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V REDEALGAS WORKSHOP (RIO DE JANEIRO, BRAZIL)

Extracts of seaweeds as potential inhibitors of quorum sensing and bacterial growth

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Abstract Macroalgae are an important source of antimicrobial compounds. However, it is unclear if these compounds are produced by the algae themselves, by their associated bacteria, or by both. The main aim of this study was to investigate the potential of macroalgae and their associated microorganisms to inhibit bacterial quorum sensing (QS) and growth. Before extraction, half of the algal specimens were treated with 30% ethanol to remove surface associated bacteria. *Canistrocarpus cervicornis* extracts were able to inhibit QS of the reporter *Chromobacterium violaceum* CV017, where extracts with associated bacteria were more efficient than those without bacteria. However, not all algal extracts that inhibited QS of CV017 were able to inhibit bacterial attachment of *Pseudomonas aeruginosas* PA01, showing specific activity of algal metabolites. Only 58% of the extracts showed antibacterial activity against eight marine fouling and pathogenic bacterial strains tested. Our data suggests that algae and their associated microbiota are important sources of antimicrobial compounds which potentially can be used in future biotechnological applications.

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Introduction

Marine biofouling is a process in which there is undesirable colonization and growth of bacteria, algae, and sessile invertebrates on submerged surfaces, both natural (rock, wood, marine organisms) and man-made (piers, decks, hulls, buoys, platforms) (Wahl 1989; Da Gama et al. 2009; Holm 2012). Biofouling causes huge problems to the naval industry by increasing vessel drag force leading to loss of speed, higher fuel consumption and increased metal corrosion (Yebra et al. 2006; Schultz et al. 2011). In addition to economic losses, biofouling promotes the introduction of non-indigenous marine species worldwide which can result in a reduction in native biodiversity, alterations in species interactions, and nutrient cycles (Carlton and Geller 1993; Gollasch 2002; Blackburn et al. 2014).

Several antifouling technologies have been developed to prevent biofouling on man-made structures (Chambers et al. 2006). In general, fouling on boats and vessels is prevented by the use of antifouling paints that contain toxic biocides. Paints containing organotin compounds, such as tributylin (TBT), have been used for many years to prevent biofouling (Coelho et al. 2006). However, several studies have shown that TBT can cause many adverse ecotoxicological effects against marine invertebrates and vertebrates (Abarzua and Jakubowski 1995; Coelho et al. 2006; Grondin et al. 2007). As result, the use of TBT has been prohibited worldwide since 2008 (Hellio 2010) and new non-toxic antifouling solutions are urgently required.

As an alternative to conventional antifouling paints, natural products extracted from marine organisms are a promising

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research field (Abarzua et al. 1999; Bhadury and Wright 2004). Antifouling activity from marine algae has been reported by several authors (Schmitt et al. 1995; De Nys et al. 1996; Steinberg et al. 1998; Hellio et al. 2001; Da Gama et al. 2002). Macroalgae are a rich source of natural bioactive products which present antibacterial, antialgal, antifungal, antiprotozoan, and antimacrofouling properties (Bhadury and Wright 2004). These biogenic agents are generally produced by marine algal species and/or their associated bacteria as secondary metabolites (Egan et al. 2000). For example, it has been shown that not only the green alga, *Ulva reticulata* (Chlorophyta) produces antifouling compounds (Harder et al. 2004), but also the thallus-associated bacteria (Dobretsov and Qian 2002). Thus, the true biosynthetic origin of isolated molecules from algae is not yet well understood (Goecke et al. 2010). There is growing interest in investigating the role of microorganisms associated with algae as a source of natural bioactive substances (Egan et al. 2008).

Marine microorganisms present advantages as source of bioactive compounds compared to other marine organisms. Microorganisms can produce compounds more rapidly and in larger amounts than macroorganisms; moreover, they can be easily modified genetically and chemically in order to increase the yield of the compound and its bioactivity (Dickschat et al. 2005; Cho et al. 2012). Furthermore, it has been shown that bacteria have a mechanism that allows coordination of various functions such as biofilm formation and production of the secondary metabolites through a process termed quorum sensing (Dobretsov et al. 2009).

Quorum sensing (QS) is a cell-to-cell communication mechanism that is based on the production, release, and perception of membrane-diffusible signal autoinducer molecules (Waters and Bassler 2005; Antunes et al. 2010). The most studied QS molecules are acyl-homoserine lactones (AHLs), produced by Gram-negative bacteria (Waters and Bassler 2005; Galloway et al. 2011). Various macroalgae are able to stimulate, inhibit, or inactivate bacterial QS signals (Maximilien et al. 1998; Joint et al. 2007; Kanagasabhapathy et al. 2009). For example, the red macroalga *Delisea pulchra*, secretes halogenated furanones which are structural analogs to AHLs. These furanones protect the algal surface by interfering with AHL-regulated processes and inhibiting bacterial colonization and biofilm formation (Maximilien et al. 1998; Rasmussen et al. 2000; Manefield et al. 2002). Similarly, Kanagasabhapathy et al. (2009) reported that certain epibiotic bacteria from the brown macroalga *Colpomenia sinuosa* may play a role in defense mechanisms, preventing the settlement of other competitive bacteria by producing QS inhibitors (QSI). Recently, Batista et al. (2014) showed that 20 out of 22 polar extracts of macroalgae from Arraial do Cabo, Brazil, inhibited the QS of the reporter *Chromobacterium violaceum* CV017. Algae with associated bacteria demonstrated higher QSI bioactivity than those without them. However, only 11 species of macroalgae were evaluated and there are many other macrolgae species (Brasileiro et al. 2009) to be investigated.

In the present study, we investigated and compared QSI, biofilm formation, and antibacterial activity of the crude extracts of six marine algae from the Brazilian coast with associated microbiota versus ones without them. The aim of this study was to investigate if biosynthetic origin of QSI compounds from macroalgae is produce by associated microorganisms, being a potential source for the paint industry.

Material and methods

Marine macroalgae were collected manually from the intertidal zone in Arraial do Cabo, Rio de Janeiro, southeast Brazil (22° 57′ 56.65″ S, 42° 01′ 40.75″ W). This region is strongly affected by coastal upwelling of the South Atlantic Central Water which has high nutrient concentrations and increases the primary productivity of the region (Gonzalez-Rodriguez 1994). A total of six abundant algal species were sampled: *Canistrocarpus cervicornis* (Kützing) De Paula and De Clerck, *Sargassum vulgare* C. Agardh, *Colpomenia sinuosa* Mertens ex Roth Derbès and Solier, *Padina* sp. (Ochrophyta) and *Spyridia aculeata* (C. Agardh ex Decaisne) Kützing, *Pterocladiella capillacea* (S.G. Gmelin) Santelices and Hommersand (Rhodophyta). The voucher specimens were deposited in the scientific collection of Instituto de Estudos do Mar Almirante Paulo Moreira under the numbers 001154, 001155, 001156, 001158, 001159, and 001160, respectively. After collection, epiphytes were removed and samples were rinsed with sterile seawater to remove associated debris.

Preparation of extracts

The cleaned material was surface dried by briefly pressing it between sheets of paper towels and air dried in the shade at room temperature for 24 h. Half of the specimens were washed with 30% ethanol for 10 min to remove microorganisms associated with the macroalgae (Hellio et al. 2001; Kientz et al. 2011). This method was proven to be effective in bacterial removal in our previous study (Batista et al. 2014). The other half of the samples was not treated. Algae were cut into small pieces and immediately extracted in methanol and dichloromethane (mixture 1:1) for 24 h at room temperature. Crude extracts were evaporated under nitrogen by using Zymark *TurboVap* II (Concentration Workstation, Caliper Life Sciences, USA). The concentrated extracts were transferred into small vials and evaporated to dryness. The dry weight of extracts was determined using an analytical balance to the nearest 0.001 g (Table 1). The extracts were kept in the fridge at +4 °C until used in bioassays.

QS inhibition bioassays

QSI activities of extracts were tested using the reporter strain *Chromobacterium violaceum* CV017. This biosensor strain produces N-hexanoyl homoserine lactone (C6-HSL), which induces production of the purple pigment violacein (Chernin et al. 1998). Inhibition of coloration in the assay in the presence of extracts indicates that they are inhibiting short-chain QS activity.

In order for all the algae to be subject to the same concentration of the extract, the weight of each algal specimen was divided by the weight of the lightest specimen. The concentration tested was 2.4 mg mL^{-1}. The assay was performed according to Choo et al. (2006). Before the experiment, the reporter *C. violaceum* CV017 was grown overnight in Luria-Bertani (LB, Sigma) broth. The extracts were applied to sterile microtiter plates (Nunc, Denmark). Antibiotic streptomycin (Sigma-Aldrich) at 100 mg mL^{-1} was used as a positive control and wells with 10 μL of the 1:1 methanol and dichloromethane were used as a negative control. Each treatment was replicated four times. Samples were evaporated till dryness and 100 μL the reporter *C. violaceum* CV017 culture together with LB broth was applied to each well. The plates were incubated overnight at 30 °C. The bacterial cultures were centrifuged (8000 rpm, 5 min); then, the pelleted cells were lysed with 0.1% sodium dodecyl sulfate (SDS, Sigma) and the pigment violacein was extracted with 1 mL of dimethyl sulfoxide (DMSO, Sigma). The absorbance was read at 595 nm using a spectrophotometer. An average inhibition (%) in comparison with the solvent control was calculated. The experiment was repeated three times to check results reproducibility. Only one experiment was used for statistical analysis and figures.

To test possible toxicity of extracts, the strain *C. violaceum* CV026 was used (Choo et al. 2006). This is a mutant strain of *C. violaceum* which does not produce C6-HSL. Briefly, the bacterial reporter *C. violaceum* CV026 was grown in LB broth (Sigma) before the experiment. The extracts of macroalgae were added to individual wells of 96-well microplates (Nunc) and tested at the same concentration as in the experiment with CV017. 100 μL of the reporter mixed with soft LB agar was added to each well with extracts. Wells with 10 μL of the solvent (1:1 methanol/dichloromethane) were used as negative controls. The plates were incubated overnight at 30 °C. The bacterial growth was determined visually. The clear zone free from bacterial culture was an indicator of extract toxicity.

QSI was also tested using *Pseudomonas aeroginosa* PA01, which uses long-chain AHLs for attachment and biofilm formation (Davies et al. 1998). Bacterial attachment assays were performed in 96-well plates (Nunc) according to Batista et al. (2014). Firstly, the bacterium was cultured in LB broth (Sigma-Aldrich) until the exponential phase of growth. In each well, 100 μL of bacterial culture was inoculated at an optical density of 0.01 at 600 nm (OD_{600}) together with the extracts at the concentrations reported in Table 1. Each treatment was replicated four times. After 2 h of incubation at 27 °C, the wells were emptied and washed with distilled water. Bound cells were stained with 0.2% (wt/vol) crystal violet solution in ethanol at room temperature for 10 min. Then, wells were washed with distilled water three times and dried at room temperature. The dye was solubilized with 95% ethanol. Alteration between the control and the treatment color indicated differences in attachment of tested bacteria. An average inhibition (%) of *P. aeruginosa* attachment in comparison with the solvent control was calculated. The experiment was repeated three times to check results reproducibility. Only one experiment was used for statistical analysis and figures.

Antibacterial bioassays

A disk diffusion assay (Devi et al. 2011) was performed with the Gram-negative marine bacteria strains that are involved in marine fouling (*Vibrio aestuarianus*, *Pseudoalteromonas elyakovii*, *Polaribacter irgensii*, and *Pseudomonas fluorescens*) and pathogenic strains (*Vibrio communis*, *Vibrio alginolyticus*, and *Vibrio coralliilyticus*). Additionally, the same assay was performed with a Gram-positive bacterium strain *Shewanella putrefaciens*, responsible for the corrosion of metal due to its ability to reduce a variety of metals such as Fe and Mn by anaerobic respiration (Nealson and Myers 1992; DiChrisitina and Delong 1993). The marine bacteria were obtained from the collection of the University of Portsmouth.

Before the bioassay, each bacterium was grown in peptone (5 g L^{-1}) at an optical density (O.D) of 1.5–2.0 at 630 nm. Sterile paper disks (diameter = 5 mm) made of Whatman No. 1 paper with extracts (for concentrations see Table 1) were used in the bioassay. The extracts were applied to disks and solvent were evaporated prior the bioassay. The standard antibiotic rifampicin (Sigma-Aldrich) was used as a positive control at concentrations of 1 g L^{-1}. 1:1 methanol/dichloromethane was used as a negative control at a concentration of 10 μL $disk^{-1}$. After 24 h of incubation at 30 °C, the diameter of the inhibition zone around the paper disks was measured with a ruler to the nearest 0.5 mm.

Statistical analysis

The means and the standard error were determined from four replicates of each treatment. The homogeneity and normality of the data were analyzed with Cochran C, Hartley, Bartlett, and Shapiro-Wilk's *W* test, respectively, at a confidence level of 95%.

Statistical significance was determined using bifactorial analysis of variance (ANOVA). Values with $p < 0.05$ were considered significant. Tukey's post hoc test was employed to compare differences between extracts with/without bacteria and also among species.

Table 1 Macroalgae species and their dry biomass used for obtaining the crude extracts, extract yield, and concentrations used in this study

Macroalgae species	Dry weight (g)		Extract yield (%)		Concentration tested (mg mL^{-1})	
	With bac.	Without bac.	With bac	Without bac.	With bac	Without bac.
Canistrocarpus cervicornis	6.1	8.4	13.9	5.6	400	400
Spyridia aculeata	18.8	19.0	5.9	2.0	350	350
Sargassum vulgare	20.9	23.6	8.0	4.0	650	650
Colpomenia sinuosa	34.5	29.5	3.6	2.6	800	800
Padina sp.	19.1	14.6	8.5	4.0	500	500
Pterocladiella capillacea	10.1	10.0	6.3	3.0	250	250

Results

QS inhibition and toxicity bioassays

Figure 1 shows that inhibition of violacein pigment production of *C. violaceum* CV017 varied significantly among treatments (ANOVA, $F = 2.85$, $p < 0.001$). Although most of the extracts with microbes had higher percentage of violacein pigment inhibition production of *C. violaceum* CV017 than without ones, no significant differences were found, with exception extracts of *C. cervicornis* and *S. vulgare* (Tukey, $p < 0.05$). QS inhibition of *C. violaceum* CV017 varied significantly among species, in which both extracts of *C. cervicornis* inhibited significantly the production of violacein when compared with the extract of *S. vulgare* without microorganisms (Tukey, $p < 0.05$). No significant differences were observed among all others extracts of *S. aculeata*, *C. sinuosa*, *Padina* sp., and *P. capillacea* due to the high variability (Tukey, $p > 0.05$) (Fig. 1). In addition, none of the extracts were toxic against the strain *C. violaceum* CV026 in the tested concentrations (data not shown).

Figure 2 shows that attachment of *P. aeruginosa* PA01 varied significantly between treatments with and without associated microorganisms (ANOVA, $F = 19.01$, $p < 0.001$). Extracts of *C. sinuosa* and extracts without microbes of *C. cervicornis* and *Padina* sp. interfered significantly on attachment of *P. aeruginosa* PA01 (Tukey, $p < 0.05$). In contrast, both extracts of *P. capillacea* as well as extracts with associated bacteria of *C. cervicornis* and *S. vulgare* did not inhibit the attachment of the bacteria *P. aeruginosa* PA01 when compared with all other extracts (Tukey, $p > 0.05$) (Fig. 2). Extracts of *C. cervicornis* and *Padina* sp. without associated bacteria significantly inhibited the attachment of *P. aeruginosa* PA01 when compared to extracts with bacteria (Tukey, $p < 0.05$) (Fig. 2). There were also significant differences among the different species of algae, in which extracts without bacteria of *Padina* sp. had a significantly higher inhibition effect than extracts with bacteria of *C. cervicornis* and *S. vulgare* (Tukey, $p < 0.05$), as both extracts of *S. aculeata* and *P. capillacea* (Tukey, $p < 0.05$).

Antibacterial bioassays

The antibacterial potential varied among the crude extracts of macroalgae; however, no significant differences were found between treatments with and without bacteria, except *S. vulgare* (ANOVA, $F = 6.41$, $p < 0.001$) (Table 2). Only the macroalga *C. cervicornis* inhibited the growth of the bacterium *S. putrefaciens*. Extracts without associated bacteria of *S. vulgare* and both extracts of *C. cervicornis* were efficient in inhibiting the growth of the bacterium *V. aestuarians* (Tab. 2). The largest inhibition zones were observed with the antibiotic control and for the extracts of *C. cervicornis* without associated bacteria (17.5 ± 1.7 mm and 9.0 ± 0.9 mm, respectively). None macroalgae extract was able to inhibit the growth of bacteria *P. elyakovii*, *P. irgensii*, *P. fluorescens*, *V. comunis*, and *V. coralliilyticus* (Tab. 2). No activity was found for the extracts of *S. aculeata* and *P. capillacea*. Only extracts with and without bacteria of *S. vulgare* (3.2 ± 1.9 mm and 3.0 ± 1.7 mm) and *C. sinuosa* (6.2 ± 0.2 mm and 5.0 ± 1.7 mm) were effective in inhibiting the growth of the bacterium *V. alginolyticus*.

Discussion

Numerous studies has shown that macroalgae produce various bioactive compounds (Da Gama et al. 2002; Hellio et al. 2009; Plouguerné et al. 2008; Persson et al. 2011). Currently, it has been observed that their associated microsymbionts play an important role in QSI. Kanagasabhapathy et al. (2009) showed that 12% of bacteria isolated from the surface of the macroalga *C. sinuosa* were able to produce compounds inhibitors of bacterial QS. Similarly, Romero et al. (2011) showed that almost 40% of the strains of bacteria isolated from the brown seaweed

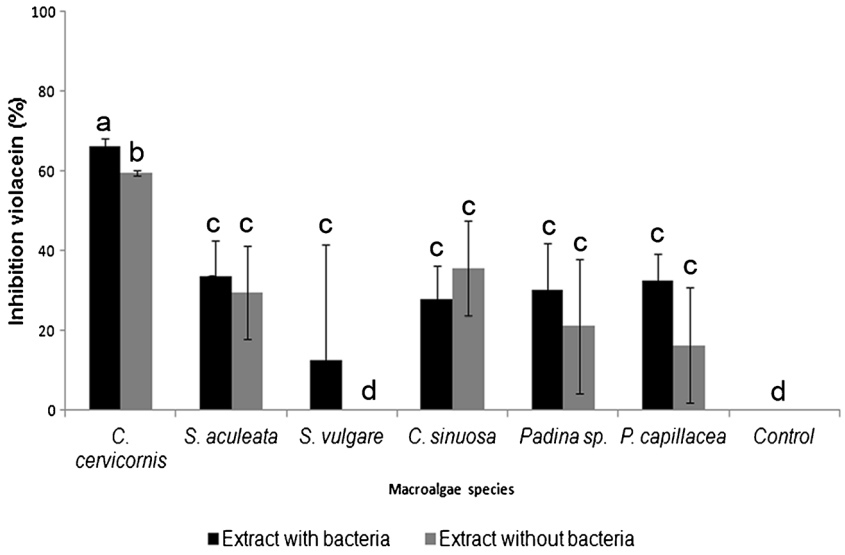

Fig. 1 Inhibition of production of violacein pigment (%) of *Chromobacterium violaceum* CV017 using crude extracts (2.4 mg mL^{-1}) of six macroalgal species. Groups identified by the same *letters* do not differ significantly (Tukey test, $P > 0.05$). Each *bar* represents the mean ± standard error ($n = 4$). Control represents the normal growth of the strain *C. violaceum* in the absence of extracts

Fucus vesiculosus were capable to degrade AHLs, interrupting QS. The present study evaluated the potential of macroalgae with and without microsymbionts as the source of natural non-toxic antifouling compounds. Out of the six algal species tested, only *C. cervicornis* extracts with associated microbes showed significantly higher ability to inhibit QS of *C. violaceum* CV017 when compared to extracts without symbiotic microrganisms. This suggests that mostly macroalgae are responsible for production of QS inhibitory compounds. *C. cervicornis* is known to produce several secondary metabolites exhibiting a wide variety of functions (Bianco et al. 2015a, b). Other studies observed the important role of the macroalgae as source of compound QSI. Halogenated furanones produced by the red macroalga *D. pulchra* (de Nys et al. 1995), 2-dodecanoyloxyethanesulfonate of *Asparagopsis taxiformis* (Jha et al. 2013), and oxidized halogen compounds of *Laminaria digitata* (Borchardt et al. 2001) were found to inhibit bacterial QS.

Recently, Batista et al. (2014) investigated inhibition of QS by extracts of 11 macroalgae with and without microsymbionts collected in the same area as the present study. The investigators found that the extracts of algae with microorganisms but not without them had the higher QSI activity, including species investigated in the present study (*P. capillacea* and *Spyridia* sp.). Differences between our and previous results could be due to differences in the sex of seaweeds and phases of their life cycle (Vegés et al. 2008). Another possibility is seasonal variations of bioactivity of seaweeds and their symbionts (Maréchal et al. 2004; Saha and Wahl 2013; Rickert et al. 2015). Batista et al. (2014) sampled macroalgae during the season with highly pronounced upwelling phenomenon, which lowered water temperature and increased salinity, abiotic factors known to change seasonal production of chemical compounds produced by macroalgae (Sudatti et al. 2011). Possible impact of environmental factors on the production of bioactive compounds by macroalgae and their symbionts should be studied in the future.

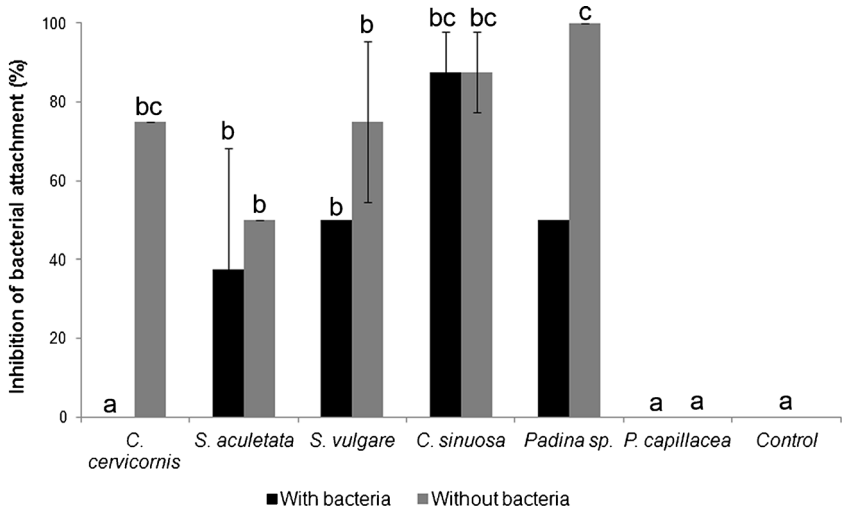

Fig. 2 Mean attachment inhibition (%) of *Pseudomonas aeruginosa* PA01 using crude extracts at tissue-level concentrations ($n = 4$). Groups identified by the same *letters* do not differ significantly (Tukey test, $P > 0.05$). Each *bar* represents the mean ± standard error. Note: The extracts were tested at concentrations shown in Table 1

Table 2 The effect of the macroalgae crude extracts on marine bacteria growth. Extracts were tested at concentrations shown in Table 1. Additionally, 1:1 dichloromethane/methanol was used as a negative control at a concentration of 10 μL disk^{-1}. Antibiotic rifampicin was used at a concentration of 1 g L^{-1} as a positive control

	Macroalgae extracts and controls													
	C. cervicornis		*S. aculetata*		*S. vulgare*		*C. sinuosa*		*Padina* sp.		*P. capillacea*		Control solvent	Control antibiotic
Bacteria species	EWB	ENB	EWB	ENB	EWB	ENB	EWB	ENB	EWB	ENB	EWB	ENB		
Shewanella putrefaciens	6.7 ± 0.2^{b}	8.2 ± 0.5^{b}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	16.2 ± 0.6^{a}
Vibrio aestuarianus	6.7 ± 0.5^{b}	9.0 ± 0.9^{b}	0^{c}	0^{c}	0^{c}	4.5 ± 1.5^{b}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	15.5 ± 2.5^{a}
Pseudoalteromonas elyakovii	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	15.2 ± 1.3^{a}
Polaribacter irgensii	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	10.0 ± 0.4^{a}
Pseudomonas fluorescens	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	10.5 ± 0.8^{a}
Vibrio communis	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	2.0 ± 2^{c}	0^{c}	0^{c}	4.2 ± 4.2^{c}	0^{c}	0^{c}	0^{c}	0^{c}	17.0 ± 1.0^{a}
Vibrio alginolyticus	0^{c}	0^{c}	0^{c}	0^{c}	3.2 ± 1.9^{b}	3.0 ± 1.7^{b}	6.2 ± 0.2^{b}	5.0 ± 1.7^{b}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	16.0 ± 1.2^{a}
Vibrio coralliilyticus	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	17.5 ± 1.7^{a}

Data are the mean diameters ($n = 4$) ± SE of inhibition zones. (0) No activity. Groups identified by the same letters do not differ significantly (Tukey test, $P > 0.05$)

EWB extracts with bacteria, *ENB* extracts without bacteria

None of the extracts at tested concentrations were toxic against *C. violaceum* CV026, indicating that the inhibition effect of the extract was due to interference with bacterial communication rather than killing or inhibition of bacterial growth. Thus, it was indicated that these macroalgae have really potential to producer compound QSI; bacterial communication can be interrupted by different mechanisms: reducing the activity of the AHL cognate receptor protein or AHL synthase, inhibiting the production of QS signal molecules, degradation of the AHL, and mimicking the signal molecules primarily by using synthetic compounds as analogs of signal molecules (Kalia 2013; Dobretsov et al. 2009). More experiments are needed in order to determine the mechanism of QSI of investigated algal species.

Eight out of the 12 macroalgal extracts were seen to be efficient in inhibiting the attachment of *P. aeruginosa*, which uses long-chain AHLs for this process (Davies et al. 1998; Kievit et al. 2001). Considering that attachment is the initial step in the formation of a biofilm (O'Toole et al. 2000), it suggests that the algae *C. cervicornis*, *S. aculeata*, *S. vulgare*, *C. sinuosa*, and *Padina* sp. can prevent biofilm formation of this pathogen. In contrast, as previously observed by Batista et al. (2014), *P. capillacea* did not inhibit the bacterial attachment of *P. aeruginosa*. Probably, this intraspecific difference is related to seasonal variation in the production of compounds by seaweed (Maréchal et al. 2004; García-Bueno et al. 2014), since collection was performed in distinct period of the year where upwelling influence varied.

Macroalgae are also being widely investigated as an important source of antibacterial compounds (Hellio et al. 2000; Bhadury and Wright 2004; Thabard et al. 2009, 2011; Persson et al. 2011). However, in the present study, it was shown that few algal species are able to produce antibacterial compounds against some fouling and pathogenic bacteria. *C. cervicornis* was the most effective one and inhibited the growth of the bacteria *S. putrefacies* and *V. aestuarianus*. It is known that this alga produces mainly dolastane and secodolastane diterpenes, which exhibit antiviral (Vallim et al. 2010), antiophidian (Santos et al. 2011), and antileishmanial (Moura et al. 2011) activities. Furthermore, *C. cervicornis* produces diterpenes capable of inhibiting mussel byssal formation (Bianco et al. 2009). In our study, *Padina* sp. and *C. sinuosa* inhibited the growth of the bacteria *V. communis* and *V. alginolyticus*, respectively. It has been previously reported that some brown algae are capable of inhibiting growth of various bacteria, such as *Bacillus* (Val et al. 2001), *Vibrio*, *Pseudoalteromonas*, and *Polaribacter* species (Thabard et al. 2011). *Sargassum vulgare* inhibited the growth of the *Vibrio* species suggesting that this species possesses active compounds which protect them against fouling and pathogenic bacteria. This genus is known to produce many antibacterial compounds against fouling and corrosive bacteria (Plouguerné et al. 2010a, b).

Our results suggest that macroalgae and their microsymbionts from Arraial do Cabo have a promising potential for both QSI and antibacterial activity. More detailed analysis of the microbiota associated with macroalgae along with isolation and identification of bioactive compounds from algae and their symbionts is required.

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