Marine Environmental Research 129 (2017) 68–75

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Experimental evidence of chemical defence mechanisms in Antarctic bryozoans

Blanca Figuerola [a, *], Carlos Angulo-Preckler [a], Laura Núñez-Pons [b, c], Juan Moles [a], Laura Sala-Comorera [d], Cristina García-Aljaro [d], Anicet R. Blanch [d], Conxita Avila [a]

[a] Department of Evolutionary Biology, Ecology, and Environmental Sciences and Biodiversity Research Institute (IrBIO), University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Catalonia, Spain
[b] Department of Biology and Evolution of Marine Organisms (BEOM) Stazione Zoologica 'Anton Dohrn' (SZN), Villa Comunale 80121, Naples, Italy
[c] Smithsonian Tropical Research Institute (STRI), Bocas del Toro Labs, Panama
[d] Department of Genetics, Microbiology and Statistics, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Catalonia, Spain

ARTICLE INFO

Article history:
Received 13 January 2017
Received in revised form
19 April 2017
Accepted 26 April 2017
Available online 27 April 2017

Keywords:
Chemical repellence
Substrate preference
Antifouling
Marine invertebrates
Polar biology
Preference assays

ABSTRACT

Bryozoans are among the most abundant and diverse members of the Antarctic benthos, however the role of bioactive metabolites in ecological interactions has been scarcely studied. To extend our knowledge about the chemical ecology of Antarctic bryozoans, crude ether extracts (EE) and butanol extracts (BE) obtained from two Antarctic common species (*Cornucopina pectogemma* and *Nematoflustra flagellata*), were tested for antibacterial and repellent activities. The extracts were screened for quorum quenching and antibacterial activities against four Antarctic bacterial strains (*Bacillus aquimaris*, *Micrococcus* sp., *Oceanobacillus* sp. and *Paracoccus* sp.). The Antarctic amphipod *Cheirimedon femoratus* and the sea star *Odontaster validus* were selected as sympatric predators to perform anti-predatory and substrate preference assays. No quorum quenching activity was detected in any of the extracts, while all EE exhibited growth inhibition towards at least one bacterium strain. Although the species were not repellent against the sea star, they caused repellence to the amphipods in both extracts, suggesting that defence activities against predation derive from both lipophilic and hydrophilic metabolites. In the substrate preference assays, one EE and one BE deriving from different specimens of the species *C. pectogemma* were active. This study reveals intraspecific variability of chemical defences and supports the fact that chemically mediated interactions are common in Antarctic bryozoans as means of protection against fouling and predation.

1. Introduction

The Antarctic benthos is predominantly subjected to constant environmental conditions below the maximum depth of iceberg disturbance (>30 m depth), and it is composed by rich assemblages of sessile, filter-feeding communities, mainly sponges, cnidarians, soft corals, ascidians, and bryozoans (Dayton et al., 1974). Under these peculiar conditions, generalist macroinvertebrate predators, such as sea stars and amphipods (densities > 300,000 individuals m^{-2} off the Western Antarctic Peninsula; Huang et al., 2007; Amsler et al., 2008), occupy high trophic levels, whilst fish and decapods —characteristic top-predators in warmer marine ecosystems— are scarce (Clarke et al., 2004). Sea stars and amphipods exhibit a broad-spectrum diet, including sponges, bryozoans, and cnidarians (Coleman, 1989; Dearborn et al., 1983; Dauby et al., 2001; Nyssen et al., 2005; Amsler et al., 2005). Benthic communities in such scenario seem to be mainly influenced by biotic interactions (e.g. predation, competition for space, and fouling) rather than environmental drivers, thus favouring the evolution of a wide array of physical and chemical defensive mechanisms (see Avila et al., 2008; McClintock et al., 2010; Núñez-Pons and Avila, 2015).

Although some Antarctic benthic phyla (e.g. sponges, soft corals, ascidians) have been somewhat investigated regarding their anti-predatory properties, other taxa like the bryozoans have been less studied (Winston and Bernheimer, 1986; McClintock and Baker, 1997; Amsler et al., 2001; Avila et al., 2008; McClintock et al., 2010; Figuerola et al., 2013; Taboada et al., 2013; Núñez-Pons and Avila,

* Corresponding author.
E-mail address: bfiguerola@gmail.com (B. Figuerola).

http://dx.doi.org/10.1016/j.marenvres.2017.04.014
0141-1136/

2014; Moles et al., 2015). To the best of our knowledge, our recent studies are the first investigating chemically-mediated interactions in Antarctic bryozoans. Such studies have revealed a very chemically protected phylum, with extended repellent and antibacterial activities (Figuerola et al., 2013, 2014a, 2014b). At the moment, no natural products (NPs) are known from Antarctic species, even though 35 NPs have been isolated from cold-water (~0 °C) bryozoans (Lebar et al., 2007).

Bryozoans are an important component of the Antarctic biodiversity, with a total number of species estimated on ~390 (De Broyer et al., 2011). These sessile invertebrates are often characterized by circumpolar distributions and broad bathymetrical ranges (Figuerola et al., 2012). Their colonies exhibit a wide range of shapes and sizes, from encrusting to rigid structures, usually conforming three-dimensional structures, providing complex habitats used by small organisms (e.g. amphipods) as structural and/or chemical shelter (De Broyer et al., 2001). Moreover, bryozoans often host rich communities of microorganisms (Peters et al., 2003).

In particular, cheilostomes, the most successful living order of bryozoans, have developed a remarkable variety of skeletal structures at both zooidal and colonial levels (e.g. calcified spines, avicularia or vibracula) and chemical mechanisms (NPs) used for different roles: defence against macro- and micropredators, prevention of fouling, and infection by pathogenic microorganisms (Hayward, 1995; Sharp et al., 2007). In fact, antibacterial activities (i.e. killing or inhibiting bacterial growth by interfering with essential processes; e.g. DNA and protein synthesis) and compounds involved in quorum quenching (QQ) (i.e. compounds interfering with bacterial cell-to-cell communication and consequently affecting gene expression related to growth) have been previously found in cheilostome species (Sharp et al., 2007). The literature available on bryozoan NPs (see review Sharp et al., 2007) suggests that erect cheilostomes, which are more fragile and vulnerable to predation than encrusting species (McKinney et al., 2003), are more prone to yield active NPs. Macropredators, such as sea stars, can cause major injuries on bryozoans, to the point of compromising their survival (Winston, 2009). Besides, smaller zooid-level predators such as amphipods, which may feed directly or accidentally on bryozoans, can also have significant sub-lethal effects on their colonies, and/or cause potential infections (Winston, 2009). These factors promote different kinds of defensive strategies, especially in less physically protected groups, like anascan cheilostomes (i.e. without calcified frontal shield) for being easily penetrated and vulnerable to predation (McKinney et al., 2003). Therefore, we hypothesize that the chemical defensive arsenal known for Antarctic bryozoans at the moment might be in part the result of a high predation pressure (Figuerola et al., 2013).

The aim of the present study was to assess the defensive potential of bryozoan-derived extracts towards bacterial fouling, substrate preference of small grazers and sympatric predation by macro- and meso-predators. For this purpose, quorum quenching and antibacterial activity against a diverse array of sympatric bacterial strains isolated from pebbles, sediment, and a sponge species were assessed. Repellence assays using keystone sympatric predators against three ether (EE) and three butanol (BE) extracts from two common, erect, anascan, cheilostome bryozoan species were also conducted.

2. Materials and methods

2.1. Collection of assay bryozoan samples

Three Antarctic bryozoan colonies, belonging to two widely distributed Antarctic species (Hayward, 1995), two classified as *Cornucopina pectogemma* (Goldstein, 1882) and one corresponding to *Nematoflustra flagellata* (Waters, 1904), were collected by scuba in Livingston Island (62°39′ S, 60°37′ W; Punta Hanna, Livingston Island, South Shetland Islands) at 15 m depth during the ACTIQUIM-3 (January-February 2012) cruise. The two colonies of *C. pectogemma* were collected in the same location and they were of similar size and coloration. All colonies were preserved at −20 °C prior chemical processing.

2.2. Collection of experimental sea star and amphipod predators

The sea star *Odontaster validus* Koehler, 1906 and the amphipod *Cheirimedon femoratus* (Pfeffer, 1888), considered model predators for their abundance and ubiquitous eurybathic distributions, were selected as in previous studies (Avila et al., 2000; Núñez-Pons et al., 2012c). Around 80 sea stars (~3–5 cm in radius) were sampled by scuba at depths ranging from 5 to 15 m, and ~200 amphipods were collected between 2 and 7 m depth at Port Foster Bay in Deception Island, South Shetland Islands (62°59.36′ S, 60°33.42′ W), during the Antarctic campaign ACTIQUIM-4 (January–February 2013). All individuals were maintained alive in large tanks (~1000 L) at environmental temperature (1–2 °C), with seawater pumped directly from the sea, at the Antarctic Spanish Base “Gabriel de Castilla” facilities during the experiments, and afterwards they were returned to the sea.

2.3. Chemical extractions of bryozoans

The bryozoan colonies were thawed and exhaustively extracted with acetone, which was subsequently evaporated in vacuo. The remaining aqueous residue was sequentially partitioned into diethyl ether (EE) and *n*-butanol (BE) extracts. All fractionation steps were repeated thrice, except for the butanol fraction, which was done once overnight. Organic solvents were evaporated, yielding dry EE and BE and aqueous residues. Dry EE (i.e. comprising the most apolar, lipophilic metabolites) and BE (i.e. polar, the most hydrophilic metabolites) were weighted and used to calculate the total sample dry weight (TDW = DW + EE + BE, where DW is the dry weight of the solid remains of the extracted sample). We used dry weight to calculate the extract's natural concentrations, as in previous studies (Figuerola et al., 2013), because this reference parameter avoids deviations related to water content, which is highly variable in aquatic samples. The natural concentration was calculated by dividing dry EE or BE partitions by the TDW and was used to perform the assays.

2.4. Antibacterial testing

Four bacterial strains were isolated from different substrates (pebbles, sediment, and the sponge *Haliclona* sp.) in Deception Island during two cruises: ACTIQUIM-3 and -4 (Table 1). Bacteria isolated from the pebbles and sponges were collected by swabbing the surface. Instead, strains isolated from the sediment were collected directly using sterile plastic tubes. One mL of all samples in seawater was added to Difco™ marine broth 2216 (Difco Laboratories, Sparks, MD, USA), left for 24 h at 18–20 °C, and subsequently cultured on Difco™ marine agar plates. A subsample of the selected strain colony was frozen at −20 °C and shipped to the University of Barcelona for further identification. These bacterial strains were maintained on marine agar slopes at 4 °C and/or frozen at −80 °C in marine broth containing 15% (v/v) glycerol (870 g/L) for further analyses.

The agar disk diffusion method (Acar, 1980, M2-A11 Performance Standards for Antimicrobial Disk NCLS 2012) was used to test antibacterial activity of extracts. A bacterial suspension from isolated colonies was prepared in saline buffer to achieve turbidity