

## Biochemical Identification of Bacteria in the Polluted Water Fish

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### ARTICLE INFO

#### Article History:

Accepted: 01 Jan 2024

Published: 12 Jan 2024

#### Publication Issue :

Volume 11, Issue 1

January-February-2024

#### Page Number :

08-13

### ABSTRACT

The bacteria are the most common naturally occurring organisms in an aquatic medium which are important for the degradation of the aquatic wastes, chemical substances and pollutants.. The normal bacteria of the fish is the flora of the medium in which they live. The heavy mortality that occurs in the cultured and the feral fish population is commonly caused by the bacterial diseases and affects fish living in the polluted waters. Therefore, the present study was aimed at studying the bacteria which can be easily identified by using biochemical parameters.

During the present study bacteria of fish collected from the polluted water body was identified using various biochemical tests and compared with that of the fish from normal or comparatively less polluted water body .The polluted water fish are more prone to the bacterial ,fungal ,viral and parasitic infections than the fish living in the less contaminated waters. Therefore, a comparative study involving fish bacterial catalase ,peroxidase, and oxidase activities was qualitatively detected from fish obtained from both the normal water body and the polluted water body . The catalase activity was interpreted as positive for example Staphylococcus species and allied ones and negative for the streptococcus and related species. Peroxidase also showed similar results. The oxidase activity was found to be less than the other two enzyme activities. The difference in the enzyme activities are due to the differential breakdown of the pollutants by the bacteria present over the surface of the body of the fish and in the body fluids and more so in case of oxidase enzyme due to the low levels of oxygen as observed in the present study.

Keywords: Adriamycin, d-limonene, AST, Histopathology, Hepatotoxicity

## I. INTRODUCTION

The heavy mortality that occurs in the cultured and the feral fish population is commonly caused by the bacterial diseases. The saprophytic bacteria are the most common naturally occurring organisms which are important for the degradation of the aquatic environment and also play a role in the synthetic pathways of the medium. It is an established fact that the normal flora of the fish is nothing but the flora of the medium in which they live. However, the bacteria of the polluted aquatic medium that infect fish are more and varied due to different pollutants. To degrade the various pollutants the different bacteria may be present. Changes which are occurring in the freshwater ecosystems are known to be fundamental in the development of all microorganisms, including those which are pathogenic to fish. This is especially evident in recent years during which dynamic variations in bacterial fish pathology have been observed. However, because of close interaction between the fish pathogens and the aquatic environment, fish culturing has raised issues related to fish health.

Understanding the causes, symptoms, and appropriate management strategies for fish diseases is crucial for maintaining the health and sustainability of fish populations (Bruno, D. W. (2012)). In the aquatic environment bacteria are found ubiquitously and may cause mortality in wild as well as cultured fish due to diverse diseases triggered by various bacterial species (Apun K, Yusof AM, Jugang K (1999)). Generally most of the bacterial pathogens are thought to be a part of the common micro-flora that are found in the aquatic environment, but these are also considered to act as opportunistic or secondary pathogens. Bacteria are ubiquitous in the aquatic environment and almost all bacterial fish pathogens can live outside the fish also. These bacteria capable of causing disease are considered by some to be saprophytic in nature. The bacteria become pathogens

only when fishes are physiologically imbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, fluctuations in temperature, and access to water deposition which is congenial for bacterial infections to proceed.

## II. MATERIAL AND METHODS

Fish are procured from the selected water bodies in and around the local vicinity along with the water to test for the physico-chemical parameters to assess the pollution levels. The physical parameters analysed were the pH, temperature, turbidity, total dissolved solids and chemical parameters like dissolved oxygen, that are important and can affect the health of the fish along with other pathogens and the pollutants. The methodology adopted for water analysis was as described by APHA, 2017. The source of bacteria from fish are taken from the mucus on the skin surface, and the gut. The bacteria were grown that is collected from different regions of the fish using suitable media (Erwin Amlacher, 2005) as and when needed. The bacteria belonging to each strain was used to test for the biochemical parameters following specific methods as below.

The catalase activity was tested by running 1ml of hydrogen peroxide over the bacteria in a petri dish and was examined immediately and after 5 minutes for the emanating gas bubbles.

The peroxidase activity was also similarly done but with addition of guaiacol. (Guaiacol is used to differentiate it from catalase.)

Oxidase activity was tested by placing a piece of filter paper in a petri dish with 2-3 drops of the oxidase reagent with the smear culture of the bacterium.

The bacteria were identified using gram stain and then differentiated into gram positive and gram negative strains based on the staining.

### III. Results

The physico-chemical parameters of the water in the polluted water body showed wide variations when compared to the water from the normal water body. The changes in pH and temperature are the main factors that can alter the bacterial composition in the waters of any aquatic medium, as reflected in the present study by having different bacterial species. The pH and temperature levels were 4.5 and 38 degrees respectively in polluted water and in normal water it

was 8.5 and 27 degrees. The dissolved oxygen levels were much lesser in the polluted waters than in the normal water at around 0.03mg/litre and 1.5mg/litre. Table -1. The catalase and the peroxidase activities were high compared to the normal water body bacteria. The oxidase activity was diminished in the polluted water bacteria as observed in terms of intensity of the colour than in the normal bacteria and the bacteria that were identified by gram stain are given in the table (Table.2).

Table 1.

Normal water	Polluted water	
pH	8.5	4.5
Temperature(Degrees)	27	38
Dissolved Oxygen(mg/L)	12.5	0.03

Values are average of concurrent readings

Table.2

Gram Positive	Gram Negative	Catalase	peroxidase	Oxidse
Streptococcus species	Aeromonas	--	--	--
Catalase ++ Peroxidase++ Oxidase++	Flavobacterium	--	--	--
	Pseudomonas	--	--	--

### IV. Discussion

Different types of free radicals considered as harmful products are now known to have cellular functions. The imbalance if any, in their production leads to varied diseases, putting the burden of this stress on anti-oxidant enzymes like catalases and peroxidases. These enzymes play an important role in antioxidant defense system of living organisms and are actively involved in free radical oxidation. Several mechanisms have been suggested to explain their varied expressions and different functions in different organisms. Making use of these enzymes along with oxidases in microbes proves to be an important means to check for the pollution in an aquatic medium and also the fauna like fish. It is with the help of these enzymes the bacteria can be identified that degrade the various chemicals present in the water bodies.

When pathogens infests fish it affects the immunological activity of the fish, in which immune activity or response failure leads to the death of the species. As mentioned above simple experimentation involving biochemical parameters ,specific bacterial diseases can be identified. Delay in identification of pathogen bacterial strain and other types of diseases within a short period of time leads to the wider infestation within fingerlings, leading to mass infestation or decreased productivity at the time of harvesting. Therefore, easy methods in curbing the menace be employed.

### V. Conclusion

With the help of above experimentation, specific bacterial pathogen can be identified. In addition to the gram stain the biochemical methods are easy to test bacteria qualitatively and based on these results

further tests with gram stain can be used to differentiate between the gram positive and the gram-negative bacteria strains. Increased mortality rate within the small- and large-scale fishery industries leading to not just loss in productivity but also economic losses can be addressed by thorough understanding of the microbial communities involved in the bioremediation process to ascertain the pollution levels in a water body.

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### Cite this article as :

K. Y. Chitra, "Biochemical Identification of Bacteria in the Polluted Water Fish ", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 11 Issue 1, pp. 90-93, January-February 2024.

Journal URL : <https://ijsrst.com/IJSRST5241111>