

Phylogeny and Antagonistic Activities of Culturable Bacteria Associated with the Gut Microbiota of the Sea Urchin (*Paracentrotus lividus*)

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Abstract In this study, we have investigated the phylogeny and the antagonistic interactions of culturable bacteria isolated from the sea urchin *Paracentrotus lividus* collected from Aber and Morgat, both located in Crozon peninsula, France. Bacteria were isolated from the gastrointestinal tracts of ten specimens by using conventional culture-dependent method and then investigated by using phylogenetic analysis based on 16S rRNA gene sequence comparisons. Assays for antagonistic interactions among the bacterial strains were performed; bacteria (including at least one strain representative of each OTU identified) were screened for antimicrobial substance production. So, 367 bacterial strains were isolated on marine-agar. On the basis of morphological characteristics, 180 strains were sequenced and 94 OTUs were classified. The dominant phyla were Proteobacteria, Firmicutes and Actinobacteria, with a high abundance of the strains belonging to the genus *Psychrobacter*. From the antagonistic interactions assays, it could be determined that 22.7% strains were positive for at least one antagonism interaction, 18.3% of them isolated from the sea urchins collected in Morgat. We hypothesize that the bacteria isolated in this study may represent the transitory microbiota of the gastrointestinal tract of *P. lividus*, and

that this microbiota may be related to the diet of this marine invertebrate. Furthermore, our results suggest that chemical antagonism could play a significant role in shaping the bacterial communities within gastrointestinal tract of the sea urchins. In addition, most isolated bacteria may have promising biotechnology applications.

Keywords Antimicrobials · Biotechnology · Culture-dependent approach · Gut microbiome · *Paracentrotus lividus* · *Psychrobacter*

Introduction

The intestinal microbiota maintains a functional relationship with its host animal, and the metabolic capacity of this bacterial community is more versatile than the host [43]. In case of heterotrophic animals, food digestion is the key process for providing nutrients necessary for development, growth, and reproduction. In order to maximize the digestive efficiency, animals rely on symbiotic interactions with bacteria residing in their intestine [45]. This gut microbiota represents an internal ecosystem, composed of a wide range of bacterial species that interact in complex and dynamic networks. This ecosystem includes persistent as well as transient members that were formerly introduced from the local environment [24]. The composition of the bacterial communities in the gut and its complexity are, therefore, strongly influenced by environmental factors (e.g. diet) [13, 44, 47] and the genetic makeup of the host [5, 23].

The sea urchin is one of many invertebrates with ecological and commercial importance in the industry of sea food [1, 30]. Furthermore, it is a model organism for studies in developmental biology [28], and for the investigation of the effects in nutrient cycling on the community structure in

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the ecosystem they inhabit [40–43]. However, so far, little attention has been paid to the bacterial ecology residing in the intestine of the sea urchin and their potential effects on its health and other aspects of the associated community [2–4, 22].

The habitat of the sea urchin *Paracentrotus lividus* is primarily hard substrata (e.g. pebbles, boulders, rocks) covered with encrusting algae as well as seagrass beds, mostly of *Cymodocea* and *Posidonia*. *P. lividus* is a scraper that feeds on attached material on hard substrata and on seagrass [6, 38]. In the field, when the resource is non-limited, analysis of gut contents indicate that *P. lividus* is basically herbivorous, with food preferences including mainly multicellular photosynthetic organisms and some fungi [7].

In this study, we describe the culturable aerobic, facultative anaerobic, and aerotolerant bacteria present in the gastrointestinal tract (pellets of the stomach and intestine) from the sea urchin *P. lividus* collected from Crozon peninsula (Brittany, France) at two different sites. Taxonomic identification was achieved through 16S rRNA gene sequencing followed by comparative sequence analyses. Bacteria were grouped according to their relative phylogenetic position. Furthermore, the composition of the obtained fractions of the *P. lividus* gastrointestinal tract (stomach and intestine) was compared between the sea urchins from the different collection sites. We also tested the hypothesis that chemical antagonism is common among bacteria and could play a role in shaping the bacterial communities within gastrointestinal tract of the sea urchins. In this regard, assays for antagonistic interactions among the bacterial strains were performed, in which bacteria were screened for antimicrobial substance production.

Materials and Methods

Collection and Sample Processing

Ten adults specimens of *P. lividus* (Lamarck, 1816) were collected by hand in February 2013, five in Aber (48.23522, -4.487218) and five in Morgat (48.235665, -4.45579), both located in Crozon peninsula (southern Brittany, France). In both sites, the water temperature was 6–7 °C and air temperature 2–4 °C. In Aber there was rare presence of the dominant algal species *Ulva lactuca*, while in Morgat were observed pinkish encrusting algae, *Lithophyllum incrustans* and *Lithotamnium* spp.. Moreover, there were the presence of laminaria and unidentified Rhodophyceae, *Fucus serratus*, brown algae (*Cytoseira* spp., *Bifurcaria bifurcata*) and a small unidentified bushy alga.

These sites differ according to their hydrodynamic conditions, because Aber is a wave-exposed intertidal site and Morgat is a protected subtidal site by its general situation in

a bay, and by a high rock wall and a dyke. The intertidal site, Aber, was characterized by coarser sand; the urchins were more scattered and more deeply buried than in the subtidal site, Morgat. Intertidal urchins were bigger than subtidal urchins but showed similar resource allocation. More information about Crozon peninsula can be found in a recent publication [34].

The specimens were immediately dissected, their gastrointestinal tracts with its content was split in two sections: upper (stomach) and lower (intestine). Samples of each section of each specimen, were placed separately in Falcon tubes containing 5 ml of marine broth (Marine 2216, Difco) added of 10 µg/ml cycloheximide, stored on ice, and taken to the laboratory within 24 h.

In the laboratory, samples of gastrointestinal tracts (stomach or intestine) from each individual specimen were ground with a mortar and pestle. The material was vortexed for 1 min and tubes were allowed to stand for 1 min. From the resulting supernatant, serial 10-fold dilutions were made and plated (in duplicate) on marine-agar added of 10 µg/ml cycloheximide.

Isolation and Cultivation of Bacteria

Bacterial cultures were incubated for up to 7 days at 25 ± 2 °C and were examined daily for growth and colony morphology. Bacteria were purified from the primary culture and kept in slant cultures at -20 °C. The strains were named with the P letter; plus 1–5 for bacteria isolated from the sea urchin samples collected from Aber or 6–10 from Morgat; plus E for bacteria isolated from the stomach or I for bacteria isolated from the intestine of the sea urchins; finally followed by the order of isolation of colony-forming units (CFUs). For example, strain P6E-5 was the 5th CFU isolated from the stomach of the sea urchin collected in Morgat.

16S rRNA Sequence Analysis of Bacterial Isolates

Bacterial DNA was recovered by a thermal lysis protocol consisting in resuspending cellular material from each colony in 25 µl sterile PCR grade water and boiling the suspension at 100 °C for 15 min. PCR amplification was performed by adding 3 µl of DNA solution to 47 µl containing 1 × buffer GO TAQ Green master mix (Promega), 0.4 mg/ml of BSA (Sigma), 0.05% of Igepal (Sigma), and 20 pmol of each universal primer, 27F (5'-GAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') [46]. Cycle conditions consisted in an initial denaturation step at 94 °C for 6 min, followed by 30 cycles at 94 °C for 30 s, 55 °C for 1 min 30 s and 72 °C for 2 min 30 s, and a final elongation step at 72 °C for 5 min.

PCR products were analyzed by electrophoresis on a 0.8% agarose gel, purified using the QIAquick PCR Purification

Kit (Qiagen), and sequenced using the universal primer 338F (5'-ACTCCTACGGGAGGCAGC-3') at Beckman Coulter Genomics (Takeley, UK). 16S rRNA gene sequences obtained for the isolates were aligned and classified using the online portal of the SILVA SINA alignment service of the ARB-Silva database (<http://www.arb-silva.de/aligner/>). Sequence data have been deposited in the GenBank database under the accession numbers KX688049, KX688053-KX688080, KX898814-KX898960, KY922742-KY922772, and KY953150-KY953151 (Table_S1).

Raw sequences were subjected to quality filtering and removal of homopolymers and chimeras with Amplicon-Noise [35]. The filtered sequences were analyzed using the QIIME (Quantitative Insights Into Microbial Ecology) [11]. Briefly, 16S rRNA gene sequences were clustered into OTUs (Operational Taxonomic Unit) at 97% similarity. OTU picking was done by an open reference method and chimera check was done using chimera-slayer. So, from OTU cluster, a single representative sequence was selected and used for taxonomical identification. Taxonomic assignments were carried out using Greengenes 13.8 database and UCLUST algorithm. The resulting OTU table including all OTUs was checked and used for downstream analyses. These comprised the phylogenetic composition of the culturable community, determination of specific and common symbionts across gastrointestinal tract (stomach and/or intestine) and/or collected site (Aber and/or Morgat) by OTU networking and Venn diagrams.

Antagonistic Interactions Among Bacterial Isolates

Assays for antagonistic interactions were performed in triplicate among the bacterial strains taxonomically identified, and the bacteria were screened for antimicrobial substance production using a method previously described [25]. Each strain from the stomach or intestine and from Aber or Morgat sites was tested against the other strains for cross-inhibition.

Hereafter, bacterial strains tested for antimicrobial substance production will be termed “producer” strains, whereas those used as targets will be called “indicator” or “inhibited” strains. Briefly, 10^7 cells of each producer strain were spotted onto marine-agar and incubated at 25 °C until colony diameter reached 4–7 mm. In parallel, each indicator strain was grown in marine broth at 25 °C. Then 10^5 cells of the latter mixed with 3 ml of marine soft agar were poured over the plates. Plates were incubated at 25 °C for 24 h and the diameter of the inhibition zone around the spotted strain was measured. An indicator strain was considered sensitive to the activity of the producer strain when it exhibited a clear inhibition zone (and was then considered “inhibited”). The antagonistic relationships were illustrated in network graphs using the program Cytoscape 3.1.0 (<http://www.cytoscape.org>).

Results

Isolation and Phylogenetic Affiliation of Isolates

Marine-agar was used for the isolation of bacteria from the gastrointestinal tract of ten sea urchin samples, five from Aber and five from Morgat sites. A selection of 367 bacteria was made, based on size and colony appearance—smooth or rough—and presence of pigments. Mucoid and pigmented colonies prevailed among the strains isolated. Among the chosen isolates from the sea urchins, 208 strains (P1–P5) from the Aber site, being 85 from the stomach (E) and 123 from the intestine (I) sections; and 159 strains (P6–P10) from the Morgat site, being 85 from E and 74 from I sections.

On the basis of morphological characteristics, 180 strains were selected and sequenced based on 16S rRNA gene sequences (targeting the V3–V5 region). Just one sequence (P7E-10 strain) could not be classified by ARB-Silva database (Table_S1). Among the sequences obtained, 167 were approved by QIIME quality control, being shared into four samples, 36 from E and 52 from I sections of the Aber site, and 38 from E and 41 from I sections of the Morgat site. Thus, these sequences were selected to perform a phylogenetic analysis based on 16S rRNA gene sequences. Ninety-four OTUs were clustered at a 97% sequence similarity from the trimmed sequences of the respective samples using UCLUST (Table_S1). Twenty bacterial OTUs remained unassigned at the phylum level and 74 OTUs were assigned to three bacterial phyla: Proteobacteria (64), Firmicutes (9), and Actinobacteria (1). The most abundant bacterial phylum, class, order, family, and genus overall were Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae and *Psychrobacter*, respectively. The relative abundances of the taxa identified to the most resolvable taxa (class, order, family, and genus) across all four samples: bacteria isolated from the sea urchins collected in Aber, stomach (E) and intestine (I); and in Morgat, stomach (E) and intestine (I), are elaborated in Fig. 1.

Among 94 OTUs, 36 and 32 were exclusive in the samples from Aber and Morgat, respectively. In relation to the samples collected in Aber, 19 OTUs were specific from stomach and 17 from intestine of the sea urchins. Considering the specific OTUs among the samples collected in Morgat, 17 were found from the stomach and 15 from the intestine of the sea urchins. More details are presented in Table_S2. In general, OTU networking analyses showed that most bacterial OTUs found in the gastrointestinal tract were specific with only a few OTUs shared to each sample (Fig. 2). The determination of specificities and commonalities of these OTUs were also showed by Venn diagrams (Fig. 3a–f). The Aber samples showed only four common OTUs (Fig. 3a), all belonging to the class Gammaproteobacteria: one Enterobacteriaceae,

Fig. 1 Phylogenetic composition of the bacteria culturable community from the sea urchin *P. lividus* collected from Aber and Morgat, France. Stacked column bar graph depicting the relative abundances and distribution of the most highly abundant resolved taxa across the four samples of this study. The gastrointestinal tract consisted mainly of Phylum Proteobacteria and Class Gammaproteobacteria. Relative abundances were performed through QIIME and graphs were generated using Microsoft Excel software. Bacteria isolated from the sea urchins collected in: Aber, stomach (E) and intestine (I); Morgat, stomach (E) and intestine (I). (Color figure online)

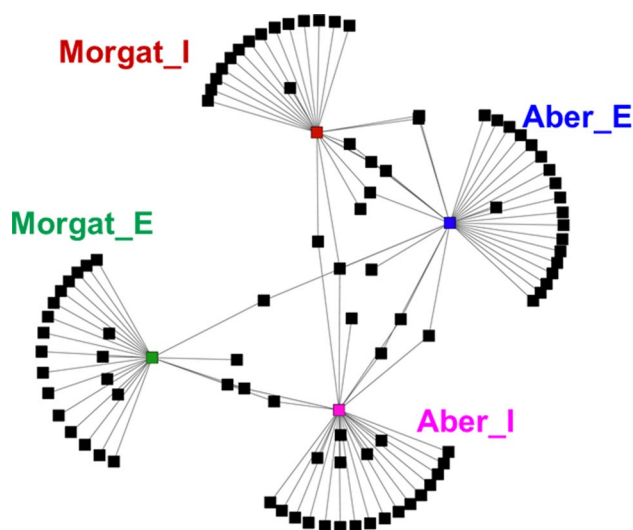
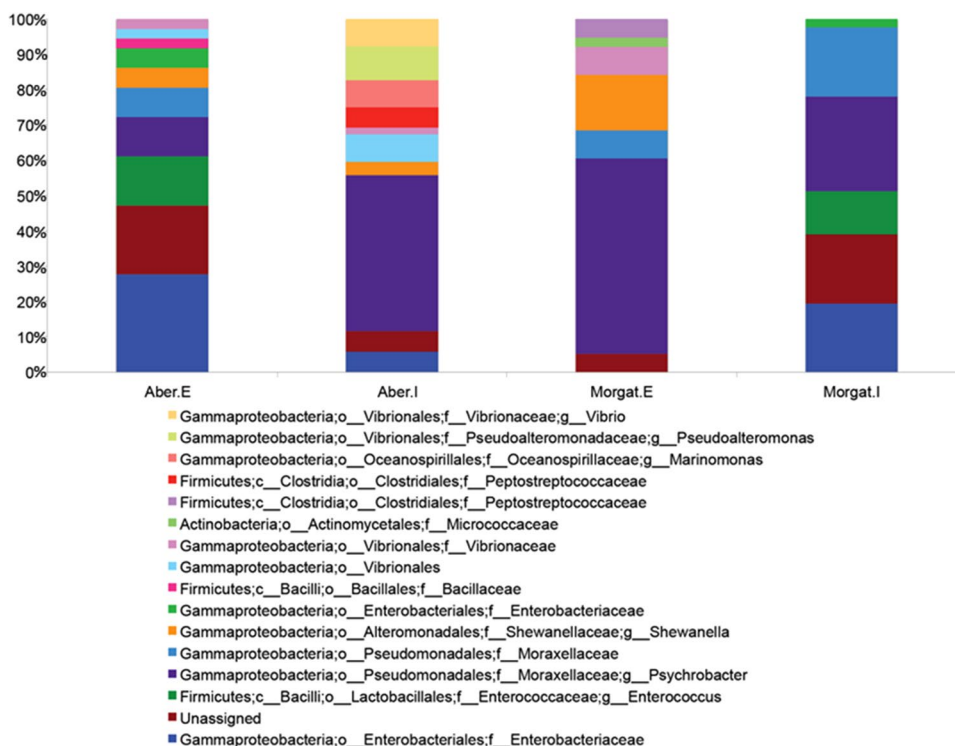


Fig. 2 Bacterial OTU networking showing the specific and common symbionts across gastrointestinal tract of the sea urchin *P. lividus*. All OTUs detected across four samples are included in the analysis. In the network diagram, Aber samples and their corresponding OTUs from the intestine are shown in pink and from the stomach are in blue; Morgat samples and their corresponding OTUs isolated from the intestine are presented in red and from the stomach are in green. (Color figure online)

one *Vibrionales* and two *Psychrobacter*. The bacterial family and class specific to the stomach were: *Enterococcaceae* (Firmicutes), *Moraxellaceae* (Gammaproteobacteria), and *Enterobacteriaceae* (Gammaproteobacteria). On the other

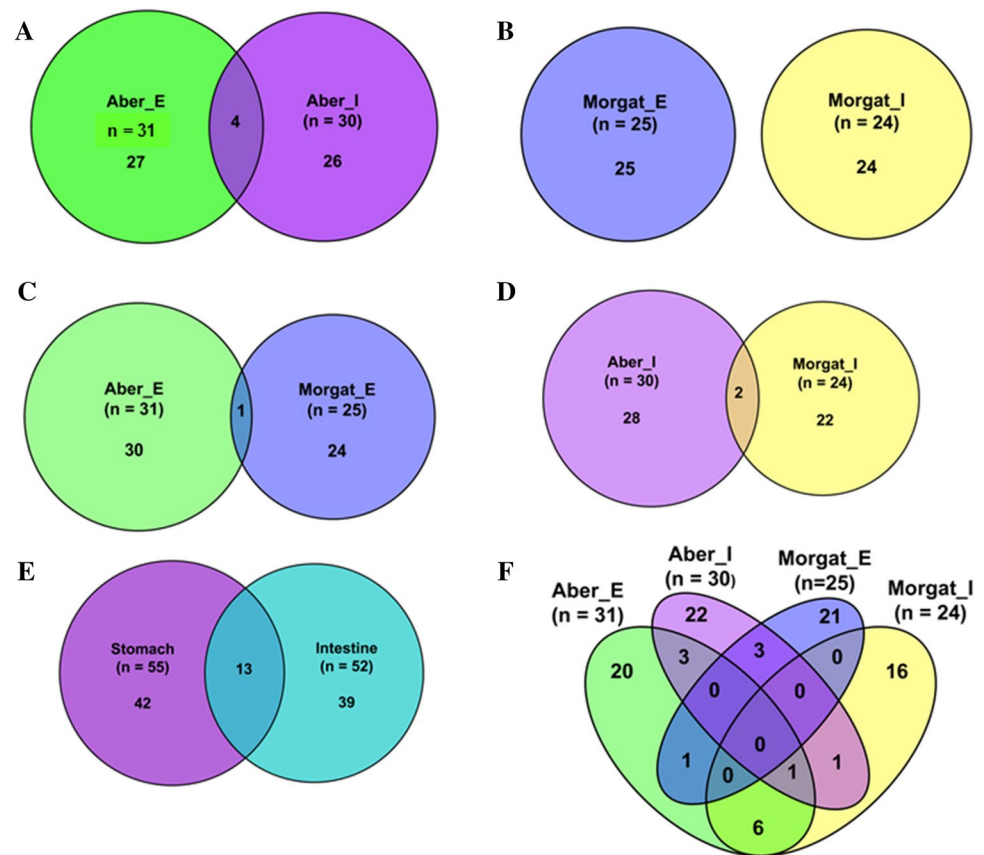
hand, in the intestine sections from Aber, Gammaproteobacteria was dominant, the bacteria mostly belonging to the genera *Psychrobacter*, *Pseudoalteromonas*, *Marinomonas* and *Vibrio*, and just one belonging to the family *Peptostreptococcaceae* (Firmicutes).

Interestingly, none of the OTUs is shared within Morgat samples, 25 OTUs were specific from the stomach and 24 OTUs were specific to the intestine sections (Fig. 3b). In both, the class Gammaproteobacteria was dominant, with the bacteria from the stomach sections belonging to the genera *Psychrobacter* and *Shewanella*, and to the families *Moraxellaceae* and *Vibrionaceae*, and class Firmicute (family *Peptostreptococcaceae*). In the intestine sections were found OTUs belonging to the family *Moraxellaceae*, genus *Psychrobacter*, genus *Enterococcus* (Firmicutes).

When the OTUs were analyzed from bacteria of the stomach of the sea urchins from both collected sites (Fig. 3c), one single OTU was shared, identified as the family *Vibrionaceae* (Gammaproteobacteria). From Aber, OTUs belonging to *Enterococcus* (Firmicutes) and to the families *Moraxellaceae* and *Enterobacteriaceae* (Gammaproteobacteria) were observed. From the Morgat samples were identified the genera *Psychrobacter* and *Shewanella*, the families *Vibrionaceae* and *Moraxellaceae*, all belonging to the class Gammaproteobacteria, and one to the family *Peptostreptococcaceae* (Firmicutes).

Considering the OTUs identified from the intestine sections (Fig. 3d), only two OTUs, both genus *Psychrobacter*, were shared between the sea urchins collected. From Aber,

Fig. 3 The numbers of common and unique bacterial OTUs presented in the gastrointestinal tract (stomach and intestine) from the sea urchin *P. lividus* collected from Crozon peninsula (Brittany, France) at two different sites. Venn diagrams were constructed considering all detected bacterial OTUs. Diagrams enumerate OTUs common and exclusive to stomach and intestine of the sea urchins collected in Aber (a) and Morgat (b). The bacterial OTUs isolated common and exclusive of the stomach (c) and intestine (d) of the sea urchins from both collected sites. Diagram (e) displays the extent of OTU shared and exclusive between stomach and intestine from all sea urchins. Diagram (f) shows the numbers of common and unique OTUs presented among the gastrointestinal tract samples from the sea urchins. (Color figure online)



28 specific OTUs were found, belonging to the genera *Psychrobacter*, *Pseudoalteromonas*, *Marinomonas* and *Vibrio*, and belonging to the families *Enterobacteriaceae* and *Peptostreptococcaceae*. From the Morgat samples, 22 specific OTUs were identified as belonging to the genus *Enterococcus* (Firmicutes), to the families *Moraxellaceae* and *Enterobacteriaceae* and finally one sequence unassigned.

When the distribution of OTUs was analyzed between the sections of the gastrointestinal tract, 13 were shared, 42 OTUs were specific to the stomach and 39 OTUs were found only in the intestine (Fig. 3e). The Fig. 3f shows OTUs shared by both gastrointestinal tract sections and both collect sites of the sea urchins.

Antagonistic Interactions Among Bacterial Isolates

The 180 bacterial strains (Table_S1) were selected and screened for antimicrobial substance production by cross-inhibition tests among bacteria isolated from the sea urchins collected from the same location, Aber (P1–P5 strains) or Morgat (P6–P10 strains). The results observed from the bacterial strains of Aber and Morgat showed notable differences. In general, bacterial strains isolated from P6–P10 (Morgat) were more involved in antagonistic interactions (as

producer or inhibited), mainly those strains isolated from sea urchin stomach.

Among isolates (P1–P5) from Aber, eight of the strains isolated of intestine were involved in at least one antagonistic interaction: four strains as antimicrobial producers and four strains as inhibited by the activity of at least one other strain (Fig. 4a, Table_S3). Two strains, P5I-23 (OTU_32) and -29 (OTU_25), both affiliated to the genus *Psychrobacter*, were strong producers against strains isolated from other sea urchin samples, *Peptoclostridium* P2I-2 (OUT_Unassigned) and P3I-5 (OTU_11) and *Vibrionaceae* P3I-12 (OTU_8). No strains from sea urchin stomach was observed as producer or inhibited.

Among strains isolated (P6–P10) from Morgat, 35 were involved in at least one antagonistic interaction, being 13 strains as antimicrobial producers and 30 strains as inhibited by the activity of at least one other strain (Fig. 4b, Table_S4). The genera *Exiguobacterium*, *Kocuria*, *Psychrobacter*, and *Shewanella* were involved in a large number of antagonistic interactions, with emphasis on the strains *Shewanella* P9E-9 and P10E-5. Such tests also revealed that antimicrobial production and sensitivity were not mutually exclusive: some active strains had their growth inhibited by the antimicrobial activity of at least one other strain. Besides that, self-inhibition from five strains was also observed. Curiously, all

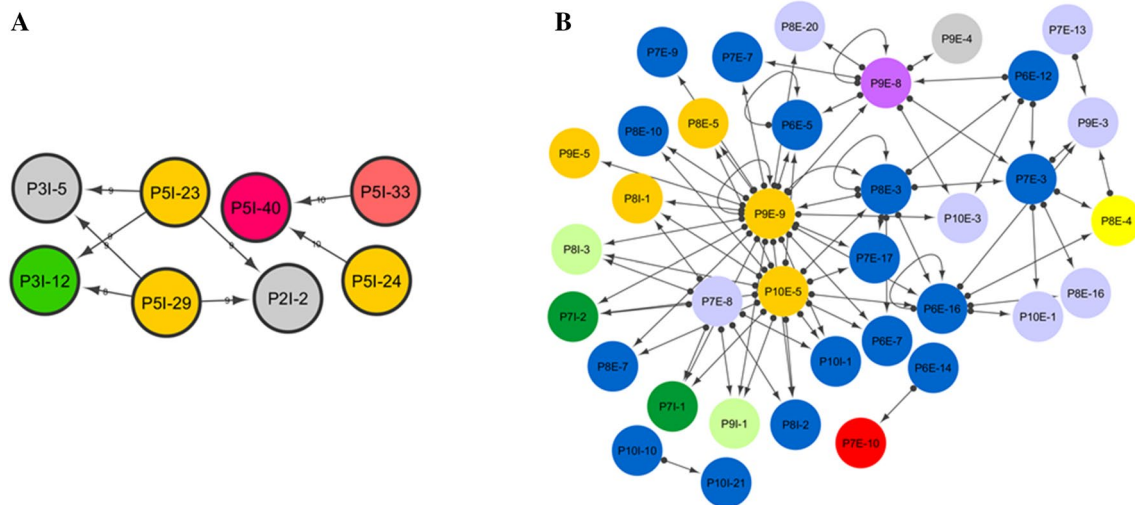


Fig. 4 Network analysis of antagonistic interactions among bacteria isolated from the gastrointestinal tract of the sea urchins. **a** 91×91 array of tests among bacteria isolated from the stomach and intestine of the sea urchins collected in Aber. **b** 89×89 array of tests among bacteria isolated from the stomach and intestine of the sea urchins

collected in Morgat. Each node represents a bacterial strain. Each line (connection) represents an antagonistic interaction from an active strain (dot) towards a sensitive strain (arrow). Strains isolated from the same bacterial genus have the same fill color. (Color figure online)

strains isolated from the intestine sections were only inhibited, while eight strains isolated from the stomach sections were classified as active and inhibited. These were affiliated to the genera *Psychrobacter* (five strains), *Exiguobacterium*, *Kocuria*, and *Shewanella* (one strain of each).

Discussion

In studies of bacterial cultures, it is essential to know which species are present in the original samples. Therefore, the bacterial community profiles of the sea urchins were analyzed with 367 isolates from the gastrointestinal tracts of ten specimens of *P. lividus*. Based on morphological characteristics, the strains were selected, sequenced and classified into 94 OTUs. The networking analyses showed that most bacterial OTUs found in the gastrointestinal tract were specific for each section (stomach and intestine). The dominant phyla were Proteobacteria, but Firmicutes and Actinobacteria were also identified. In sea urchins, large numbers of bacteria in the center of digestive balls have been observed. This microflora would be transitory, ingested with food and therefore not symbiotic [14]. However, there are still very few studies about their gut microbiota.

The presence of bacteria in the gastrointestinal tract of marine invertebrates has advantages for both, bacteria and host. The microorganisms may provide alternative sources of carbon and energy to the host cells and the hydrolysis of complex organic compounds and production of microbial biomass could provide protein to the host cells [27]. In

return, the host supplies the microorganisms with a steady environment free of predators [29].

Paracentrotus lividus (Lamarck 1816) is common in the temperate areas, with winter water temperatures of around $11\text{--}12\text{ }^{\circ}\text{C}$ and summer temperatures ranging from $18\text{--}25\text{ }^{\circ}\text{C}$ [7]. This species has largely been used as a model animal to study the impact of toxicants or natural toxins [33]. Its gonads have been appreciated as seafood and it has been intensely harvested in France, Italy, Spain and parts of Croatia [7]. In addition to the transitory microbiota received by food, the sea urchins are constantly exposed to high concentrations of microorganisms of which some are pathogenic. For example, a disease of *P. lividus* reported in the late 1970s and early 1980s in the Atlantic (Brittany) known as the “bald sea urchin disease”, is due to pathogens which are bacteria of the genera *Aeromonas* and *Vibrio* [7]. However, this microbiota may therefore play an important role in the biocontrol of the host, protecting it from disease [32].

Based on the inferred phylogeny of the retrieved sequences from bacteria isolated, the sea urchin’s gastrointestinal tract is mainly characterized by bacteria belonging to Gammaproteobacteria, most probably originating from the surrounding environment. Gammaproteobacteria were also the most class bacterial isolated by Meziti and colleagues [29] and Nelson and colleagues [31]. Furthermore, no specific relationship was observed between the bacterial taxonomy and the gastrointestinal tract section analyzed. It suggests that at least most of the isolated bacteria make up a transitory microbiota of these invertebrates. The stomach and intestine revealed isolated strains that belonged to

Moraxellaceae, with predominance to the genera *Psychrobacter* sp. Over the past years, this genus has been better characterized after massive isolation of bacteria from different environmental sources and consecutive 16S rRNA gene sequencing. The results obtained indicate that the genus *Psychrobacter* is evolutionary successful and widely distributed. Therefore, the investigation of its biology may elucidate important aspects of adaptation and survival in different settings [9]. Since many *Psychrobacter* species produce lipases, they can catabolize some substrates ubiquitous in nature but not normally used by Gram-negative bacteria. In addition, many species *Psychrobacter* can thrive on uric acid as sole carbon and nitrogen source [8].

Bacteria identified as belonging to *Enterobacteriaceae*, *Micrococcaceae* and *Vibrionaceae*, and to the genera *Shewanella*, *Enterococcus*, *Exiguobacterium*, *Micrococcus* and *Peptoclostridium* were also isolated from the stomach of the sea urchins. Since the majority of their closest relatives are aerobic species, the anaerobic species which were isolated could be putative symbionts of the *P. lividus* stomach, in which anaerobic conditions also prevail. A similar profile was found in the intestine of the sea urchins, where also bacteria from the Oceanospirillaceae family and the genera *Marinomonas* and *Pseudoalteromonas* sp. were present. However, strains of *Exiguobacterium* and *Micrococcus* could not be observed in intestinal sections. The bacteria detected were either ingested during feeding or are either transient or resident part of sea urchin's gut microbiome. Like Firmicutes and Actinobacteria, the major part of these bacteria can be commonly isolated from the environment or from other marine animals [10].

The present study analyzed the antagonistic interactions among bacteria isolated from the gastrointestinal tract (pellets of the stomach and intestine) from the sea urchin *P. lividus*. The results observed from the bacterial strains of Aber (intertidal site) and Morgat (subtidal site) showed notable differences and these may be related to an acclimation ability of *P. lividus* [12]. The differences among the isolates may be due to the type of feed (algae species and its microbiota) and to the environmental conditions of each site. In Aber there was rare presence of the dominant algal species *Ulva lactuca*, while in Morgat there was some diversity of algal species (at least seven) in the collect period. Based on the literature, the density of bacteria on some healthy algae varies from 10^4 to 10^8 bacteria/g (wet mass). This amount is equivalent to the average density of bacteria found in the digestive tract of sea urchins [32]. A separation between intertidal and subtidal benthic communities is observed, although there are linkages between these two habitats. The relationships between inter- and subtidal benthic communities mainly depend on migrations in relation to species-specific life cycles (reproduction, feeding), behaviors (predator avoidance) or interactions (competition) [34].

Thus, bacteria belonging to the main isolated genera were capable of presenting an antimicrobial activity. Some of these genera are known to possess inhibitory activity. *Bacillus*, *Escherichia* and *Kocuria* are well-known bioactive compound producers [39] as well as *Shewanella* [16, 21, 26, 37]. Antibacterial activity produced by *Psychrobacter* also has been previously found [15, 19, 21]. Interestingly, in the samples from Aber, strains belonging to the family *Vibrionaceae* (associated to the "bald sea urchin disease", see above) were inhibited by strains of the genus *Psychrobacter*. Here, in addition to these genera, *Exiguobacterium* strains were also very active against the other marine strains.

Our data demonstrated that the antagonistic interaction is present among the isolates from all sea urchin samples; however, this activity was more intense among isolates from Morgat, with emphasis on the strains *Shewanella* P9E-9 and P10E-5. Bacteria isolated from the stomach of the sea urchins were more active (producer) and those from the intestine sections were more inhibited. These antagonistic interactions were independent of the bacterial taxonomic classification. Bacteria from a wide range of marine environments, including sediments, seawater, biofilms, and tissues/surfaces of invertebrates and algae have been shown to possess antagonistic activities [20, 21]. In most cases, these bacteria are members of complex communities in which competition for limited space and resources can be intense [18]. Antagonistic interactions may play an important role in structuring these communities, where the evolutionary advantages afforded by an effective chemical defence may be crucial for survival.

In this study, the genus *Psychrobacter* was identified as the most isolated from gastrointestinal tract of ten specimens of *P. lividus* collected in Aber and Morgat, Crozon peninsula (southern Brittany, France). At the same time, it was possible to observe a biotechnological potential of the bacteria isolated. The genera *Exiguobacterium*, *Kocuria*, *Psychrobacter* and *Shewanella* were strong producers of antimicrobial substances against other marine bacteria. These are aquatic microorganisms widely distributed in marine and fresh water environments [17]. Some have adapted to life in extreme and varied environments and they have shown great potential for remediation of various environmental pollutants such as radionuclides, toxic elemental waste, halogenated organic compounds, and cyclic nitramines. They have also shown potential for use in biofuel cell applications [17], production of enzymes which have broad applicability in industry [36] and production of antimicrobial substances with promising biotechnology applications [39].

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Compliance with Ethical Standards

Conflict of interest No conflict of interest is declared.

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