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Antimicrobial activity of Antarctic bryozoans: An ecological perspective with potential for clinical applications

Blanca Figuerola [a], [*], Laura Sala-Comorera [b], Carlos Angulo-Preckler [a], Jennifer Vázquez [a], M. Jesús Montes [c], Cristina García-Aljaro [b], Elena Mercadé [c], Anicet R. Blanch [b], Conxita Avila [a]

[a] *Department of Animal Biology (Invertebrates) and Biodiversity Research Institute (IrBIO), Faculty of Biology, University of Barcelona, Barcelona, Catalunya, Spain*
[b] *Department of Microbiology, Faculty of Biology, University of Barcelona, Barcelona, Catalunya, Spain*
[c] *Department of Health Microbiology and Parasitology, Faculty of Pharmacy, University of Barcelona, Barcelona, Catalunya, Spain*

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ABSTRACT

The antimicrobial activity of Antarctic bryozoans and the ecological functions of the chemical compounds involved remain largely unknown. To determine the significant ecological and applied antimicrobial effects, 16 ether and 16 butanol extracts obtained from 13 different bryozoan species were tested against six Antarctic (including *Psychrobacter luti*, *Shewanella livingstonensis* and 4 new isolated strains) and two bacterial strains from culture collections (*Escherichia coli* and *Bacillus cereus*). Results from the bioassays reveal that all ether extracts exhibited antimicrobial activity against some bacteria. Only one butanol extract produced inhibition, indicating that antimicrobial compounds are mainly lipophilic. Ether extracts of the genus *Camptoplites* inhibited the majority of bacterial strains, thus indicating a broad-spectrum of antimicrobial activity. Moreover, most ether extracts presented activities against bacterial strains from culture collections, suggesting the potential use of these extracts as antimicrobial drugs against pathogenic bacteria.

1. Introduction

The Antarctic continental shelves, with a relatively stable environment below the limit of ice scour and anchor ice, are characterized by the presence of a high diversity and abundance of sessile suspension feeders (Dayton et al., 1974). This stability leads to the sessile suspension feeder communities being mainly influenced by biotic interactions, favouring the evolution of chemical defences (Avila et al., 2008; McClintock et al., 2010). Among these benthic organisms, bryozoans are an important component of Antarctic biodiversity, with a total number of species estimated at over 390, with new species still being discovered (Kuklinski and Barnes, 2009; Hayward and Winston, 2011; De Broyer et al., 2011; Figuerola et al., 2013a). Antarctic bryozoans are often characterized by having circumpolar distributions and broad bathymetric ranges (Hayward, 1995). In particular, cheilostome bryozoans, the most successful living order of bryozoans, have developed a wide range of physical (e.g. avicularia or spines) and chemical mechanisms (natural products) used for roles, such as the defence against predators and prevention of settlement of epibionts fouling (Al-Ogily and Knight Jones, 1977; Winston, 1986, 1991; Walls et al., 1993; Lindquist, 1996; Iyengar and Harvell, 2002; Lopanik et al., 2006; Sharp et al., 2007; Carter et al., 2010; Figuerola et al., 2013b, 2014). Although thirty-five natural products have been isolated from cold-water (temperatures near zero degrees Celsius) bryozoans, none of these reports cite Antarctic species (Lebar et al., 2007). In fact, the absence of epibionts on the surface of many bryozoans is normally thought to be an evidence for the presence of these defences (Wahl, 1989; Shellenberger and Ross, 1998; Krug, 2006).

For instance, fouling is a common life-history trait in marine organisms, initiated by bacterial colonization (first stages) and, subsequently, by the settlement of unicellular and multicellular epibionts (Wahl, 1989). As marine organisms are constantly exposed to high concentrations of bacteria (Jenkins et al., 1998), some antimicrobial activity may be useful in limiting later successional fouling stages (i.e. settlement of epibionts) through prevention of “surface conditioning” by early stage bacteria (Wahl,

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

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1989). In the case of filter-feeding organisms that feed on bacteria, like bryozoans, surface colonization by microorganisms, including opportunistic pathogens, is favoured by their concentration from the water column during the feeding process (e.g. Winston, 1977; Bergquist, 1978). In fact, bryozoans often host communities of microorganisms and small invertebrates within their colony structures (Peters et al., 2003; Carter, 2008). In contrast, other microorganisms are inhibited by antimicrobial secondary metabolites produced by some bryozoan species, such as *Bugula dentata* Lamouroux, 1816, and *Flustra foliacea* Linnaeus, 1758 (Matsunaga et al., 1986; Wright, 1984).

Microbial biodiversity in Antarctic waters is also thought to be high but is still poorly explored (around $0.37 \cdot 10^6$ cells per ml; Zdanowski, 1995; Bej et al., 2010). In the present study, the bryozoans might potentially be in contact with the Antarctic bacterial strains tested, as diverse Antarctic bacterial strains of the genera *Bacillus* Cohn 1872, and *Micrococcus* Cohn 1872, have been found in sediment samples collected from the sea adjacent to King George Island, an island close to our study area (Zhou et al., 2013). Likewise, the genus *Paracoccus* Davis 1969 has been isolated from diverse Antarctic regions (e.g. Michaud et al., 2004; Heindl et al., 2012). In particular, *Bacillus aquimaris* Yoon et al., 2003, *Shewanella livingstonensis* Bozal et al., 2002, and *Oceanobacillus* sp. Lu et al., 2002, have all been isolated from Antarctic sponges in the Weddell and Ross Seas (Xin et al., 2011; Papaleo et al., 2012). Moreover, several species of the genus *Psychrobacter* Juni and Heym 1986 are widespread in Antarctic environments and some of them have been isolated from Antarctic sea ice and krill (Bowman et al., 1997; Denner et al., 2001). Interestingly, this genus has also been found associated with internal tissues of an ascidian collected in the Indian Ocean (Romanenko et al., 2002). With regard to bacteria isolated from bryozoan species belonging to the genus *Bacillus*, *Microccocus*, *Psychrobacter* and *Shewanella* MacDonell and Colwell 1986 have been found them associated with the common boreal bryozoan species *F. foliacea* in the North Sea (Pukall et al., 2001). Additionally, species belonging to the genus *Bacillus*, *Paracoccus*, *Psychrobacter* and *Shewanella* have been isolated from the cosmopolitan bryozoan *Membranipora membranacea* Linnaeus, 1767 (Heindl et al., 2012). *Shewanella* has also been found associated with other bryozoan species from the North and Baltic Seas (Peters et al., 2003; Heindl et al., 2010). Singularly, this genus is considered an opportunistic pathogen of humans (e.g. Brink et al., 1995) and aquatic animals (Aguirre et al., 1994).

Regarding pathogenic bacteria, the evolution of antibiotic-resistant bacteria led to the search for potential antimicrobial agents from unexplored marine natural areas, such as Antarctica, which is considered a potential reserve of novel active compounds (Lebar et al., 2007; Avila et al., 2008). In particular, compounds from some bryozoans have demonstrated to present pharmacological properties, such as antitumour activity (e.g. de Vries and Beart, 1995). The active secondary metabolites from bryozoans could potentially be used as antimicrobial drugs against common bacteria, such as *Bacillus cereus* Frankland and Frankland 1887, and *Escherichia coli* (Migula 1895), which were tested in this study. On the one hand, *Bacillus* species are ubiquitous and diverse both in the terrestrial and marine ecosystems (Oguntoyinbo, 2007); *B. cereus* has been associated with fresh and pasteurised food products due to their ability to generate heat-resistant spores under adverse environmental conditions and it has been recognised as a food poisoning causative agent linked to food borne emetic and diarrhoeal syndromes (Ghelardi et al., 2002). On the other hand, some *E. coli* strains have been associated with food borne illness caused by consumption of raw or undercooked meat (Fischer et al., 2007).

To our knowledge, there is little information about the ecological and applied prospective roles of secondary metabolites as antimicrobials in Antarctic or any other bryozoans. Considering that the global bryozoan species richness is around 5869 spp and that now we know that diverse Antarctic species possess active compounds (e.g. Figuerola et al., 2012b; Bock and Gordon, 2013; Taboada et al., 2013; Figuerola et al., 2013b, 2014), there is room for more secondary metabolites as antimicrobials to be discovered. The aim of the present study is to assess the role and the potential pharmacological interest of bryozoan-derived natural products by investigating the antimicrobial activity of 16-ether and 16-butanol extracts from Antarctic bryozoans against a diverse array of sympatric bacterial populations and bacterial strains from culture collections.

2. Material and methods

2.1. Collection of bryozoan samples and identification

Sixteen abundant Antarctic bryozoan colonies (13 species) were collected in the eastern Weddell Sea (Antarctica) between 273.6 m and 351.6 m depth during the ANT XXI/2 (November 2003–January 2004) cruise on board the R/V Polarstern (Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany) using an Agassiz Trawl and a Bottom Trawl (Tables 1 and 2). These species show wide bathymetric ranges in their distributions in the Eastern Weddell Sea (Figuerola et al., 2012a). Bryozoan species offering a high diversity of potential

Table 1
Samples and data from the stations in the Weddell Sea.

Species	Latitude (S)	Longitude (W)	Depth (m)
Bostrychopora dentata Waters, 1904	70° 57.00′	10° 31.61′	284.4
Camptoplites angustus (1) Kluge, 1914	70° 50.75′	10° 28.01′	281.2
Camptoplites angustus (2) Kluge, 1914	70° 50.78′	10° 28.51′	273.6
Camptoplites bicornis Busk, 1884	70° 56.83′	10° 32.61′	338
Camptoplites tricornis (1) Waters, 1904	70° 56.67′	10° 32.05′	302.4
Camptoplites tricornis (2) Waters, 1904	70° 57.33′	10° 33.86′	351.6
Dakariella dabrowni Rogick, 1956d	70° 57.00′	10° 33.02′	332.8
Isoschizoporella secunda Hayward and Taylor, 1984	71° 06.44′	11° 27.76′	277.2
Isosecuriflustra tenuis Kluge, 1914	70° 52.75′	10° 51.24′	294.8
Klugella echinata Kluge, 1914	70° 56.83′	10° 32.61′	338
Melicerita obliqua Thornely, 1924	71° 06.44′	11° 27.76′	277.2
Nematoflustra flagellata Waters, 1904	70° 56.42′	10° 31.61′	284.4
Notoplites drygalskii (1) Kluge, 1914	70° 57.11′	10° 33.32′	337.2
Notoplites drygalskii (2) Kluge, 1914	71° 04.30′	11° 33.92′	308.8
Smittina antarctica Waters, 1904	71° 06.44′	11° 27.76′	277.2
Systenopora contracta Waters, 1904	71° 06.44′	11° 27.76′	277.2