG medium and incubated at 25 °C for 7 days. Furthermore, *Planctomycetota* cell suspensions had to have an optical density at 600 nm of 0.7 A.U. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were employed as quality controls.

3. Results and Discussion

3.1. Planctomycetota Isolation

In this study, four oyster samples, three from local markets (samples A, B and C) and one acquired directly from a farm producer (sample D) allowed the isolation of 65 *Planctomycetota*, exclusively from the biofilm of the oysters' shells (Figure 1, Table 1). It was not possible to retrieve any *Plancyomycetota* from the oyster's edible content. Several possible explanations may be pointed out: (i) since *Planctomycetota* live mainly in biofilm association, and are seldom present in the planktonic water column (usually only as marine snow) [38], the levels of these microorganisms inside the filter-feeding oysters should be probably low; (ii) different areas of production might have different microbial profiles, both in amount and diversity, resulting in a potentially low concentration of *Planctomycetota* in the studied oysters, a fact already observed by Singh et al. [39] in the study of the gill microbiome signatures in oysters from different areas; (iii) the absence of these microorganisms from the water column might be due to seasonal variations and, as a consequence, the oyster microbial community varies according to seasonality along the year [40]; (iv) the methodology used for the *Planctomycetota* isolation comprised an enrichment step which might have favored the fast-growing bacteria, instead of the slow-growing *Planctomycetota* [5]; and (v) although DNA from *Planctomycetota* can be detected, cells may not be viable, making their culture unfeasible.

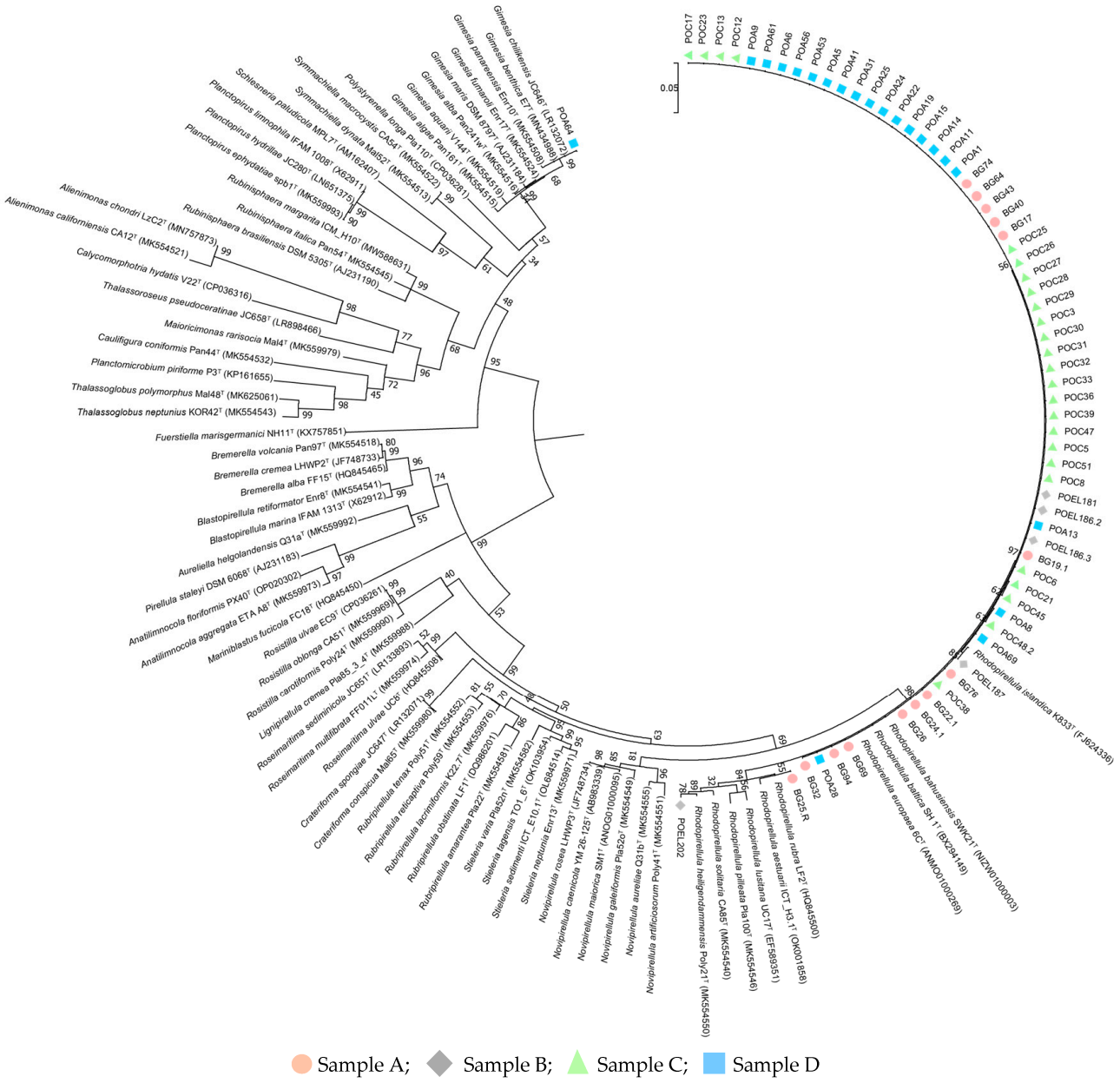

Figure 1. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences showing the evolutionary relationships of the isolated bacteria. Bootstrap values based on 1000 replicates are shown at branch nodes. *Phycisphaera mikurensis* NBRC 102666^{T} (AB447464.1) was used as the outgroup.

Table 1. Phenotypic features and origin of the *Planctomycetota* isolates.

Species	No of Isolates	Origin of Production	Colony Color	Cell Shape
Rhodopirellula baltica	62	Portuguese/French	Pink to red	Ovoid, ellipsoidal or pear-shaped
Rhodopirellula rubra	1	French	Red	Pear- or club-shaped
Rhodopirellula heiligendammensis	1	Portuguese	Coral pink	Ovoid to pear-shaped
Gimesia chilikensis	1	Portuguese	Orange	Ovoid to pear-shaped

Despite the absence of growth from the oyster edible content, the existence of an association between *Planctomycetota* and the oyster microbiome has been reported by several authors [23–29]. Nevertheless, all these studies applied molecular-based methods

and, in none of them, cultural approaches were attempted. Those techniques are more sensitive and detect a low amount of microbial DNA, which allows for, in a more effective way, the detection of *Planctomycetota* members in oysters.

Four different *Planctomycetota* species, belonging to the families *Pirellulaceae* and *Planctomycetaceae*, were identified. These species were *Rhodopirellula baltica* (n = 62; 13 from sample A, 4 from sample B, 25 from sample C and 20 from sample D), *Rhodopirellula rubra* (n = 1 from sample A), *Rhodopirellula heiligendammensis* (n = 1 from sample B) and *Gimesia chilikensis* (n = 1 from sample D) (Figure 1, Table 1). Table 1 presents evidence concerning some of the morphological characteristics of these isolates. Typical *Planctomycetota* colonies can have a pink, orange, or a pale-white pigmentation. Further optical microscopy complements the selection of all suspicious colonies, due to their distinctive morphology (aggregation in rosettes, spherical to ovoid-shaped or pear-shaped cells and cell division by budding).

The family *Pirellulaceae* includes the well-reported genus *Rhodopirellula*, which comprises the majority of the isolated strains within this family [41]. Bacteria belonging to this genus have been detected in the soft tissues of oysters using molecular methods [26,42]. In the present study, 95% of the isolates were identified as *R. baltica*, which is in accordance with the well-known cosmopolitan distribution of this species, already found in macroalgae, mussel shell biofilm, marine sediments and also in the water column [8,31,43,44]. *Rhodopirellula rubra* was initially isolated from the surface biofilm of the macroalga *Laminaria* sp. in Portugal [18,31] and, curiously, it was isolated in our study from the Pacific oyster samples from a French aquaculture production system (sample A). Moreover, *R. heiligendammensis*, primarily described from plastic surfaces (polyethylene particles) submerged in the Baltic Sea, Germany [45], was here obtained from a Portuguese oyster sample (sample B). This study seems to be the second report of the isolation of this species and the first isolation of *R. heiligendammensis* in Portugal.

Gimesia chilikensis, belonging to the family *Planctomycetaceae*, was initially isolated from sediments collected in the Chilika lagoon, in India [46]. This species was also found in aquatic marine environments, namely in the biofilm of macroalgae and sediments [47], and from sediments in Portugal [8]. Nevertheless, to the best of our knowledge, this is also the first study reporting the association of this species with Pacific oysters.

Further studies on members of this bacterial group are fundamental in order to understand their association with Pacific oysters and their role in aquaculture production systems. Since members of this bacterial group seem to contribute to the removal of organic matter [44], they might also contribute for the depuration of water quality in oyster aquaculture farm systems.

3.2. Antimicrobial Susceptibility Patterns of Planctomycetota

Data on the antimicrobial susceptibility of *Planctomycetota* are scarce and restricted to three papers [6,48,49]. In order to complement this information with species never characterized, the evaluation of the antimicrobial susceptibility patterns of the two newly isolated *Planctomycetota* species, *R. heiligendammensis* strain POEL202 and *G. chilikensis* strain POA64, was performed. As the available interpretative criteria for the inhibition zone diameters (from CLSI and EUCAST guidelines) are mainly toward species with clinical impact, reference values for environmental species, like the *Planctomycetota*, are unavailable. The antimicrobial susceptibility profiles obtained for both *Planctomycetota* species are presented in Table 2. If no inhibition zones occurred, the strain was considered resistant. The antibiotics that induced any degree of inhibition of growth were considered active and the strains susceptible.

Similar antibiotic susceptibility patterns were obtained for the two *Planctomycetota* isolates. Both strains showed resistance to β-lactams, fosfomycin, aminoglycosides, and nalidixic acid and susceptibility to clindamycin, erythromycin, polymyxin B, ciprofloxacin, chloramphenicol, doxycycline, and tetracycline. A variable antibiotic susceptibility pattern was obtained for nitrofurantoin. The observed resistance of *G. chilikensis* strain POA64 and

R. heiligendammensis strain POEL202 to β-lactams is in accordance with the well-reported resistance behavior of *Planctomycetota* to this class of antibiotics [5,6,47,48]. Although several β-lactamase-encoding genes have been found in the genomes of members of this bacterial group [50,51], no study has yet proved their role as a main mechanism of resistance, and, thus, the resistance to β-lactams remains enigmatic.

Table 2. Antibiotic resistance/susceptibility profile obtained for the tested *Planctomycetota* strains.

Target	Class	Antibiotic	Disk Content	Diameter (mm) of Inhibition Zone	
				Gimesia chilikensis POA64	*Rhodopirellula heiligendammensis* POEL202
Cell wall biosynthesis	Beta-lactams	Amoxycillin	10 µg	0	0
		Amoxycillin/Clavulanic acid	30 µg	0	0
		Aztreonam	30 µg	0	0
		Cefotaxime	30 µg	0	0
		Cefoxitin	30 µg	0	0
		Ceftazidime	30 µg	0	0
		Imipenem	10 µg	0	0
		Meropenem	10 µg	0	0
		Piperacillin	100 µg	0	0
		Piperacillin/Tazobactam	110 µg	0	0
	Fosfomycin	Fosfomycin	50 µg	0	0
	Glycopeptides	Teicoplanin	30 µg	ND	0
		Vancomycin	30 µg	ND	0
Structure of cell membrane	Polymyxins	Polymyxin B	300 IU	27	18
Protein synthesis	Aminoglycosides	Amikacin	30 µg	0	0
		Gentamicin	10 µg	0	0
		Kanamycin	30 µg	0	0
		Tobramycin	10 µg	0	0
	Amphenicol	Chloramphenicol	30 µg	10	33
	Lincosamide	Clindamycin	2 µg	38	53
	Macrolides	Erythromycin	15 µg	>50	40
	Tetracyclines	Doxycycline	30 µg	>50	26
		Tetracycline	30 µg	>50	12
DNA replication/ Protein synthesis	Nitrofuran	Nitrofurantoin	300 µg	0	35
DNA replication	Quinolones	Ciprofloxacin	5 µg	33	47
		Nalidixic acid	30 µg	0	0

ND—Not determined.

These two strains also showed resistance to fosfomycin, a molecule involved in the inhibition of the initial step of the peptidoglycan synthesis [52], and similar pattern was previously reported for other *Planctomycetota* [6,49].

In Gram-negative bacteria, resistance to glycopeptides is intrinsic, as these antibiotics are incapable of passing through the outer membrane [53]. As so, the result of resistance for this antibiotic obtained in both strains was accordant.

This study confirms the previously reported resistance of *Planctomycetota* to aminoglycosides [6,48,49], which are often employed to limit the growth of different bacteria during the isolation of members of this phylum [5]. Susceptibility to chloramphenicol was observed for both strains, a similar result to that previously obtained by Godinho et al. [6] who identified susceptibility profiles in two *Rhodopirellula* spp. Curiously, in another study [47], *Gimesia maris* showed resistance to chloramphenicol, a phenotype probably associated with efflux pumps. The other tested antibiotics targeting the protein synthesis showed inhibitory activity against both *G. chilikensis* strain POA64 and *R. heiligendammensis* strain POEL202, which is consistent with the results from Cayrou et al. [48] and Godinho et al. [6] for members of these genera.

Both strains were susceptible to polymyxin B. Polymyxins bind anionic lipopolysaccharides (LPS) molecules and displace calcium and magnesium cations, damaging LPS's three-dimensional structure, consequently altering the integrity of the cell membrane and causing cell lysis and death [54,55].

Regarding quinolones that target DNA gyrase and topoisomerase IV, both strains exhibited resistance to nalidixic acid but susceptibility to ciprofloxacin. These variable susceptibility profiles to quinolones in *Planctomycetota* were also observed in other studies [6,48]. Different patterns of susceptibility to nitrofurantoin in the *Planctomycetota* strains were observed in this study, which is consistent with the findings reported by Godinho et al. [6] who also observed either resistance or susceptibility profiles for the studied strains.

Both strains exhibited a multidrug-resistant phenotype, i.e., resistance to at least one antimicrobial agent from three different classes of antibiotics. Although the mechanisms behind resistance phenotypes in *Planctomycetota* are still far from being clarified, some in silico studies already showed that *Planctomycetota* possessed encoding genes for several multidrug-resistance efflux pumps, beta-lactamases and *cfr* genes, enabling the methylation of the 23S rRNA [49–51,56]. As already known for the glycopeptide vancomycin, intrinsic resistance mechanisms may also justify some resistance patterns. Additionally, members of the *Planctomycetota* have been reported to biosynthesize antimicrobial compounds [57–59], and it was hypothesized that the production of these bioactive molecules might confer innate defense mechanisms to protect them from their own secondary metabolites [6,57]. For instance, for most of the already studied *Planctomycetota*, it was possible to identify, by in silico analysis, the presence of several encoding genes responsible for the production of antibiotic molecules such as the 2-deoxystreptamin glucosyltransferase enzyme involved in the biosynthesis of kanamycin [6,60].

Planctomycetota can be, thus, a reservoir/source of antibiotic resistance genes that, by horizontal gene transfer events, might be spread to other environmental and/or clinical pathogenic-relevant bacteria. These bacteria may affect public heath as they may reach humans through the food chain or by direct contact in the aquatic environments.

4. Conclusions

This study represents the first report of the cultured diversity of *Planctomycetota* in association with oysters, confirming that oysters represent a natural reservoir of distinctive *Planctomycetota* as already assessed by molecular-based methods. Although *R. baltica*, *R. rubra* and *G. chilikensis* have already been identified from diverse habitats in Portugal, this study reports the first isolation of *R. heiligendammensis* in our country.

The antibiotic susceptibility of two *Planctomycetota* species was unveiled, contributing to fulfill the scarce existing information and to better understand their behavior on antimicrobial resistance. In fact, as this group of bacteria are resistant to several antibiotics with therapeutic impact, they can be a threat to public health by (i) being a source of antibiotic-resistance genes, which, by horizontal gene transfer events, may reach pathogenic bacteria; and (ii) possibly emerging as opportunistic agents. Moreover, since the members of this phylum are known to produce secondary metabolites with antimicrobial activity, and being part of the oyster's holobiont, they can support the oyster in its defense against pathogenic bacteria. Furthermore, since *Planctomycetota* play a role in the removal of organic matter, they might contribute to the depuration of water quality in oyster's aquaculture production systems. All these aspects, gathering potentially public health threats, but, at the same time, several beneficial impacts in the ecosystem, by the *Planctomycetota*, reflect well the concept behind the One Health approach.

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References

1. Wagner, M.; Horn, M. The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. *Curr. Opin. Biotechnol.* **2006**, *17*, 241–249. [CrossRef]
2. Wiegand, S.; Jogler, M.; Jogler, C. On the maverick Planctomycetes. *FEMS Microbiol. Rev.* **2018**, *42*, 739–760. [CrossRef] [PubMed]
3. Lage, O.M.; van Niftrik, L.; Jogler, C.; Devos, D.P. Planctomycetes. In *Encyclopedia of Microbiology*, 4th ed.; Schmidt, T.M., Ed.; Academic Press: Oxford, UK, 2019; pp. 614–626.
4. Mahajan, M.; Seeger, C.; Yee, B.; Andersson, S.G.E. Evolutionary remodelling of the cell envelope in bacteria of the Planctomycetes phylum. *Genome Biol. Evol.* **2020**, *12*, 1528–1548. [CrossRef] [PubMed]
5. Lage, O.M.; Bondoso, J. Bringing Planctomycetes into pure culture. *Front. Microbiol.* **2012**, *3*, 405. [CrossRef] [PubMed]
6. Godinho, O.; Calisto, R.; Øvreås, L.; Quinteira, S.; Lage, O.M. Antibiotic susceptibility of marine Planctomycetes. *Antonie van Leeuwenhoek* **2019**, *112*, 1273–1280. [CrossRef] [PubMed]
7. Kuenen, J.G. Anammox bacteria: From discovery to application. *Nat. Rev. Microbiol.* **2008**, *6*, 320–326. [CrossRef]
8. Vitorino, I.; Santos, J.D.N.; Godinho, O.; Vicente, F.; Vasconcelos, V.; Lage, O.M. Novel and conventional isolation techniques to obtain Planctomycetes from marine environments. *Microorganisms* **2021**, *9*, 2078. [CrossRef] [PubMed]
9. Buckley, D.H.; Huangyutitham, V.; Nelson, T.A.; Rumberger, A.; Thies, J.E. Diversity of Planctomycetes in soil in relation to soil history and environmental heterogeneity. *Appl. Environ. Microbiol.* **2006**, *72*, 4522–4531. [CrossRef]
10. Kulichevskaya, I.; Pankratov, T.; Dedysh, S. Detection of representatives of the Planctomycetes in Sphagnum peat bogs by molecular and cultivation approaches. *Microbiology* **2006**, *75*, 329–335. [CrossRef]
11. Wang, J.; Jenkins, C.; Webb, R.I.; Fuerst, J.A. Isolation of *Gemmata*-like and *Isosphaera*-like planctomycete bacteria from soil and freshwater. *Appl. Environ. Microbiol.* **2002**, *68*, 417–422. [CrossRef]
12. Storesund, J.E.; Øvreås, L. Diversity of Planctomycetes in iron-hydroxide deposits from the Arctic Mid Ocean Ridge (AMOR) and description of *Bythopirellula goksoyri* gen. nov., sp. nov., a novel Planctomycete from deep sea iron-hydroxide deposits. *Antonie Van Leeuwenhoek* **2013**, *104*, 569–584. [CrossRef] [PubMed]
13. Elcheninov, A.G.; Podosokorskaya, O.A.; Kovaleva, O.L.; Novikov, A.A.; Toshchakov, S.V.; Bonch-Osmolovskaya, E.A.; Kublanov, I.V. *Thermogemmata fonticola* gen. nov., sp. nov., the first thermophilic planctomycete of the order Gemmatales from a Kamchatka hot spring. *Syst. Appl. Microbiol.* **2021**, *44*, 126157. [CrossRef] [PubMed]
14. Andrew, D.R.; Fitak, R.R.; Munguia-Vega, A.; Racolta, A.; Martinson, V.G.; Dontsova, K. Abiotic factors shape microbial diversity in Sonoran Desert soils. *Appl. Environ. Microbiol.* **2012**, *78*, 7527–7537. [CrossRef] [PubMed]
15. Brümmer, I.H.M.; Fehr, W.; Wagner-Döbler, I. Biofilm community structure in polluted rivers: Abundance of dominant phylogenetic groups over a complete annual cycle. *Appl. Environ. Microbiol.* **2000**, *66*, 3078–3082. [CrossRef] [PubMed]
16. Chouari, R.; Paslier, D.L.; Daegelen, P.; Ginestet, P.; Weissenbach, J.; Sghir, A. Molecular evidence for novel Planctomycete diversity in a municipal wastewater treatment plant. *Appl. Environ. Microbiol.* **2003**, *69*, 7354–7363. [CrossRef] [PubMed]
17. Pimentel-Elardo, S.; Wehrl, M.; Friedrich, A.B.; Jensen, P.R.; Hentschel, U. Isolation of Planctomycetes from Aplysina sponges. *Aquat. Microb. Ecol.* **2003**, *33*, 239–245. [CrossRef]
18. Lage, O.M.; Bondoso, J. Planctomycetes and macroalgae, a striking association. *Front. Microbiol.* **2014**, *5*, 267. [CrossRef] [PubMed]
19. Gill, S.R.; Pop, M.; Deboy, R.T.; Eckburg, P.B.; Turnbaugh, P.J.; Samuel, B.S.; Gordon, J.I.; Relman, D.A.; Fraser-Liggett, C.M.; Nelson, K.E. Metagenomic analysis of the human distal gut microbiome. *Science* **2006**, *312*, 1355–1359. [CrossRef]
20. Costello, E.K.; Lauber, C.L.; Hamady, M.; Fierer, N.; Gordon, J.I.; Knight, R. Bacterial community variation in human body habitats across space and time. *Science* **2009**, *326*, 1694–1697. [CrossRef]
21. Maldonado-Contreras, A.; Goldfarb, K.; Godoy-Vitorino, F.; Karaoz, U.; Contreras, M.; Blaser, M.J.; Brodie, E.L.; Dominguez-Bello, M.G. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. *ISME J.* **2011**, *5*, 574–579. [CrossRef]
22. Cayrou, C.; Sambe, B.; Armougom, F.; Raoult, D.; Drancourt, M. Molecular diversity of the *Planctomycetes* in the human gut microbiota in France and Senegal. *Apmis* **2013**, *121*, 1082–1090. [CrossRef] [PubMed]
23. Fernandez-Piquer, J.; Bowman, J.; Ross, T.; Tamplin, M. Molecular analysis of the bacterial communities in the live Pacific oyster (*Crassostrea gigas*) and the influence of postharvest temperature on its structure. *J. Appl. Microbiol.* **2012**, *112*, 1134–1143. [CrossRef] [PubMed]
24. King, G.M.; Judd, C.; Kuske, C.R.; Smith, C. Analysis of stomach and gut microbiomes of the eastern oyster (*Crassostrea virginica*) from Coastal Louisiana, USA. *PLoS ONE* **2012**, *7*, e51475. [CrossRef] [PubMed]
25. Pierce, M.L.; Ward, J.E. Gut microbiomes of the eastern oyster (*Crassostrea virginica*) and the blue mussel (*Mytilus edulis*): Temporal variation and the influence of marine aggregate-associated microbial communities. *mSphere* **2019**, *4*, e00730-19. [CrossRef] [PubMed]

26. Clerissi, C.; de Lorgeril, J.; Petton, B.; Lucasson, A.; Escoubas, J.-M.; Gueguen, Y.; Dégremont, L.; Mitta, G.; Toulza, E. Microbiota composition and evenness predict survival rate of oysters confronted to Pacific Oyster mortality syndrome. *Front. Microbiol.* **2020**, *11*, 311. [CrossRef]
27. Stevick, R.J.; Post, A.F.; Gómez-Chiarri, M. Functional plasticity in oyster gut microbiomes along a eutrophication gradient in an urbanized estuary. *Anim. Microbiome* **2021**, *3*, 5. [CrossRef]
28. Unzueta-Martínez, A.; Welch, H.; Bowen, J.L. Determining the composition of resident and transient members of the oyster microbiome. *Front. Microbiol.* **2022**, *12*, 828692. [CrossRef]
29. Liu, M.; Li, Q.; Tan, L.; Wang, L.; Wu, F.; Li, L.; Zhang, G. Host-microbiota interactions play a crucial role in oyster adaptation to rising seawater temperature in summer. *Environ. Res.* **2023**, *216*, 114585. [CrossRef]
30. Pathak, A.; Stothard, P.; Chauhan, A. Comparative genomic analysis of three Pseudomonas species isolated from the eastern oyster (*Crassostrea virginica*) tissues, mantle fluid, and the overlying estuarine water column. *Microorganisms* **2021**, *9*, 490. [CrossRef]
31. Guedes, B.; Godinho, O.; Lage, O.M.; Quinteira, S. Microbiological quality, antibiotic resistant bacteria and relevant re-sistance genes in ready-to-eat Pacific oysters (*Magallana gigas*). *FEMS Microbiol. Lett.* **2023**, *370*, fnad053. [CrossRef]
32. Lage, O.M.; Bondoso, J. Planctomycetes diversity associated with macroalgae. *FEMS Microbiol. Ecol.* **2011**, *78*, 366–375. [CrossRef] [PubMed]
33. Lane, D.J. 16S/23S rRNA Sequencing. In *Nucleic Acid Techniques in Bacterial Systematic*; Stackebrandt, E., Goodfellow, M., Eds.; John Wiley and Sons: New York, NY, USA, 1991; pp. 115–175.
34. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef] [PubMed]
35. Yoon, S.-H.; Ha, S.-M.; Kwon, S.; Lim, J.; Kim, Y.; Seo, H.; Chun, J. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 1613–1617. [CrossRef] [PubMed]
36. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [CrossRef] [PubMed]
37. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef] [PubMed]
38. DeLong, E.F.; Franks, D.G.; Alldredge, A.L. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnol. Oceanogr.* **1993**, *38*, 924–934. [CrossRef]
39. Singh, P.; Williams, D.; Velez, F.J.; Nagpal, R. Comparison of the gill microbiome of retail oysters from two geographical locations exhibited distinct microbial signatures: A pilot study for potential future applications for monitoring authenticity of their origins. *Appl. Microbiol.* **2023**, *3*, 1–10. [CrossRef]
40. Offret, C.; Paulino, S.; Gauthier, O.; Château, K.; Bidault, A.; Corporeau, C.; Miner, P.; Petton, B.; Pernet, F.; Fabioux, C.; et al. The marine intertidal zone shapes oyster and clam digestive bacterial microbiota. *FEMS Microbiol. Ecol.* **2020**, *96*, fiaa078. [CrossRef]
41. Vitorino, I.R.; Lage, O.M. The biology of Planctomycetia: An overview of the currently largest class within the phylum Planctomycetes. *Antonie Van Leeuwenhoek* **2022**, *115*, 169–201. [CrossRef]
42. Dugeny, E.; de Lorgeril, J.; Petton, B.; Toulza, E.; Gueguen, Y.; Pernet, F. Seaweeds influence oyster microbiota and disease susceptibility. *J. Anim. Ecol.* **2022**, *91*, 805–818. [CrossRef]
43. Hieu, C.X.; Voigt, B.; Albrecht, D.; Becher, D.; Lombardot, T.; Glöckner, F.O.; Amann, R.; Hecker, M.; Schweder, T. Detailed proteome analysis of growing cells of the planctomycete *Rhodopirellula baltica* SH1^{T}. *Proteomics* **2008**, *8*, 1608–1623. [CrossRef] [PubMed]
44. Lage, O.M.; Bondoso, J.; Viana, F. Isolation and characterisation of Planctomycetes from the sediments of a fish farm wastewater treatment tank. *Arch. Microbiol.* **2012**, *194*, 879–885. [CrossRef] [PubMed]
45. Kallscheuer, N.; Wiegand, S.; Jogler, M.; Boedeker, C.; Peeters, S.H.; Rast, P.; Heuer, A.; Jetten, M.S.M.; Rohde, M.; Jogler, C. *Rhodopirellula heiligendammensis* sp. nov., *Rhodopirellula pilleata* sp. nov., and *Rhodopirellula solitaria* sp. nov. isolated from natural or artificial marine surfaces in Northern Germany and California, USA, and emended description of the genus Rhodopirellula. *Antonie van Leeuwenhoek* **2020**, *113*, 1737–1750. [CrossRef] [PubMed]
46. Kumar, D.; Gaurav, K.; Pk, S.; Uppada, J.; Ch., S.; Ch.V., R. *Gimesia chilikensis* sp. nov., a haloalkali-tolerant planctomycete isolated from Chilika lagoon and emended description of the genus *Gimesia*. *Int. J. Syst. Evol. Microbiol.* **2020**, *70*, 3647–3655. [CrossRef]
47. Wiegand, S.; Jogler, M.; Boedeker, C.; Pinto, D.; Vollmers, J.; Rivas-Marín, E.; Kohn, T.; Peeters, S.H.; Heuer, A.; Rast, P.; et al. Cultivation and functional characterization of 79 planctomycetes uncovers their unique biology. *Nat. Microbiol.* **2020**, *5*, 126–140. [CrossRef] [PubMed]
48. Cayrou, C.; Raoult, D.; Drancourt, M. Broad-spectrum antibiotic resistance of Planctomycetes organisms determined by Etest. *J. Antimicrob. Chemother.* **2010**, *65*, 2119–2122. [CrossRef] [PubMed]
49. Ivanova, A.A.; Miroshnikov, K.K.; Oshkin, I.Y. Exploring antibiotic susceptibility, resistome and mobilome structure of Planctomycetes from *Gemmataceae* family. *Sustainability* **2021**, *13*, 5031. [CrossRef]
50. Jeske, O.; Schüler, M.; Schumann, P.; Schneider, A.; Boedeker, C.; Jogler, M.; Bollschweiler, D.; Rohde, M.; Mayer, C.; Engelhardt, H.; et al. Planctomycetes do possess a peptidoglycan cell wall. *Nat. Commun.* **2015**, *6*, 7116. [CrossRef]
51. Aghnatios, R.; Drancourt, M. *Gemmata* species: Planctomycetes of medical interest. *Future Microbiol.* **2016**, *11*, 659–667. [CrossRef]
52. Michalopoulos, A.S.; Livaditis, I.G.; Gougoutas, V. The revival of fosfomycin. *Int. J. Infect. Dis.* **2011**, *15*, e732–e739. [CrossRef]

53. Blair, J.; Webber, M.A.; Baylay, A.J.; Ogbolu, D.O.; Piddock, L.J. Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **2015**, *13*, 42–51. [CrossRef] [PubMed]
54. Andrade, F.F.; Silva, D.; Rodrigues, A.; Pina-Vaz, C. Colistin update on its mechanism of action and resistance, present and future challenges. *Microorganisms* **2020**, *8*, 1716. [CrossRef] [PubMed]
55. Mohapatra, S.S.; Dwibedy, S.K.; Padhy, I. Polymyxins, the last-resort antibiotics: Mode of action, resistance emergence, and potential solutions. *J. Biosci.* **2021**, *46*, 85. [CrossRef] [PubMed]
56. Faria, M.; Bordin, N.; Kizina, J.; Harder, J.; Devos, D.; Lage, O.M. Planctomycetes attached to algal surfaces: Insight into their genomes. *Genomics* **2018**, *110*, 231–238. [CrossRef] [PubMed]
57. Graça, A.P.; Calisto, R.; Lage, O.M. Planctomycetes as novel source of bioactive molecules. *Front. Microbiol.* **2016**, *7*, 1241. [CrossRef]
58. Gimranov, E.; Santos, J.; Vitorino, I.; Martín, J.; Reyes, F.; Moura, L.; Tavares, F.; Santos, C.; Mariz-Ponte, N.; Lage, O.M. Marine bacterial activity against phytopathogenic Pseudomonas show high efficiency of Planctomycetes extracts. *Eur. J. Plant Pathol.* **2022**, *162*, 843–854. [CrossRef]
59. Vitorino, I.R.; Lobo-da-Cunha, A.; Vasconcelos, V.; Vicente, F.; Lage, O.M. Isolation, diversity and antimicrobial activity of Planctomycetes from the Tejo river estuary (Portugal). *FEMS Microbiol. Ecol.* **2022**, *98*, fiaa078. [CrossRef]
60. Park, J.W.; Park, S.R.; Nepal, K.K.; Han, A.R.; Ban, Y.H.; Yoo, Y.J.; Kim, E.J.; Kim, E.M.; Kim, D.; Sohng, J.K.; et al. Discovery of parallel pathways of kanamycin biosynthesis allows antibiotic manipulation. *Nat. Chem. Biol.* **2011**, *7*, 843–852. [CrossRef]