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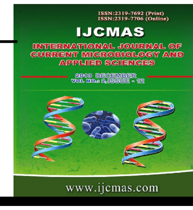

Original Research Article

Antagonistic activity of seaweed associated bacteria against human pathogens

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A B S T R A C T

Keywords

Antibacterial; bacteria; epiphytes; seaweed; pathogens.

Marine bacteria have become an important target for the biotechnology industry because of the large number of bioactive compounds recently discovered from them. In this present study, the antagonistic strains were isolated from seaweeds in the Gulf of Mannar, South east coast of India. Totally 126 bacteria were isolated from five different seaweeds. Ethyl acetate extracts of bacterial supernatant were screened for antibacterial activity. As a result, ten bacterial species have the wide spectrum antibacterial activity against human pathogens. Mostly the antagonistic strains were belongs to the genera Pseudomonas, Alteromonas, Pseudoalteromonas and Bacillus. In this study concluded that the seaweed associated bacteria have the ability to produced bioactive compounds may be useful in biomedical applications.

Introduction

Seaweeds are one of the large and diverse ecosystems; it plays an essential role in marine environment. It is mainly involved in global primary production and providing food and shelter for variety of organisms. Seaweeds surface supplies protected and nutrient rich conditions for the bacterial growth. (Armstrong *et al*., 2000), so compare to other multicellular organisms, seaweed haven a rich diversity of associated microorganisms, these microorganisms maybe beneficial or harmful to the seaweeds. In particular, epiphytic bacterial communities have been reported as vital for morphological development of seaweeds, and bacteria with antibacterial properties are thought to protect the seaweeds from pathogens and surface colonization of other competition organisms. Since the late 1980s, more than 50,000 bioactive natural products have been discovered from marine microorganisms. Among these compounds more than 8,000 had bactericidal activity (Betina 1983, Berdy, 1989). Marine bacteria often produce bactericidal compounds for maintaining the relationships between epiphytic bacterial communities and inhibiting microbial pathogens (Avendaño-Herrera *et al*., 2005). Some bacterial species show host specificity and bactericidal activity against specific pathogens; this specificity engage complex biochemical interactions between seaweed and bacteria (Strobel, 2003). In this present study focused on the preliminary screening of the antibiotic producing bacteria associated with different seaweeds in coral reef areas of Gulf of Mannar, South east coast of India.

Materials and Methods

Five seaweed samples such as *Gracillaria corticata, Hypnea musiformis, Geledium pussilum, Padina gymnosphora* and *Valoniopsis pachynema* were collected in situ at 0.5–2 m depth during Nov 2010 from the distinct sites of Mandapam coastal regions, Southeast coast of India (latitude 9°17 ′N, longitude 79°22 ′E). The Seaweed samples was picked with hand and immediately washed with seawater to remove the foreign particles, sand particles and macro epiphytes. The samples were transport to the laboratory on ice. The seaweed samples appeared to be healthy at the time of collection.

A sterile swab was used to rub the seaweed surface; the swab is dipped in sterile seawater, and serially diluted with saline water. The serial diluted samples were spread on Zobell marine agar 2216 plates. The plates were incubated for 24-48 h at 28°C. After incubation the bacterial colonies were counted using colony counter.

The bacteria were separated based on colony morphology, cell morphology and staining. The pure bacterial colonies were identified using morphology, biochemical and physiological tests.

Crude extract preparation

Overnight bacterial culture (100 ml) with the O.D of 0.6-0.9 was centrifuged at 4˚C at 7000rpm for 20min. The supernatant was collected and extracted with ethyl acetate. Organic layer was concentrated with vacuum and the concentrated powder was dissolved in deionized water and used for bioassay against several human pathogens such as *E.coli, Staphylococcus sp. Staphylococcus sp. Klebshilla pneumonia, Pseudomonas aeroginosa, Micrococcus sp. Salmonells sp. Vibrio cholera, Shigella dysenteriae* and *Serratia sp.*

Disc diffusion method

Mueller-Hinton agar was prepared and poured in petriplates under aseptic conditions. The concentrated powder was diluted with deionized water and prepared 50µg/ml concentrations. Using sterile discs (Himedia) are prepared and are immersed in vials and stand for 30 min. the pathogenic cultures are swabbed on petriplates. Then the discs were placed on the Mueller-Hinton agar using forceps and the petriplates were incubated overnight without inverting at optimum temperature of 37 ˚C for 24 hours. The experiments were repeated thrice with duplicate.

Results and Discussion

Seaweed and their surface associated microbial communities form complex and highly dynamic ecosystems (Holmstrom *et al*., 2002). Several new bacterial species and genera have been described from seaweeds suggesting that seaweeds represent an interesting biotic environment for discovery of new bacterial taxa, even if the origin does not necessarily indicate a specific association (Goecke *et al*., 2010;

Seyedsayamdost *et al*., 2011; Hollants *et al*., 2012). Several phylogenetic studies have provided insights into the complex epiphytic bacterial communities associated with seaweeds (Penesyan *et al*., 2009). Although comprehensive assessments of whole bacterial communities on seaweed surfaces are still relatively scarce (Burke *et al*., 2011), the available data suggest that bacterial communities associated with seaweed are in some degree specific to the seaweed host.

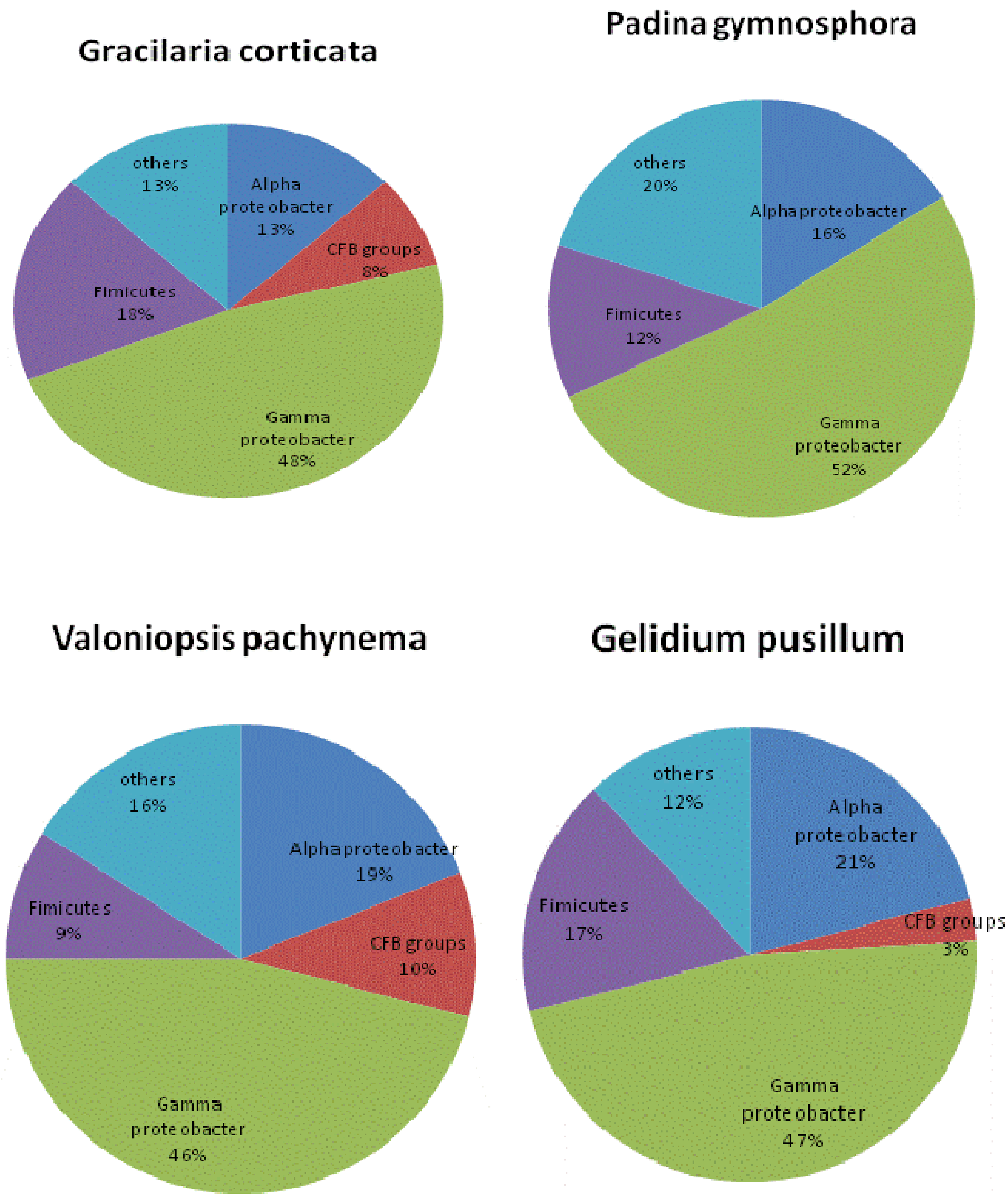
Seaweeds are typical habitat to a diverse group of bacteria with densities varying from 10^2 to 10^7 cells cm^2 depending on the host species, and season (Armstrong *et al*., 2000; Bengtsson *et al*., 2010). In this present study, the number of viable bacteria on the seaweeds surface was investigated; the enumeration of culturable bacteria is calculated as CFUs. The bacterial counts on the surface of the seaweed samples were 2.8 X 10^3 CFU per cm^2 (*Gracillaria corticata*), 3.7 X 10^3 per cm^2 (*Padina gymnosphora*), 6.2 X 10^3 per cm^2 (*Valoniopsis pachynema*), 5.6 X 10^3 per cm^2 (*Gelidium pusillum*) and 3.2 X 10^3 per cm^2 (*Hypnea musiformis*) (Figure 1). The common taxa have been identified on seaweed surfaces although mostly at the phylum level. The common epiphytic bacteria include members of Alphaproteobacteria, Gammaproteo bacteria, Bacteroidetes, and Cyanobacteria was reported in different red, green, and brown seaweeds (Hollants *et al*., 2012). Similarly in this present study totally 126 marine bacteria were isolated from the seaweeds surface. In this, more than 50% of bacteria are coming under gamma proteobacteria. The alpha proteobacteria and firmicutes are also rich in these epiphytic communities because proteobacteria is common and in many cases dominant in aquatic environments (Cottrell & Kirchman 2000a, b; Biegala *et al*. 2002; Simonato *et al*. 2010). As a result of Gram's classification >60% of the epiphytes are gram negative bacteria. Nakanishi *et al*. (1996) have reported that several bacterial genera are concerned in morphogenesis of *U. pertusa* including, *Flavobacterium, Vibrio, Cytophaga, Pseudomonas, Escherichia*, and Gram-positive cocci.

Numerous studies have previously reported on antimicrobial compounds of seaweed origin (Paul & Ritson-Williams, 2008; Goecke *et al*., 2010). In this study the preliminary screening of antibiotic producing bacteria was studied against the human pathogens. The bacteria allied with five seaweeds surfaces were isolated using Zobell marine agar that better reflect the nutrients available to seaweed epiphytes in their natural environment. These bacteria were then cultured in Zobell marine broth and the supernatant was extracted with ethyl acetate (Boyd *et al*., 1999) and using a disc diffusion method against human pathogens such *as E.coli, Staphylococcus sp. Staphylococcus sp. Klebshilla pneumonia, Pseudomonas aeroginosa, Micrococcus sp. Salmonells sp. Vibrio cholera, Shigella dysenteriae* and *Serratia sp*. (Table 1). Among the antibiotic producing bacteria *Pseudomonas, Pseudoalteromonas* and *Alteromonas sp*. have the strong antibacterial activity against most of the pathogens. These ethyl acetate extracted antibacterial compounds are maybe Quinolinol/ pyrrole/ organic compounds or a polysaccharide (Jayanth *et al*., 2002). In previous investigations, Ismail-Ben Ali *et al*. (2012) isolated the bacterial genera *Pseudomonas, Pseudoalteromonas, Paracoccus* and *Bacillus* from brown alga *Padina pavonica* having the antibacterial activity. The present result showed that the bacteria

Table.1 Zone of inhibition formed by seaweed epiphytic bacteria against human pathogens

Seaweed	Pathogens	Human pathogens									
		E.coli	*Staphylococcus sp.*	*Streptococcus sp.*	*Klebshilla pneumonia*	*Pseudomonas aeroginosa*	*Micrococcus sp.*	*Salmonells sp.*	*Vibrio cholera*	*Serratia sp.*	*Shigelle dycentriae*
Gracillaria corticata	*Pseudoalteromonas sp.*	12.2 mm	8.6mm	8.3mm	3.2mm	-	-	9.6mm	12.6mm	-	7.6mm
	Pseudomonas sp.	13mm	9.8mm	7.3mm	-	-	6.8mm	6.5mm	-	7.8mm	7.3 mm
	Pseudomonas sp.	6.0mm	7.1mm	11.7mm	12.6mm	3.2mm	-	-	15mm	-	-
Padina gymnosphora	*Alteromonas sp.*1	4.8mm	16mm	14mm	11.8mm	13.2mm	11.2mm	-	-	-	12.7mm
	*Alteromonas sp.*2	6.8mm	-	-	8.38mm	13.3mm	-	12.6mm	15.2mm	9.3mm	11.2mm
Valoniopsis pachynema	*Alteromonas sp.*3	3.1mm	8.9mm	9mm	2.6mm	3mm	11.6mm	-	-	-	-
	Oceanobacillus sp.	13.6mm	-	-	12.6mm	11.6mm	-	12mm	8.4mm	12.6mm	11.8mm
	Bacillus sp 1	13mm	7.6mm	12.1mm	-	-	11.8mm	-	4.6mm	8.9mm	10.4mm
Valoniopsis pachynema	*Bacillus sp.* 2	15mm	-	-	12.7mm	11.8mm	-	13.2mm	16mm	12.8mm	14.4mm
Hypnea musiformis	*Rhodobacter sp.*	10.2mm	-	-	9.6mm	8.5mm	10 mm	8.6mm	11.6mm	8mm	5.2mm

Figure.1 Epiphytic bacterial community of different seaweeds



released compounds into the supernatant had antimicrobial activity. It is confirmed by Rungprom *et al*. (2008) findings i.e. a natural antimicrobial cyclotetrapeptide compounds have been isolated from the supernatant of seaweed *Diginea sp.* associated *Pseudomonas sp.* and *Pseudoalteromonas sp.* (Dahiya and Gautam 2011) Furthermore, seaweed associated Bacillus isolates have been found to produce peptide compounds with antimicrobial activity (Jaruchoktaweechai *et al*., 2000).

Burgess *et al*. (2003) isolated some bacteria with high antibacterial activity against fouling microbes and found that most of these bacteria belonged to *Bacillus sp*. and the brown kelp *S. latissima* (previously Laminaria saccharina) have more than 100 different antimicrobial strains covering the phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria (Wiese *et al*., 2009). Jensen *et al*. (2005) and Macherla *et al*. (2005) reported that, 35 bacterial strains isolated from six seaweeds, it was possible to phylogenetically identify 33, located within the phyla Firmicutes, Proteobacteria and Actinobacteria and these are considered excellent producers of bioactive secondary metabolites. Finally this study agreed Zheng *et al*. (2005) report, totally 341 bacterial isolates were cultured from marine sources. In these 42 isolates are having antimicrobial activity, among these, 11% of antibacterial strains are isolated from seaweeds surface, were belongs to the genera *Alteromonas*, *Pseudomonas, Bacillus* and *Flavobacterium*. This study concluded that the seaweeds associated bacteria are found to have a bactericidal activity against several human pathogens and further investigations are required for the identification of different bioactive compounds from seaweed associated bacteria.

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References

Armstrong, E., A. Rogerson, and Leftley, J.W. 2000. The abundance of heterotrophic protists associated with intertidal seaweeds. Estuar .Coast. Shelf. Sci. 50: 415–424.

Avendaño-Herrera, R., M. Lody, and Riquelme, C.E. 2005. Producción de substancias inhibitorias entre bacterias de biopelículas en substratos marinos. Revista de Biología Marina y Oceanografía. 40(2): 117-125.

Bengtsson, M., K. Sjøtun and Øvreas, L.2010 Seasonal dynamics of bacterial biofilms on the kelp Laminaria hyperborea. Aquat Microb Ecol. 60: 71–83.

Berdy, J., 1989. The discovery of new bioactive microbial metabolites: screening and identification. In: Bushell ME & U Grafe (eds). Bioactive metabolites from microorganisms. Prog. Industrial Microbiol. 27: 3-25.

Betina, V. 1983. The chemistry and biology of antibiotics, 590 pp. Elsevier, Amsterdam.

Biegala, I.C., G. Kennaway, E. Alverca, J.F.Lennon, D. Vaulot and Simon,N. 2002. Identification of bacteria associated with dinoflagellates (Dinophyceae) Alexandrium spp. using tyramide signal amplification–fluorescent in situ hybridization and confocal microscopy. J Phycolo. 38: 404–411.

Boyd, K.G., D.R. Adams and Burgess, J.G. 1999. Antibacterial and repellent activities of marine bacteria associated with algal surfaces. Biofouling. 14: 227–236.

Burgess, J.G., K.G. Boyd, E. Armstrong , Z. Jiang, L.YanL, M. Berggren, U. May, T. Pisacane, A. Granmo and Adams, D.R. 2003. The development of a marine natural product-based antifouling paint. Biofouling. 19:197–205.

Burke, C., T. Thomas, M. Lewis, P. Steinberg and Kjelleberg, S. 2011. Composition, uniqueness and variability of theepiphytic bacterial community of the green alga Ulvaaustralis. ISME J. 5: 590–600.

Cottrell, M.T. and Kirchman, D.L. 2000a. Natural assemblages of marine Proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. Appl . Environ. Microbiol. 66: 1692–1697.

Cottrell, M.T. and Kirchman, D.L. 2000b. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl. Environ. Microbiol. 66: 5116–5122.

Dahiya, R and Gautam, H. 2011. Toward the synthesis and biological screening of a cyclotetrapeptide from marine bacteria. Mar Drugs. 9:71–81.

Goecke, F., A. Labes, J. Wiese, and Imhoff, J. 2010. Chemical interactions between marine macroalgae and bacteria. Mar Ecol Prog Ser. 409: 267–300.

Hollants, J., F. Leliaert, O.De Clerck and Willems, A. 2012. What we can learn from sushi: a review on seaweed–bacterial associations. FEMS Microbiol Ecol. 83: 1–16.

Holmstrom, C., S. Egan, A. Franks, S. McCloy and Kjelleberg, S. 2002. Antifouling activities expressed by marine surface associated Pseudoalteromonas species. FEMS Microbiol Ecol. 41: 47–58.

Ismail-Ben Ali, A., M. El Bour, L. Ktari, H. Bolhuis, M. Ahmed , A. Boudabbous and Stal, L. J. 2012. Jania rubens-associated bacteria: molecular identification and antimicrobial activity. J Appl. Phycol.24:525–534.

Jaruchoktaweechai, C., K. Suwanborirux, S. Tanasupawatt, P. Kittakoop and Menasveta, P. 2000. New macrolactins from a marine *Bacillus sp.* Sc026. J Nat Prod. 63:984–986.

Jayanth, K., G. Jeyasekaran and jeyashakila, R. 2002. Isolation of marine bacteria, antagonistic to human pathogens. Ind j Mar sci. 31(1) : 30-44.

Jensen, P.R., T. Mincer, P.G.Williams and Fenical, W. 2005. Marine actinomycete diversity and natural product discovery. Antonie Van Leeuwenhoek. 87(1): 43-48.

Macherla, V.R., S.S. Mitchell, R.R. Manam, K.A. Reed, Chao,T.H. *et al*. 2005. Structure-activity relationship studies of Salinosporamide A (NPI-0052), a novel marine derived proteasome inhibitor. J.Medic. Chem. 48(11): 3684-3687.

Nakanishi, K., M. Nishijima, M. Nishimura, K. Kuwano and Saga, N. 1996. Bacteria that induce morphogenesis in Ulva pertusa (chlorophyta) grown under axenic conditions. J Phycol. 32: 479 – 482.

Paul, V.J., and Ritson-Williams, R. 2008. Marine chemical ecology. Nat. Prod. Rep. 25: 662–695.

Penesyan, A., Z. Marshall-jones, M.C.Holmstro, S. Kjelleberg and Egan, S. 2009. Antimicrobial activity observed among cultured epiphytic bacteria reflects their potential as source of new drugs. FEMS Microbiol Ecol. 69: 113–124.

Rungprom, W., E.R.O. Siwu, L.K. Lambert, C. Dechsakulwatana, *et al*. (2008) Cyclic tetrapeptides from marine bacteria associated with the seaweed *Diginea sp.* and the sponge *Halisarca ectofibrosa*. Tetrahedron. 64:3147–3152.

Seyedsayamdost, M.R., R.J.Case, R. Kolter and Clardy, J. 2011. The Jekyll-and Hyde chemistry of *Phaeobacter gallaeciensis*. Nature Chem. 3: 331–335.

Simonato, F., P.R.Gomez-pereira, B.M. Fuchs and Amann, R. 2010. Bacterioplankton diversity and community composition in the Southern Lagoon of Venice. Syst. Appl. Microbiol. 33: 128–138.

Strobel, G., 2003. Endophytes as sources of bioactive products. Microbes and Infect. 5(6): 535-544.

Wiese J, V. Thiel, K. Nagel, T. Staufenberger and Imhoff J. 2009. Diversity of antibiotic-active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea. Mar Biotechnol. 11: 287–300.

Zheng, L., X. Han, H. Chen, W. Lin and Yan, X.2005. Marine bacteria associated with marine macroorganisms : potential antimicrobial resources. Ann. Microbiol. 55(2):119-124.