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Antibacterial Activity of Two Species of Bryozoans from Northern Puget Sound

Abstract

For the first time, bryozoan species from northern Puget Sound have been shown to contain antibacterial compounds. The antibacterial activity of two local marine cheilostome species was tested against six strains of local marine bacteria and against stock cultures of *Vibrio anguillarium*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. The filter paper disc method was used to test for antibacterial activity. A crude extract made from the bryozoan *Bugula pacifica* inhibited the growth of two marine isolates, as well as *B. subtilis*, *S. aureus*, and *E. coli*. A crude extract made from the bryozoan *Tricellaria occidentalis* inhibited the growth of *B. subtilis*. Preliminary scanning electron microscopy data indicate that *Tricellaria occidentalis* had higher densities of surface bacteria than *Bugula pacifica*. This inverse relationship between antibacterial activity and surface fouling may indicate an antifouling role for these bryozoan secondary metabolites. The presence of antibacterial compounds may allow bryozoans to manipulate the microbial film growing on them, and may influence the types of organisms that are able to settle near or on them. The ability to manipulate microbial films may also enable bryozoans to make the substrate nearby more suitable for the settlement of their own larvae.

Introduction

In the marine environment, most surfaces are eventually colonized by sessile organisms such as bacteria, diatoms, protozoans, algae, and invertebrates. However, the surfaces of many of these organisms remain remarkably free of epibionts. This may be due to adaptations to prevent fouling. Possible antifouling mechanisms include mechanical, physical, and chemical defense mechanisms,which may be used alone or combined (Wahl, 1989). This present study was concerned with the chemical defenses of two local, marine bryozoans.

Bryozoans (Ectoprocta) are sessile, colonial, filter-feeding organisms widely distributed throughout the world's marine and freshwater environments. Many bryozoans are common fouling organisms on marine facilities. There are about 4,000 extant species most of which are marine. The class Gymnolaemata encompasses two orders; Ctenostomata and Cheilostomata comprising 3,000 or more living species (Ryland, 1970).

Many marine invertebrates, including sponges (Thompson et al., 1985; Becerro et al., 1994), ascidians (Davis, 1991), jelly fish, sea anemones, bryozoans, and corals (Bhakuni and Jain, 1990) produce secondary metabolites that have antibacterial properties. Study of the antibacterial properties of these substances is unevenly spread across the taxonomic groups. There is a large amount of literature on sponges, less on ascidians and cnidarians, and even less on bryozoans. However, during the past decade, a number of secondary metabolites from marine bryozoans have been isolated and identified. With a few exceptions, the natural products identified from bryozoans so far are either alkaloids, sterols, or bryostatins (Pettit et al., 1982; Anthoni et al., 1990; Morris and Prinsep, 1996; Newman, 1996). Some of the alkaloids have been found to confer antibiotic activity to the bryozoans that produce them. For example: a tetrapyrrole alkaloid isolated from *Bugula dentata* is antimicrobial against Gram-positive and Gram-negative bacteria (Matsunaga et al., 1986); a flustramine alkaloid isolated from *Flustra foliaceae* L. showed strong activity against the bacterium *Bacillus subtilis* (Wright, 1984); antibacterial alkaloids have been isolated from *Phidolopora pacifica* (Tischler et al., 1986); and amathamide alkaloids produced by *Amathia wilsoni* are antibacterial (Walls et al., 1993). Antibacterial secondary metabolites have been found in bryozoans from Tasmania (Walls et al., 1993), the Mediterranean (Uriz et al., 1991), south India (Nair, 1993), the United Kingdom (Al-ogily and Knight-Jones, 1977), Japan (Matsunaga et al., 1986), Scandinavia (Wright, 1984), and the west coast of Canada (Tischler et al., 1986). To date, bryozoans from northern Puget Sound have not been assayed for antibacterial secondary metabolites.

Once it is known that an animal such as a bryozoan contains antibacterial secondary metabolites the question arises of how these secondary metabolites affect the ecology of the animal. Antibiotic compounds may allow bryozoans to manipulate the composition of the bacterial film in their immediate vicinity. This could provide the bryozoans with some control over the types of organisms that are able to settle around them or on them (Al-ogily and Knight-Jones, 1977; Uriz et al., 1991; Walls et al., 1993) or may make the substrate more suitable for the settlement of bryozoan larvae (Brancato and Woolacott, 1982; Maki et al., 1989) or less suitable for the settlement of larvae from bryozoan competitors (Maki et al., 1989; Kon-ya et al., 1994).

The bryozoans, *Bugula pacifica* and *Tricellaria occidentalis*, were collected from northern Puget Sound and tested for the presence of antibacterial secondary metabolites. If these compounds were present, their antibacterial properties were measured along with the population of bacteria on the surface of the colonies. The hypothesis tested was that bryozoans which exhibited antibacterial activity would have a lower density of bacteria on their surfaces than bryozoans that did not contain antibacterial secondary metabolites.

Methods

Specimen collection

Erect bryozoans with little or no calcification of the zooecia were collected for this research, because they are easier to extract. Suitable species were found on buoys at two local marinas. On 23 July, 28 July, 4 August, 13 August, and 20 August 1995, *Bugula pacifica* (Figure 1) colonies were collected from the bottom of buoys in Cap Sante Marina, Anacortes, Washington (48° 30' N, 122° 40' W, Figure 2). These collections were made a week apart so as to determine if the concentration of antibacterial compounds in *B. pacifica* changes over time and if the number of bacteria growing on its surfaces changes over time. On 25 August 1995, *B. pacifica* colonies were collected from the bottom of buoys in Point Hudson Marina, Port Townsend, Washington (48° 10' N, 122° 50' W, Figure 2). On 23 July 1995, another suitable bryozoan species, *Tricellaria occidentalis* (Figure 3), was found by snorkeling during a spring low tide at Larrabee State Park (48° 40' N, 122° 30' W, Figure 2). Colonies of this bryozoan were collected from a rock wall. The population of *T. occidentalis* was not large enough for multiple collections.

Back at the laboratory, a dissecting microscope was used to view each bryozoan colony, and fouling organisms were removed using forceps. During this procedure, the bryozoans were kept in cold sea water. After this cleaning procedure, a few colonies from each collection were placed in 95% ethanol for species identification and scanning electron microscope work. The sea water was then drained off the remaining colonies, and they were stored at -20 °C until extracted.

Bacteria used in assays

Twenty-nine strains of marine bacteria were cultured from sea water collected from Cap Sante Marina and Larrabee State Park on 13 August 1995. The water was collected close to where the bryozoan colonies were found using sterile polycarbonate containers, which were opened under water to collect the water samples. The water samples were transported to the lab in a cooler. 10^{-1}, 10^{-2}, and 10^{-3} dilutions were made from each water sample using sterile Bacto Marine Broth 2216 (Difco). 0.1 ml of each dilution were plated on Bacto Marine Agar 2216 (Difco). The plates were incubated in the dark at room temperature, and within two to four days, individual colonies formed. The 10^{-2} dilution yielded the most isolated colonies. The colonies were initially characterized by their size, morphology, growth rate, and color. The individual colonies were then plated, tested for purity and characterized by Gram stain, cell size, and cell morphology. Using hanging drop slides, motility was determined only for the bacteria used in the antibacterial assays. The bacteria were maintained in Bacto Marine Broth 2216 and on slants of Bacto Marine Agar 2216. Five strains of marine bacteria were randomly selected from the 29 marine isolates for use in the antibacterial assays (Table 1). The sixth isolate used, Larrabee State Park 9, was chosen because it was the only Gram-positive isolate.

Four commercial cultures were also used in the antibacterial assays. They were chosen for their Gram stain. *Bacillus subtilis* and *Staphylococcus aureus* are Gram-positive and *Escherichia coli* and *Vibrio anguillarum* are Gram-negative. *V. anguillarum* was also chosen, because it is a

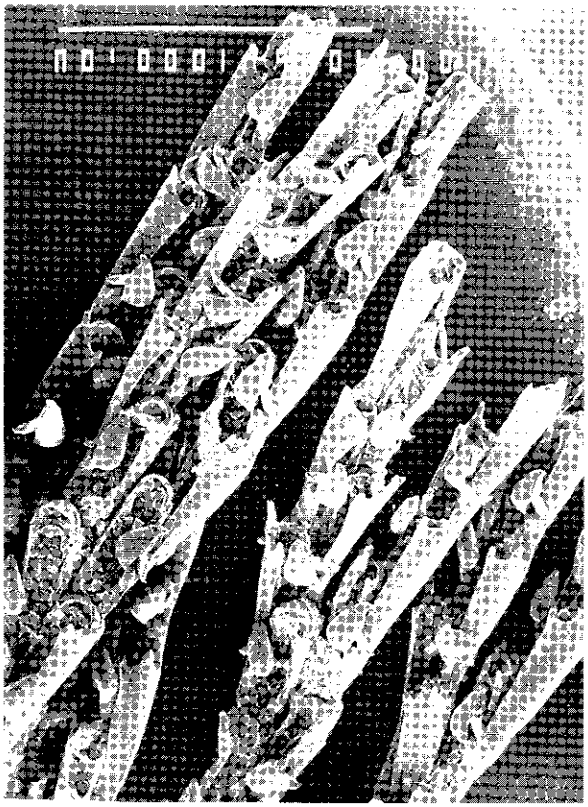

Figure 1. Scanning electron micrograph of *Bugula pacifica*. The scale bar is 1 mm and the magnification is X40.

marine bacterium. The nonmarine cultures were maintained in Nutrient Broth (Difco) and on Nutrient Agar (Difco) slants, and the *V. anguillarium* was maintained in Bacto Marine Broth 2216, and on Bacto Marine Agar 2216 slants.

Extraction

A preliminary extraction indicated that the samples of *Bugula pacifica* collected over time from Cap Sante Marina were too small to detect changes in antibacterial activity over time, so those samples were combined for extraction. The colonies were ground in liquid nitrogen and then lyophilized. During the lyophilization, the containers with the ground bryozoan samples were placed in liquid nitrogen, because the vacuum produced by the lyophilizer was not sufficient to prevent the samples from melting. Each sample was extracted overnight three times with distilled dichloromethane (DCM) in a proportion of 0.1 g dry weight in 10 ml of solvent (Walls et al., 1993; Becerro et al., 1994). The crude extracts were evaporated under reduced pressure and the residue was weighed. The residues were stored at -20 °C until tested for antibacterial activity.

Antibacterial testing

For antibacterial testing, 3 ml of DCM were added to the Cap Sante Marina extract of *Bugula pacifica*, and 2 ml were added to the Larrabee State Park extract of *Tricellaria occidentalis* and to the Point Hudson Marina extract of *Bugula pacifica*. Less DCM was added to the last two samples, because fewer colonies used were to make the extracts. This procedure should have maximized the antibacterial activity of the extracts and should have equalized the concentration of antibacterial compounds in all three extracts. DCM is highly volatile, so 2 ml were the minimum of DCM that was needed to soak filter paper discs for all three

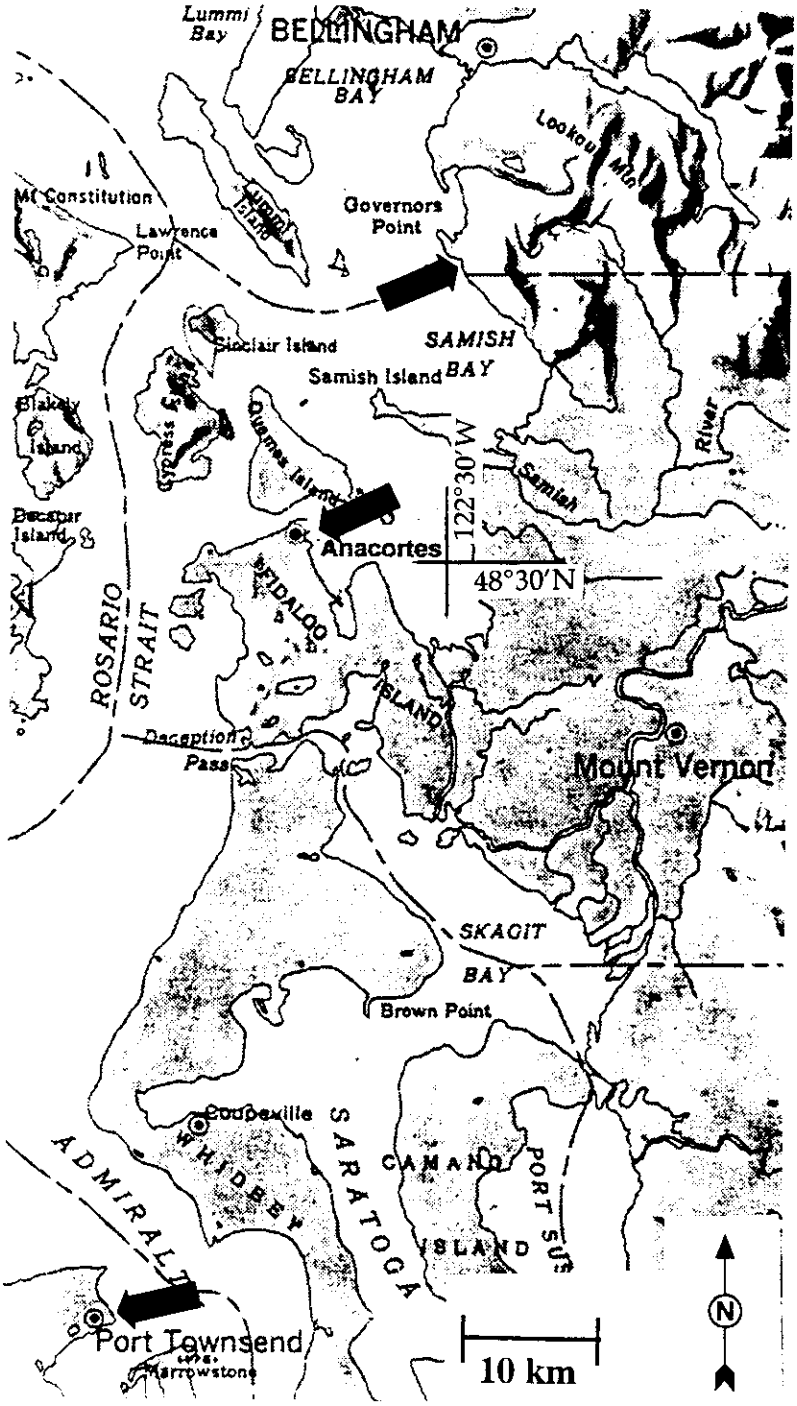

Figure 2. Map showing the three collection sites in northwest Washington State. The northernmost site is a small harbor at Larrabee State Park, the second site is Cap Sante Marina, Anacortes, and the southernmost site is Point Hudson Marina, Port Townsend. Collection sites are marked with arrows.

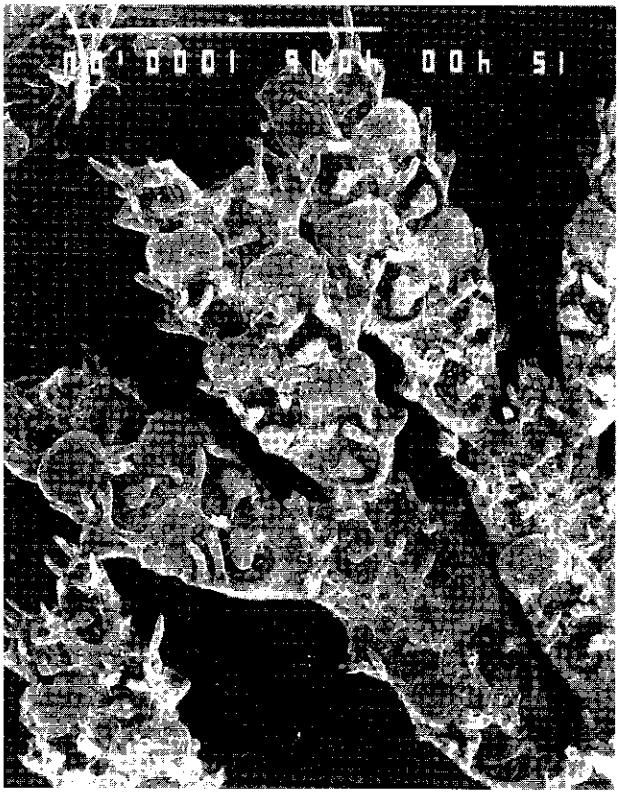

Figure 3. Scanning electron micrograph of *Tricellaria occidentalis*. The scale is 1 mm and the magnification is X40.

replicates. Each extract was tested against the six strains of marine bacteria and against the four commercial cultures. The antibacterial assays were performed using the filter paper disc method developed by Berquist and Bedford (1978). A lawn of each species of bacteria was spread on petri plates prepared with Bacto Marine Agar 2216. Four filter paper discs (6 mm diameter) were impregnated with 5 µl of extract, and one control disc was impregnated with 5 µl of DCM. The discs were placed on the bacterial lawns, and the plates were incubated for 24 h at 24 °C. Three replicates were run, and the average diameter of zones of inhibition (including the disc) was recorded.

Scanning electron microscopy

Bryozoan colonies of *Tricellaria occidentalis* from Larrabee State Park and the weekly collections of *Bugula pacifica* colonies from Cap Sante Marina were examined at the University of Washington's Friday Harbor Laboratories using a JEOL JSM 35 scanning electron microscope (SEM). The colonies were stored in 95% ethanol for 5 months at room temperature before being examined by SEM. For the SEM work, the colonies were then dehydrated through an alcohol series, treated with hexamethyldisalizane for 15 min., air dried, mounted on stubs with double sided-tape and silver paint, and sputter coated with gold and palladium. The magnification of the SEM was adjusted to produce a display size of 100 μm^2. The number of bacteria and their cell morphology was recorded on 12 spine tips and 12 zooecia on the weekly bryozoan samples of *Bugula pacifica* from Cap Sante Marina, and they were recorded on 12 spine tips and 12 scuta on the bryozoan colonies of *Tricellaria occidentalis* from Larrabee State Park. Therefore, the total area viewed per location on each colony was 1200 μm^2.

Bryozoan species identification

An initial identification of each species was made as the bryozoans were collected. Colonies which

TABLE 1. Marine bacteria isolated from water at Cap Sante Marina and Larrabee State Park. The isolates were characterized according to Gram stain, size, and morphology. The isolates were characterized according to Gram stain, size, and morphology. The isolates used in the antibacterial assays were also tested for motility, and are marked with an asterisk (*). Size refers to length if bacillus or spirillum and to diameter if coccus.

Collection site	Stain no.	Gram stain	Size (μm)	Morphology	Motility
Cap Sante Marina	1*	–	2.5-5	bacillus	+
	2*	–	4	bacillus	+
	3	–	0.5	bacillus	n.a.
	4	–	2.5-7.5	coccus	n.a.
	5	–	1.25	coccus	n.a.
	6	–	2.5	coccus	n.a.
	7*	–	6.25	bacillus	–
	8	–	2.5	bacillus	n.a.
	9	–	2.5	coccus	n.a.
	10	–	2.5	bacillus	n.a.
	11*	–	1.88	bacillus	+
	12	–	2-5	bacillus	n.a.
	13	–	3	bacillus	n.a.
	14	–	1	bacillus	n.a.
	15	–	2.5	bacillus	n.a.
	16*	–	2.5	spirillum	+
	17	–	1.88	coccus	n.a.
Larrabee State Park	1	–	2.5-5	bacillus	n.a.
	2	–	1.25	bacillus	n.a.
	3	–	2.5	bacillus	n.a.
	4	–	2.5	bacillus	n.a.
	5	–	2.5	bacillus	n.a.
	6	–	1.88	coccus	n.a.
	7	–	1.25	coccus	n.a.
	8	–	2.5	bacillus	n.a.
	9*	+	2.5	bacillus	–
	10	–	3.5	coccus	n.a.
	11	–	2.5	coccus	n.a.
	12	–	2.5	coccus	n.a.

had been stored in ethanol were later verified to species using keys by Bergey and Denning (1987), Gordon (1986), and Osburn (1950). Scanning electron micrographs were also used in keying out the species.

Numerical methods

Zones of inhibition were used only as an indication of antibacterial activity, and no attempt was made to compare them using statistics, because the chemical nature of the bryozoan secondary metabolites tested is unknown and presumably different. Therefore, they would diffuse in agar at different rates and to different extents. Thus, zones of inhibition cannot be used to compare directly the potencies of different chemical compounds.

Because the number of bryozoan colonies used to estimate the density of surface bacteria was small, parametric statistics were not used to analyze the data. Standard errors were used to provide an indication of the precision of the estimation of the population mean (Sokal and Rohlf, 1981; Zar, 1974). Thus, counts of bacteria are reported as means with standard errors, and if the error bars in the histograms reported here do not overlap, the means are probably different.

Results

At both Cap Sante Marina and Point Hudson Marina, *Bugula pacifica* was found on the bottom of buoys which were in the early phases of fouling. *B. pacifica*'s main competitor for space on the buoys was the compound ascidian *Distaplia*

occidentalis, which was the only animal observed to overgrow *B. pacifica*. The *B. pacifica* colonies collected from Cap Sante Marina on 21 August were fouled with algae and had many caprellid amphipods living on them. There were also many caprellid amphipods living on the *B. pacifica* colonies collected from Point Hudson Marina on 25 August. *Tricellaria occidentalis*, collected at Larrabee State Park, was found attached either directly to a vertical rock face, or to holdfasts of the green alga *Ulva* and the bases of the red alga *Prionitis*, both of which were also attached to the rock face. In general, the colony bases of both bryozoan species had more marine debris on them than the colony tips. The *B. pacifica* colonies were 3-5 cm tall, and the *T. occidentalis* colonies were 1-2 cm tall.

All the marine bacteria isolates used in the antibacterial assays were Gram-negative, except one (Table 1). Their size ranged from 1.88-6.25 μm. Five of the six marine isolates used in the antibacterial assays were bacillus and one was spirillum. Four of the six were motile.

During the extraction of *B. pacifica*, the DCM turned a pea soup green color, and it was cloudy. During the extraction of *T. occidentalis*, the DCM turned a yellowish-green color, but was not cloudy. After the DCM was evaporated off the extracts, the *B. pacifica* residue was oily and green, and the *T. occidentalis* residue was oily and yellowish-brown. The residue from the *B. pacifica* colonies collected from Cap Sante Marina was 0.024 % of the sample dry weight, and the residue from the *B. pacifica* colonies collected from Point Hudson Marina was 0.050 % of the sample dry weight. The residue from the *T. occidentalis* colonies was 0.026 %. These residue weights are approximate, because some sediment remained in the final extracts, especially in the extracts made from the bryozoans collected at Point Hudson Marina and Larrabee State Park.

The extract made from the *B. pacifica* colonies from Cap Sante Marina inhibited two cultures of marine bacteria: CS 2 and CS 16, and three nonmarine bacteria: *B. subtilis*, *S. aureus*, and *E. coli* (Table 2). The extract made from the *B. pacifica* colonies from Point Hudson Marina inhibited one marine bacterium: CS 16, and two nonmarine bacteria: *B. subtilis* and *S. aureus*. The extract made from the *T. occidentalis* colonies inhibited the nonmarine bacterium *B. subtilis*.

From the SEM analysis, the growth of surface bacteria on *B. pacifica* varied over time. The mean surface density of bacteria on *B. pacifica* on 28 July 1995 was 0.7 ± 0.2 bacteria/100 μm^2 (Table 3). The density increased to 5.0 ± 1.4 bacteria/100 μm^2 on 4 August 1995, and it decreased slightly to 4.4 ± 0.8 bacteria/100 μm^2 on 13 August 1995. By 21 August 1995, the mean surface density of bacteria on the surface of *B. pacifica* had decreased to 2.3 ± 0.8 bacteria/100 μm^2.

The density of surface bacteria on *T. occidentalis* (collected on 23 July 1995) was three times greater than that found on the *B. pacifica* colonies (collected on 28 July 1995). *T. occidentalis* had a mean surface density of bacteria of 2.1 ± 0.8 bacteria/100 μm^2 compared to a mean surface density of bacteria of 0.7 ± 0.2 bacteria/100 μm^2 on *B. pacifica* (Table 3). In both bryozoan species, the mean surface density of bacteria on the spine

TABLE 2. Assay results for crude extracts from two species of marine bryozoans collected in July and August, 1995 in northern Puget Sound, Washington. The assay for antibacterial activity of extracts was scored doubly positive (++) if the zone of inhibition was between 1 and 2 mm from the edge of the disc, positive (+) if the zone was ≤ 1 mm from the edge of the disc, and negative (–) if there was no inhibition of bacterial growth. CS—Cap Sante Marina; PH—Point Hudson Marina; LSP—Larrabee State Park.

	Marine bacteria						Nonmarine bacteria			
Bryozoan and (collection site)	CS 1	CS 2	CS 7	CS 11	CS 16	LSP 9	*Vibrio anguillarum*	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
Bugula pacficia (CS)	–	++*	–	–	+	–	–	++	+	+
Bugula pacifica (PH)	–	–	–	–	+*	–	–	+	+	+
Tricellaria occidentalis (LSP)	–	–	–	–	–	–	+	–	–	

*Zone of inhibition only seen in the third replicate.

TABLE 3. The density of surface bacteria on different areas of *Bugula pacifica* and *Tricellaria occidentalis*. Counts were made using scanning electron microscopy. Values are the mean number of bacteria/100 μm^2 with standard errors.

Species		Location	Count	Filamentous	Mean Cocci	Mean Bacilli
B. acificia	28-Jul-95	Spine tip	12	no	0	
		Zooecium	12	no		
	4-Aug-95	Spine tip	12	no		
		Zooecium	12	no		
	13-Aug-95	Spine tip	12	yes		
		Zooecium	12	yes		
	21-Aug-95	Spine tip	12	yes		
		Zooecium	12	yes		
T. occidentalis	23-Jul-95	Spine tip	12	no	0	
		Scutum	12	yes		

tips was lower than that on the zooecium or scutum (Table 3).

The growing tips of the branches of *B. pacifica* usually had a lower surface density of bacteria than the bases of the branches. The growing tips of *T. occidentalis* did not have any bacteria on them. In *B. pacifica*, the distance from the growing tip to the base of a branch is 3-5 mm, and in *T. occidentalis* it is 2 mm (3 zooecia).

Rod-shaped bacteria were the dominant type of bacteria on the surfaces of *B. pacifica* and *T. occidentalis*. They were also the most dominant type of bacteria isolated from the water surrounding the bryozoans. A small amount of filamentous bacteria was observed on the *B. pacifica* colonies on 13 August 1995. By 21 August 21. 1995, there were many more filamentous bacteria present on the colony surface. Filamentous bacteria were also observed on *T. occidentalis*.

Discussion

The results from this study indicate that both *Bugula pacifica* and *Tricellaria occidentalis* do contain antibacterial compounds and that the extracts made from *B. pacifica* exhibited more antibacterial activity than the extract made from *T. occidentalis*. These results are particularly exciting when they are combined with the observation that the surfaces of *B. pacifica* had less bacteria growing on them than the surfaces of *T. occidentalis*, suggesting that *B. pacifica* was possibly more effective in deterring microbial epibionts.

Other researchers have also found an inverse correlation between antimicrobial activity and surface fouling, suggesting that antibacterial activity may help a sessile organism such as a bryozoan prevent the growth of epibionts (McCaffrey and Endean, 1985; Walls et al., 1993). Because space is a limiting resource for benthic organisms, they will often settle on each other (Jackson and Buss, 1975). Studies have shown that a surface film of microorganisms (bacteria being the first to arrive) is often required for the settlement of many fouling invertebrates including hydroids, polychaetes, bivalves, gastropods, barnacles, echinoderms, and bryozoans (MacGinitie and MacGinitie, 1949; Crisp, 1984; Pawlik, 1992). Therefore, preventing the growth of epibionts is important, especially for animals like bryozoans which are filter feeders, because for efficient filtration to occur, the colony surface must be kept clean. Macroepibionts can be especially harmful, because they can deplete incoming water of nutrients if they are also filter feeders, or they may alter current patterns at the colony surface (Wahl and Lafargue, 1990). In addition to promoting larval settlement, microbial films may be deleterious in other ways. They may cause chemical attack of the colony surface by slime mold metabolism, they may promote further fouling by creating attachment points or nutrient-rich microenvironments, or they may form an insulating layer between settling larvae and the toxins produced by the organism (Wahl, 1989).

Kon-ya et al. (1994) showed that an extract made from the bryozoan *Zoobotryon pellucidum* inhibited the settlement of barnacle and mussel larvae. This suggests that, in addition to inhibiting the growth of bacteria, the secondary metabolites produced by bryozoans may directly influence

larval settlement behavior. However, to date, the allelochemicals produced by bryozoans have not been measured in the water surrounding them.

It is possible that *B. pacifica* from Cap Sante Marina showed more antibacterial activity not because it has stronger secondary metabolites, but because the extract made from those colonies was more concentrated. The Cap Sante Marina extract of *B. pacifica* contained about four times as many colonies as the extracts of *B. pacifica* from Point Hudson Marina and *T. occidentalis* from Larrabee State Park. However, the samples of *B. pacifica* from Point Hudson Marina and *T. occidentalis* from Larrabee State Park were approximately the same size, and *B. pacifica* from Point Hudson Marina inhibited more species of bacteria than *T. occidentalis*.

There were two cases where zones of inhibition were only seen in the third replicate (Table 2). This is probably due to the highly volatile nature of DCM; 0.6 ml of each extract were used for the antibacterial assays, but by the time each assay was finished, at least twice that much DCM had evaporated from the extracts. Thus, the extracts became more concentrated over time.

The use of the filter paper disc method to measure the amount of antibacterial activity has its limitations, because lipid soluble compounds in the DCM extract do not diffuse into agar well, and other compounds may diffuse at different rates and amounts. Therefore, this method cannot be used to compare directly the strength of different compounds. Nevertheless, this method is widely used, because it is an easy and accurate method for detecting the antibacterial activity of various compounds (Berquist and Bedford, 1978; Walls et al., 1993).

The number of bacteria on the surface of *B. pacifica* did change over time. However, the decrease in the number of bacteria on August 21 was not expected. On the contrary, an increase in the number of bacteria throughout the sample period was expected. Because it was not possible to test for changes in antibacterial activity over time, we do not know if the changes in the number of bacteria that were observed are linked to variable production of antibacterial compounds by the bryozoan or if they are due to natural, seasonal cycles of bacterial growth.

It is possible that the differential distribution of bacteria between the spine tips, which are above the zooid surface, and the zooecium or scutum is due to a differential distribution of antibacterial compounds within the zooid. However, a more likely explanation is that it is related to zooid morphology. The spine tips are more exposed to water currents than the rest of the zooid. This may make them less suitable for the settlement and growth of bacteria.

The differential distribution of bacteria between the growing tips and more proximal portions of the colony may be due to a differential distribution of antibacterial compounds within the colony. This was shown to be true for the bryozoan *Amathia wilsoni*. In *A. wilsoni*, higher concentrations of antibacterial compounds were measured in the growing tips than in the basal portions of the colony, and samples taken midcolony had intermediate concentrations. The distribution of bacteria on the surface of *A. wilsoni* was patchy and could be related to the distribution of antibacterial compounds within the colony (Walls et al., 1991; Walls et al., 1993). Again, morphology may also be a factor. The growing portions of the colony are more exposed to water currents, and so they may not be favored by bacteria for settlement and growth. Age may be another factor influencing the distribution of bacteria on the colony. Proximal portions of the colony are older, so bacteria have had more time to settle and to grow on these parts of the colony.

We were unable to determine if the bryozoan extracts contained alkaloids. However, in most cases, the secondary metabolites isolated from bryozoans have been found to be alkaloids (Christophersen, 1985; Anthoni et al., 1990; Prinsep and Morris, 1996). It is likely that the active secondary metabolites isolated from *B. pacifica* and *T. occidentalis* have an alkaloid component.

This study initiates efforts to characterize the chemical nature of bryozoans from Pacific Northwest waters and to understand the role of these metabolites. However, much remains to be done. The question of whether or not the concentration of antibacterial compounds in these bryozoans changes over time remains unanswered. Unfortunately, the small size of these animals limits the amount of extract that can be made from them, so a more precise way to measure antibacterial activity than the filter paper disc method may be needed to answer this question. An attempt needs to be made to measure biologically active

secondary metabolites in the water surrounding *B. pacifica* and *T. occidentalis*. This may be possible using a submersible apparatus developed by Coll et al. (1982). The apparatus permits *in situ* sampling of allelochemicals released from sessile marine organisms. It would also be interesting to see if extracts from these bryozoans can inhibit the settlement of larvae from their competitors or perhaps enhance settlement of their own larvae. The active components of the extracts need to be isolated and characterized using HPLC. More extensive SEM work would be useful, especially if counts of bacteria were made on colonies collected over a longer period of time. Studies of this type have the potential to lead to a greater understanding of the ecology of fouling communities. The presence of chemical defenses within even a small proportion of sessile organisms in a community may influence the community structure by influencing patterns of grazing, larval settlement, and adult interrelationships.

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