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Review

Bacteria Associated with Marine Benthic Invertebrates from Polar Environments: Unexplored Frontiers for Biodiscovery?

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Abstract: The ecological function of bacteria-invertebrate interactions in Polar areas remains poorly understood, despite increasing evidence that microbial metabolites may play pivotal roles in host-associated chemical defense and in shaping the symbiotic community structure. The metabolic and physiological changes that these organisms undergo in response to adapting to extreme conditions result in the production of structurally and functionally novel biologically active molecules. Deepening our knowledge on the interactions between bacteria and their invertebrate host would be highly helpful in providing the rationale for why (e.g., competition or cooperative purpose) and which (whether secondary metabolites, enzymes, or proteins) bioactive compounds are produced. To date, cold-adapted bacteria associated with marine invertebrates from the Arctic and Antarctica have not been given the attention they deserve and the versatility of their natural products remains virtually unexplored, even if they could represent a new attractive frontier in the search for novel natural compounds. This review is aimed at showcasing the diversity of cold-adapted bacteria associated with benthic invertebrates from Polar marine areas, highlighting the yet unexplored treasure they represent for biodiscovery.

Keywords: diversity; biotechnological potential; cold-adapted bacteria; Arctic; Antarctica

1. Introduction

Studies of bacterial communities living in association with marine benthic invertebrates have largely focused on sponges, corals, bryozoans, and crustaceans from temperate and tropical climates [1–5]. The associated communities generally differ from those in the water column, thus displaying host-specificity, and they often vary between habitats and seasons [4,6,7]. Several factors, both environmental and biological (including the age and health state of the host, the production of organic metabolites and extracellular polymers), may influence the colonization of living surfaces, both inner and outer portions. Bacterial epi- and endobiotic associations play an important role in the development and evolution of an organism. These associations can be positive or negative, depending on the benefits or damage incurred by one of the organisms during association (reviewed in [8,9]). This is particularly true in extreme and isolated environments, such as Antarctica and, at a lesser extent, the Arctic, where the host and the symbionts often evolve together, establishing peculiar and strict interactions.

One would imagine that at low temperatures, life is sporadic or somewhat static, remaining in a suspended state. However, this is not the case in polar environments. Despite the incredibly harsh

conditions in extremely cold habitats that preclude life in most of its forms, microorganisms become dominant in terms of biodiversity and biomass by adopting peculiar survival strategies [10]. Not only microbes, but also invertebrates, can reproduce and complete their life cycle at low temperatures [11]. As an example, even if benthic organisms living in some Antarctic coastal regions experience periodic disruption by iceberg scour, the benthic community appears to be relatively stable from an environmental and biological point of view, being structured in large part by predatory and competitive interactions [12–14].

Bioprospecting is an emerging and fascinating branch of marine research, which aims at exploring biological matrices as sources of new natural compounds with biological activity and possible commercial exploitation [15–17]. The search for new molecules with biocompatibility and safety features has been strongly encouraged to avoid the use of synthetic compounds that could be very deleterious for the ecosystems [16]. In comparison to land resources, marine environments remain largely underexplored for bioprospecting aims, despite their undisputed ecological value as harboring system of highly diversified communities of living being. Moreover, the possibility of finding new biologically active molecules with thermal stability and specificity is higher in organisms from (marine) extreme environments due to the development of unusual metabolic and physiological adaptations [18]. The exploration of such biologically diverse resources can lead to the identification of novel natural products or chemical scaffold(s) with biotechnologically relevant bioactivity [15–17].

This review aims at highlighting current knowledge on the fraction of cold-adapted bacteria that live in association with Polar marine benthic invertebrates as a yet underexplored frontier for biodiscovery.

2. Polar Marine Environments

About 85% of the biosphere is permanently exposed to temperatures below 5 °C throughout the year [19]. Cold habitats include bathy- and abysso-pelagic zones, permafrost, sea-ice, glaciers and, to a lesser extent, cold soils, groundwater, deserts, lakes, and shallow subterranean regions. Polar regions (Arctic and Antarctica) represent 14% of the total biosphere (Figure 1). Although cold areas were previously considered to be uniform environments, recent advancements in ground equipment and satellite-guided systems have recently highlighted that they include a variety of geological variations (e.g., different sediment textures, a mixture of ice and snow with different degrees of salinity, nutrients, and thermal values) [20]. Antarctica and the Arctic greatly differ from a geographic point of view. Antarctica is an ice-covered continent surrounded by the Southern Ocean and very distant from populated areas (the tip of the Antarctic Peninsula is >1000 km from the Southern tip of South America), whereas the Arctic is an ice-covered ocean, surrounded by tree-less permafrost (often covered with snow and ice), whose boundaries are generally considered to be north of the Arctic Circle (66°33′ N) [21]. Desiccation, osmotic stress, ice-covering, high salinity, low biochemical activity (due to low temperatures and the Q10 effect, i.e., a measure of the rate of change of a biological system as a consequence of increasing/decreasing the temperature by 10 °C), limited nutrient availability, adverse solar radiation (e.g., high levels of UVB radiation under the Antarctic ozone hole), and a highly variable photoperiod (from no light at all to continuous light during a 24-h period) are among the main environmental stresses characterizing, alone or in combination, low-temperature environments [10]. In particular, Polar marine environments are mainly characterized by temperatures below 0 °C. The temperature of Antarctic surface seawater ranges annually from −1.86 °C to +0.3 °C, with Arctic surface seawater temperature that ranges from <0 °C to +10 °C [22]. It is therefore unsurprising that the macro and microorganisms inhabiting these harsh environments possess many adaptive mechanisms to assist in their survival and proliferation.

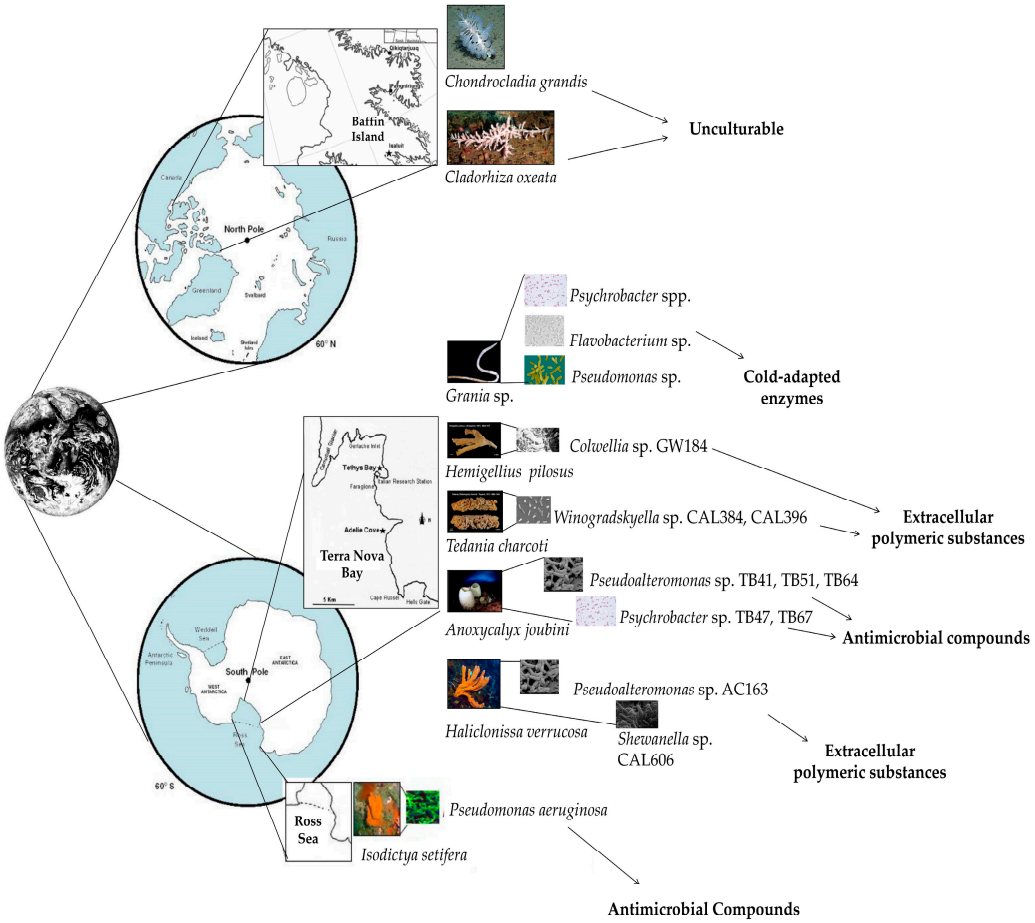

Figure 1. Polar benthic invertebrates as a source of bacterial diversity and biodiscovery. A number of studies have demonstrated that cold-adapted bacteria from Antarctic invertebrates produce bioactive compounds (e.g., enzymes, extracellular polymeric substances, and molecules with antimicrobial activity). To date, cold-adapted bacteria from Arctic holobionts have only been explored for biodiversity.

3. Cold-Adapted Bacteria

3.1. Definition

According to the definition given by Morita [23], cold-adapted bacteria can be classically distinguished as psychrophiles (cold-loving) and psychrotrophs (cold-tolerant or psychrotolerant). Psychrophiles have an optimal growth temperature of 15 °C or below, with no growth occurring above 20 °C, while psychrotrophs grow over a wide temperature range and exhibit the fastest growth rates above 20 °C. As a consequence, true psychrophilic microorganisms, being more heat-sensitive, frequently occur in permanently cold habitats. Conversely, a widespread distribution characterizes psychrotolerant microbes that can be overrepresented in environments undergoing diurnal or seasonal thermal fluctuations [10,19]. From an ecological point of view, the terms eurypsychrophile (i.e., facultative psychrophiles or psychrotrophs) and stenopsychrophile (i.e., true or obligate psychrophiles) can be used to indicate microorganisms with a wide and narrow tolerance to temperature, respectively [24,25]. The term "cold-adapted bacteria" will be used throughout this chapter to collectively indicate bacteria indigenous to cold environments.

3.2. Survival Strategies

Cold-adapted bacteria are subjected to restrictive environmental conditions on a long timescale. To surmount the negative effects of low temperature (alone or in concomitance with further

environmental constraints), they have successfully evolved a variety of structural and physiological modifications [10]. Most phenotypic changes in cold-adapted bacteria may be permanent and genetically regulated, and not merely driven by a short-term acclimatization [26]. Recently, the application of novel "multi-omic" approaches (including metagenomic, metatranscriptomic, and metaproteomic analyses) evidenced the presence of a highly diverse set of metabolic features to thrive in the cold, by the acquisition of information related to the functionality of genotypic traits. As a general conclusion, cold-adapted bacteria downregulate primary metabolism under cold conditions and activate non-classical metabolisms for a cold lifestyle [27,28]. Some examples are discussed in the following text.

Cell envelopes constitute a dynamic interface between the external and internal environments of the cell. Hence, external factors that first affect the cell envelope physical characteristics would in turn affect some vital cell functions, such as those played by membrane proteins which are involved in respiration and transport processes [26]. Thus, the composition and structure of the cell membrane are recognized as important factors to withstand cold shock [28]. At low temperature, the maintenance of membrane fluidity is gained by the incorporation of a higher amount of polyunsaturated (PUFAs) and methyl branched fatty acids, and carotenoids in cell membranes [24,29,30]. More importantly, cold-adapted bacteria are able to modulate the protein content of the membrane, the fatty acid chain length, the proportion of *cis* to *trans* fatty acids, and the type of carotenoids synthesized. It was demonstrated that the synthesis of polar carotenoids, as membrane fluidity modulators, by the Antarctic bacteria *Sphingobacterium antarcticus* and *Micrococcus roseus*, increases under cold conditions of growth with a concomitant decrease in the synthesis of non-polar carotenoids, which instead increase the fluidity of the membrane [29,30].

Although the structural modifications of the cell membrane at low temperatures have been intensively investigated, changes in other bacterial envelope components have been less considered [28]. The genomes of *Pseudoalteromonas haloplanktis* and *Psychrobacter arcticus* harbor a higher percentage of cell envelope genes as a specialized adaptation to cope with the cold [28,31,32]. Lipopolysaccharides (LPS) are constitutive components, together with phospholipids and proteins, of the outer membrane in Gram-negative bacteria. A recent study by Benforte et al. [33] describes LPS as an essential feature of cold-adapted bacteria for their active growth at low temperature. The authors analyzed a *Pseudomonas extremaustralis wap*H mutant strain, encoding a core LPS glycosyltransferase. The *wap*H deficiency resulted in a lower flexibility and higher turgor pressure of the cell envelope, cell permeability, and surface area to volume ratio (S/V). The cell envelope of Gram-positive bacteria includes the cell wall and the inner membrane, with both having a fundamental role in cold adaptation by protecting the cell against ice-caused disruption and/or osmotic pressure. The expression of genes encoding for carbonic anhydrase, responsible for the mineralization of calcium carbonate, by *Planococcus halocryophilus* Or1, increases under low temperature conditions, evidencing microbial-mediated calcium carbonate precipitation at subzero temperatures [34]. Further, membrane fluidity is preserved by the increase in fatty-acid saturation with decreasing temperature, by the inactivation of fatty-acid desaturases [35,36].

Among primary cell processes, transcription and translation are inhibited by low temperature as the activity of enzymes involved in such reactions can be reduced. To overcome this, the main challenge that is faced by cold-adapted bacteria is therefore to maintain an appropriate rate for enzyme-catalyzed reactions. The activity of cold-adapted enzymes is up to 10 times higher at low temperatures than that of their mesophilic homologues, even if they suffer from thermolability [24]. This is because they benefit from structural adjustments to achieve a less compact 3D structure in the whole protein and the destabilization of the active site (i.e., residues in loops bordering the active site are deleted and bulky side chains are replaced with smaller groups at the entrance to the active site) [24]. In particular, proline and arginine residues (which stabilize the protein by restricting backbone rotations and forming multiple hydrogen bonds and salt bridges, respectively) are reduced in number. Conversely, the occurrence of glycine residues, which essentially have no side chains,

allows a localized chain mobility [24]. These structural modifications allow the catalytic center to be more flexible at temperatures that tend to freeze molecular motions, and are often larger and more accessible to ligands at low energy costs. In turn, the release and exit of the reaction products are facilitated. Hence, in cold environments, the selective pressure is strongly addressed to highly active enzymes instead of stable proteins [24].

In addition to enzymes, other proteins, such as cold-shock proteins (CSPs), cold-acclimation proteins (CAPs), and heat shock proteins (HSPs), play an important role in bacterial survival under extreme conditions. The family of CSPs includes small, single-stranded nucleic acid binding proteins. They are involved in the regulation of a number of cellular processes (including transcription, translation, protein folding, and membrane fluidity). Many of the mesophilic CSPs act as CAPs in psychrophiles, as they are constitutive rather than transiently expressed at low temperatures, as in mesophiles [37,38]. Different from these latter organisms, the response to a cold-shock by cold-adapted bacteria includes the lack of repression of housekeeping protein synthesis and the presence of CAPs. The major HSPs, such as the chaperones DnaK and Hsp90, and the chaperonin GroEL, are strongly cold-repressed in the proteome of *P. haloplanktis*, but overexpressed at elevated temperatures, which have been shown to induce cellular stress in cold-adapted bacteria [39,40].

The synthesis of cryoprotective compounds avoids the formation of ice crystals inside cells (resulting in cellular damage and osmotic imbalance) or acts at the translation or transcriptional level, stabilizing the mRNA [17,22,23]. Glycine, betaine, sucrose, and mannitol are among compatible solutes that are accumulated by cold-adapted bacteria to lower the cytoplasmic freezing point and avoid desiccation [41]. Antifreeze proteins (AFPs) are ice-binding proteins controlling ice crystal growth and recrystallization [42], while antinucleating proteins (ANPs) facilitate ice crystal formation at temperatures close to melting point by preventing the supercooling of water [43].

Among the substances involved in cold adaptation and cryoprotection, extracellular polymeric substances (EPSs) and polyhydroxyalkanoates (PHAs) can also be considered. EPSs are high-molecular weight polymers mainly consisting of carbohydrates. They may be homo- or hetero-polysaccharides and may contain several different organic and inorganic substituents (e.g., sulfate, phosphate, acetic acid, and acetylate). EPSs can occur in two forms, i.e., capsular polysaccharides (in which the polymers are covalently bound to the cell surface) and slime polysaccharides (which are loosely bound to the cell surface or released into the environment) [18]. In addition to the role played in trapping water, nutrients, and metals, as well as in cellular aggregation and biofilm formation, EPSs protect extracellular enzymes against cold denaturation and autolysis, and lower the freezing point and ice nucleation temperature of water [44]. Some of these roles have been proven, for example, for *Pseudoalteromonas* isolates from Antarctic sea-ice [45] and seawater [46].

A number of cold-adapted bacteria produce polyhydroxyalkanoates (PHAs). Such polymers represent a dynamic reservoir of carbon and reducing equivalents [28]. The accumulation of polyhydroxybutyrate (PHB), a short chain length PHA, by *Pseudomonas extremaustralis*, seems to influence biofilm formation and motility under cold conditions [47,48], thus representing an adaptive advantage for surface colonization in those environments.

All the complex and sophisticated survival strategies and unusual features discussed above often rely on the synthesis of still undisclosed natural biomolecules with unique properties for adapting to extreme environmental constrains, thus making cold-adapted bacteria valuable resources for biotechnological purposes [27,49].

3.3. Biotechnological Relevance

A number of investigations have dealt with the production of biotechnologically exploitable molecules by cold-adapted bacteria deriving from abiotic matrices of both the Arctic and Antarctica (reviewed by) [50], whereas biological matrices have only been seldom considered (see Section 5). However, cold-adapted microbes could furnish an array of unique natural products that are produced for their defense and survival in harsh and competitive environments. Some examples are given below.

PUFAs from cold-adapted bacteria have been proposed as an economic alimentary source for aquaculture industries [51]. Among PUFAs, eicosapentaenoic acid (EPA) and docosaesaenoic (DHA) acids (both omega-3 fatty acids) can find application in the pharmaceutical industry as they act favorably on cholesterol and triglyceride transport, plaque aggregation, inflammatory processes, and nervous cells [52]. As an example, PUFAs with promising properties have been obtained from several *Shewanella* isolates from Antarctic marine matrices [52–54].

Cold-enzymes, thanks to their high activity and stability at low temperatures, can be exploited in industrial large-scale processes with economic benefits, as no expensive heating of reactors is needed. For example, in the food industry, the utilization of cold-enzymes (such as amylases, β-galactosidases, pectinases, and xylanases) prevents contamination and spoilage, allows the retention of labile and volatile flavor compounds, and minimizes undesirable chemical by-product reactions that may occur at higher temperatures [55]. The development of detergents effective at ambient temperatures leads to the reduction of the environmental and economic impact by reducing washing temperatures and carbon dioxide emissions, and protecting the colours of fabrics of clothes. Among cold-adapted enzymes that are of interest for cold-water household and industrial laundry, as well as dishwasher detergents, there are lipases, proteases, amylases, cellulases, mannanases, and pectinases (with these two latter enzymes being mainly exploited in the laundry and dishwashing industry) [55].

The application of bacterial EPSs spans from industry (e.g., textile, dairy, cosmetics), health, and the environment. For example, in the food industry, they are used as thickening, emulsifying, and stabilizing additives [56]. Some EPSs have been proven to possess antitumor, antiviral, and immunostimulant activities, thus finding application in the pharmaceutical field [57]. Finally, they can be applied in the remediation of heavy metal contaminated environments, also at low temperature, as recently reported by Caruso et al. for a *Pseudoalteromonas* isolate [46].

4. Polar Marine Benthic Invertebrates as Bacterial Hosts

Marine invertebrates can be intensively colonized by bacteria, in addition to other microbes, with some of them serving as beneficial symbionts or being pathogenic, or having no effect [58]. Contrary to inanimate surfaces, which are rapidly colonized by a diverse assemblage of marine microbes, biotic surfaces frequently harbor species-specific microbial communities that can greatly differ from those inhabiting the surrounding environment [58]. Antarctic benthic communities mainly include members in the phyla Porifera, but also Cnidaria (e.g., orders Actiniaria, Gorgonaria, Alcyonacea, Pennatulacea, and class Hydrozoa), Bryozoa, Brachiopoda, Anellida (class Polychaeta), and Cordata (class Ascidiacea) [59,60], with some taxa that often dominate at the local scale. However, bacteria-invertebrate associations in Polar areas have been rarely investigated and analyses have been performed by applying both culture-independent and culture-dependent approaches. In particular, the isolation and characterization of marine microorganisms remain a fundamental approach in obtaining more exhaustive information on communities that cannot be obtained directly from culture-independent methods alone. With this aim, the methods for the cultivation of yet unculturable bacteria are always improving, paving the way toward the identification of novel organisms. As an example, a novel *Psychrobacter* sp. was cultivated after repeated coculture using helper strains together with tissue culture inserts and agar plates [61,62]. The helper strains produced essential growth factors, facilitating growth of the *Psychrobacter* strain. This was an important finding for the cultivation of an uncultivable strain with artificial media.

Our current knowledge on cold-adapted bacteria associated with Polar benthic invertebrates remains quite scarce and fragmented as it is limited to few organisms, i.e., several species of Porifera (Section 4.1), the soft coral *Alcyonium antarcticum*, the sea-urchin *Sterechinus neumayeri*, and the oligochaete *Grania* sp. (Section 4.2), as reviewed in the following sections and summarized in Table 1.

Table 1. Invertebrate hosts analyzed to date for the associated bacterial community.

Phylum	Invertebrate Host	Culturable/Unculturable Bacterial Community	Sampling Site	Reference(s)
Porifera	*Anoxycalyx (Scolymastra) joubini*	Culturable	Terra Nova Bay (Ross Sea, Antarctica)	[63,64]
	Chondrocladia grandis	Unculturable	Baffin Island (Qikiqtaaluk Region, Nunavut, Canadian Arctic)	[65]
	Cladorhiza oxeata	Unculturable	Baffin Island (Qikiqtaaluk Region, Nunavut, Canadian Arctic)	[65]
	Clathria sp.	Unculturable	Fildes Bay (King George Island, South Shetlands, Antarctica)	[66]
	Haliclona (Gellius) sp.	Unculturable	Fildes Bay (King George Island, South Shetlands, Antarctica)	[66]
	Haliclonissa verrucosa	Culturable	Terra Nova Bay (Ross Sea, Antarctica)	[63]
	Hemigellius pilosus	Culturable	Terra Nova Bay (Ross Sea, Antarctica)	[67]
	Homaxinella balfourensis	Culturable/Unculturable	McMurdo Sound (Ross Sea, Antarctica); Weddell Sea (Antarctica)	[68,69]
	Hymeniacidon torquata	Unculturable	Fildes Bay (King George Island, South Shetlands, Antarctica)	[66]
	Kirkpatrickia variolosa	Unculturable	McMurdo Sound (Ross Sea, Antarctica); Fildes Bay (Antarctica)	[66,68]
	Latrunculia apicalis	Unculturable	McMurdo Sound (Ross Sea, Antarctica)	[68]
	Leucetta antarctica	Unculturable	Fildes Bay (King George Island, South Shetlands, Antarctica)	[66]
	Lissodendoryx (Ectyodoryx) nobilis	Culturable	Terra Nova Bay (Ross Sea, Antarctica)	[63,64,70]
	Megaciella annectens	Unculturable	Fildes Bay (King George Island, South Shetlands, Antarctica)	[66]
	Mycale acerata	Unculturable	McMurdo Sound (Ross Sea, Antarctica)	[68]
	Myxilla (Burtonanchora) sp.	Unculturable	Fildes Bay (King George Island, South Shetlands, Antarctica)	[66]
	Myxilla mollis	Culturable	Weddell Sea (Antarctica)	[69]
	Myxodoryx hanitschi	Culturable	Terra Nova Bay (Ross Sea, Antarctica)	[70]
	Phorbas glaberrimus	Culturable	Terra Nova Bay (Ross Sea, Antarctica)	[70]
	Radiella antarctica	Culturable	Weddell Sea (Antarctica)	[69]
	Rossella nuda	Culturable	Weddell Sea (Antarctica)	[69]
	Rossella racovitzae	Culturable	Weddell Sea (Antarctica)	[69]
	Sphaerotylus antarcticus	Unculturable	McMurdo Sound (Ross Sea, Antarctica)	[68]
Echinodermata	*Sterechinus neumayeri*	Culturable	Maxwell Bay (King George Island, South Shetlands, Antarctica)	[71]
Cnidaria	*Alcyonium antarcticum*	Culturable/Unculturable	McMurdo Sound (Ross Sea, Antarctica)	[72]
Annelida	*Grania* sp.	Culturable	Maxwell Bay (King George Island, Antarctica)	[73]

4.1. Bacteria Associated with Porifera

To date, the whole prokaryotic community associated with Arctic and Antarctic sponges has been investigated by culture-independent methods by Webster et al. [68], Rodríguez-Marconi et al. [66], and Verhoeven and Dufour [65] at sites in McMurdo Sound (Ross Sea, Antarctica), Fildes Bay (King George Island, South Shetlands, Antarctica), and Baffin Island (Qikiqtaaluk Region, Nunavut, Canadian Arctic), respectively.

Webster et al. [68] analyzed five sponge species, i.e., *Kirkpatrickia variolosa*, *Latrunculia apicalis Homaxinella balfourensis*, *Mycale acerata*, and *Sphaerotylus antarcticus*, by the 16S rRNA sequencing of cloned DNA fragments, and Denaturing Gradient Gel Electrophoresis. The bacterial communities were sponge-species related regardless of the collection site, and primarily composed of *Gamma-* (e.g., genera *Vibrio* and *Alteromonas*) and *Alphaproteobacteria* (mainly *Roseobacter* spp.) and the Cytophaga/Flavobacterium (CF) group of *Bacteroidetes* (mainly *Polaribacter* spp.). Further, most bacterial sequences were more closely related to sequences previously retrieved from Antarctic seawater and sea-ice than from other sponge-derived microorganisms. This finding suggests that bacteria associated with the analyzed sponges were probably not specialized symbionts, mainly belonging to species that possess the ability to thrive in low temperatures.

Recently, the whole microbial community (Bacteria, Archaea, and Eukaryotes) associated with Antarctic sponges was analyzed in more depth by Rodríguez-Marconi et al. [66], using high through-put sequencing technologies of ribosomal genes. The sponges *Myxilla (Burtonanchora)* sp., *Clathria* sp., *Kirkpatrickia variolosa*, *Haliclona (Gellius)* sp., *Megaciella annectens*, *Hymeniacidon torquata*, and *Leucetta antarctica* were taken into consideration. Overall, previous observations by Webster et al. [68] were confirmed, with the associated bacterial community that was predominantly represented by Proteobacteria, followed by Bacteroidetes, Verrucomicrobia, and Planctomycetes. Thanks to the adopted approach, Rodríguez-Marconi et al. [66] gained new information incorporating additional taxa found in minor proportions, thus increasing the number of phyla detected in Antarctic sponges to 25. According to these authors, only a few phylotypes were common in seawater and sponge communities. This again highlights a possible host specificity and the role played by sponges as a diversity reservoir in the Antarctic marine ecosystem. Finally, Rodríguez-Marconi et al. [66] suggested a peculiar signature for the Antarctic sponge-associated microbial community, which differed at the phylum level from that previously reported for temperate and tropical ecosystems.

Additional information on the bacterial communities associated with Antarctic sponges, different from those analyzed by Webster et al. [68] and Rodríguez-Marconi et al. [66], derives from culturable bacteria. Altogether, Papaleo et al. [63] and Mangano et al. [64,67] reported on the identification of 207 bacterial strains isolated from the Antarctic sponges *Anoxycalyx (Scolymastra) joubini*, *Lissodendoryx (Ectyodoryx) nobilis*, *Haliclonissa verrucosa*, and *Hemigellius pilosus*. Gammaproteobacteria and Actinobacteria predominated within the cultivable bacterial community, followed by Alphaproteobacteria, the CF group of Bacteroidetes, and Firmicutes. Interestingly, the culturable bacterial fraction from sponges [including additional sponge species such as *Calyx arcuarius*, *Haliclona* (*Rhizoniera*) *dancoi*, *Haliclona* (*Gellius*) *rudis*, *Haliclona virens*, *Tedania charcoti*, and *Trachytedania spinata*] in the Terra Nova Bay mostly differed from that isolated from seawater (Lo Giudice A., *personal communication*) [74], confirming observations by Webster et al. [68] and Rodríguez-Marconi et al. [66] for the whole prokaryotic communities associated with Antarctic sponges in McMurdo Sound and Fildes Bay, respectively. Moreover, phylotypes related to the genera *Psychrobacter*, *Pseudoalteromonas*, and *Arthrobacter* were generally common to all sponge species. Conversely, *Sphingopyxis*, *Octadecabacter*, and *Pseudomonas* members were only isolated from A. *joubini*, and *Marinobacter*, *Colwellia*, *Rhodococcus*, *Gillisia*, *Staphylococcus*, and *Oceanobacillus* only from *H. verrucosa*. Furthermore, Xin et al. [69] analyzed the diversity of Gram-positive bacteria associated with the deep-sea Antarctic sponge species (from depths between 150 and 4790 m) *Rossella nuda*, *Rossella racovitzae*, *Myxilla mollis*, *Radiella antarctica*, and *Homaxinella balfourensis* collected from the Weddell Sea (Antarctica). Bacterial isolates belonged to Actinobacteria (24 strains in the genera *Streptomyces*, *Nocardiopsis*, *Pseudonocardia*, *Dietzia*, *Brachybacterium*, and *Brevibacterium*) and Firmicutes (22 strains in the genera *Bacillus* and *Virgibacillus*). This study added

two more genera (i.e., *Dietzia* and *Brevibacterium*) to cultivable Actinobacteria from marine sponges worldwide. In particular, the dominant Actinobacteria were members of the genus *Streptomyces*, which were broadly distributed in four out of five of the Antarctic sponges analyzed. Along with other previous reports, overall results by Xin et al. [69] indicated that Gram-positive bacteria are rather unspecialized with respect to their sponge hosts, with some strains from two sponge classes (i.e., Demospongiae and Hexactinellida) that form sponge-specific clusters across different Poriferan taxa.

With regard to Arctic sponges, Verhoeven and Dufour [65] examined and compared the bacterial communities associated with two carnivorous species, *Chondrocladia grandis* and *Cladorhiza oxeata*, sampled from Baffin Island (Canadian Arctic). The two sponge species hosted distinct bacterial communities, probably reflecting differences in trophic adaptability, specialization, and overall reliance of associated bacteria. In line with results from Antarctic sponges, several bacterial taxa were consistently recovered in multiple host individuals from geographically distant sites, indicating a high level of specificity. Interestingly, in the case of *C. grandis*, certain bacterial taxa were enriched in particular anatomical regions of the sponge specimens with unique assemblages of bacteria within the root and root tip samples, suggesting the occurrence of a species-specific core microbial assemblage involved in functional roles in carnivorous sponge metabolism or other biological processes. The vast majority (93%) of sequences were assigned to either Proteobacteria (mainly Gamma- and Alphaproteobacteria) or Bacteroidetes (mainly Flavobacteria). Bacterial genera common to both species were *Tenacibaculum*, Candidatus *Branchiomonas*, *Fulvivirga*, and *Pseudahrensia*, as well as all identified biomarkers in all anatomical regions for which taxonomy was resolved to the genus level, which were *Robiginitomaculum*, *Colwellia*, *Maritimimonas*, *Granulosicoccus*, *Mycobacterium*, *Anderseniella*, *Reichenbachiella*, and *Nitrospira*.

All these findings strengthened the idea that sponge-associated bacterial communities might also be sponge-specific in Polar regions and that the host probably gains benefits from the interaction with selected bacteria, thus stably maintaining the associated bacterial community. Both antagonistic relations, acting as an effective control of the different bacterial populations inhabiting the sponge tissue [64], and the production of N-Acyl homoserine lactones, involved in the quorum sensing [70], might play a key role in profiling the bacterial community associated with Antarctic sponges. Additionally, by the production of bioactive metabolites, the host can be directly involved in the selection of symbiotic bacteria [75]. Furthermore, the hologenome concept asserts that the holobiont, with its hologenome, acts as a unique biological entity and therefore also as a level of selection in evolution [76]. Such theory places importance on both major symbionts and enormously diverse associated microbiota (only recently partially uncovered using molecular techniques) [76]. Both the host genome and the associated microbiome can be transmitted between generations, thus maintaining the unique properties of the holobiont. This is because microbes and their hosts tightly interact, affecting the fitness of the holobiont (in terms of morphology, development, behavior, physiology, and resistance to disease). For this reason, the theory considers the holobiont as a single dynamic entity in which the genetic information and variability is contributed by the microorganisms. Since the microbiome can react more rapidly than the host genome to environmental changes, it plays a fundamental role in the adaptation and evolution of the holobiont [76]. Thus, changes in the host genome and/or in any of the associated microbial genomes allow the evolution of the holobiont, relying on the cooperation between the genomes within the holobiont, as much as on the competition with other holobionts.

4.2. Bacteria Associated with Other Benthic Invertebrates

At the time of writing, a unique paper exists on bacteria associated with the Antarctic sea urchin *Sterechinus neumayeri* (Meissner, 1900) (Phylum Echinodermata), which is frequently found in the shallow subtidal zone down to a 500 m depth around the Antarctic continent [77]. González-Aravena et al. [71] isolated 42 bacterial strains from *S. neumayeri* specimens collected in the Maxwell Bay (King George Island, South Shetland Islands). Isolates were predominantly affiliated to Gammaproteobacteria (with the genera *Pseudoalteromonas*, *Psychrobacter*, *Shewanella*, and *Pseudomonas*), Flavobacteria among the CF group of Bacteroidetes, and Actinobacteria. As it was observed by

Mangano et al. [67] for the Antarctic sponge *H. pilosus*, isolates from *S. neumayeri* were mainly heavy metal- and antibiotic-resistant. Despite the fact that both heavy metals and antibiotics can derive from natural processes, their concentration levels in the Antarctic environment, including benthic organisms, are increasing due to continuous anthropogenic inputs [49]. Bacteria from Antarctica, such those isolated by González-Aravena et al. [71] and Mangano et al. [67], could represent a reservoir of resistance genes in such a pristine environment.

Within the phylum Cnidaria, corals from tropical and temperate climates have frequently been the subject of microbiological studies. However, a single report exists on the description of the bacterial community associated with the Antarctic soft coral *Alcyonium antarcticum* (Wright and Studer 1889) at McMurdo Sound (Ross Sea) [72]. The authors adopted both culture-dependent and culture-independent methods to describe not only the microbial species composition, but also the stability of the host–bacterium associations between individuals, and the spatial variability in coral-derived bacterial communities. The results highlighted the predominance of Gammaproteobacteria, but also the occurrence of Alpha- and Betaproteobacteria, Bacteroidetes, Planctomycetes, Chlorobi, Gram-positive members (within Firmicutes and Actinobacteria), and sulfate-reducing bacteria. As it was observed for other benthic invertebrates, a specific coral–microbial interaction was observed, with a stable microbial community across replicate coral samples within a site and between sites. Ten bacterial isolates were obtained from *A. antarcticum* specimens and they were affiliated to the genera *Pseudomonas*, *Psychrobacter*, and *Shewanella* (among Gammaproteobacteria), *Psychroserpens* and *Algoriphagus* (among Bacteroidetes) and, finally, *Corynebacterium* (among Actinobacteria).

Within the phylum Annelida, gut-associated bacteria of the oligochaete *Grania* sp. was recently described by Herrera et al. [73] for specimens collected in the Artigas Beach at Maxwell Bay (King George Island, South Shetland Islands, and Maritime Antarctica). A total of 28 bacterial isolates were obtained and mainly identified as belonging to the genera *Flavobacterium*, *Pseudomonas*, *Psychrobacter*, and *Salinibacterium*. An Enterobacteriaceae member was also isolated. The authors suggested that bacteria probably invaded *Grania* sp. along with algae, which are their main food source. Further, they may be involved in the degradation of algae through their ability to hydrolyze a number of organic substrates (e.g., cellulose, starch, proteins, and triacylglycerols), thus probably contributing to nutrient recycling in the Antarctic ecosystem.

5. Polar Benthic Invertebrates as a Source for Bacterial Biodiscovery

Marine benthic invertebrates are exposed to significant predation, fouling (micro)organisms, and opportunistic pathogens from the surrounding environment and, further, they must often compete for space and substrates upon which to settle. To contrast the colonization of their surfaces by unwanted microorganisms and little invertebrates, benthic organisms can adopt chemically-mediated defensive strategies [14,78]. As a result, several invertebrates have been proven to possess great pharmaceutical potential as producers of anti-viral, anti-inflammatory, anti-proliferative, anti-tumor, and antimicrobial activities [16,79–82]. With regard to the Polar Regions, the production of bioactive molecules has been reported for diverse Arctic (e.g., [58,83]) and Antarctic (e.g., [58,84–91]) marine benthic organisms (including sponges, corals, echinoderms, tunicates, molluscs, and bryozoans) (see for review [75]). Even if the number of natural products from Polar invertebrates remains lower than that recorded elsewhere, they have shown bioactivity levels comparable to those recorded in temperate and tropical marine environments, supporting the plausible theory that polar regions have likely potential for the bioprospecting of natural products, particularly Antarctica [82]. Interestingly, some recent reports suspect that a number of metabolites obtained from host invertebrates may be produced by their microbial symbionts [17,92,93]. The huge diversity within the microbial communities harbored by benthic organisms, even in Polar areas, implies a rich and mainly unexploited source for the biodiscovery of natural products with unusual features. However, the biotechnological relevance of cold-adapted bacteria associated with Polar benthic invertebrates has only been rarely investigated, and only for Antarctica. Data available at the time of writing are reviewed in the following sections and summarized in Table 2.

Table 2. Cold-adapted bacteria isolated from Antarctic marine benthic invertebrates as a source of biomolecules.

Invertebrate Host (Species, Phylum)	Antarctic Location	Bacterial Isolate(s)	Bioactive Compound(s) Produced	Reference
Lissodendoryx nobilis,Porifera	Terra Nova Bay	*Arthrobacter* sp. TB23	*Antimicrobial compounds*	[63]
Lissodendoryx nobilis, Porifera	Terra Nova Bay	*Gillisia* sp. CAL575		[94]
Haliclonissa verrucosa, Porifera	Terra Nova Bay	*Pseudoalteromonas* sp. AC163		[95]
Lissodendoryx nobilis, Porifera	Terra Nova Bay	*Pseudoalteromonas* sp. TB13		[96]
Lissodendoryx nobilis, Porifera	Terra Nova Bay	*Pseudoalteromonas* sp. TB25		[96]
Anoxycalyxjoubini, Porifera	Terra Nova Bay	*Pseudoalteromonas* sp. TB41		[96]
Anoxycalyx joubini, Porifera	Terra Nova Bay	*Pseudoalteromonas* sp. TB51		[96]
Anoxycalyx joubini, Porifera	Terra Nova Bay	*Pseudoalteromonas* sp. TB64		[96]
Isodictya setifera, Porifera	Ross Island	*Pseudomonas aeruginosa*		[97]
Anoxycalyx joubini, Porifera	Terra Nova Bay	*Psychrobacter* sp. TB47		[96]
Anoxycalyx joubini, Porifera	Terra Nova Bay	*Psychrobacter* sp. TB67		[96]
Lissodendoryx nobilis, Porifera	Terra Nova Bay	*Shewanella* sp. TB4		[98]
Hemigellius pilosus, Porifera	Terra Nova Bay	*Colwellia* sp. GW185	*Extracellular polymeric substances*	[46]
Haliclonissa verrucosa, Porifera	Terra Nova Bay	*Shewanella* sp. CAL606		[46]
Tedania charcoti, Porifera	Terra Nova Bay	*Winogradskyella* sp. CAL384		[46]
Tedania charcoti, Porifera	Terra Nova Bay	*Winogradskyella* sp. CAL3396		[46]
Grania sp., Annelida	Maxwell Bay	*Flavobacterium* spp.	*Cold-adapted enzymes*	[73]
Grania sp., Annelida	Maxwell Bay	*Pseudomonas* spp.		[73]
Grania sp., Annelida	Maxwell Bay	*Psychrobacter* spp.		[73]

5.1. Antimicrobials

Bacterial colonization of a living surface lies in physiological and structural features, enabling them to survive and produce a prolific number of antimicrobial compounds that help to protect against competitive microorganisms (i.e., pathogenic and fouling organisms), resulting in the shape of the bacterial community structure. Thus, bacterial symbionts are a potential source of molecules with yet unrevealed activities covering a wide range of biological functions. To the best of our knowledge, the antibiotic activity of bacteria associated with benthic invertebrates from Polar areas has only been reported for Antarctic sponges. In a pioneer study, Jayatilake et al. [97] reported on the production of diketopiperazines and two phenazine alkaloid antibiotics from the bacterium *Pseudomonas aeruginosa* isolated from the sponge *Isodictyas etifera* (Ross Island). The structure of a novel diketopiperazine was determined to be cyclo(L-proline-L-methionine) (Figure 2). Antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus luteus* was observed in culture broth from *I. setifera*-derived *P. aeruginosa*. However, diketopiperazines were inactive as antibiotics or cytotoxins. Conversely, phenazine alkaloids inhibited the growth of *Bacillus cereus*, but were less active against *M. luteus* and *S. aureus*.

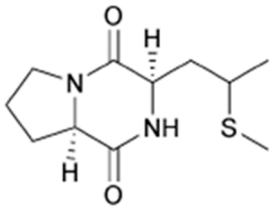

Figure 2. A bioactive compound, cyclo(L-proline-L-methionine), produced by *P. aeruginosa* [97].

A total of 132 bacterial strains from the Antarctic sponge *L. nobilis*, *A. joubini*, and *H. verrucosa* from the Terra Nova Bay were screened for antibiotic activity against opportunistic pathogens, including those affecting cystic fibrosis (CF) patients [63,94]. Most sponge-associated bacteria were able to completely inhibit the growth of bacteria belonging to the *Burkholderia cepacia* complex (Bcc), one of the most important pathogens in CF, whereas they showed no activity against *P. aeruginosa* or *S. aureus*. A further in-depth analysis was carried out on selected isolates, i.e., *Pseudoalteromonas* spp. TB41 and AC163, *Shewanella* sp. TB4, *Psychrobacter* spp. TB47, and TB67 by Romoli et al. [98]. The solid phase micro extraction gas-chromatography mass-spectrometry technique revealed that some of the produced antimicrobial compounds were very likely volatile organic compounds (VOCs), which are a well-known regulatory factor in the interactions among different organisms in microbial ecosystems, and that their synthesis was constitutive, as it was not induced by the presence of target strains [99]. More interestingly, VOCs seemed to be more effective in inhibiting the growth of Bcc bacteria than most of the commonly used antibiotics. The volatile profiles under aerobic conditions of *Psychrobacter* spp. from *A. joubini* [64] were investigated by Papaleo et al. [100]. The results suggest that the exhibited antimicrobial activity might rely on a (complex) mixture of VOCs, including sulfur-containing ones, whose relative concentration may vary depending on the growth conditions (presence/absence of oxygen and growth media used) [49].

Nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) are multi-enzymatic, multi-domain megasynthases involved in the biosynthesis of nonribosomal peptides and polyketides, often with antibiotic activities [101]. *Pseudoalteromonas* sp. TB41, as well as *Arthrobacter* sp. TB23 [64], also possessed some genes belonging to the nrps–pks cluster, providing evidence of the potential biosynthesis of bioactive compounds [63]. The biosynthetic potential by the screening for PKS genes was also evaluated for 46 Gram-positive strains from deep-sea Antarctic sponges [69], revealing that most isolates harbored the genes encoding polyketide synthases.

More recently, the ability of several *Pseudoalteromonas* strains from different Antarctic matrices (including sponges) to synthesize antimicrobial compounds effective against Bcc bacteria was

investigated under different growth conditions [96]. The results also suggested the production of non-volatile compounds by the isolates, in addition to VOCs, and were supported by the occurrence of genes involved in the biosynthesis of antimicrobial compounds in the core genome, e.g., polyketides, bacteriocins, and siderophores [95,102].

5.2. Extracellular Polymeric Substances

Extracellular polymeric substances (EPSs) are involved in bacterial cell aggregation, flocculation and biofilm formation, cell recognition, and water retention to avoid desiccation, and can act as a protective barrier [103]. In this latter case, EPS production by cold-adapted bacteria also represents a survival strategy to thrive in low temperatures, avoiding cell damage and the attenuation of cell viability in environments such as cold Polar regions, where freeze-thaw cycles are frequent, by maintaining a protective microhabitat around microorganisms. To date, biological matrices have rarely been employed for bacterial extracellular polymer production, even if they may play a key role in bacterial adhesion to living surfaces [8], and a single report on EPS-producing bacteria isolated from Antarctic sponges exists [104]. The production of EPSs by *Winogradskyella* spp. CAL384 and CAL396 from the sponge *T. charcoti*, *Colwellia* sp. GW185 from *H. pilosus* [67], and *Shewanella* sp. CAL606 from *H. verrucosa* [34] was enhanced by a step-by step approach varying the carbon source, substrate and NaCl concentrations, temperature, and pH for bacterial growth [104]. Interestingly, no EPSs were previously described for cold-tolerant *Winogradskyella* isolates. A sub-optimal incubation temperature (4 °C) seemed to be more effective in terms of EPS production, supporting the cryoprotective role played by these molecules under low temperature stressful conditions. This finding was confirmed by the analysis of cell-viability after repeated freeze-thaw cycles [104].

Produced EPSs were chemically characterized, resulting in a moderate carbohydrate content (range 15–28%), and the presence of proteins (range 3–24%) and uronic acids (range 3.2–11.9%) [104]. The carbohydrate portion was characterized by galactose, glucose, galactosammine, and mannose as principal constituents. The high protein content in the EPSs produced by *Winogradskyella* sp. CAL384 was probably responsible for the excellent emulsifying activity towards hydrocarbons, with a stable emulsion index (E24) higher than those recorded for synthetic surfactants. The authors also reported on the ability of Antarctic sponge-associated isolates to grow in the presence of higher concentrations of mercury and cadmium when growing with the supplement of sugars that enhanced EPS production. This was probably dependent on the presence of uronic acids and sulfate groups, as observed in extracted EPSs, in the EPS molecules, which can act as ligands for cations.

5.3. Cold-Enzymes

Enzymes are key molecules in bacterial adaptation to the cold. A number of structural modifications promote their flexibility at the active site, low substrate affinity, and high specific activity at low temperatures [105]. Altogether, these peculiar features make cold-adapted enzymes promising candidates in a broad range of industrial, agricultural, and medical applications aimed at decreasing energy consumption and preventing undesirable chemical side reactions (which often occur at high temperatures). However, only a small portion of enzymes of marine origin have reached the commercialization stage [106]. With regard to polar microbes, the synthesis of cold-adapted enzymes has been intensely studied in bacteria isolated from abiotic matrices [28,107]. To date, the oligochaete *Grania* sp. has been the only benthic invertebrate from a Polar marine environment to be used as a source of bacteria producing hydrolytic enzymes [73]. Bacteria associated with the gut of *Grania* sp. showed the ability to produce extracellular proteases, esterases, amylases, cellulases, and agarases. They belonged to the genera *Pseudomonas* (14 isolates), *Flavobacterium* (10 isolates), and *Psychrobacter* (four isolates).

6. Concluding Remarks

Polar microorganisms could represent an answer to the rising needs of novel and effective bioactive compounds for contrasting human diseases, and protecting human health and the environment [108]. In the coming decades, chemists and biologists will be required to closely collaborate with each other by cross-disciplinary interactions to provide innovative approaches and promising candidates within Polar bioprospecting activities. In addition to the screening and characterization of compounds, a further effort should also be made to address mechanisms driving their activities [108]. Finally, as it is well known, bioprospecting is regulated by various international law regimes worldwide. It has considerable scientific and economic repercussions, and includes the research and discovery of bioactive compounds with a potentially high value for human and environmental health [109–112]. It must be pointed out that, in the case of Antarctica, the exploitation of biological resources must follow the directions within the Antarctic Treaty, which includes a number of ethical principles to be followed for all activities in this region to preserve Antarctic life. This aspect further encourages the deepening of knowledge about bioprospecting perspectives of Antarctic-associated bacteria, more easily treatable under in vitro conditions, and whose utilization could reduce the risky exploitation of Antarctic higher organisms in the future, by preserving their biodiversity.

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