Chelonian Conservation And Biology

Vol. 18 No. 2 (2023) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2023.12(2).1844.1853

STUDY OF BACTERIAL PATHOGENS ASSOCIATED WITH LABEO CALBASU IN MARATHWADA, MAHARASHTRA: IMPLICATIONS FOR ENVIRONMENTAL FACTORS ON PSEUDOMONAS SP.GROWTH"

Uttam Dileeprao Pawde and Ravi Dhondiraj Barde*

Department of Zoology, SGB College, Purna Dist Parbhani

*Corresponding author

ABSTRACT:

In this study, an extensive survey was conducted to evaluate the presence of diverse bacterial pathogens in Labeo calbasu specimens sourced from various water bodies and fish landing centers across all eight districts of the Marathwada region in Maharashtra. Samples exhibiting visible symptoms were collected for analysis.

A screening process revealed the isolation of thirteen pathogenic bacteria from the fish samples, encompassing Aeromonas sp., Bacillus sp., E.coli , Enterobacteria sp., Flavobacterium sp., Lactobacillus sp., Micrococcus sp., Proteus sp., Pseudomonas sp. Salmonella sp., Shigella sp., Staphylococcus sp., Streptococcus sp., and Vibrio sp.These bacteria were identified based on their specific growth characteristics on culture media. Notably, Pseudomonas sp. emerged as the most prevalent among the isolated pathogens.

Further investigation delved into examining the influence of environmental factors—such as pH, temperature, and salinity—on the growth of Pseudomonas sp.The findings highlighted that optimal growth for this pathogen occurred at pH levels between 7 and 8, temperatures ranging from 27 to 37°C, and a salinity range of 0.1% to 0.5%.

This study sheds light on the presence of various bacterial pathogens in Labeo calbasu and underscores the impact of environmental factors on the growth patterns of specific pathogens, particularly highlighting the optimal conditions for Pseudomonas sp. proliferation."

Keywords: Labeo calbasu, Pseudomonas sp. Marathwada region.

INTRODUCTION

Labeo calbasu stands out as a prominent carp species alongside the major Indian carps like Labeo rohita, Catla catla, and Cirrhinus mrigala, thriving in freshwater habitats. Highly valued for both its culinary appeal and sporting characteristics, this fish has also found its way into domestic and

international ornamental fish markets. However, due to overfishing and various human-induced factors, its population has significantly dwindled in recent times. While it's categorized as low risk in India and Bangladesh, in certain areas, it's considered an endangered species due to looming threats.

Previous research has delved into various aspects of Labeo calbasu, exploring topics such as its dietary habits, reproductive biology, and even its use in aquaculture. Considered an important choice for the table, numerous studies have focused on farming techniques and the artificial cultivation of wetlands, where this carp exhibits more robust growth compared to indigenous carps under controlled experimental settings (CIFA (2004).

Fish are susceptible to two distinct categories of ailments: non-infectious diseases, stemming from environmental, nutritional, or genetic factors, and infectious diseases caused by microorganisms (FAO, 2005). The latter pose a significant threat to aquaculture and the fish industry, being transmissible and potentially reaching human consumption, thus posing risks to public health. These infectious diseases can be attributed to various pathogenic organisms such as bacteria, fungi, viruses, and protozoa, either present in the environment or transmitted via other fish. The interplay between the pathogen, host, and environment leads to the development of infectious diseases (Kumar and Day, 1992).

Among fish pathogens, bacteria stand out as a major group, posing substantial threats globally to aquaculture and the fish industry. These microscopic agents cause severe fish diseases, displaying specific pathogenicity towards certain fish species. Infections caused by these bacterial pathogens can also affect humans, often arising from facultative bacteria capable of causing disease in both fish and humans, whether or not the fish show outward signs of illness (Khatun et al., 2011).

Pathogenic microorganisms thrive in environments tailored to their specific metabolic needs. External environmental factors, notably salinity, temperature, and pH, play crucial roles in microbial growth and the course of infection. Favorable environmental conditions enable these microorganisms to reach their full growth potential, either within the environment or within the host.

pH serves as a critical environmental factor affecting the growth and multiplication of pathogenic microorganisms. Extreme pH conditions—either highly acidic or alkaline—lead to the denaturation of membrane proteins and swift cell death. While most eukaryotes thrive in neutral pH close to 7.0, acidophilic bacteria require acidic environments, and alkaliphilic bacteria necessitate alkaline conditions.

Temperature influences the structural integrity of nucleic acids, proteins, and enzymes, impacting the rate of metabolic reactions in organisms. Abnormal temperatures—either below or above the norm—slow down metabolic reactions or cause irreversible damage to cell structures, leading to cell death.Salt concentration, primarily sodium chloride (NaCl), also plays a role in bacterial

survival, with bacteria thriving in moderate NaCl levels but exhibiting better growth in its absence (Barde, 2023a and 2023b).

Despite limited scientific exploration on bacterial diseases in fish in the Marathwada region, a few attempts have been made in Maharashtra to evaluate the bacterial population in the aquatic environment and their involvement in causing fish diseases. This study aims to identify prevalent bacterial diseases and pathogens in freshwater fishes within this region.

MATERIALS AND METHODS

Sample Collection:

Various sources including fish farms, seed farms, cold storage, and markets across different districts of Marathwada, Maharashtra, provided the fish samples exhibiting visible disease symptoms on different parts of their bodies. Upon collection, these fish samples were transported to the laboratory for further analysis.

Determination of Total Viable Count and Bacterial Isolation from Infected Portions of Fish:

Fish samples displaying visible symptoms in the infected areas were meticulously washed using sterile distilled water. Swabbing was conducted on these infected portions, and the swabs were suspended in a saline solution (0.1% NaCl). Serial dilutions were performed using the saline solution, and dilutions of 10-5, 10-6, and 10-7 were plated on nutrient agar (HiMedia). The agar plates were then incubated at room temperature (27±2°C) for 24 hours. Subsequently, the total viable count, measured as colony forming units (cfu) per milliliter for each dilution, was determined, and the average number of colonies was recorded.

Identification of Bacterial Isolates Based on Morphological and Biochemical Characteristics:

The morphological characteristics of the bacteria isolated from the diseased fish samples were determined following established protocols (Collins et al., 1989; Cappuccino and Sherman, 2002). Detailed observations on cell and colony morphology were recorded, encompassing colony characteristics such as size, shape, color, margin, elevation, consistency, and opacity. Furthermore, biochemical characterization was conducted in accordance with Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984) to accurately identify and classify the bacterial isolates.

Study of Environmental Factors' Impact on Bacterial Pathogens:

To assess the influence of environmental factors on bacterial pathogens, the bacterial isolates were cultured in nutrient broth for 24 hours to create an inoculum. This inoculum was adjusted to a cell density corresponding to an absorbance of approximately 0.05 at 600 nm before the initiation of the experiment.

The experiment was carried out in 250 ml flasks, each containing 50 ml of nutrient broth. The incubator shaker was set at 200 rpm, and the temperature was regulated as per the experiment's

requirements. The absorbance of the culture suspension was measured at 600 nm using a UV-Vis spectrophotometer (Shimadzu, Japan) at three-hour intervals until reaching the stationary phase.

Determining Optimal pH:

For this phase, an inoculum volume of 1 ml was introduced into 250 ml flasks, each holding 50 ml of nutrient broth with varied pH levels (5, 6, 7, 8, and 9). These flasks were incubated in an incubator shaker at 200 rpm and room temperature (27±2°C), maintaining other conditions constant. The absorbance of the culture suspension was monitored throughout the experiment.

Determining Optimal Temperature:

Similarly, 1 ml of the inoculum was introduced into 250 ml flasks containing 50 ml of nutrient broth adjusted to a pH of 7. The flasks were subjected to different temperatures (22°C, 27°C, 32°C, 37°C, and 42°C) within the incubator shaker at 200 rpm. The absorbance of the culture suspension was measured while keeping other conditions consistent.

Determining Optimal Salinity:

For this assessment, an inoculum volume of 1 ml was added to 250 ml flasks containing 50 ml of nutrient broth adjusted to a pH of 7. Various NaCl concentrations (0.1%, 0.5%, 1%, 2%, and 5%) were added to the nutrient broth in each flask. These flasks were incubated at room temperature (27±2°C) in the incubator shaker at 200 rpm, maintaining consistent conditions. The absorbance of the culture suspension was recorded throughout the duration of the experiment.

RESULTS AND DISCUSSION

Marathwada region spans across an area of 64,813 square kilometers, encompassing Aurangabad, Jalna, Parbhani, Nanded, Hingoli, Latur, Beed, and Osmanabad. It is bordered by the Vidarbha region to the North, Telangana to the East and Southeast, Karnataka to the South, and Western Maharashtra to the West. This region's altitude ranges from 300 to 650 meters above mean sea level, sloping gradually from west to east.

The primary fishing season occurs in October, while the least activity takes place during the rainy season. The surge in fish catch during summer months is often linked to water loss due to evaporation. Human activities, alterations in water diversion methods, and changes in land utilization have induced stress on the aquatic environment, impacting the fish population significantly (Ubarhande and Sonawane, 2015).

Isolation, Screening, and Identification of Bacterial Isolates – Total Viable Count:

The total viable count of bacteria (cfu/ml) obtained from fish samples was notably high, attributed to swab isolation from the fish body surface housing a substantial population of both pathogenic and non-pathogenic bacteria. Parbhani samples exhibited the highest viable count of bacteria, whereas the lowest count was observed in samples from Aurangabad (Table 1). The elevated total

viable count (cfu) of bacteria in Parbhani samples could be linked to the contamination of the aquatic environment by domestic waste and sewage from the city.

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

Samples	Colony forming units/ ml		
	cfu x 10^{-5}	cfu x10^{-6}	cfu x10^{-7}
Aurangabad	72.3	66.6	49.5
Jalna	146.0	125.0	104.5
Parbhani	136.0	114.0	72.5
Hingoli	87.3	68.0	54.8
Nanded	80.0	51.0	34.8
Nanded 2	78.7	56.3	32.5
Latur	110.3	76.6	35.5
Beed	85.6	62.3	40.5
Osmanabad	115.3	82.3	53.8

Identification of the bacterial isolates by morphological and biochemical Characterization

Bacterial colonies displaying distinct appearances were specifically chosen for further analysis regarding their morphological and biochemical traits. These distinct colonies were identified as thirteen different types. These thirteen bacterial isolates were then meticulously selected for comprehensive characterization and identification. Following Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984), thorough examinations were conducted to determine cell and colony morphology, gram staining characteristics, and various biochemical features of these isolates. The isolates were categorized as Gram-negative rods, Gram-positive cocci, and rods (Table 2). Through biochemical analysis, the isolates were identified 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)

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

The isolates in this study are commonly known fish and human pathogens that have been characterized up to species level (Chauhan & Singh, 2019).

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 Pseudomonas sp. Pseudomonas 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.

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.

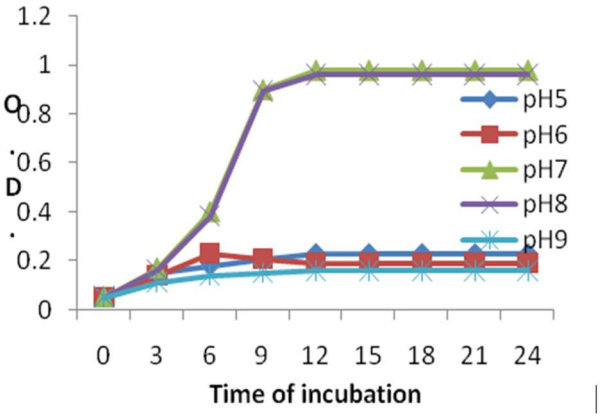

Figure 1. Effect of pH on growth of Pseudomonas sp.

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. Pseudomonas 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 earlier reports on growth response of bacterial fish pathogens with reference to pH (Aberoumand, 2010).

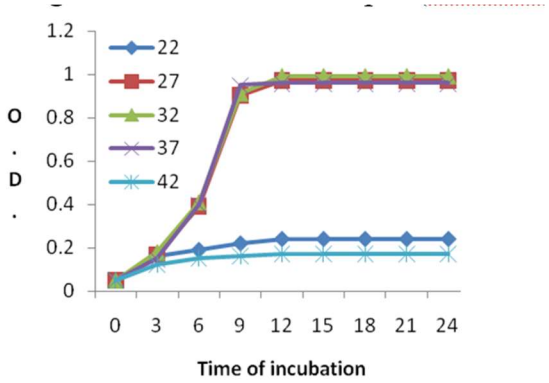

Figure 2. Effect of temperature on growth of Pseudomonas sp.

3.3.2 Optimal temperature for growth

The growth of the bacterial pathogen Pseudomonas sp.was assessed over a wide range of temperatures. Pseudomonas 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 Pseudomonas sp.were favoured in the incubation temperatures ranging from 27 °C to 37°C (Surendran et al., 1995). (Fig.2).

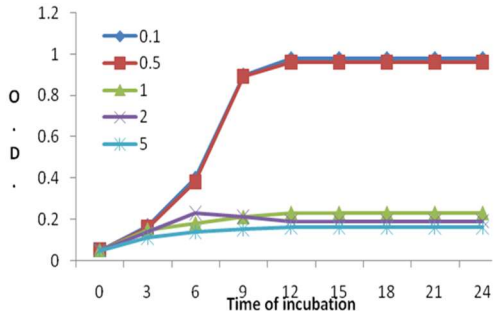

Figure 3. Effect of salinity on growth of Pseudomonas sp.

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 Pseudomonas sp.The pathogen showed significant growth at 0.1% and 0.5 % where NaCl levels exceedingly thereafter inhibited the growth. (Fig.3).

Mesophilic Pseudomonads are halotolerant and are associated with direct discharges to the sea or via rivers and streams. In their study, Kumar et al., (2013). concluded that although Pseudomonas sp.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 Pseudomonas sp.at 5 °C is an interesting observation (da Costa et al., 2021; Zhang et al., 2021).

CONCLUSION

The bacterial genera Pseudomonas sp. is a ubiquitous facultative parasite and are potential pathogen posing a serious threat to freshwater aquaculture and fish industry. It forms an 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. Pseudomonas sp. causes disorder in most fishes where it occurs in abdominal dropsy, ulcerative and generalized hemorrhagic septicemia. The disease is encounter worldwide infecting cultured cyprinids and other cultured fishes. The infected fishes appear abnormally dark with large subcutaneous hemorrhages with distended abdomen. Pseudomonas sp. caused a severe disease outbreak in cultured fish.

[V] REFERENCES

Aberoumand, A. 2010. A review on Pseudomonads in marine fish. World J.Fish Mar. Sci. 2 (4): 291-296.

Barde, R. D. (2023). Exploring the Disease-causing Potential of Aeromonas Hydrophila Obtained from Freshwater Carp-Cirrhinus Mrigala. Journal of Survey in Fisheries Sciences, 10(3S), 1725-1732.

Barde, R. D. (2023). Investigation into the Virulence of Psuedomonas fluorescens associated with the diseased fresh water Cirrhina mrigala. Journal of Survey in Fisheries Sciences, 10(3S), 1733-1742.

Cappuccino JG and Sherman N (2002). Microbiology a laboratory manual (Vol. 1). New york: Benjamin Cumming.

Chauhan, A., & Singh, R. (2019). Isolation and evaluation of putative probiotic strains from different teleost to prevent Pseudomonas aeruginosa infection in Cyprinus carpio. Aquaculture Research, 50(12), 3616-3627.

CIFA (2004). Annual Report Central Institute of Freshwater Aquaculture. Bhubaneswar, CIFA.

Collins CH, Lyne PM and GM Grange (1989). Microbiological Methods, 6th Ed. Butterworth, London,

da Costa, A. R., Chideroli, R. T., Chicoski, L. M., de Abreu, D. C., Favero, L. M., Ferrari, N. A., & Pereira, U. P. (2021). Frequency of pathogens in routine bacteriological diagnosis in fish and their antimicrobial resistance. Semina: Ciências Agrárias, 42(6), 3259-3272.

FAO (2005). FISHSTAT Plus Version 2.30 for 2003 statistics.

Khatun, H., Hossain, M D., Jahan, S N and Khanom, D A. 2011. Bacterial Infestation In Different Fish At Rajshahi J. Sci. Foundation, 9(1&2): 77-84,

Kreig, N. R. and J. G. Holt, 1984. Bergey's Manual of Systematic Bacteriology. Volume-1. Williams and Wilkins. 964pp.

Kumar, A., Singh, P., & Danish, M. (2013). Changes in proximate, biochemical and microbiological characteristics of dried Labeo gonius fillets during storage at room temperature. African Journal of Biotechnology, 12(20).

Kumar, D. and R. K. Day, 1992. Outbreak of epizootic ulcerative syndrome of fishes in India. A preliminary report. In: Aquaculture Research needs for 2000 AD. Oxford and IBH publishing co. New Delhi India. pp. 233-242.

Surendran, P. K., Thampuran, N., & Gopakumar, K. (1995). Microbial profile of cultured fishes and prawns viz a viz their spoilage and contamination. FAO Fisheries Report, 514, 1-12.

Ubarhande, S. and Sonawane, S. Freshwater fish population estimation of major carps (Cyprinadie family) from Marathwada Region, Maharashtra, India by Delury (1947) catch- Effort Method. International Journal of Fisheries and Aquatic Studies 2015; 2(4): 58-68

Zhang, W., Lv, X., Liu, Z., & Ni, L. (2021). The spoilage and adhesion inhibitory effects of Bacillus subtilis against Shewanella and Pseudomonas in large yellow croaker (Pseudosciaena crocea). Food Science and Technology, 42, e02721.