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Antibacterial Potential of Marine Cyanobacteria against Bacterial Fish Pathogens isolated from *Catla catla, Lobeo rohita, Channa gachua*

Riyazulla Azeez[1,*], Kumarasamy Anbarasu[2], Dhanalakshmi P. K[1] and Thangaraju Nallamuthu[1]
[1]Centre for Advanced Studies in Botany, University of Madras, Maraimalai Campus, Guindy, Chennai-25.
[2]Department of Marine Biotechnology, Bharathidasan University, Tiruchirapalli.

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ABSTRACT

Aquaculture is growing more rapidly than all other animal food producing sectors. In order to meet out the global rising animal protein demand, aquaculture plays an important role. Fishes are constantly exposed to wide variety of diseases includes bacteria, viral, fungal, and parasites in all water bodies. Bacterial fish diseases constitute one of the major limiting factors for sustainable aquaculture production all over the globe. Environmental conditions such as pollution, poor water quality parameters, intensive culture practice, integrated fish management, etc are the major factors to cause diseases. In the present study diseased fish such as *Catla catla, Lobeo rohita, Channa gachua* showing symptoms were identified, brought to the laboratory for isolation of bacterial pathogens. Total of 11 isolates were identified based on colony morphology, gram staining, catalase, oxidase reaction. Biochemical test 11 different isolates were reveals heterogeneous characteristics belong to *Pseudomonas* sp, *Aeromonas* sp and *Vibrio sp.* All the 11 different isolates were examined for Antibiogram Assay against commercially available antibiotics. Moreover efficacy of crude Cyanobacterial extracts was tested against this isolates. The antibacterial potency was evaluated using various concentration 50, 100, 150, 200 µg in paper disc. Among these only *Pseudomonas* sp was sensitive to *Synechococcus* sp, *O. weilli* BDU141621 BDU110791 and *Lyngbya* sp. All other bacterial isolates were resistant to all the cyanobacterial strains *O. weilli* BDU130781, BDU110791, BDU141621, BDU13791, BDU130411 *Phormidium* sp, *Lyngbya* sp, *Synechococcus* sp strains examined in the present study.

Introduction

Aquaculture is growing more rapidly than all other animal food producing sectors. In order to meet out the global rising animal protein demand, aquaculture play important role. It is one of the fast emerging and promising food production sector to supply proteinaceous food, women employment, rural development, waste land development, employment, foreign exchange, bioremediation etc. The contribution to the global supply of fish, crustaceans, and molluscans has increased steadily from 3.9% in 1970 to 29% in 2001. Capture fisheries exploit much of the world's oceans and pressure on this resource will increase as the human population expands from 6 billion in

2000 to an estimated 9-10 billion by 2050 (FAO, 2002). It is estimated that aquaculture has grown at an average rate of 8.8% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial meat production sectors including cattle and poultry. According to FAO statistics, aquaculture provided nearly 50% of the annual world fisheries production of 120 million metric tons in 2004 (FAO, 2006). Of this, half is comprised of finfish, a quarter is aquatic plants and the remaining quarter is crustaceans such as shrimp, prawns, lobster, crabs, oysters and mussels.

Crypnoids, Tilapia and Catfish are the major freshwater fishes cultured in India and contribute about 72% of the total aquaculture production in Asia (Csavas, 1994). The major cultured marine fish includes the red fish, basses, congers, jacks, mullets, and suries. Based on the statistics its clear that the aquaculture industry is mainly depends on fresh water ecosystem and the capture fisheries are depend on marine ecosystem. One of the major limiting problems in the fish production in Asia is loss due to the diseases. Environmental factors such as pollution, sudden climatic changes, flood out, malnutrition, intensive fish farming, are the other phenomena for loss of fish production. As per FAO survey (2004) 3 billion US dollar loss annually to Asian aquaculture industry. Invariably, all fish species and water bodies such as freshwater, brackish water, and marine environments are susceptible to number of bacterial, fungal, viral and parasitic diseases. Among these, in the present study aimed to indentify and characterize bacterial pathogens that cause disease to fishes.

Cyanobacteria (Blue Green Algae) has been identified as one of the most promising group of organism from which novel active natural compounds were identified as antimicrobial, antioxidant, colorant, etc. The medicinal qualities of marine cyanobacteria was appreciated as early as 1500 B.C. Prior to 1990 limited investigations were undertaken on the isolation of biologically active natural products from cyanobacteria (Burja et al., 2001). After 1990 the potency of so many Cyanobacterial metabolites has been recognized by the scientist and in recent years. So many laboratories are working on this aspect. In our lab we are interested in identify bioactive compounds against fish diseases. The present work is an attempt

Tele: +91 97894 30467, +91 88830 95789
E-mail addresses: riyazscientist@gmail.com

to identify potential marine Cyanobacteria against bacterial fish pathogens.

Materials and methods

Sampling site

Diseased fish such as *Catla catla, Labeo rohita* and *Chana gachua* were collected from the River Cauvery at Mukkombhu (latitude of 10º 35 N and longitude of 78º 46 E) and Kallanai about 25 km apart in Trichirapalli, Tamil Nadu. The fishes showing diseased symptoms such as skin lesion on the body surface, tail rot, eye haemorrhage, ulcerative syndrome) were transferred to laboratory in live *via* road as soon as possible to the laboratory.

Processing of diseased fish

The diseased fish was washed with sterile water followed by wiped the dorsal surface in ethanol dipped filter paper and the diseased portion were cut with the sterile knife: The tissues were transferred into sterile vials, minced, diluted and inoculated in to the nutrient broth and then streaked onto the nutrient agar plates.

Presumptive identification tests

Kovac's oxidase test

Whattman No. 1 filter paper strips were impregnated with 1% solution of tetra methyl Para phenylene diamine dihydrochloride were used to test oxidation. A small amount of young cultures (16 - 18 h) were streaked on the filter paper and observed for colour change. The appearance of an intense purple colour within 5 - 10 seconds indicated a positive reaction while presence or absence of colour after 11 - 60 seconds indicated a negative reaction.

Catalase test

A small amount of culture was placed over a clean slide. A drop of 3 % H_2O_2 was placed over the culture and observed for effervescence. The production of effervescence showed the ability to produce the enzyme catalase.

Gram staining

Bacterial cultures of 16 - 18 h were smeared on clean grease free glass slides, heat fixed and stained as follows. The slide was flooded with crystal violet for a min, drained and rinsed with water followed by grams iodine for one min, drained and rinsed with water. Decolourised with acetone alcohol for 30 sec, counter stained with safranin for one min and observed under an oil immersion microscope. Violet or purple cells were identified as Gram positive and pink colour as Gram negative.

Biochemical tests

Triple sugar iron tests

TSI slants ware prepared and inoculated with the selected isolates by stab and streak method. The inoculated slant was incubated at room temperature for about 24 h. The change in color of the slant and butt was recorded. The yellow butt and red slant (A/K) indicates formation of glucose, yellow butt and slant (A/A) indicates the formation of lactose or sucrose and red butt and red slant (K/K) indicates no sugar formation. Gas production was noticed by bubble formation in butt and H_2S production by black color formation in the butt.

Oxidation - Fermentation test (Hugh and Leifson's method)

Oxidation - Fermentation discs with physiological saline were inoculated and incubated at 24 oC in the interval of 6 hours. Colour change from white to yellow showed fermentation.

Indole test

Tryptone broth was inoculated with the test isolates and incubated at 24oC for 48 - 72 h. About 0.2 - 0.3 ml of Kovac's reagent was then added to the test tube, shaken and allowed to stand. The formation of a red ring on the broth surface was confirmed the production of indole.

Methyl red test

MR-VP broth was inoculated with isolates, incubated for 24 h at 24 - 30 ^oC. The appearance of a red colour on addition of methyl red solution was considered as positive.

Voges - Proskauer test

MR-VP medium was inoculated with culture isolates and incubated at 24°C for 24 - 48 h. After incubation 3 ml of Barrits reagent A and 1 ml of Barrits reagent B were added. The tubes were shaken and allowed to stand for 15 min and observed for colour change. Pink colour development was considered as positive.

Test for H_2S production and glucose utilization

Triple sugar iron agar slants were inoculated (stabbed and streaked) with the test organism and incubated at 24 ^{o} C for 24 - 48 h. The change in colour of the medium from red to yellow indicated the production of acid from glucose. Blackening of the medium was indicated the production of H_2S. Cracks/Breaks in the medium were showed as production of gas from glucose.

Citrate utilization test

Simmons's citrate agar slants were inoculated with the selected isolates by stabbing and streaking method. The inoculated slants were incubated at room temperature for about 24 h. Blue color indicates the positive and green color indicates the negative result.

Urease test

Christensen's medium slant was prepared, inoculated with the selected isolates and incubated at room temperature for about 24h. Presence of pink color indicates the positive result and absence of pink indicates the negative result.

Carbohydrate Fermentation test

The carbohydrate fermentation test was used to distinguish the organism upto species level identification. The glucose, sucrose, lactose, fructose, sorbitol, starch and mannitol were used in the present study to check the fermentation of each isolates. The media were prepared, sterilized and dispensed in tubes with inverted Durham's tube. After 48 hours of incubation, results were noted to determine the acid and gas production.

Test For Hemolysis on Blood agar media

Hemolytic activity was determined for all the isolates by direct blood agar plate assay.

Blood agar plate procedure

About 5 ml of human blood was collected from jugular vein with sterile 3.8% trisodium citrate (1 ml citrate solution for 10 ml blood). Simultaneously prepare the basal medium nutrient agar, sterilized and cooled to 50 - 55^o C in water bath. Add aseptically with the sterile pipette10 ml of blood to 90 ml of the basal mix and pour the plates care must be taken to avoid air bubbles during pouring (if the Air bubbles present pass the Bunsen burner flame over the medium to break it) allow it to set for solidification.

Direct hemolysin assay

Hemolytic activity was determined on blood agar plates. A zone of hemolysin around the colonies on the blood agar plates were noted, after 24 h incubation at 24 ^{o} C.

Pigment production

This test is used for the direct identification of various *Pseudomonas* sp. It consists of two types, such as phyocyanin pigment and pyoveridin pigment production. For pigment production the culture were inoculated on medium A and B and

incubated at 24°C for 24 -72 h. The production of green pigment indicates the identification of *Pseudomonas* sp.

Antimicrobial activity

Antibiogram studies

Eleven isolates were examined for their *in vitro* susceptibility to six antibiotics namely Ampicillin (10 mcg), Amoxicillin (10 mcg), Cefalaxin (30 mcg), Penicillin (10 units), Gentamycin (10 mcg), Streptomycin (10 mcg).Antibiotic susceptibility was determined by antibiogram disc assay on Muller Hinton Agar (Hi media) by Kirby - Bauer method.

The test cultures were grown in 5 ml of broth, the log phase culture was swabbed onto the media after 10 minutes, and antibiotic discs were kept 20 mm apart. After 24 hrs at 37 °C measure the zones of inhibition was to determine sensitive, resistant and intermediate characteristics of different isolates. This was done according to the recommendation national committee for clinical laboratory standards.

Cyanobacteria biomass

Eight strains of marine cyanobacteria namely *Synechococcus* sp, *Oscillatoria* sp, *Lyngbya* sp, *Phormidium* sp obtained from "National Facility for Marine Cyanobacteria" Bharathidasan University, Tiruchirapalli were cultivated on a large scale for biological activity screening. For the biomass production the collected strains were grown in of ASN-III medium. With the following culture condition, cultures were maintained at 25o C, at a light intensity of 10 µmol m-2 s-1 provided by cool white fluorescent tubes and with a light/dark cycle of 14 h / 10 h. Cells were harvested after one-month by centrifugation. Samples were frozen at -20o C for further analysis.

Cyanobacterial extract

For antimicrobial activity, extracts were obtained from 8 cyanobacteria cultures (*Lyngbya* sp, *Oscillatoria* sp - 5 strains, *Synechococcus* sp, *Phormidium* sp) with organic solvent methanol prepared at a concentration of 100 mg ml-1 (freeze dried material / ml of solvent). Solutions were sonicated with an ultra-sound probe for 3 x 2 min on ice. The solutions were centrifuged at 10000 g for 10 min, the supernatants recovered, dried and resuspend in methanol at 100 mg/ml-1 (freeze dried material / ml of solvent) concentration.

Paper disc assay

Sterilized petri dishes containing about 20 ml medium had been inoculated with the test culture by spread plate method, sterilized paper discs (0.5cm) loaded with cyanobacterial compounds with varying concentrations (50, 100, 150, 200 µ g) were placed over the agar surface. The plates were left at 24°C for two hours, then incubated at 24°C for 24 h and examined for zones of inhibition around the disc.

Results and Discussion

Diseased fish samples were collected with the symptoms of skin lesion all over the body, tail rot, haemorrhage in eye, fin rot in a live / moribund condition in river Cauvery at Mukkombhu and Kallanai in Tiruchirapalli.

Processing of Samples

Diseased fish were wiped with sterile water followed by ethanol dipped tissue paper for removal of the surface contaminants, the infected tissues were scraped with the sterile scalpel or knife. The tissues were inoculated on to the nutrient broth; it was serially diluted from 10-1 to 10-5 and spread plate on nutrient agar plate. Pure isolated colonies were collected and labelled as A.R.F.P 1 – 26.

Cultures were maintained as stock and master cultures.

These cultures were routinely inoculated on to the nutrient agar plates as well as MacConkey agar, for morphological, biochemical and selective media for identification. The cultures were identified for various assays for characterization incubated at 24 – 30ºC for 24 – 48 h depends on the methods.

Presumptive tests

All 26 isolates were subjected to gram staining for studying the morphology of the selected isolates. Most of the cultures were showing as gram–ve rods (fig-1) and few isolates such as A.R.F.P.9 and A.R.F.P. 13 were identified as gram+ve rods.

Colony characterization on selective media

MacConkey agar is one of the best selective media to differentiate whether the bacteria is a lactose fermentor or not. In the present study, the selective isolates were grown on the MacConkey agar overnight and seen the colour of the colonies. The results show that colourless colony was observed after overnight incubation which indicates that the isolates are non- lactose fermenters.

Starch Ampicillin agar is a selective medium for *Aeromonas* sp. On Starch Ampicillin agar *Aeromonas* sp grows as honey or yellow color colony, *Pseudomonas* sp produce pink and blue color colony and *Vibrio* sp grows as yellow colour colonies. Thiosulfate citrate bile salt sucrose agar (TCBS) is used for the selective identification of *Vibrio* sp. In TCBS agar *Aeromonas* sp and *Pseudomonas* sp produce blue color colony, *Vibrio* sp produce yellow colour colony.

Blood agar is also an effective medium for characterization of bacteria. Based on the degradation of blood the organism can be categorized into α, β, γ hemolytic organisms. β shows the complete hemolysis, α shows the partial hemolysis and γ shows no hemolysis. In the present study, *Aeromonas* sp and *Vibrio* sp showed as β hemolytic colonies, and *Pseudomonas* sp shows partial hemolysis when grown at 30 °C for 24 – 48 hrs.

Based on the biochemical and colony characterization on selective agar 11 isolates were identified as *Pseudomonas* sp, *Aeromonas* sp, and *Vibrio* sp. The detailed biochemical and colony characterization were referred in fig-1.

Antibiogram assay

The selected 11 isolates of bacterial fish pathogens were checked for antibiotic assay by Kirby-Bauer method against 6 commercially available antibiotics such as Gentamycin, Streptomycin, Ampicillin, Amoxicillin, Cefalaxin, Penicillin (Hi- media). The results show that all the 11 isolates are highly resistant to Penicillin and Ampicillin antibiotics and highly sensitive to Gentamycin and Streptomycin antibiotics. Isolates A.R.F.P.8, A.R.F.P.16 shows moderate sensitive to Amoxicillin and Ciprofloxacin antibiotics based on Kirby Bauer Method of determination. The detailed results of 11 isolate are described in the fig-2.

Antimicrobial potential of Marine Cyanobacteria

In the present study, the antimicrobial potency of 8 marine cyanobacteria was analyzed against fish pathogens isolated from the diseased fish.

Fig 1: Morphological and biochemical characteristics of identified bacterial pathogens isolated from skin lesion, tail rot, ulcerative syndrome of *Channa gachua, Catla catla, Labeo rohita* diseased fish.

Culture No	Cell shape	Gram staining	TSI	Indole	MR	P	citrate	Urease	Catalase	Oxidase	O NPG	Pyoverdin pigment	Hemolysis on blood agar	TCBS	Starch ampicillin agar	H_2S	Glucose	Lactose	Mannitol	Arabinose
A.R.F.P.1	R	-	k/k	-			+	-	+	+	-	Green	α	blue	pink		A	-	-	-
A.R.F.P.2	R	-	k/k	-			+	-	+	+	-	Green	α	Blue	pink		-	-	-	-
A.R.F.P.7	R	-	a/a	+			-	-	+	+	-	N	β	green	Yellow		-	+/-	-	-
A.R.F.P.8	R	-	a/a	-			-	-	+	+	-	N	B	yellow	Yellow		-	+/-	-	-
A.R.F.P.10	R	-	a/a	+			-	-	-	+	+	N	β	No growth	Yellow	/-	-	+/-	-	-
A.R.F.P.11	R	-	k/k	-			+	-	+	+	-	Green	α	blue	pink		-	-	-	-
A.R.F.P.12	R	-	k/k	-			+	-	+	+	-	Green	α	blue	pink		-	-	-	-
A.R.F.P.15	R	-	a/a	-			-	-	+	+	-	N	β	yellow	No growth		-	+/-	-	-
A.R.F.P.16	R	-	a/a	+			-	-	+	+	+	N	β	No growth	Yellow		-	+/-	-	-
A.R.F.P.17	R	-	k/k	-			+	-	+	+	-	Green	α	blue	pink		-	-	-	-
A.R.F.P.18	R	-	k/k	-			+	-	+	+	-	Green	α	blue	N		-	-	A/g	-

- negative, **+/-** variable, **N** not done, **K** alkaline, **A** acid, **g** gas, **TSI** Triple sugar iron, **MR** Methyl Red, **VP** Vogues proskauer Test, **+** positive

Fig:2 Antibiogram assay for the bacterial fish pathogens isolate

Antibiotics	**ARFP 1**	**ARFP 2**	**ARFP 11**	**ARFP 12**	**ARFP 17**	**ARFP 18**	**ARFP 7**	**ARFP 8**	**ARFP 10**	**ARFP 15**	**ARFP 16**
Ampicillin	R	R	R	R	R	R	R	R	R	R	R
Amoxicillin	R	R	R	R	R	R	R	S	R	S	R
Cefelaxin	R	R	R	R	R	R	R	S	R	S	R
Gentamycin	S	S	S	S	S	S	S	S	S	S	S
Penicillin	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

S- sensitive, R- resistant

Cyanobacteria	Con (µg)	A.R.F.P.1	A.R.F.P.2	A.R.F.P.7	A.R.F.P.8	A.R.F.P.10	A.R.F.P.11	A.R.F.P.12	A.R.F.P.15	A.R.F.P.16	A.R.F.P.17	A.R.F.P.18
Lyngbya sp	50	R	R	R	R	R	R	R	R	R	R	20mm
	100	25mm	R	21mm	R	R	32mm	R	R	R	R	17mm
	150	R	10m	R	8mm	R	R	R	R	R	R	R
	200	R	R	R	R	R	R	R	R	R	R	R
O. weilli BDU141621	50	25mm	R	R	R	R	10 mm	R	R	R	R	R
	100	R	R	R	R	R	30 mm	R	R	R	R	R
	150	R	12mm	R	R	R	25 mm	R	R	R	R	R
	200	R	R	R	R	R	17 mm	R	R	R	R	R
O. weilli BDU110791	50	R	R	R	R	R	15mm	R	R	R	R	R
	100	R	R	R	R	R	R	R	R	R	R	R
	150	R	R	R	R	R	R	R	R	R	R	R
	200	19mm	8mm	R	R	R	23mm	R	R	R	R	R
Synechococcus sp	50	R	R	R	R	R	15mm	R	R	R	R	R
	100	R	R	R	8mm	8mm	40mm	R	R	R	R	R
	150	R	R	R	R	R	25mm	R	R	R	R	R
	200	R	R	R	R	R	13mm	R	R	R	R	R
Phormidium sp	50	R	R	R	R	R	R	R	R	R	R	R
	100	R	R	R	R	R	R	R	R	R	R	R
	150	R	R	R	R	R	R	R	R	R	R	R
	200	R	R	R	R	R	R	R	R	R	R	R
O. weilli BDU130781	50	R	R	R	R	R	R	R	R	R	R	R
	100	R	R	R	R	R	R	R	R	R	R	R
	150	R	R	R	R	R	R	R	R	R	R	R
	200	R	R	R	R	R	R	R	R	R	R	R
O. weilli BDU13791	50	R	R	R	R	R	R	R	R	R	R	R
	100	R	R	R	R	R	R	R	R	R	R	R
	150	R	R	R	R	R	R	R	R	R	R	R
	200	R	R	R	R	R	R	R	R	R	R	R
0.weilli BDU130411	50	R	R	R	R	R	R	R	R	R	R	R
	100	R	R	R	R	R	R	R	R	R	R	R
	150	R	R	R	R	R	R	R	R	R	R	R
	200	R	R	R	R	R	R	R	R	R	R	R

Fig 3: Antimicrobial potential of methanolic extract of marine Cyanobacteria against bacterial fish pathogen isolated from river Cauvery

In order to get the marine cyanobacteria secondary metabolites, the cyanobacteria such as *Lyngbya* sp, *Oscillatoria weilli*, *Synechococcus* sp, *Phormidium* sp were mass cultured in 25 ºC provided by cool white fluorescent tubes with light and dark cylcle1 of 4/10hrs under controlled conditions. After one month Cyanobacteria were harvested and extracted by Rosario F. Martin method. For the *Pseudomonas* sp, *Aeromonas* sp, *Vibrio* sp methanolic extract of marine cyanobacterium was checked for the antimicrobial activity using paper disk assay. The concentration of disc prepared was 50, 100, 150 and 200µ g. Of the eight Cyanobacterial extracts *Synechococcus* sp, showed maximum inhibitory zone of 40 mm at 100 µ g of against *Pseudomonas* sp (A.R.F.P.11), *O. weilli* BDU 110791, *Lyngbya* sp, *O. weilli* BDU141621 showed maximum inhibitory zones against *Pseudomonas* sp. Other cyanobacterial extract of *O. weilli* BDU130781, *O. weilli* 13791, *O. weilli* BDU130411 does not showed any inhibition against *Pseudomonas* sp upto 200 µ g concentrations fig-3.

All eight cyanobacterial extract does not showed antimicrobial activity against *Aeromonas* sp and *Vibrio* sp up to 200µ g concentration. Result shows cyanobacteria *Lyngbya* sp, *Synechococcus* sp, have potential antimicrobial compounds against fish pathogens especially *Pseudomonas* and not effective against *Vibrio* sp and *Aeromonas* sp.

Number of bacterial pathogens were isolated and identified in freshwater, brackish water and marine ecosystem since 1970 in India, however, large new emerging strains were developed due to resistant with various environmental and antibiotic resistant mechanism. This is the major reason for the failure of effective protective vaccine / antibiotics against bacterial pathogens. In our knowledge, no effective vaccines were available in our market against bacterial pathogens, however, experimental studies shows that formalin, heat killed, lipopolysaccharides provide protective and promising antibody projection against specific pathogens (Anbarasu. K and M. R. Chandran, 1998 and 2001) (Anbarasu. K and K. Thangakrishnan, Aruna. B. V and M. R. Chandran, 1998). Hence, an attempt to make to isolate the freshwater fish pathogens in the River Cauvery, to identify them in order to develop effective vaccines against them. In the present study, total of 26 isolates were collected from diseased fish *Labeo rohita*, *Catla catla*, *Channa gachua* with the symptoms of skin lesion, tail rot, ulcerative syndrome.

Of these 26 isolates 11 isolates were identified by biochemical characterization. From the 11 isolates six isolates were found to be *Pseudomonas* sp, three isolates were found to be *Aeromonas* sp and two isolates were found to be *Vibrio* sp. This identification shows increasing incidence infection to the freshwater fish pathogen during the month of July to October 2009. Also these three organisms prevalent in freshwater causing severe ulcerative syndrome, skin lesion, tail rot etc. Previous workers (Nabi *et al.*, 2000), isolated three different types of *Pseudomonas* sp from *Channa gachua*, *Pseudomonas anguilloseptica* in cultured eel (Wakabayashi, 1972) and in ayu (Nakai *et al*., 1985). *Aeromonas* sp is a motile gram negative rod shaped bacteria is responsible for causing severe septicemia in fish and shell fish and also responsible for causing ulcerative syndrome and skin lesion in wide variety of fish (Anbarasu. K *et al*., 1998). The biochemical analysis and selective media shows the effective in identification of the pathogen.

Antibiogram of *Pseudomonas* sp, *Aeromonas* sp and *Vibrio* sp shows the marked sensitivity against Gentamycin (10 mcg) and streptomycin (10 mcg). All the test isolates were showed resistant pattern against Ampicillin, (10 mcg) Penicillin, Amoxicillin and Cefalaxin antibiotics. Similar results were obtained when Saleh *et al*., 2000 *(Pseudomonas anguilloseptica* showed sensitive to Streptomycin and Gentamycin and resistance to Penicillin) similarly Nabi *et al.*, 2000 showed high sensitivity to Gentamycin however they are resistance to Streptomycin against *P. aeruginosa*, *P. putida*, *P. stuzeri* reveals the genetic diversity among various isolates tested.

Eight marine Cyanobacterial extracts were used for the antimicrobial potential against *Pseudomonas* sp, *Aeromanas* sp, *Vibrio* sp. Of these *Synechococcus* sp, *Lyngbya* sp and *O. weilli* BDU 141621 showed maximum inhibitory activity against *Pseudomonas* sp only. Other strains of *O. weilli*, *Phormidium* sp does not showed any antimicrobial potential against any of the fish pathogen upto 200µ g concentration. Moreover *Aeromonas* sp and *Vibrio* sp does not produce inhibitory zones against methanolic extract of Cyanobacteria (200µ g concentration).

Early report reveals that *Synechococcus* extract was found to be effective against three test microbes *E. coli*, *Pseudomonas* sp, *Vibrio* sp, however, it exhibited strong activity (zone of inhibition 16 mm) against *Vibrio* sp. Mild activities were observed in case of *Synechocystis* sp and *Lyngbya* sp against two of test, whereas *Oscillatoria* sp. was totally inactive. Endocellular products of *Synechocystis* sp. have been reported to be inhibitory against *Escherichia coli* and *Salmonella* sp. (Oufdou *et al.*, 1998).

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