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# Association of Bacterial Pathogens with *LABEO ROHITA* in Marathwada Region of Maharashtra

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**ABSTRACT:**

In the present study a detailed survey was carried out to assess the association of various bacterial pathogen with Labeo Rohita from Marathwada region of Maharashtra. The fishes with visible symptioms were collected from different water bodies and fish landing centre of all the eight districts of Marathwada region.

The screening could yield thirteen pathogenic bacteria from the fish samples that included *Vibrio* sp., *Aeromonas* sp., *Streptococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Lactobacillus* sp., *Flavobacterium* sp., *Alteromonas sp.*, *Proteus* sp., *Salmonella* sp. and *Shigella* sp. *Micrococcus sp., Bacillus sp., Lactobacillus sp., Vibrio sp., Aeromonas sp., Streptococcus sp., Flavobacterium sp., Vibrio sp., Proteus sp., Staphylococcus sp., Enterobacteria sp., E.coli , Pseudomonas sp.* These bacteri were identified based on their growth characteristics on specific culture media. The most dominant among these pathogens was *Aeromonas* sp. Further investigation included the effect of various environmental factors like pH, temperature and Salinity on the growth of *Aeromonas* sp. The study revealed that the pH 7-8, 27-37 °C and 0.1 to 0.5% salinity was optimum for growth on the pathogen.

**Keywords**: *Labeo rohita (rohu), Aeromonas sp, Marathwada region.*

## [I] INTRODUCTION

*Labeo rohita* (rohu) one of the widely cultivated carp in all the states of india. It is preferred over other carp owing to the high consumer preference and good growth rate [1].

Fishes suffers from two types of diseases, non-infectious diseases caused by environmental, nutritional or genetic factors and infectious diseases caused by infectious microorganisms.

The microorganisms are transmittable and create a universal threat to the aquaculture and fish industry. The infected fish ultimately reach to human food and poses a serious threat to public health. These infectious diseases may be caused by various pathogenic organisms that include bacteria, fungi, viruses and protozoa. These pathogenic microorganisms are present in the environment or transmitted by other fish. The Infectious diseases are the outcome of cascade of complex interactions among the pathogen, the host and the environment [2].

Bacteria comprise of the major group of the fish pathogens posing important threats to aquaculture and fish industry worldwide. Bacterial pathogens are smallest pathogenic agents causing devastating fish diseases where the pathogenicity is a characteristic feature associated with specific fishes as hosts.

Infections in human caused by these bacterial pathogens transmitted through fish or the aquatic environment are common and associated with the various factors of fishes and human. Such infections are often by facultative bacteria that are pathogenic to both fish and human beings. These may be isolated from fish with or without apparent symptoms of the disease [3].

The pathogenic microorganism thrives in an environment modified and adapted to their specific metabolic needs and capabilities. The aspects of the external environmental factors are numerous but the most significant and essential are salinity, temperature and pH. These factors play an important role in growth of microorganisms and course of infection. When environmental conditions are favourable for the pathogenic microorganisms, it grows to its biotic potential and survives in the environment or in the host [4].

pH is an important environmental factor that affects the growth and multiplication of pathogenic microorganisms. Extreme conditions either acidic or alkaline denature membrane proteins and quickly cause death of cells. Most of the eukaryotes are classified as neutrophiles those thrive in pH close to 7.0, whereas acidophilic bacteria require acidic and alkaliphilic requires alkaline conditions [5].

Temperature is associated with the structural integrity of nucleic acids proteins and enzymes and the rate of metabolic reactions of organisms. The survival of any organisms below the normal ambient temperature slows down metabolic reactions and results in slow growth or death. On the contrary, higher than normal temperature causes irreversible enzyme denaturation, cell membrane dissolution and ultimately cell death [6]. The naturally occurring solute over a wide concentration range is NaCl and bacteria survive over a range of salt concentration. Bacteria can grow in moderate NaCl levels but grow better in the absence of NaCl.

In Marathwada, no proper scientific research on bacterial disease in fish has been undertaken so far. Few attempts have been made in order to assess the bacterial population in aquatic environment and their involvement in causing diseases in fish in Maharashtra. The aim of the present study is to identify the common bacterial diseases and pathogen in freshwater fishes.

## [II] MATERIALS AND METHODS

### 2.1. Sample collection

The fishes were collected from various sources like fish farms, seed farms, cold storage, market etc. were brought from different districts of Marathwada, Maharashtra. These fish samples had visible disease symptoms and also on the surface of other body parts. After collection fish samples were transported to the laboratory for further studies.

### 2.2 Determination of total viable count and isolation of bacteria from infected portions of fish

The fish samples with infected portions having visible symptoms were washed thoroughly with sterile distilled water. The infected portion was swabbed and swab was suspended in saline

solution (0.1 % NaCl). Serial dilutions were made using saline solution and $10^{-5}$, $10^{-6}$ and $10^{-7}$ were plated on nutrient agar (Hi media). Agar plates were incubated at room temperature (27±2 °C) for 24 hrs.

The total viable count as colony forming units (cfu) for each ml for each dilution was determined and average of number of colonies were recorded.

**2.3 Identification of bacterial isolates based on morphological and biochemical characteristics**

The morphological characteristics of bacteria isolated from diseased fish samples were done following the method of [7, 8]. The cell as well as colony morphology were recorded. The colony characteristics size, shape, colour, margin, elevation, consistency and opacity were recorded. Biochemical characterization was carried out as per Bergey's manual and was identified according to Bergey's Manual of Systematic Bacteriology [9].

**2.4 Determination of environmental factors on growth and pathogenisis**

The effect of various environmental factors on bacterial pathogens was studied. The inoculum was prepared by growing the bacterial isolates for 24 hrs in nutrient broth. The inoculum was adjusted to the cell density of absorbance of approximately 0.05 at 600 nm at the beginning of the experiment. The experiment was conducted in 250 ml flasks with 50 ml of nutrient broth. The incubator shaker was adjusted to 200 rpm and temperature was set as required in the experiment. Absorbance of the culture suspension was recorded at 600 nm every three hour using UV- Vis spectrophotometer (Shimadzu, Japan) till stationary phase was attained.

**Determination of optimum pH**

The inoculum of volume 1m1 of was added in 250m1 flasks containing 50 ml nutrient broth NB with medium pH adjusted to 5,6, 7,8 and 9 in each flask separately. The flasks were incubated in incubator shaker with shaker speed 200 rpm and at room temperature (27±2 °C). Absorbance of the culture suspension was recorded whereas all the other conditions were kept constant.

**Determination of optimum Temperature**

The inoculum of volume 1m1 of was added in 250m1 flasks containing 50 ml nutrient broth NB with medium pH adjusted to 7 in each flask separately. The flasks were incubated in incubator shaker with shaker speed 200 rpm and at temperature (22°C, 27°C, 32°C, 37°C and 42). Absorbance of the culture suspension was recorded whereas all the other conditions were kept constant.

**Determination of optimum Salinity**

The inoculum of volume 1m1 of was added in 250m1 flasks containing 50 ml nutrient broth NB with medium pH adjusted to 7 in each flask separately. Nutrient Broth in each flask were supplemented with different NaC1 levels 0.1%, 0.5%, 1%, 2% and 5%, The flasks were incubated in incubator shaker with shaker speed 200 rpm and at room temperature (27±2 °C). Absorbance of the culture suspension was recorded whereas all the other conditions were kept constant.

## [III] RESULTS AND DISCUSSION

The total area of Marathwada region is 64,813 km and comprises of Aurangabad, Jalna, Parbhani, Nanded, Hingoli, Latur, Beed and Osmanabad. This region is bounded by Vidarbha region on the North, Telangana on the East and South East, Karnataka on the South and Western Maharashtra on the West. Marathwad region is situated at an altitude of 300-650 m above mean Sea level gradually inclined from west to east.

The main fishing season is during the month of October while least is during rainy season. The increase in fish catch during the summer can be attributed to loss of due evaporation. The stress induced due to human activities, water diversion, changes in method of water and land utilization had contributed to various impact on fish population [10].

### 3.1 Isolation, screening and identification of the bacterial isolates Total Viable Count

The total viable count of bacteria (cfu/ml) was high from the fish samples and high viable count was attributed to swabs isolation from body surface of fish carrying significant population of pathogenic and non-pathogenic bacteria. The highest viable count of bacteria was observed from the Parbhani samples whereas lowest viable count was found in the sample from Aurangabad (Table 1). The high total viable count ( cfu) of bacteria in Parbhani samples may be attributed to contamination of aquatic environment from domestic waste and sewage from city.

**Table 1** Total viable count of bacteria from infected fish samples.

| Samples | Colony forming units/ ml | | |
|---|---|---|---|
| | cfu x $10^{-5}$ | cfu x$10^{-6}$ | cfu x$10^{-7}$ |
| Aurangabad | 60.3 | 57.6 | 42.3 |
| Jalna | 134 | 116 | 97.3 |
| Parbhani | 124 | 105 | 65.3 |
| Hingoli | 75.3 | 59 | 47.6 |
| Nanded | 68 | 42 | 27.6 |
| Nanded 2 (Kinwat) | 66.7 | 47.3 | 25.3 |
| Latur | 98.3 | 67.6 | 28.3 |
| Beed | 73.6 | 53.3 | 33.3 |
| Osmanabad | 103.3 | 73.3 | 46.6 |

### 3.2 Identification of the bacterial isolates by morphological and biochemical Characterization

Bacterial colonies that were different in appearance were selected for further morphological and biochemical characterization. The different colonies were accounted to different thirteen types of colonies. These thirteen bacterial isolates were selected for characterization and identification. The cell and colony morphology, gram character, and biochemical characteristics of these thirteen were carried as per Bergey's Manual of Systematic bacteriology [9]. The isolates were Gram -ve rods, Gram +ve cocii and rods (Table 2). The biochemical characteristic leads to identification of isolates as 1. *Micrococcus sp., 2. Bacillus sp., 3. Lactobacillus sp., 4. Vibrio sp., 5. Aeromonas sp., 6. Streptococcussp., 7. Flavobacteriumsp., 8. Vibrio sp., 9. Proteus sp., 10. Staphylococcus sp., 11. Enterobacteria sp., 12. E.coli , 13. Pseudomonas sp,* (Table 3). The isolates in this study are commonly known fish and human pathogens that have been characterized up to species level [11].

**Table 2** Colony morphology and gram characters of bacteria from samples

| strain | Gram | Shape | Size | Color | margin | Elevation | opacity | lustre | Edge | Consistency |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | +ve | cocci | 1 mm | white | irregular | raised | opaque | glistening | entire | viscous |
| 2 | +ve | rods | 2 mm | white | irregular | dull | opaque | umbonate | rhizoidal | butyrous |
| 3 | +ve | rods | 2 mm | white | irregular | dull | opaque | umbonate | rhizoidal | butyrous |
| 4 | -ve | rods | 1-2 mm | pale | circular | raised | opaque | glistening | entire | viscous |
| 5 | -ve | rods | 2 mm | yellow | circular | flat | transparent | glistening | entire | butyrous |
| 6 | +ve | cocci | 1 mm | white | cicular | convex | opaque | smooth | entire | viscous |
| 7 | -ve | rods | 1-2 mm | yellow | circular | raised | opaque | glistening | entire | viscous |
| 8 | -ve | rods | 2-4 mm | pale | circular | raised | opaque | glistening | entire | viscous |
| 9 | -ve | rods | 1-2 mm | pale | circular | raised | transparent | glistening | entire | butyrous |
| 10 | +ve | cocci | 1-2 mm | pale | circular | raised | opaque | glistening | entire | viscous |
| 11 | -ve | rods | 1-2 mm | pale | circular | raised | opaque | dull | entire | butyrous |
| 12 | -ve | rods | 1-2 mm | pale | circular | raised | opaque | dull | entire | butyrous |
| 13 | -ve | rods | 1-2 mm | pale | circular | raised | opaque | smooth | entire | butyrous |

**Table 3** Biochemical characteristics of selected bacterial isolates.

| Biochemical Characters | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Catalase | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Oxidase | + | + | + | + | + | - | - | - | + | - | + | + | + |
| Ammonia FromPeptone | - | + | - | + | - | - | - | + | - | - | - | - | + |
| M.R. | + | - | + | + | - | + | - | - | - | - | - | - | + |
| V.P. | - | - | - | - | + | - | + | + | + | + | + | + | - |
| Indole | - | - | + | - | - | - | - | - | - | - | + | + | - |
| Urease | - | - | - | + | - | - | - | + | + | + | - | - | - |
| Citrate | + | + | + | + | - | - | + | + | + | + | + | + | - |
| Gelatinase | + | + | + | + | + | + | + | + | + | - | + | + | + |
| Phenylalanine Deamination | + | - | - | + | + | - | - | + | + | - | - | - | - |
| Starch Hydrolysis | - | + | + | + | + | - | - | - | - | - | - | - | - |
| Esculin Hydrolysis | + | + | + | + | - | + | - | - | - | + | + | + | - |
| Casein Hydrolysis | - | - | + | + | + | - | + | + | + | + | - | - | - |
| Lipase Activity | + | + | + | + | + | + | + | + | + | + | - | - | - |
| Nitrate Reduction | + | + | + | + | - | - | - | + | + | - | - | - | - |
| $H_2S$ Production- | - | - | - | - | - | - | - | + | + | - | - | - | - |
| T S I | - | + | + | + | + | - | - | - | + | + | + | + | - |
| OF | - | - | + | - | - | + | - | + | + | - | + | + | + |
| Growth On TCBS Agar | ++ | + | + | + | + | + | - | - | - | + | - | - | - |
| Growth On MacConkey's Agar | + | - | + | + | + | + | - | + | + | - | - | - | - |
| Growth On SS Agar | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Pigmentation On Nutrient Agar | - | - | - | - | + | - | - | + | + | + | - | - | - |

The most common and dominant bacterial pathogen was *Aeromonas* sp. *Aeromonas* sp. *is* found to be associated with various freshwater and marine fishes. So this bacterial pathogen was further selected for studying the effect of various environmental factors affecting the growth of this pathogen in the water bodies as well as during the course of infection in the fishes.

### 3.3 Optimum environmental conditions for the growth of selected bacterial Pathogen

The fitness of pathogenic bacteria depends on the adaptability of bacteria to survive and grow in non favourable condition. The growth in best suited environmental conditions plays a important role in the growth and biological activities of the bacteria.

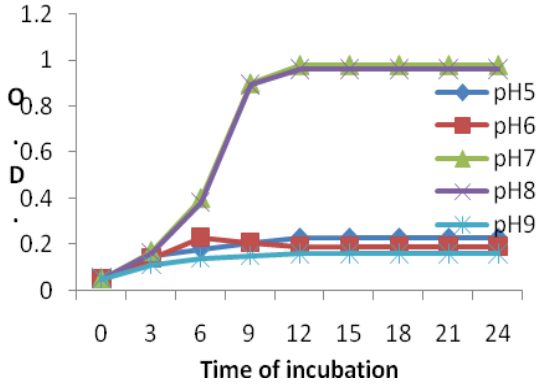


**Figure 1.** Effect of pH on growth of *Aeromonas* sp.

### 3.3.1 Optimal pH for growth

The pH of the growth medium was adjusted to 5, 6, 7, 8 and 9 to asses the adaptability over a wide range of pH comprising of acidic, neutral and alkaline range of pH. *Aeromonas* sp. strain was able to grow at the pH range neutral to slightly alkaline (7 and 8) but the growth was retarded at acidic of pH 5 and 6 and even at higher pH 9(Fig.1).

pH is an essential factor that governs survival and growth of bacteria in fish and water bodies. These results are in accordance with with earlier reports on growth response of bacterial fish pathogens with reference to pH [12].

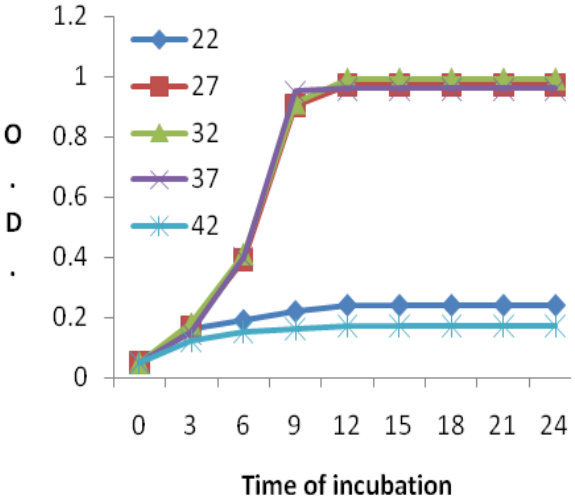


**Figure 2.** Effect of temperature on growth of *Aeromonas* sp.

### 3.3.2 Optimal temperature for growth

The growth of the bacterial pathogen *Aeromonas* sp. was assessed over a wide range of temperature. *Aeromonas* sp. growth was optimum between the temperature range of 27 °C to 37°C but the growth was inhibited at 22°C and above 42°C. Most of the environmental isolates of *Aeromonas* sp. were favoured in the incubation temperatures ranging from 27 °C to 37°C[13] (Fig.2).

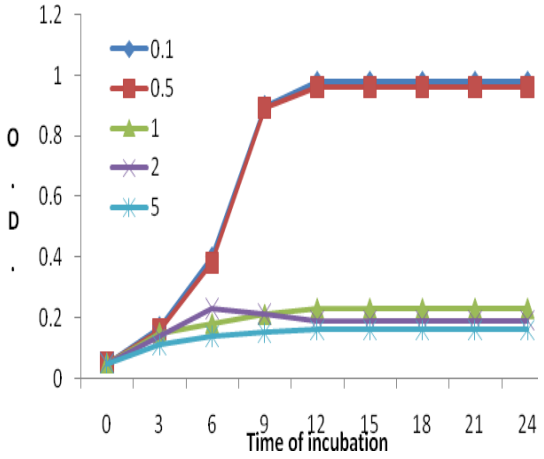


**Figure 3.** Effect of salinity on growth of *Aeromonas* sp.

### 3.3.3 Optimal salinity (NaCl) for growth

The amount of salt in solution plays an important role in growth and survival of bacterial pathogen in water bodies. The growth media were supplanted with different levels of NaC1 to determine the optimum salinity for growth of selected pathogenic *Aeromonas* sp. The pathogen showed significant growth at 0.1% and 0.5 % where NaCl levels exceeding thereafter inhibited the growth. (Fig.3).

Mesophilic *Aeromonads* are halotolerant and are associated with direct discharges to the sea or via rivers and streams. In their study, Hazan et al. [14] concluded that although *A. hydrophila* was not generally considered to be a marine bacterium, it could be found naturally in marine systems which interface with fresh water. In general, their populations in saline waters were higher than in freshwater. With preference to slightly alkaline pH, moderate growth of A. *hydrophila* at 5 °C is an interesting observation [15, 16].

## [IV] CONCLUSION

The bacterial genera *Aeromonas* is ubiquitous facultative parasites and are potential pathogen posing a serious threat to freshwater aquaculture and fish industry. It forms a essential component of normal bacterial flora of aquatic bodies,

hatcheries, fish farms and water bodies for domestic use. It is found colonizing in the skin fins, gills and intestinal lumen of fish.

*Aeromonas* causes disorder in most of fishes where it occurs in abdominal dropsy, ulcerative and generalized hemorrhagic septicaemia. The disease is encounter worldwide infecting cultured cyprinids and other cultured fishes. The infected fishes appears abnormally dark with large subcutaneous haemorrhages with distended abdomen. *Aeromonas* caused a severe disease outbreak in cultured fish.

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