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A PRELIMINARY STUDY ON BACTERIAL DIVERSITY IN AQUACULTURE FARMS OF WEST GODAVARI REGION OF ANDHRA PRADESH

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ABSTRACT

***Background**: Aquaculture is the fastest growing food sector globally and is established itself as high protein resource to fulfil the food demand since the natural exhibits over exploitation. India occupies second position in the culture fish resources. Andhra Pradesh state is called the 'Fish Bowl' of India and is endowed with rich aquatic resources, comprising Godavari, Krishna and Penna rivers. Fisheries play an important role in providing livelihood option to the rural, poor and also supporting Indian economy. Bacteria which are omnipotent exist as microflora in water and can be of particular importance in causing damage to fish farming industry. Diseases caused by the bacteria are often chronic than acute and may also cause a high percentage of mortality which is highly induced by the environmental stress. Early diagnosis of the disease and development of successful vaccines are important for the future development of aquaculture. **Objective:** The present study was aimed to isolate and possible identification of potential pathogenic bacteria from water samples of West Godavari Regions of Andhra Pradesh. **Materials and Methods:** Water samples from five different regions namely Bhimavaram, Akiveedu, Diruusamarru, Palokollu and Veeravasaram of West Godavari district. Samples collected were identified, analysed and the bacterial load of the samples were determined for the possible indicator organisms by implicating agar plate method. Differentiations and characterizations of various bacterial isolates were identified based on biochemical reactions and gram -staining technique. **Results:** Preliminary study reported that possible enterococci were identified in the majority of the water samples. Other bacteria including Staphylococcus aureus, Bacillus subtilis, Enterobacter; Klebsiella, Micrococcus and E. coli were also identified in the selected fish ponds. **Conclusion:** It is revealed that the ponds were grossly contaminated with pathogenic microorganisms especially bacteria which possess high risk to the cultured fishes.*

KEY WORDS

Aquaculture, Bacterial diversity, Pathogenic bacteria, Bacterial load, Bacterial characterization.

INTRODUCTION

Aquaculture is emerging as the fastest growing food-producing industry in the world because of the increasing demand for food fish consumption and it has showed a rapid growth for the past few decades. Globally it has a greater importance which serves a variety of purposes majority of which include producing high nutritional food value for human consumption and contributes rural income and employment through farming and related activities. India is the potential land for aquaculture with its 5700 km coastline. Due to its International demand aquaculture in India became a very important economic activity and a flourishing sector Bacteria always present in the water environment, multiply and invade to fish and spread the disease when reaching the suitable condition. *Pseudomonas* was originally described as the causative agent of Bacterial Hemorrhagic Septicemia (BSH) disease of pond-cultured fish. It is considered as a primary pathogen of freshwater fish and opportunistic pathogen for different fish species cultured in marine and brackish waters worldwide. It is reported that *P. fluorescens* has been isolated from eye surface and mouth lesions of diseased Rajpunti (*Barbodes gonionotus*) along with some other fish pathogenic bacteria. Isolation and characterization of *P. fluorescens* from gills of silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), African magur (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) has also been reported.

Andhra Pradesh stands first in the production of coastal aquaculture and also occupied second place in the production of fresh water fish in India. West Godavari is one of top regions in fish production in Andhra Pradesh for major carps like *Catla catla, Labeo rohita, Cirrhinus mrigala,* prawns like *Penaeus monodon, Macrobrachium rosenbergii* and fishes like *Pangasius hypophthalmus*. It is estimated that 40% of the total available resources in India have been utilized for aquaculture because it has both technical and market access issues and there is a lot of scope provided for the development of fish culture. When fishes are stocked under high densities they exhibit stress, over stocking and overcrowding often trends adversely and it affects the health of the cultured fishes by making them vulnerable to the severe infectious disease out breaks (Gultepe et al., 2012). As a result, fishes are evolved with a number of protective adaptations with several unique traits against pathogenic bacteria and some external organs like fins, scales, and mucous which covers the body of the fishes along with the internal organs like gills and liver secrete juices possesses antibiotic resistance properties (Foster et al., 2013).

Diseases in fishes are characterized by the appearance of clinical signs of the bodily alterations and also depend on and various factors like physical, chemical, and biological risks which causes physiological alteration of the organism (Huicab-Perch et al., 2016). Fishes are highly susceptible to the contamination with different bacteria because of their highly perishable protein content in their body. Most of the pathogenic bacteria can reside in the environment or on/in apparently attacks normal fishes. Thus, infections are often precipitated by some stress that upsets the natural defences against the causative agents (e.g. overcrowding, over stocking, stress, low DO, high ammonia). Diseases in fishes are characterized by various factors like physical, chemical and biological risks which cause physiological alterations in the organism (Huicab-Perch et al., 2016). Outbreaks of bacterial diseases in fishes are highly responsible for huge mortality both in wild and farm-cultured fishes which causes economically devasting losses to the aquaculture fish farmers. The presence of bacteria in fish could play a diverse role in such a way that some may be beneficial to the fish while others leads to adverse health conditions.

Bacterial diseases remain like a hindrance to the aquaculture expansion globally. Infectious diseases like bacteria are considered as one of the major impediments and causes significant economic losses to the aquaculture industry and these infections are caused by various bacterial, viral and fungal pathogens. They are well characterized as bacterial infections by their symptoms in fish culture farms (Carbone and Faggio, 2016). The present study was focused on isolation, screening and identification of potential pathogenic bacteria from fish ponds of the West Godavari region in Andhra Pradesh.

MATERIALS AND METHODS

Sample collection:

This study was conducted in West Godavari district, were five different functional aquaculture pond sites were selected from different localities like Bhimavaram, Akiveedu, Dirusumarru, Palakollu and Veeravasaram. Water samples were collected at a depth of 25-35 cm below the surface of the fish pond, at the time period from 10:00 am to 12:00 pm aseptically using sterile water sampling bottles from the fresh oxygenated ponds, once a month during the study period. All the samples were collected aseptically and appropriately labelled with the permanent marker. The pH of each water sample was measured using pH meter and the temperatures of the samples were also measured with the help of a mercury thermometer by immersion in to the water samples. Salinity was also recorded on the spot itself. All these samples were brought to the microbiological laboratory in an ice box for observation and immediate analysis.

Microbial analysis of the sample:

The microbial load of the water samples from the fish ponds was determined by performing a tenfold serial dilution in the test tubes containing sterile distilled water for inoculation on nutrient agar medium. The total viable bacterial count was determined by using the pour plate technique cultured in duplicates. The petri dishes were inverted to prevent condensation dropping from the lid in to the agar and incubated in the incubator at 37°C for 24-48 hours. Overriding colonies were selected and picked from the nutrient agar and again streaked for locating pure cultures. These pure cultures of the bacterial isolates were further subjected to various phenetic, biochemical and microscopic characterization studies (Cheesbrough, 2006).

Table (1): Physico-chemical analysis of fish pond water collected in different locations of West Godavari Regions of Andhra Pradesh

Sample	Pond	Temperature (°C)	Salinity (ppt)	pH
Narasapur	A1	27	3	8.3
(colourless)	A2	27	0	8.5
	A3	27	2	8.3
	A4	27	0	8.0
	A5	27	4	8.1
Bhimavaram	B1	28	3	7.5
(colourless)	B2	29	2	8.1
	B3	27	3	8.3
	B4	26	4	8.2
	B5	25	2	8.1
Palakollu	C1	26	4	8.1
(Pale yellow–light green)	C2	25	4	8.2
	C3	25	5	8.1
	C4	25	5	8.2
	C5	27	4	8.1
Dirasumurra	D1	27	0	7.4
(colourless)	D2	26	0	8.4
	D3	26	0	7.8
	D4	25	0	8.3
	D5	25	0	8.3
Akiveedu	E1	26	0	7.9
(pale yellow to dark green)	E2	26	0	8.0
	E3	25	0	7.9
	E4	25	0	8.1
	E5	26	0	8.1

Table 2. Phenotypic characterization of bacteria isolated in fish ponds in different locations

Sample	Pond	Culture	Formation	Colour	Gram Staining	Shape	Arrangement
Narasapur (1)	A1	Opaque	Irregular	White	-	Rods	Single
	A2	Opaque	Round	White	+	Cocci	Chain
	A3	Translucent	Round	White	+	Rods	Pairs &Chain
	A4	Opaque	Irregular	White	-	Rods	Single & chain
	A5	Translucent	Irregular	Pale yellow	-	Cocci	Single
Bhimavaram (2)	B1	Translucent	Round	White	+	Rods	Groups
	B2	Transparent	Irregular	White	+	Rods	Single & chain
	B3	Transparent	Irregular	White	+	Cocci	Single & chain
	B4	Opaque	Irregular	White	+	Rods	Single & chain
	B5	Opaque	Round	White	-	Rods	Single & chain
Pallakolu (3)	C1	Transparent	Irregular	Pale yellow	+	Rods	Single
	C2	Opaque	Round	White	+	Cocci	Group
	C3	Opaque	Round	White	-	Cocci	Group
	C4	Opaque	Filamentous	White	-	Cocci	Group
	C5	Translucent	Round	White	-	Rods & cocci	Group
Dirasumurra (4)	D1	Translucent	Round	White	-	Rods	Single
	D2	Translucent	Round	White	+	Cocci	Chain
	D3	Translucent	Round	White	+	Rods	Single & chain
	D4	Irregular	Irregular	White	+	Cocci	Group
	D5	Irregular	Irregular	White	+	Cocci	Group
Akkiveedu (5)	E1	Transparent	Irregular	White	+	Rods	Single
	E2	Transparent	Irregular	Pale yellow	-	Rods	Group& chain
	E3	Transparent	Irregular	White	+	Cocci	Group& chain
	E4	Opaque	Round	Yellow	+	Cocci	Single
	E5	Translucent	Opaque	White	+	Cocci	Single

In order to estimate the bacteria in numbers, the agar plates were incubated for 18-24 hrs at 37°C. Duplicates were prepared for each dilution. After the incubation, the total number of colony forming unit (CFU) was determined and the representative colonies which were obtained were again sub-cultured the accurate identification of the bacteria. Bacterial numbers were calculated as the average of each set of duplicates and expressed as CFU/ml of the homogenate. Finally, the bacteria were isolated by the random collection of the colonies from the agar plates. The bacterial colonies were purified by the repeated sub-cultures.

Colonial characteristics were also determined with the instrument of digital colony counter magnifying lens, which was also used to count the number of colonies in each plate. Further clarification was also done to identify the morphological characters with the help of light microscope. The colour, shape, size, diameter and arrangement of the colonies were examined and recorded. Gram's staining was also performed to identify the bacteria according to Fawole and Oso (1988).

Different biochemical tests were carried out for the possible identification of bacteria from the water samples. The cultural morphological and biochemical characteristics of the respective isolates which are obtained were compared with the criteria in Begey's manual of Determinative Bacteriology (1994). The biochemical tests used in the identification and characterization of the isolates includes: catalyse test, Voges proskauer (VP) test, indole production test, methyl red test, Simmons's citrate utilization agar test, sugar fermentation test, glucose fermentation test, hydrogen production test, bacteria motility test.

Table (3): Biochemical characterization of bacteria isolated in fish ponds

Test	Bacteria*						
	A	B	C	D	E	F	G
Catalase test	-	+	-	-	+	+	+
Coagulase test	-	+	-	-	-	-	-
Indole production test	-	-	-	-	-	-	+
Methyl red test	+	-	+	+	-	-	+

Look-like bacteria: A= *S. aureus*; B = *B. subtilis*; C= *B. subtilis*; D= *Enterobacter*; E= *Klebsiella*; F= *Micrococcus*; G= *E. coli

RESULTS AND DISCUSSION

In this study, microbiological examination on the selected fish pond waters in the West Godavari region for the detection of various bacteria and their population could be found in different fish culture ponds. All the purified isolates were observed for cell shape, motility, flagellation, spores and encapsulation followed by Gram staining. Based on the phenetic and biochemical characterization, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterobacter, Klebsiella*, *Micrococcus* and *E. coli* look-like bacteria were identified in this study. Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. In the present study, marked variations in its physico-chemical parameters, such as water temperature, pH, dissolved oxygen, alkalinity, hardness and chloride have been observed. It seems that these variations in the water quality parameters may favour for outbreak of the disease in fish culture farms (20). It has been observed that infectious disease is one of the most important constraints to efficient the sustainable aquaculture production which mainly focuses on food security, socio-economic development, trade and market and profitability (21). The persistence of pathogens in the aquatic environment is also considered as one of the most crucial factors for the transmission of the infections which finally results in the acute outbreak of the disease in the fish culture ponds (Mlejnkova and Svova, 2012). Water samples collected from different areas of West Godavari region which were adjoining to each other so that surrounding physic chemical factors of the fish ponds regulate the quality and quantity of microorganisms which in turn reflects the microbial load assessment. The pH for any water body is to be neutral and it actually ranges from 7.5 to 8.5. Fish have their own tolerable limits and any fluctuations in pH results in the mass mortality. The pH is slightly high during summer and it is suitable enough for the survival of the fishes (1). Dissolved oxygen concentration is also another important parameter used in judging the suitability of the water body which helps to support the fish community (2). Temperature is another crucial factor which has greater impact for an aquatic ecosystem and the optimum temperature for any increased fish productivity was found to be 20-30°C. But the temperature obtained from this study ranged from 25-27°C. Diseases reported by the fish farmers were found to be very high due to the lack of awareness of fish farmers about the different types of fish diseases, lack of reporting places, or a particular diagnostic laboratory for detecting the appropriate diseases and other support services. Especially during winter season, fish consumes less food because of their physiological conditions becomes gradually weak making the fishes vulnerable to various bacterial infections. Therefore, aqua farmers are educated by giving sufficient information to follow some active preventive measures of spraying chemicals like potassium permanganate mixed with copper sulphate, application of lime and salt to the drained ponds, disinfecting the equipment and supply of fresh aerated water, etc. (Faruk et al., 2004b). Finally, efforts should be made as much as possible to curtail the indiscriminate discharge of untreated sewage and industrial effluents in to the culture ponds which in turn increase the microbial load in water and consequently induces stress on fishes and other aquatic organisms. Therefore, good hygienic practices and proper water quality parameters must be an excellent source to prevent any type of microbial diseases in aquaculture ponds. It may be noted that the outbreak of bacterial diseases occurs only at the time of increase in the bacterial load of fish culture systems. It is also suggested that the culture species become more susceptible due to increased bacterial load in the pond water. Hence, good cultural management practices and continuous monitoring of benthic microbes are the key factors to avoid bacterial outbreaks in most of the fish cultured ponds.

Temperature has been identified as the primary abiotic factor controlling key physiological, biochemical and life history process of the fish**,** which have greater importance for any aquatic ecosystem. The temperature of a pond is a reflection of the hotness or coldness of its external environment. When a pond is directly impacted by the sun, it leads to an increase in the temperature of the pond. Also, heat losses at night lead to a corresponding drop in the temperature of the pond. Generally, biologic activities have been observed to double for each 10^0C rise in temperature. Temperature is therefore an important parameter in water as it impacts on the biotic life of the pond and also on the chemical and physicochemical characteristics of water.

Dissolved oxygen is the most important chemical parameter in aquaculture. Dissolved oxygen plays a vital to aquatic life, as it is needed to keep organisms alive. This is because low levels of dissolved oxygen lead to fish morbidity and mortality, the amount of oxygen that dissolves in water decreases at higher temperature and decreases with increase in altitude. Dissolved oxygen level less than 5mg/l causes stress to fish and levels less than 2 mg/l will result in death. The dissolved oxygen obtained from this investigation ranged from 4-8 mg/L. DO was lowest in the sample containing fish pond water plus fish feed in addition to the fish droppings. The low DO recorded in fish ponds might be attributed due to elevated temperature, as a result of this, increased organic and microbial load takes place and finally it leads to decrease in the metabolic activities of the fish in the pond (FAO, 2005). Depletion of dissolved oxygen could suppress respiration, leading to the severe mortality of fishes, depress feeding or affect embryonic development and hatching success takes place due to oxygen starvation (Clark, 1996).

The pH is an important limiting chemical factor for aquatic life. The pH of any natural waters is greatly influenced by the concentration of carbon dioxide which is considered as acidic gas (Boyd, 1979). In this study, pH recorded in all the ponds were within range required for aquaculture (8.1-8.5) which is suitable for fish production. Measurement of pH helps to determine if the water is a proper environment for fish although most fish can tolerate pH as low as 5.0. Ekubo and Abowei, (2011) suggested that to avoid overcrowding which cause stress to fishes in turn leaves fishes become susceptible to infections. Hence it is important to maintain optimum pH ranges from 4.0 - 6.5. According to the reports given by Njoku et al (19), the optimum pH required for the aquaculture ranges from 7.0 to 10.0.

Salinity has been considered as one of the most important variables influencing the utilization of organisms almost in all the estuaries (Marshall and Elliot, 1998). Salinity is defined as the total concentration of electrically charged ions (cations – Ca++, Mg++, K +, Na+; anions – CO3 - , HCO3 - , SO4 - , Cl and other components such as NO3 - , NH4 + and PO4-). The salinity recorded in fish water samples ranges from 2-5 ppt. Fish are sensitive to the salt concentration of their waters and have evolved a system that maintains a constant salt ionic balance in its bloodstream through the movement of salts and water across their gill membranes. Both fresh water and saltwater fish species generally show poor tolerance to large changes in water salinity. Often salinity limits vary from species to species level. The desirable range is 2 ppt for common carp. Barman et al. (3) gave a standard level of 10 ppt which is considered to be suitable for *Mugil cephalus* and Garg et al. (13) suggested that 25 ppt is required to maintain for *Chanos chanos* (Forsskal). Salinity is considered as a major driving factor that affects the density and growth of aquatic organisms in fish culture populations.

Bacteria always present in the water environment, multiply and invade to fish and spread the disease when reaching the suitable condition. *Pseudomonas* was originally described as the causative agent of Bacterial Hemorrhagic Septicemia (BSH) disease of pond-cultured fish. It is considered as a primary pathogen of freshwater fish and opportunistic pathogen for different fish species cultured in marine and brackish waters worldwide. It is reported that *P. fluorescens* has been isolated from eye surface and mouth lesions of diseased Rajpunti (*Barbodes gonionotus*) along with some other fish pathogenic bacteria. Isolation and characterization of *P. fluorescens* from gills of silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), African magur (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) has also been reported.

CONCLUSION

The present study has provided an important understanding on some of the most important pathogenic bacterial strains that causes severe bacterial infections in fishes of West Godavari region of Andhra Pradesh. *Staphylococcus aureus*, *Bacillus subtilis*, *Enterobacter*; *Klebsiella*, *Micrococcus* and *E. coli* looking like were identified in water samples of fish ponds selected for this study. Further studies required to observe activities and dynamics of bacterial pathogens in aquaculture farms. This knowledge may help fish farmers for the successful improvement of fish productions and ultimately reflects the economy of the farmers and nation as a whole.

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Conflict of the interest:

The authors declare no conflict of interest.

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