J Ind Microbiol Biotechnol (2003) 30: 433–439
DOI 10.1007/s10295-003-0068-7

ORIGINAL PAPER

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Antibiotic activity of lectins from marine algae against marine vibrios

Received: 14 February 2003 / Accepted: 8 May 2003 / Published online: 23 July 2003

Abstract Saline and aqueous ethanol extracts of marine algae and the lectins from two red algal species were assayed for their antibiotic activity against marine vibrios. Experimental studies were also carried out on the influence of environmental factors on such activity, using batch cultures. The results indicated that many of the saline extracts of the algal species were active and that the activity was selective against those vibrios assayed. The algal extracts were active against *Vibrio pelagius* and the fish pathogen *V. vulnificus*, but inactive against *V. neresis*. Algal lectins from *Eucheuma serra* (ESA) and *Galaxaura marginata* (GMA) strongly inhibited *V. vulnificus* but were inactive against the other two vibrios. The antibacterial activity of algal extracts was inhibited by pretreatment with various sugars and glycoprotein. Extracts of the two red algae, *E. serra* and *Pterocladia capillacea,* in saline and aqueous ethanol, inhibited markedly the growth rate of *V. vulnificus* at very low concentrations. Culture results indicated that metabolites active against *V. vulnificus* were invariably produced in *P. capillacea* over a wide range of temperature, light intensity, and nutritional conditions. Enhanced antibacterial activity occurred when *P. capillacea* was grown under higher irradiance, severe nutrient stress and moderate temperature (20 °C), reflecting the specific antibiotic characteristics of this alga. The strong antibiotic activity of lectins towards fish pathogenic bacteria reveals one of the important roles played by algal lectins, as well as the potential high economic value of those marine algae assayed for aquaculture and for biomedical purposes.

Keywords Lectin · Antibiotic activity · Marine algae · Marine vibrios · Environmental factors

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Introduction

Many marine algae produce antibiotic substances capable of inhibiting bacteria, viruses, fungi, and other epibionts. It appears that the antibiotic characteristic is dependent on many factors, including the particular alga, the microorganisms, the season, and the growth conditions [5, 14, 15, 25]. The antibacterial activity of marine algae is generally assayed using extracts in various organic solvents, e.g., acetone [15], methanol–toluene [4], ether [6], ethanol [25], and chloroform-methanol [5]. Several extractable compounds, such as cyclic polysulfides and halogenated compounds, are toxic to microorganisms and therefore responsible for the antibiotic activity of some marine algae [9, 23, 31]. However, an antibiotic assay of extracts in organic solvents probably does not reflect adequately the antibacterial activity of marine algae under natural conditions. In earlier investigations, phenolics were found to be released into seawater from fucoid thalli, and these served as antifouling substances [22, 28]. Other studies indicated that released organic substances from juvenile forms of the red alga *Chondrus crispus* had an inhibitory effect on growth of adjacent diatoms [18], while those from the brown alga *Fucus spiralis* exhibited a stimulatory effect [16]. Assays using fragments of thalli from various marine algae also demonstrated remarkable antibiotic activity [14]. Moreover, water-extractable substances from various marine algae had antifouling activity towards the green alga *Enteromorpha prolifera* [7]. A sulfated polysaccharide from the cell wall of *Porphyridium* sp. recently was found to display pronounced antiviral activity [17]. Bioactive substances that

appear on the surface of thalli or as exudates from thalli during growth in saline solution are thus probably more biologically and ecologically significant in such alleopathic interactions.

Lectins are proteinaceous substances that are widely distributed in animals, plants, and microorganisms [8]. Since the report of Boyd et al. [3], numerous studies have indicated that marine algae contain lectins capable of binding specific carbohydrates to produce unique biological activities, such as the aggregation of erythrocytes, yeasts, bacteria, and various unicellular algae [12, 13, 26]. In both freshwater and marine algae, lectins play an important role in recognition and adherence of gametes during sexual reproduction [1, 21, 27]. Other biological and ecological activities of algal lectins are probably involved in symbiosis and defense as exhibited by lectins of land plants [8], marine mussels, and other invertebrates [10, 20, 24, 29, 30]; however, this has not been clearly proven. It has recently been suggested that carrageenan oligosaccharides mediate the association of a red alga and its green algal pathogen [2].

In this study, we assayed the antibacterial activity of both saline and aqueous ethanol extracts of marine algae against marine vibrios. Lectins purified from two marine red algae were assayed for their activity against a pathogenic vibrio. The growth rate of the vibrio pathogen in the presence of lectin-containing algal extracts, as well as the inhibitory activity of marine algae in relation to growth condition, was also determined using batch cultures.

Materials and methods

Algal materials and bacterial clones

Thirteen species of marine algae were collected from the northeastern coast of Taiwan. Marine vibrios, *Vibrio neresis* (ATCC 25916), *V. pelagius* (ATCC 25917), and *V. vulnificus* (CPC1-2, CPC1-7), were examined for their responses to marine algal extracts and purified lectins. The pathogenic bacterium *V. vulnificus*, which is capable of causing infectious diseases in fish and humans, was isolated from the northeastern coastal waters and identified by one of the authors (W.-Y. S.). This bacterium appeared in seawater having a salinity of 5.0–37.0‰ and temperatures of 14.0–30.3 °C. All bacterial clones were grown in 80% seawater, containing 0.3% bacto-peptone and 0.1% yeast extract, at 25 °C.

Preparation of algal extracts and lectins

After removal of epibionts and other contaminants, the harvested thalli were cleaned in filtered seawater using an ultrasonic cleaner and then in previously distilled Milli Q ion-exchanged water. The cleaned thalli were freeze-dried, ground into powder, and maintained at −20 °C until used. The powdered algae were extracted at a 1:10 (w:v) ratio [12, 19] in either 50 mM phosphate-buffered saline (PBS; pH 7.2, containing 0.8% NaCl) or in 20% ethanol. The algal lectins, referred to as ESA and GMA, were isolated and purified from the red algae *Eucheuma serra* and *Galaxaura marginata,* respectively, by one of the authors (W.R.L.). Detailed isolation and purification procedures, together with the biochemical characteristics, will be reported elsewhere. Additionally, two lectins from land plants, namely Con A and WGA, were obtained from a commercial source (Amersham Pharmacia Biotech, Uppsala, Sweden) and were used for comparison in the antibacterial assay.

Antibacterial assay

Marine vibrios were seeded separately onto the surface of agar plates, followed by the placement of a 12-mm filter membrane disc on the agar surface of each plate. An aliquot (20 µl) of algal extract was then added to each disc. For each algal extract, triplicate agar plates were used and each replicate had two agar plates for the assay. Bacterial cells were allowed to grow at 25 °C for 48 h. The antibacterial activity of algal extracts was assayed based on the presence and diameter of an inhibition zone around the discs.

As described above for crude extracts, 20-µl aliquots of ESA and GMA, containing 102 and 184 µg of purified lectin, respectively, were transferred separately to discs covering agar plates seeded with *V. vulnificus* cells. Similarly, 20-µl aliquots containing 120 and 800 µg of Con A and WGA, respectively, were applied separately to the discs for assaying. For each combination of lectin and bacterial clone, the assay was carried out in triplicate at 25 °C for 48 h.

Inhibition of antibacterial activity

Inhibition of the antibacterial activity of four strongly reactive species was assayed as described above using various carbohydrates and glycoproteins. An aliquot (50 µl) of 0.1 M sugar or glycoprotein was mixed with an equal volume of algal extract at room temperature for 30 min. Then, 50 µl of the mixture was added to each membrane disc on an agar plate seeded with *V. vulnificus*. Antibiotic activity was examined after incubation. The inhibitors used in the assay were the monosaccharides and their derivatives: arabinose, D (+) fucose, D (+) glucose, sialic acid, *N*-acetyl-D-galactosamine (GalNAc) and N-acetyl-D-glucosamine (GlcNAc); the oligosaccharide D (+) maltose; and the glycoprotein fetuin.

Bacterial growth rate with algal extracts

Both saline and aqueous ethanol extracts of *E. serra* and *Pterocladia capillacea* were tested separately against the pathogen *V. vulnificus* (clone CPC 1-2). The vibrio suspension was added to test tubes containing PY broth and algal extract of serial twofold dilutions, and then incubated at 25 °C for 48 h. The initial vibrio concentration in each tube (at the start, T_0) was 10^5–10^6 CFU/ml. PY broth with added PBS or aqueous ethanol instead of algal extracts was used as the control assay. Each assay had two replicates in serial dilutions. The bacterial growth rate in each dilution was determined based on the number of bacterial colonies formed on the agar surface, following the procedures described above. Inhibition of vibrio growth by the algal extracts was expressed as a percentage of the growth rate in the control.

Environmental factors influencing the antibacterial activity of marine algae

Undamaged healthy plants (with holdfast) of *P. capillacea* were cleaned in sterile seawater immediately after being collected and brought to the laboratory as described above and then examined using an optical microscope to aid in the removal of epiphytes and small animals, if any. The plants were then soaked in sterile seawater at 25 °C overnight. During the experiments, light was supplied by cool-white fluorescent lights in a light–dark cycle of 12 h on and 12 h off. The light intensity was measured at the base of cultures with a photometer, Model LI-185B, (LI-COR, Lincoln, Neb., USA). The culture medium used was silicon-free half-strength f medium [11]. Seawater collected nearby the sampling

sites for *P. capillacea* was used to prepare the experimental medium. The ambient inorganic nitrogen, (including nitrate, nitrite and ammonium), and phosphate concentrations in the seawater were 22.48 μM and 0.46 μM, respectively. The culture medium was replaced every 2 days. Variability in measuring the inhibitory activity against the pathogen *V. vulnificus* was assessed by using triplicate experimental cultures. Experiments were carried out at different temperatures, light intensities, and nutrient (N/P) ratios for 2 weeks to determine whether the antibiotic activity of marine algae was influenced by a change in the growth environment. At the end of the experiments, *P. capillacea* thalli were extracted in aqueous ethanol and assayed for their antibacterial activity, as described above.

Temperature and light

Whole plants (3.0–3.4 g) were placed into a 1-l flask containing 300 ml enriched medium and allowed to grow at a temperature of 5, 10, 20, 25 or 30 °C and a light intensity of 150 μmol photons m^{-2} s^{-1}, or at light intensities of 50, 100, 150, 200 or 250 μmol photons m^{-2} s^{-1} and a temperature of 20 °C.

Nutrients

Plants were also grown in the above enriched seawater medium, but supplemented with different concentrations of nitrate to give N/P ratios of 0.04, 0.44, 2.21, 4.43, 22.13, 44.26, 132.78 and 221.25. Algal cultures were maintained at 20 °C and an irradiance of 150 μmol photons $m^{-2}s^{-1}$.

Results

Antibacterial assay with algal extracts

Most of the red and green species assayed were inhibitory to *V. pelagius*; *E. serra* was the most active, followed by *Sarcodia ceylanica*, *G. marginata,* and *Microdictyon nigrescens* (Table 1). By contrast, nearly all the algal extracts were inactive against *V. neresis*. Notably, *E. serra, G. marginata,* and *Ulva conglobata* strongly inhibited the pathogenic vibrio *V. vulnificus*. The brown alga *Sargassum duplicatum* showed pronounced antibacterial activity of saline extract against *V. pelagius* and of ethanol extract against *V. vulnificus*, but was inactive towards *V. neresis*. More species exhibited a positive reaction against *V. pelagius* in their saline extracts than in their ethanol extracts.

Inhibition test of antibacterial activity

Those species that were strongly active against *V. vulnificus* became inactive after being pretreated with GalNAC, GlcNAC, and the glycoprotein fetuin (although the latter did not inhibit the activity of *U. conglobata*) (Table 2). Fucose and glucose were inhibitory to saline extracts of *E. serra* and *U. conglobata* but not to ethanol extracts. Arabinose, maltose, and sialic acid did not inhibit the antibacterial activity of either extract of the assayed algae.

Antibacterial assay with purified lectins

With the purified red algal lectins ESA and GMA, the inhibitory activity against *V. vulnificus* was notable (Table 3). However, these two algal lectins were inactive toward *V. neresis* and *V. pelagius*. Con A and WGA from land plants did not inhibit any of the vibrios assayed.

Bacterial growth rate with algal extracts

The growth rate of *V. vulnificus* was markedly inhibited in the presence of *E. serra* and *P. capillacea* extracts at relatively low concentrations. The growth rates were reduced by 44–57% and 11–36% by aqueous ethanol and saline extracts, respectively, at a dilution ratio of 1/16 (Fig. 1). However, the bacterial growth rate increased at higher dilution ratios. Generally, the vibrio pathogen was

Table 1 Antibacterial activity of marine algal extracts in phosphate-buffered saline (*P*) and aqueous ethanol (*E*). *Numbers* indicate width of inhibition zones in mm,— no inhibition, *n.d.* not determined

Algal species	*Vibrio pelagius*		*Vibrio neresis*		*Vibrio* vulnificus (CPC1-2)	
	P	E	P	E	P	E
Rhodophyta						
Carpopeltis maillardii	1.1	—	—	—	n.d.	n.d.
Eucheuma serra	4.3	4.5	—	—	2.5	10.0
Galaxaura marginata	1.5	2.5	1.0	2.5	7.0	4.0
Gracilaria lemaneiformis	1.3	—	—	—	5.0	—
Halymenia ceylanica	2.5	—	—	—	n.d.	n.d.
Helminthocladia australis	< 1.0	—	—	—	n.d.	n.d.
Pterocladia capillacea	2.3	—	—	—	2.0	3.5
Sarcodia ceylanica	3.9	—	—	—	n.d.	n.d
Chlorophyta						
Microdictyon nigrescens	2.6	—	—	—	n.d.	n.d.
Ulva conglobata	1.0	—	—	—	6.0	3.5
U. fasciata	1.1	< 1.0	—	—	n.d.	n.d.
Phaeophyta						
Endaracne binghamiae	1.2	—	—	—	n.d.	n.d.
Sargassum duplicatum	3.6	—	—	—	—	9.0

Table 2 Inhibition by sugars and glycoprotein of the antibacterial activity of marine algal extracts in phosphate-buffered saline (*PBS*) and aqueous ethanol (*E*) tested against *V. vulnificus* (CPC1-2). + Inhibition, — no inhibition, *n.d.* not determined

Extract	Sugar or glycoprotein							
	Arabinose	Fucose	Glucose	GalNAc	GlcNAc	Sialic acid	Maltose	Fetuin
Eucheuma serra								
PBS	—	+	+	+	+	—	—	+
E	—	—	—	+	+	—	—	+
Galaxaura marginata								
PBS	—	—	—	+	+	—	—	+
E	—	—	—	+	+	—	—	+
Pterocladia capillacea								
PBS	—	—	—	+	+	—	—	+
E	—	—	—	+	+	—	—	+
Ulva conglobata								
PBS	—	+	+	+	+	—	—	—
E	—	—	+	+	+	—	—	—

more susceptible to *E. serra* extracts than to *P. capillacea* extracts in aqueous ethanol, and, conversely, to *P. capillacea* extracts than to *E. serra* extracts in saline.

Table 3 Antibacterial activity of lectins from marine red algae and higher plants. *Numbers*indicate width of inhibition zones in mm,— no inhibition

Lectin	*V. neresis*	*V. pelagius*	*V. vulnificus* (CPC1-2)	*V. vulnificus* (CPC1-7)
ESA	—	—	8.3	2.7
GMA	—	—	8.8	2.0
Con A	—	—	—	—
WGA	—	—	—	—

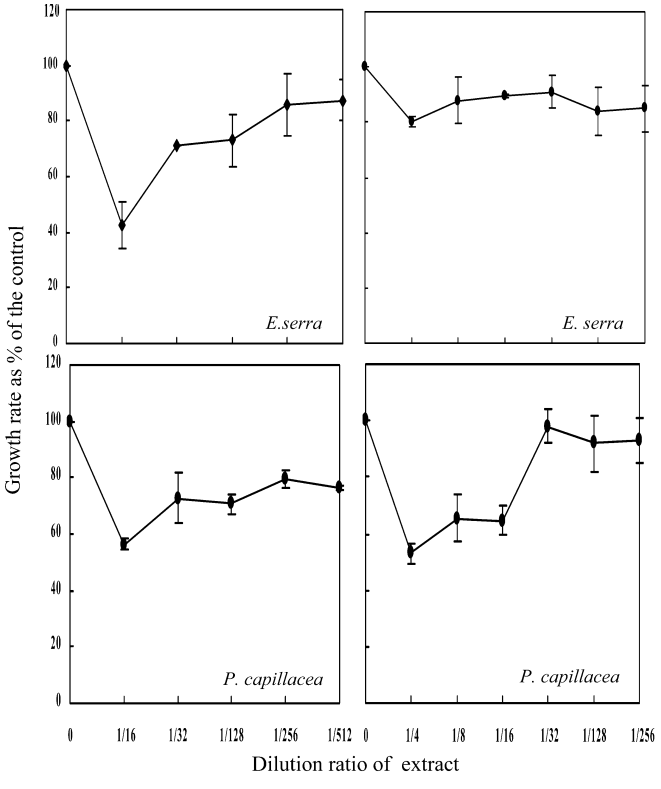

Fig. 1 Growth rate of *Vibrio vulnificus* in the presence of aqueous ethanol (*left*) and saline (*right*) extracts of *Eucheuma serra* and *Pterocladia capillacea* at various dilution ratios

Environmental factors influencing the antibacterial activity of marine algae

At all given temperatures, light intensities, and N/P ratios, extracts of *P. capillacea* inhibited the growth of *V. vulnificus*, as revealed by the formation of zones of inhibition on the agar surface of the culture plates. However, while algal antibacterial activity did not vary significantly ($p > 0.05$) over the temperature range used, it increased remarkably from 1 to 20 °C and then decreased at higher temperatures, i.e. 25 and 30 °C (Fig. 2). The activity of extracts either increased (for vibrio clone CPC 1-7) or decreased (for vibrio clone CPC 1-2) with an increase in the light intensity under which *P. capillacea* was grown ($p < 0.05$). At low light intensities (50 and 100 μmol photons $m^{-2}s-^{1}$) (Fig. 3), the activity toward the two clones varied greatly—being higher for CPC 1-2 and lower for CPC1-7, and differing by ca. two-fold. A change of the N/P ratio caused a dramatic change in the antibacterial activity of the assayed alga: activity was maximal when the alga was grown at lower and higher N/P ratios, i.e., 0.4 and 221.2,

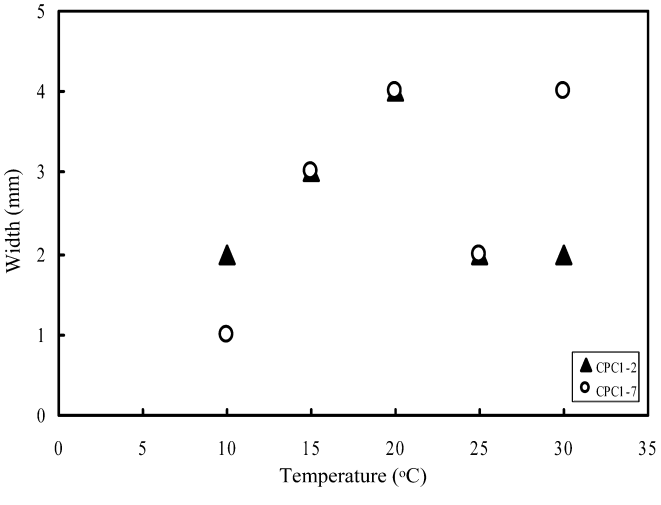

Fig. 2 Variation of antibiotic activity of *P. capillacea* grown at various temperatures; activity was assayed against *V. vulnificus* and is expressed as the width of the inhibition zone around the disc

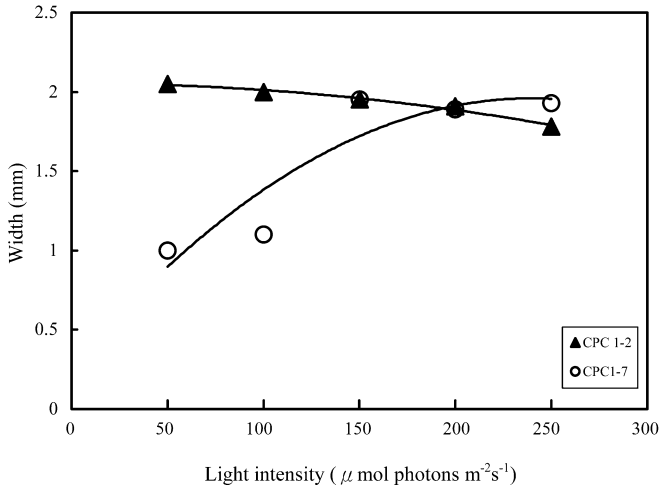

Fig. 3 Variation of antibiotic activity of *P. capillacea* grown at various light intensities; activity was assayed against *V. vulnificus* and is expressed as the width of the inhibition zone around the disc

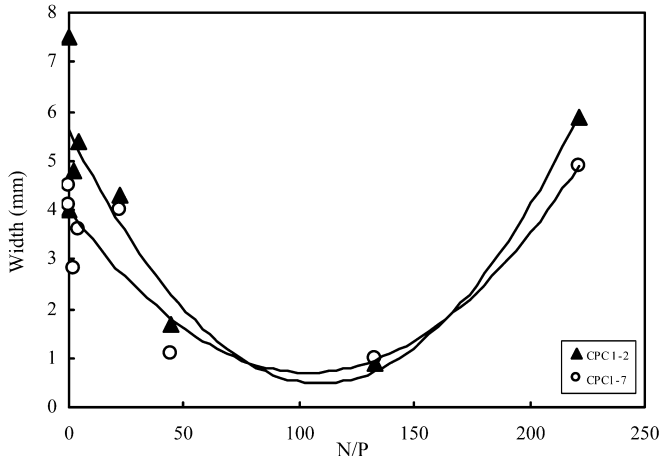

Fig. 4 Variation of antibiotic activity of *P. capillacea* grown in media supplemented with nitrate and phosphate in various concentration ratios (N/P ratio); activity was assayed against *V. vulnificus* and is expressed as the width of the inhibition zone around the disc

and minimal at ratios around 115 ($p < 0.005$) (Fig. 4). Algal antibacterial activity correlated with light intensity and N/P ratio in a quadratic function generally defined by $f(x) = ax^2 + bx + c$, where a or $b < 0$.

Discussion

Many marine algae exhibit antibiotic activity that varies between species and even within the same thalli [4,6,15,25,31]. In the present study, specific inhibition of the pathogenic vibrio *V. vulnificus* by marine algae was demonstrated.

Water-soluble bioactive compounds that appear on the surface of thalli, or as exudates from the thalli of marine algae, have substantial effects on the association of microorganisms. This has been demonstrated in fragments of thalli and juvenile plants of various marine algae [14,16,18], induced by phenolic compounds from fucoid algae [22,28], and, more recently, by sulfated polysaccharides from *Porphyridium* sp. [17] and by water-extractable fractions from various marine algae [7]. However, in a previous study, we showed that both PBS and aqueous ethanol extracts of the assayed algae strongly agglutinated human and animal erythrocytes. The hemagglutinating activity was inhibited by various mono- and oligosaccharides and glycoproteins (unpublished data). Similarly, in this work, it was shown that extracts of strongly active species became inactive against *V. vulnificus* after pretreatment with carbohydrates and glycoproteins. Assays with the purified lectins ESA and GMA, from two red algae, strongly inhibited growth of *V. vulnificus*, confirming the finding of lectins as antibacterials. This suggests that lectins or lectin-like compounds, rather than toxic compounds per se, are involved in the antibiotic reaction displayed in the reported assays. Similar results were obtained in *Alexandrium cohorticula*, a dinophycean alga, which was shown to secrete agglutinins from its cell surface into a culture medium to cause aggregation of erythrocytes [13].

Lectins in higher plants defend against pathogenic bacteria and fungi by recognizing and immobilizing the infecting microorganisms via binding, thereby preventing their subsequent growth and multiplication [8]. Lectins from bivalves and other invertebrates act in a similar manner to eliminate invading pathogenic bacteria [10,20,24,29,30]. This type of defense mechanism is little known in algae, mainly due to the limited number of lectins available from marine algae and the small amounts present. Some known algal lectins recognize and bring about adherence of sexual gametes during reproduction, e.g., *Chlamydomonas reinhardii* , *Fucus serratus* and *Aglaothamnion oosumiense* [1,21,27]. In addition, lectins from marine algae appear to aggregate fungi, bacteria, blue-green algae, diatoms, dinoflagellates and other unicellular algae, as well as erythrocytes [3,12,13]. In the present study, we observed the immobilization and aggregation of cells of *V. vulnificus* (but not of two other vibrios) following reaction with the assayed lectins (Fig. 5). Thus, algal lectins can mediate the allelopathy of bacterial–algal associations through recognition and binding of the complementary carbohydrates on either the cell walls or plasma membranes of the associated bacteria. Such conjugate reactions between proteins and carbohydrates or glycoproteins provide a defense mechanism for marine algae. We believe that specific carbohydrate-containing receptors of algal lectins are probably not uncommon on the cell surface of marine pathogenic vibrios, in addition to *V. vulnificus*. Similar molecular links are also present in the *Chondrus crispus–Acrochaete operculata* association, in which carrageenan released from *C. crispus* mediated *A. operculata* recognition and subsequent pathogenic association [2].

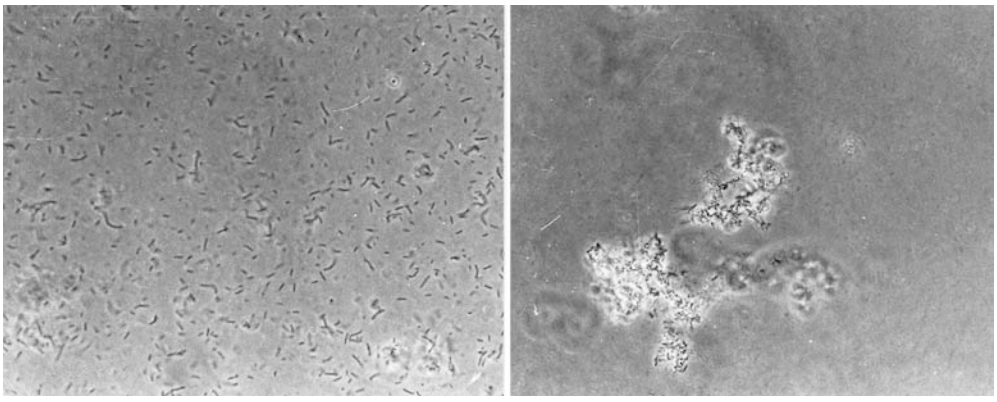

Fig. 5 Photographs showing *V. vulnificus* -free cells in the control (*left*) and aggregated cells after treatment with lectin ESA (*right*)

The growth rate of *V. vulnificus* was inhibited markedly in the presence of *E. serra* or *P. capillacea* extracts at very low concentrations.With increased irradiance, the red alga *Spyridia filamentosa* increased production of antibiotically active metabolites against pathogenic bacteria [5]. As seen in *P. capillacea*, and possibly in some other marine algae, metabolites acting against the pathogen *V. vulnificus* are produced over a wide range of temperatures, light intensities, and N/P ratios. Moreover, the N/P ratio had a greater effect on activity than either light intensity or temperature, and antibacterial activity increased dramatically when the alga was under severe nutrient stress, i.e., either highly N-limited or highly P-limited conditions. Higher light intensity also enhanced the antibacterial activity of algae, although to a lesser extent, whereas temperature affected the activity in a different manner. Therefore, the antibacterial activity of *P. capillacea* is a function of the N/P ratio and light intensity, but not necessarily of temperature.

The present study exemplifies one of the important roles played by algal lectins and suggests that many marine algae have anomalous properties in antibiosis. Much work remains to explore the biochemical characteristics of lectins in relation to defense as well as symbiosis between marine algae and their associated organisms. No doubt there are other marine algae that contain lectins with similar characteristics.

Acknowledgements This study was supported by the National Science Council and Council of Agriculture of the Republic of China.

References

1. Bolwell GP, JA Callow, ME Callow, LV Evans (1979) Fertilization in brown algae. II. Evidence for lectin-sensitive complementary receptors involved in gamete recognition in *Fucus serratus*. J Cell Sci 36:19–30
2. Bouarab K, P Potin, F Weinberger, J Correa, B Kloareg (2001) The *Chondrus crispus* - *Acrochaete operculata* host-pathogen association, a novel model in glycobiology and applied phycopathology. J Appl Phycol 13:185–193
3. Boyd WC, LR Almodovar, LG Boyd (1966) Agglutinins in marine algae for human erythrocytes. Transfusion 6:82–83
4. Caccamese S, R Azzolina, G Fumari, M Cormaci, S Grasso (1980) Antimicrobial and antiviral activities of extracts from Mediterranean algae. Bot Mar 23:285–288
5. Centeno POR, DL Ballantine (1999) Effects of culture conditions on production of antibiotically active metabolites by the marine alga *Spyridia filamentosa* (Ceramiaceae, Rhodophyta). I. Light. J Appl Phycol 11:217–224
6. Chesters CG, JA Stott (1956) Production of antibiotic substances by seaweeds. Proc. 2nd International Seaweed Symposium. Pergamon , New York, pp.49–54
7. Cho JY, EH Kwon, JS Choi, SY Hong, HW Shin,, YK Hong (2001) Antifouling activity of seaweed extracts on the green alga *Enteromorpha prolifera* and the mussel *Mytilus edulis*. J Appl Phycol 13:117–125
8. Etzler ME (1986) Distribution and function of plant lectins. In: Liener IE, Sharon N, Goldstein IJ (eds),The lectins. Properties, function, and applications in biology and medicine. Academic, Orlando, Florida, pp 371–435
9. Fenical W (1975) Halogenation in the Rhodophyta. A review. J Phycol 11:245–259
10. Fisher WS, AR DiNuzzo (1991) Agglutination of bacteria and erythrocytes by serum from six species of marine molluscs. J Invertebr Pathol 57:380–394
11. Guillard RRL, JH Ryther (1966) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229-239
12. Hori K, K Miyazawa, K Ito (1986) Preliminary characterization of agglutinins from seven marine algal species. Bull Jap Soc Fish 52:323–331
13. Hori K, T Ogata, H Kamiya, M Mimuro (1996) Lectin-like compounds and lectin receptors in marine microalgae: hemagglutination and reactivity with purified lectins. J Phycol 32:783–790
14. Hornsey IS, D Hide (1974) The production of antimicrobial compounds by British marine algae. I. Antibiotic producing algae. Br Phycol J 9:353–361
15. Hornsey IS, D Hide (1976) The production of antimicrobial compounds by British marine algae. II. Seasonal variation in production of antibiotics. Br Phycol J11:63–67
16. Huang R, AD Boney (1984) Growth interactions between littoral diatoms and juvenile marine algae. J Exp Mar Biol Ecol 81:21–45
17. Huleihel M, V Ishanu, J Tal, A Shisgaba (Malis) (2001) Antiviral effect of red microalgal polysaccarides on *Herpes simplex* and *Varicella zoster* virus. J Appl Phycol 13:127–134
18. Khfaji AH, AD Boney (1979) Antibiotic effects of crustose germlings of the red alga *Chondrus crispus* Stackh. on benthic diatoms. Ann Bot 43:231–232
19. Kawakubo A, H Makino, JI Ohnishi, H Hirohara, K Hori (1997) The marine red alga *Eucheuma serra* J. Agardh, a high yielding source of two isolectins. J Appl Phycol 9:331–338
20. Kilpatrick, DC (2002) Animal lectins: a historical introduction and overview. Biochim Biophys Acta 1572:187–197

21. Kim SH, GH Kim (1999) Cell-cell recognition during fertilization in the red alga *Aglaothamnion oosumiense* (Ceramiaceae, Rhodophyta). Hydrobiologia 398/399:81–89
22. McLachlan J, JS Craigie (1964) Algal inhibition by yellow ultraviolet absorbing substances from *Fucus vesiculosus*. Can J Bot 42:287–292
23. Ohta K (1979) Chemical studies on biologically active substances in seaweeds. Proc Int Seaweed Symp 9:401–411
24. Olafsen JA (1996) Lectins: models of natural and induced molecules in invertebrates. Adv Comp Environ Physiol 24:49–76
25. Pesando D, B Caram (1984) Screening of marine algae from the French Mediterranean coast for antibacterial and antifungal activity. Bot Mar 27: 381–386
26. Rogers DJ, Fish BC (1991) Marine algal lectins. In: Kilpatrick DC, van Driessche E, BØg-Hansen TC (eds) Lectin reviews, vol 1. Sigma, St. Louis, Missouri, pp129–142
27. Saito T, Y Matsuda (1984) Sexual agglutinin of mating-type minus gametes in *Chlamydomonas reinhardii*. II. Purification and characterization of minus agglutinin and comparison with plus agglutinin. Arch Microbiol 139:95–99
28. Sieburth J McN, JT Conover (1965) *Sargassum* tannin and antibiotic which retards fouling. Nature (London) 208:52–53
29. Tamplin ML, WS Fisher (1989) Occurrence and characteristics of agglutination of *Vibrio cholerae* by serum from the Eastern oyster, *Crassostrea virginica*. Appl Environ Microbiol 55:2882–2887
30. Tunkijjanukij S, A Olafsen (1998) Sialic acid-binding lectin with antibacterial activity from the horse mussel: further characterization and immunolocalization. Devel Comp Immun 22:139–150
31. Wrattens J, DJ Faulkner (1976) Cyclic polysulfides from the red alga *Chondrus californica*. J Org Chem 41:2465–2467