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Antimicrobial activity of tissue and associated bacteria from benthic sea anemone *Stichodactyla haddoni* against microbial pathogens

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Abstract: *Associated bacteria from Stichodactyla haddoni are found maximum in tentacle tissues than the body tissue. There are eight associated bacterial species viz., Alcaligenes sp, Corynebacterium sp, Aeromonas sp, Sporosarcina sp, Renibacterium sp, Carnobacterium sp1, Carnobacterium sp2 and Salinococcus sp were recorded. The culture extracts from the associated bacterial species showed sensitivity against human bacterial and fungal pathogens. However, the hexane tissue extract of sea anemone showed maximum sensitivity (24 mm dia.) against the fish bacterial pathogen Aeromonas hydrophila than the other chosen pathogens. Comparatively, the tissue extracts showed promising antimicrobial sensitivity than the cell free extracts of associated bacteria, and hence, the tissue samples from the sea anemone Stichodactyla haddoni is recommended for further exploration of novel antimicrobial drugs than the associated bacteria.*

Key words: *Stichodactyla haddoni, Sea anemone, Antimicrobial drug, Associated bacteria, Human pathogens*
PDF file of full length paper is available with author

Introduction

The marine environment is an exceptional reservoir of natural products, many of which exhibit structural features, not found in terrestrial natural products (Ireland *et al.,* 1988) but lacks an ethno medical history (Faulkner, 1992). Of the natural products isolated from marine organisms (Rao *et al.,* 1985), only less than 1% has been examined so far for pharmacological activity (Halstead, 1965; Fusetani, 2000). Much of studies are warranted to find antimicrobials in the present context of increasing need for novel drugs that can control new illness or resistant strains of microorganisms.

Marine invertebrates especially sedentary sea anemones are evolved with rich sources of bioactive metabolites, which could be used for novel animicrobial drugs.Among the marine organisms, anthozoans are ecologically important animals, which need to protect themselves against the lethal or debilitating consequences of microbial or parasitic invasion (Grigg and Dollar, 1990; Ramalingam and Ramarani, 2006). However, unlike their coelomate relatives, anthozoans have received little attention with respect to their antimicrobial and antiparasitic defenses. The ability of anthozoans to display discriminatory tissue reactions to foreign grafts has been demonstrated by many workers (Theodor, 1970; Burnet, 1971; Bigger and Hildemann,1982; Porter and Targett,1988; Jokiel and Bigger,1994; Rinkevich *et al.,* 1994; Prema *et al*., 2005). Earlier findings reported that these animals possess populations of wandering amoebocytes within the mesoglea (Young, 1974; Patterson and Landolt, 1979; Vander Vyer, 1981; Shick, 1991). However, detailed *in vitro* analyses of the associated bacteria have not been undertaken and also the cellular basis of antibacterial activity remains largely unknown. The present study is therefore aimed at identifying the associated bacteria with *Stichodactyla haddoni* and to find out the efficacy of bactericidal activity of sea anemone and associated bacteria.

Materials and Methods

(i) Collection of sea anemone and isolation of associated bacteria:

Name of sea anemone	*Stichodactyla haddoni*
Collection area	Tuticorin (Lat. 8°45'N; Long. 78°10'E); South East coast of India
Animal parts used	Body tissue, tentacle tissue
Medium used for the isolation of associated bacteria	Zobell marine agar 2216e
Method adopted for isolation and identification	Holt *et al.* (1994)

(ii) Extraction of antimicrobial compounds:

Animal samples used	Tissue sample (25 g)
Identified bacterial species	*Alcaligenes* sp, *Sporosarcina* sp, *Corynebacterium* sp, *Renibacterium* sp, *Aeromonas* sp, *Carnobacterium* sp and *Salinococcus* sp

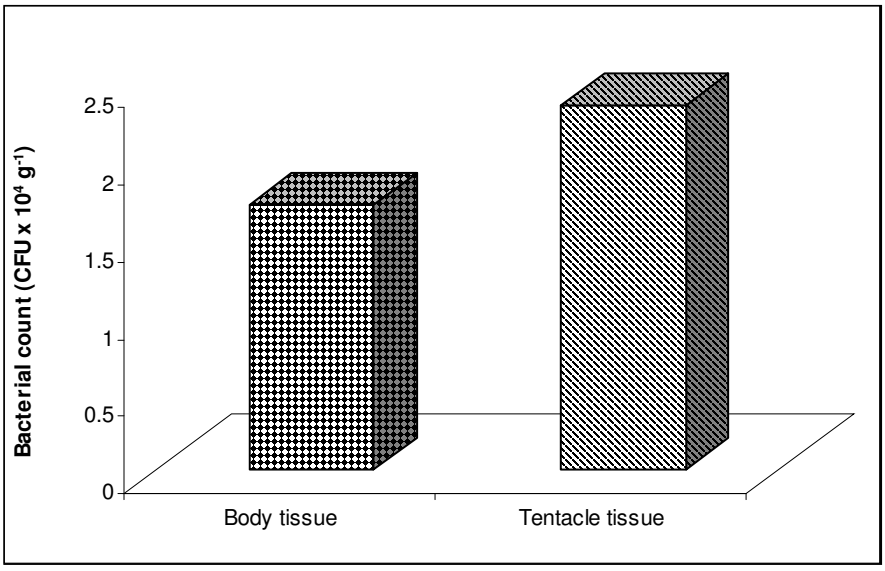

Fig. 1: Associated bacterial population of sea anemone, *Stichodactyla haddoni*

Solvents used for the extraction of antimicrobial compounds	n-butanol, methanol, hexane, chloroform and methanol - 2:1 (v/v) and ethyl acetate

(iii) Antimicrobial sensitivity assay:

Bacterial pathogens tested	Human pathogens *viz., Bacillus sphaericus, Staphylococcus aureus, Enterobacter* sp*, Salmonella typhi, Klebsiella pneumoniae, Serratia* sp*, Escherichia coli, Vibrio parahaemolyticus, Pseudomonas* sp*,* and *Citrobacter* sp*,* and fish pathogens *viz., Aeromonas hydrophila, Alcaligenes* sp*, Vibrio harveyi, Pseudomonas aeruginosa*
Method for antibacterial assay	Bauer *et al.* (1966)
Medium used for antibacterial activity	Muller Hinton Agar
Fungal pathogens tested	*Alternaria* sp*, Curvularia* sp*, Aspergillus* sp*, Trichoderma viridi, Cercospora* sp
Method for antifungal assay	Lehrer *et al.* (1991)
Medium used for antifungal activity	10 ml of molten gel solution (1% agarose and 0.3 mg of Sabouraud dextrose broth powder per ml and 10 mM sodium phosphate buffer, pH 7.4) was mixed with spores (10^2 spores ml^{-1})

Results and Discussion

Much of the biota especially the sea anemone possesses a variety of defence mechanisms with its own or derived from the associated organsms to gain a selective advantage and to cope up with competitors. Microbes have been identified as ecto and endo symbiotic organisms in many of the multicellular animals. Hence the present study was made, an attempt on the microbial species associated with the chosen sea anemone. The associated bacterial counts were maximum in tentacle tissues than in body tissue (Fig.1), which were identified as *Alcaligenes* sp*, Corynebacterium* sp*, Aeromonas* sp*, Sporosarcina* sp*, Renibacterium* sp*, Carnobacterium* sp1*, Carnobacterium* sp2 *and Salinococcus* sp To check the defensive action, the cell free extracts of isolated bacterial species and the tissue extract from sea anemone were tested for the antimicrobial sensitivity against human pathogens. Of the human pathogens tested, *Klebsiella pneumoniae* is highly inhibited (20 mm dia.) by the tissue extract of sea anemone by using chloroform and methanol as extractives (Table 1). Moreover, the inhibitory activity is still higher (24mm dia.) with the hexane tissue extract against the fish pathogen, *Aeromonas hydrophila* (Table 2) and ethyl acetate tissue extract against *Aspergillus* fungi (22 mm dia.) (Table 3). Compared with the sea anemone tissue extract, the cell free extract also showed equal sensitivity against the tested pathogens (Table 4). For instance, the diethyl ether extract from *Corynebacterium* against an opportunistic pathogen, *Escherichia coli* (23 mm dia.) showed maximum sensitivity, which is more or less equivalent to the activity of hexane tissue extract from sea anemone. A similar report has earlier been made that, the crude extracts of di-ethyl ether showed good activity against Gram positive and Gram negative bacteria (Padmini Sreenivasa Rao and Karmakar, 1986; Ravikumar *et al.,* 2002; Sureshkumar *et al.,* 2002). It is also reported by the present study that, the cell free extract of associated bacteria exerts antimicrobial sensitivity against microbial pathogens. This is also supported by the earlier studies that, the amoebocytes from the sea anemone *Actinia equina* showed considerable inhibitory activity against Gram-negative bacteria within 3 hr (Hutton and Smith, 1996). Hence, it is obvious that the secondary metabolites produced from the associated microorganisms could transport into the sea anemone tissue and hence exploitation of associated

Table - 1: Antibacterial sensitivity of sea anemone extract against human pathogens

Pathogens	Zone of inhibition in mm dia.					
	n-butanol	Methanol	Hexane	Chloroform: Methanol 2 : 1 (v/v)	Ethyl acetate	SD
Bacillus sphaericus	18	-	-	8.5	8	±7.45
Staphylococcus aureus	10	-	10	15	18	±6.84
Enterobacter sp	10.5	8	9	-	-	±5.09
Salmonella typhi	-	10.5	12.5	10	10	±4.91
Klebsiella pneumoniae	11	-	14.5	20	15	±7.48
Serratia sp	-	9.5	-	-	-	±4.24
Escherichia coli	8.5	-	-	-	-	±3.8
Vibrio parahaemolyticus	9	13	8	-	-	±5.78
Pseudomonas sp	10	-	10	18	16	±7.01
Control	-	-	-	-	-	
SD	±5.58	±5.55	±5.66	±8.31	±7.66	

' - ' = No activity, Values are average of 3 experiments with three replicates
Vertical SD shows the difference among the pathogens
Horizonal SD showsn the difference among the solvents

Table - 2: Antibacterial activity of sea anemone extract against fish pathogens

Pathogens	Zone of inhibition in mm dia.					
	n-butanol	Methanol	Hexane	Chloroform: Methanol 2 : 1 (v/v)	Ethyl acetate	SD
Aeromonas hydrophila	22	20	24	12	8	± 6.87
Alcaligenes sp	20	12	17	2	15	± 3.42
Vibrio harveyi	13	14	18	16	13.5	± 2.07
Pseudomonas aeruginosa	20	16	12	22	20	± 4
Control	-	-	-	-	-	
SD	±3.94	±3.41	±4.92	±4.72	±4.93	

' - ' = No activity, Values are average of 3 experiments with three replicates
Vertical SD shows the difference among the pathogens
Horizonal SD showsn the difference among the solvents

Table 3: Antifungal activity of sea anemone extract against fungal pathogens

Pathogens	Zone of inhibition in mm dia.			
	Chloroform : Methanol 2 : 1 (v/v)	Ethyl acetate	Hexane	SD
Alternaria sp	9	10	12	± 1.24
Curvularia sp	8	12	12.5	± 1.52
Aspergillus sp	15	22	20	± 3.6
Trichoderma viridi	-	-	9.5	± 5.48
Cercospora sp	10.5	-	-	± 6.02
Control	-	-	-	
SD	± 5.45	± 7.81	± 7.19	

' - '= No activity, Values are average of 3 experiments with three replicates
Vertical SD shows the difference among the pathogens
Horizonal SD showsn the difference among the solvents

Table - 4: Effect of cell free extracts of associated bacteria on human pathogens

Pathogens	Zone of inhibition in mm dia.																
	Alcaligenes		*Coryne bacterium*		*Aeromonas*		*Sporosacrina*		*Renibacteria*		*Carnobacterium-1*		*Carnobacterium-2*		*Salinococcus*		
	NB	DE	NB	DE	NB	DE	NB	DE	NB	DE	NB	DE	NB	DE	NB	DE	SD
Bacteria -																	
Staphylococcus aureus	13	-	8.5	12	12	-	10	-	-	7	8	-	7	8	-	12	±5.19
Bacillus sphaericus	8	14.5	10	7	15	10	10	8	9	-	9.5	7	-	-	18	-	±5.56
Serratia sp	11	20	12.5	-	11	-	12	-	-	-	-	8	8.5	-	-	17	±7.03
Vibrio parahaemolyticus	-	-	9	19	9	-	-	-	9.5	8	-	-	-	-	-	11	±5.94
Salmonella typhi	10	-	8	13.5	9	-	-	-	8	-	-	7	-	-	-	12	±5.16
Escherichia coli	10	18.5	7	23	10	14.5	10	16	-	9.5	7	-	-	8.5	-	18.5	±7.24
Klebsiella pneumoniae	10	-	-	-	10	-	10	-	7	7	11	-	-	-	-	-	±4.68
Pseudomonas sp	-	18	12.5	21	10	16	-	15.5	-	-	8.5	7	-	-	11	18	±7.75
Enterobacter sp	-	-	20	21.5	-	-	-	-	7	-	-	-	8	8	15	15	±7.87
Citrobacter sp	-	-	-	21	-	-	-	-	7	9	-	-	-	-	-	16	±6.59
Fungi -																	
Aspergillus sp	11	-	-	-	-	-	9.5	-	-	-	7	-	7	7	-	-	±4.08
Trichoderma viridi	-	-	-	-	10	-	11	-	-	-	-	-	-	-	-	-	±3.59
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
SD	±5.48	±8.82	±6.32	±9.64	±5.08	±6.24	±5.37	±6.25	±4.20	±4.22	±4.5	±3.57	±3.77	±3.89	±6.8	±7.71	

NB = n-butanol, DE = diethyl ether, '-' = No activity. Values are average of 3 experiments with three replicates
Vertical SD denotes difference among pathogens
Horizontal SD denotes difference among associates bacteria

microorganisms could solve the supply problem of raw materials *i.e*., host organisms and hence the biodiversity of marine organisms could be conserved for the future benefits. Interesting finding from the antimicrobial sensitivity of associated bacteria must play a role in host defense, and thus constitute a valuable source of immunocompetent effector cells for *in vitro* analyses. This report also added further understanding of the impact of bleaching on the bacterial infection of anthozoans as reported in reef corals (Kushmaro *et al.,* 1996).

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