ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 3(1): 389-391

www.fisheriesjournal.com
Received: 18-07-2015
Accepted: 19-08-2015

RS Dhivya
Sree Devi Kumari Women's College, Kuzhithurai, Kanyakumari district, Tamil Nadu, India.

AP Lipton
Central Marine Fisheries Research Institute, Vizhinjam, Thiruvananthapuram, India.

Correspondence
RS Dhivya
Sree Devi Kumari Women's College, Kuzhithurai, Kanyakumari district, Tamil Nadu, India.

Microbial pathogens infecting *Etroplus* species and management of pathogens using marine Natural products (MNPs)

RS Dhivya, AP Lipton

Abstract
The common pathogens such as *Vibrio* infecting pearlspot *Etroplus suratensis* and *E. maculatus* were isolated and characterized. The lethal doses of some of the common pathogens were evaluated using *E. maculatus*. Marine Natural Product was isolated from *Ulva fascita* using methanol. The crude methanol extract was used as one of the ingredients at different proportions. The growth and disease resistance towards pathogens were evaluated in *E. maculatus* and *E. suratensis*, using appropriate experimental and control sets. In the experimental set fed with Marine Natural Products, the percent relative protections (PRP) as well as survival were higher compared to the control sets. The use of Marine Natural Products in enhancing growth and disease resistance were discussed.

Keywords: Microbial pathogens, *Etroplus* species, pathogens, marine Natural products

1. Introduction
Etroplus suratenesis commonly called Green chromide and *E. maculatus* commonly called Orange chromide are common inhabitants of estuaries, lagoons and backwaters. *Etroplus suratenesis* is one of the most sought table fishes especially in Kerala, Karnataka, Goa, Tamil Nadu. and there has been an increasing demand for it in the domestic market. The Orange chromide, *E. maculatus* is an attractive aquarium fish and is considered as a biological pollution-monitoring indicator. Bacterial disease problems form a bottle neck for the commercial culture of these fishes. The major disease causing bacteria of these fishes include *Pseudomonas*, *Alcaliigenes*, *Flavobacteria*, *Moracella*, *Vibrios*, Gram positive micrococci, *Arthrobacter* and *Bacillus* sp. The Gram negative fish pathogen belonging to the genus *Vibrio* is a major threat as far as *Etroplus* sp**.** is concerned. The disease control in aquaculture relates to the preventive measures by the use of Marine Natural Products (MNPs) which form excellent source for developing potent immunostimulants. The Extract of *Ulva fasciata* having antibacterial properties (Selvin *et al*., 2004) [7, 8] could enhance the disease resistance by boosting the immune response. The present study was an attempt to isolate the estuarine *Vibrios* infecting *Etroplus sp.* and their management using diets mixed with *U. fasciata* in different concentration.

2. Materials and Methods
Etroplus species *(E. suratenesis* and *E. maculatus*) were collected from backwaters of Rajakkamangalam, Kanyakumari District. Fishes were separated and acclimated in 50 litre tanks by providing feed and other routine husbandry practices. Moribund fishes with white patches, reddening on the body and wide open mouth, were sub used to isolate pathogen. Pathogens were characterized by morphological and biochemical studies. *U. fasciata* collected from Kanyakumari coast were washed with sea water after collection and shade dried, powdered and refluxed with methanol and condensed to get the extract (Selvin and Lipton 2004) [7, 8]. Koch postulates was performed to screen and select the potential pathogen strain.
Preparation of medicated feed: Commercial feed (C.P. Nova starter) was used for preparing medicated feed for the experiments. The recommended dose of *U. fasciata* was incorporated with the feed by spraying on the surface of feed using 4.0% gelatin as binder. The *Etroplus* sp. was fed at a rate of 5.0% of the body weight daily. The diet administration was continued for a period of 30 days.

LD_{50} determination: Pathogen isolate grown in nutrient broth for 24h. One ml was centrifuged at 4000rpm for 15min, washed twice in normal saline and serially diluted. The conc. of 5 x 10^5 to 1x10^6 cells in saline was adjusted and injected intraperitoneally using 1ml tuberculin syringe.
Survival was monitored and the percentage was calculated as follows.

$$\text{Survival (\%)} = \frac{\text{Total number of fishes on initial day - Total number on day 30}}{\text{Initial number of fishes}} \times 100$$

The absolute and specific growth rate was calculated by using the formula.

$$\text{AGR (g/body weight/day)} = \frac{\text{Final body weight – Initial body weight}}{\text{Total no. of days}}$$

$$\text{SGR (\%)} = \frac{\text{Final wet weight – Initial wet weight}}{\text{Experimental duration (days)}} \times 100$$

For *E. suratenesis*, two way analysis of variance (ANOVA) was carried out following the procedure described by Zar (1974) [11].

$$\text{PRP} = 1 - \left(\frac{\text{\%Mortality in the treated group}}{\text{\% Mortality in control group}} \right) \times 100$$

Challenge experiment: Fishes challenged with LD_{50} dose of pathogen were observed for mortality.
The Percent Relative Protection was calculated by the following formula.

3. Results and Discussion

Table 2: Survival Rate of *Etroplus* Sp. Fed with the Medicated Feed

Fish	Period of growth (days)	MF-I (%)	MF-II (%)	Control (%)
Etroplus suratensis	5	100	100	100
	10	100	100	80
	20	100	90	50
	30	95	80	-
Etroplus maculatus	5	100	100	100
	10	100	100	80
	20	100	100	60
	30	100	90	-

MF I: Ulva extract 100mg + Normal diet 1000g
MF II: Ulva extract 50mg + Normal diet 1000g

Table 3: Growth of Etroplus *suratenesis* in terms of weight after 30 days of treatment

S.No.	Control (g)	Medicated I (g)	Medicated II(g)
1	4.83±1.30	9.73±1.03	5.87±1.11
2	5.00±1.28	9.47±0.54	5.83±1.48
3	4.20±0.94	9.60±0.51	6.27±0.39
4	5.03±0.84	9.23±0.73	5.30±0.44

MF I: Ulva extract 100mg + Normal diet 1000g
MF II: Ulva extract 50mg + Normal diet 1000

Table 4: Survival Rate and Percent Relative Protection (Prp) of Experimental Group of Etroplus. *Sps*

Etroplus. Sps	Feeding period	Survival rate			PRP	
		Control	MF-I	MF-II	MF-I	MF-II
Etroplus suratenesis	30 days	0%	95%	80%	95%	80%
Etroplus maculatus	30 days	0%	100%	90%	100%	90%

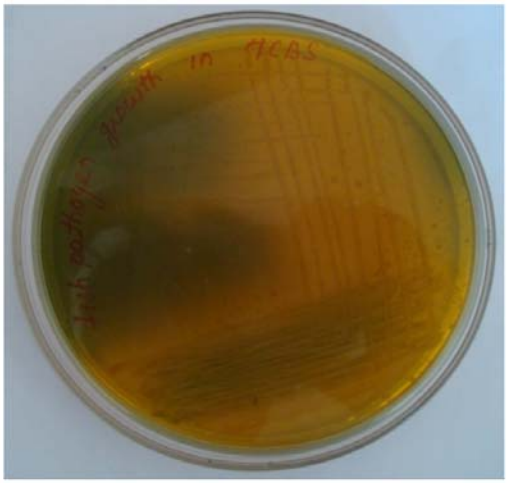
MF I: Ulva extract 100mg + Normal diet 1000g
MF II: Ulva extract 50mg + Normal diet 1000g

Etroplus sp. was screened for its susceptibility to bacterial infections and found its dependence on seasonal variations. The condition of the fish and the environment exert great influence on the outbreak of clinical disease. Sinderman (1990) [9] described that the bacteria are part of indigenous micro flora, existing as commensals on the fish and in its environment. Similarly, the fish species studied in the present investigation harbored *Vibrio* sp., and was thus affected by the disease. The biochemical identification (Table 1) confirmed that the organism belongs to *Vibrio* sp.

Table 1: Bio-chemical Characteristics

Name of the test	Result
Gram Staining	-ve (negative) rod
Growth on TCBS	Yellow colonies
Sucrose	-ve
Gas Production	-ve
Lactose	-ve
Lipase	-ve
Citrate	+ve
H_2S Production	Nil
Acid Production	Nil
Starch	-ve
Indole	+ve
Methyl Red	-ve
Gelatin hydrolysis	+ve
Caesin hydrolysis	+ve

Fig 1: Growth of *Vibrio* sp. Isolated from E. *suratensis* in TCBS agar



The bacterial disease was successfully transmitted to the apparently healthy *Etroplus* sp. by administering *Vibrio* cells intraperitoneally. Same symptoms observed earlier were seen in experimentally infected fishes. This proved the Koch

Postulate and confirmed the role of bacteria in bringing about the mortality. Earlier studies on successful laboratory infection of rainbow trout were carried out by several researchers (Egidius *et al.*, 1981) [1]. The LD50 determination was done in *E. suratensis* and the lethality dose was determined as $1x10^6$ cells and hundred percent mortality was observed in fishes, 13.25 h after injection. The variability in different species of fish with same bacterial isolate was also studied by Lipton (1987) [5] and Soltani *et al.*, (1994) [10].

The average absolute growth in *E. maculatus* was 0.37 g and 0.3 g in case of the fishes fed with the medicated feed at different doses when compared to 0.01g observed in case of control. The specific growth rate was determined as 3.7 % and 3 % as against 1% for control. The results obtained for *E. suratensis* also showed a comparative increase in the growth (Table 3). Similarly, seaweeds such as *U. fasciata* and *Sargassum wightii* enhanced measurable immune response (Felix *et al.*, 2004, Selvin *et al.*, 2004) [2, 7, 8].

Marine Natural products (MNPs) are important sources of medicine for preventing bacterial diseases of fishes (Sakai, 1999) [6]. The methanol extract of *Ulva fasciata* at two treatment levels was helpful to effectively minimize the natural mortality of the two *Etroplus* sp. and increase the growth of both compared to the control as is evident from the determination of the survival rate (Table 2) and the absolute and specific growth rates.

Future studies should be conducted which focus on different parameters which could finally establish and commercialize these *U. fasciata* with feed to be given to *Etroplus* species (*Etroplus maculatus and Etroplus suratenesis*) to increase the disesase resistance and in its successful growth.

4. References

1. Egidius E, Andersen K, Clausen E, Raa J. Cold water vibriosis (or) 'Hitra Disease' in Norwegian salmonid farming. J Fish. Dis. 1981; 4:352-354.
2. Felix S, Robins PH, Rajeev A. Immune enhancement assessment of dietary incorporated marine alga Sargassum wightii (Phacophyceae / Punctariales) in tiger shrimp penacus monodon (crustace/ penacidae) through prophenol oxidase (pro) system. India J Mar. Sci. 2004; 33(4):361-364.
3. Hjeltnes B, Roberts RJ. *Vibriosis* In: Inglis V., Roberts R.J. and Bromage N. (eds) Bacterial diseases of fish Black well Scientific Publications, London, 1993, 109-121.
4. Jeanjose J, Lipton AP, Subhash SK. Impact of Marine Secondary Metabolites from Hypnea Musciformic as an Immunostimulant on Hemogram Count and Vibrio alginolyticus Infection in the Drawn, Penacus monodon at different salinifies. The Israeli Journal of Aquaculture-Bamidgeh. 2007; 60(1):65-69.
5. Lipton AP. Studies on microbial diseases of some commercially important freshwater fishes with special reference to Aeromonarsp, and Pseudomonas sp. Thesis submitted to Madurai Kamaraj University, 1987.
6. Sakai M. Current research status of fish immunostimulants aquaculture 1999; 172(1-2):63-92
7. Selvin J, Huxley AJ, Lipton AP. Immunomodulatory potential of marine secondary metabolites against bacterial diseases of shrimp aquaculture, 2004.
8. Selvin J, Lipton AP. Biopotentials of *Ulva fasciata* and Hypnea muciformis collected from the Penisular coast of India J Mar. Sci. Tech. 2004; 12(1):1-6.
9. Sindermann CJ. Principal diseases of Marine fish and shell fish 2nd end diseases of Marine fish acad press Inc.Ny 1990; 1:521.
10. Soltani M, Burke CM. Responses of fish pathogenic cytophaga flexibacter like bacteria (CFLB) to environment conditions. Bull Eur. Ass. Fish. Pathol, 1994; 14(6):185-187.
11. Zar JH. Bio-statistical analysis. Prentice Hall. New Jersey, 1974, 620.