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THE BACTERIAL QUALITY OF *OREOCHROMIS MOSSAMBICA* IN MAJOR FISH MARKETS OF BHOPAL

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Abstract: **The bacterial quality of *Oreochromis mossambica* in major fish markets of Bhopal were assessed and related to other retail outlets. Bacteriological load were determined using the methods of pour plate and identification by morphological characteristics and biochemical tests. Wide variations and significant differences were recorded in the counts of micro-organisms in *Oreochromis mossambica* within the markets. *Oreochromis mossambica* where compared to those of other markets. Total viable counts (TVC) ranged from 2.45 to 10.8 Cfu/g for *Oreochromis mossambica*. Based on microbiological counts and profile, *Oreochromis mossambica* collected from Bittan market were significantly higher compared to other fish markets. *Pseudomonas fluorescence* were the dominant micro-organism isolated from *Oreochromis mossambica*. In a comparative analysis, market samples from Piplani and Govindpura have shown less microbial load. The market environment is more important than the processing technology in determining the quality of fish. The quality of fish in Bittan market is unsatisfactory, probably due to the mode of storage, handling, exposure, and poor environmental and sanitary conditions.**

Keywords: Oreochromis mossambica*, *Pseudomonas fluorescence, **Bittan market, Handling and Processing.**

I. INTRODUCTION

Fish is one of the best protein sources available to mankind in quality and quantity. It is used as staple food in countries with long coastlines and extensive inland water resources. It is a valuable source of vitamin A, B, iodine and oils containing polyunsaturated fatty acids. However, of all flesh foods, fish is the most susceptible to tissue decomposition, development of rancidity and microbial spoilage. Fish quality is directly related with bacterial load, which is dependent on the conditions of transport, handling and processing. The growth of micro-organisms is the major cause of spoilage of fresh fish.

II. MATERIAL AND METHODS

Sampling Method

Samples for bacteriological examination were collected by taking swabs with the help of swab sticks inserting internally as well as externally. These swabs were diluted serially (10^1,10^2,10^3). After serial dilution, the inocula were poured on different selective agars for growth.

Incubation

The temperature and time plays an important role in the incubation of bacteria for culture. The temperature range, over which different pathogens grow, differ considerably, but as a matter of practical expediency, a single incubation temperature with in the range of 15˚C to 37˚C was generally used for isolation purpose.

Purification of bacteria

Pure bacterial colonies were obtained by taking small inocula from mixed colonies of bacteria and aseptically streaked on selective agars with a sterile inoculating loop, incubated at 37˚C temperature for 24 hours. The colony which develops singly on a petri plate is further streaked on the agar plates till a pure colony is obtained.

Identification

Gram reaction test was applied for the isolate obtained from fishes.

Morphological characterization

For this purpose, following characteristics were taken into account

- Size of the colony
- Shape of the colony
- Color of the colony
- Margin of the colony
- Elevation of the colony
- Opacity of the colony

Gram Staining Reaction

This test determines the type (Gram positive/ Gram negative) of bacteria. For this test, small colonies were taken for smear preparation. Air dried and heat fixed smear was stained for 1 minute with crystal violet, rinsed in running tap water, then one drop of Gram's iodine solution was poured over the slide for 1 minute, it was rewashed with running ap water, decolourized by alcohol - acetone solution for 8 - 10 seconds and counter stained for 30 minutes with safranine. The smear was washed with tap water thoroughly and gently blotted dry prior to microscopic examination. Appearance of purple or violet colour indicated the presence of Gram positive bacteria whereas the appearance of pink or red colour indicated the presence of Gram negative bacteria.

Motility

Nutrient agar was used for the determination of motility of bacteria. A drop of culture suspension was taken on a clean grease free slide. A cover slip was placed in such a way that an air bubble was created. The slide was immediately observed at the magnification of 40x of microscope. The creation of an air bubble made the observation of motile bacteria easy. This method is used as a convenient alternative to hanging drop method.

III. BIOCHEMICAL TESTS

Oxidation/Fermentation Test

This test is related with the production of acid from glucose metabolism under aerobic conditions in the basal medium of Huge and Leifson (1953). The appearance of yellow colour, after 1, 2 and 7 days incubation was taken as an indication of oxidation reaction. In case, the yellow colour does not appear, in stipulated time, it was taken as an indication of fermentation reaction.

Oxidase Test

For this test, a piece of filter paper was placed in a petri dish and moistened with freshly prepared cold 1% w/v of tetramethyle-p-phenylenediamine dihydrocholoride solution. Subsequently, a bacterial colony was smeared over the moistened paper by means of platinum loop. Appearance of dark purple colour within 30 seconds indicated positive reaction.

Catalase Test

For this test, young colony were scraped with the help of glass rod and transferred to a drop of hydrogen peroxide on glass slide. Appearance of effervescence within 1 minute indicated positive reaction.

Indole Production Test

For this test, 1% w/v peptone water was inoculated with bacteria and incubated for 7 days and then a few drops of Kovac's reagent were added to the peptone water. Appearance of pink or cherry red colour yielded positive reaction.

Hydrogen Sulphide Production Test

For this test, 1% w/v peptone water was inoculated with bacteria and incubated for 7 days and then a lead acetate paper strip was inserted between the plug and the tube. Appearance of black colour on paper after 4 to 7 days indicated positive reaction.

Methyl red and Voges Proskauer (MR-VP) test

For methyl red test, bacteria were inoculated on methyl red medium and incubated at 30˚C for 2 to 5 days. Then 2 drops of methyl red solution were added to it. Appearance of strong red colour after one hour indicated positive reaction. It was later confirmed by Voges Proskauer test by adding 0.6 ml of napthol solution and 0.2ml of 40% KOH aqueous solution which gave strong red colour after one hour, indicated positive reaction.

Decarboxylase Test

For this test, arginine, lysine, ornithine and a control (arginine) were inoculated with bacterial colony and overlayed it with mineral oil, then tubes was incubated at 35˚C temperature for four days. The inoculated media were examined daily. The media first became yellow due to acid production from glucose and later on they changed to violet due to decarboxylation. The control tube remained yellow.

Urease Production Test

For this test, urease medium was inoculated with bacteria and incubated for 28 days. Appearance of red colour indicated positive reaction.

Coagulase test

For this test, B-P agar was inoculated with bacteria and incubated at 37˚C temperature. The coagulation of human or animal plasma in absence of calcium indicated positive reaction.

Starch Hydrolysis Test

For this test, Starch agar was inoculated with bacteria and incubated at 30˚C temperature for 5 days. Then the inoculated petri plate was flooded with lugol's iodine solution. Appearance of clear colourless zone indicated positive reaction while blue colour indicated negative reaction.

Fig. 1 Oreochromis mossambica

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IV. RESULTS AND DISCUSSION

Pseudomonas fluorescens when cultured on selective medium i.e. *Pseudomonas* isolation agar (PIA), it developed in the form of yellowish green coloured colonies with rounded edges, about 1.0 mm in diameter at 25 to 30˚C temperature within 24 to 48 hours. The isolate was observed to be Gram negative, rod, motile bacterium measuring about 1 µm in diameter.

Fig.2;showing growth of Pseudomonas f.

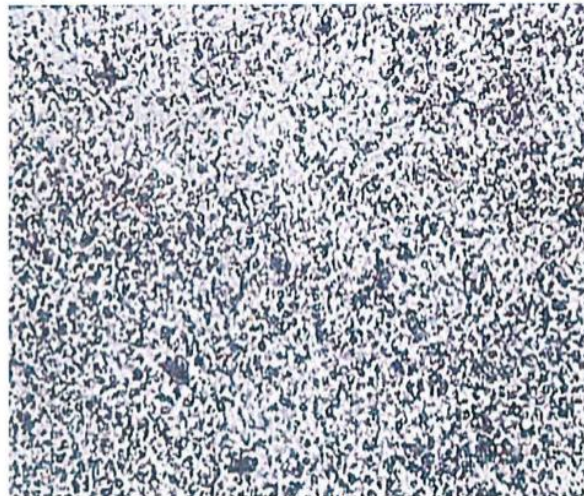

Fig.3: Micriscopic view of Pseudomonas f.

Table-1 Showing infestation of *Pseudomonas fluorescence* in *Oreochromis mossambica* collected from Bittan fish market

S.no.		Skin	Gills	Muscles	Intestine
1	*Oreochromis mossambica*	$10.1x10^3$ CFU/g	$10.5x10^3$ CFU/g	$4.5x10^3$ CFU/g	$10.8x10^3$ CFU/g

Table-2 Showing infestation of *Pseudomonas fluorescence* in *Oreochromis mossambica* collected from Piplani fish market

S.no.		Skin	Gills	Muscles	Intestine
1	*Oreochromis mossambica*	$5.1x10^3$ CFU/g	$3.5x10^3$ CFU/g	$1.5x10^3$ CFU/g	$7.8x10^3$ CFU/g

Table-3 Showing infestation of *Pseudomonas fluorescence* in *Oreochromis mossambica* collected from Govindpura fish market

S.no.		Skin	Gills	Muscles	Intestine
1	*Oreochromis mossambica*	$2.5x10^3$ CFU/g	$3.0x10^3$ CFU/g	$0.5x10^3$ CFU/g	$4.8x10^3$ CFU/g

The micro-organisms present in Bittan markets were comparatively higher due to unhygienic condition of the market. *Pseudomonas fluorescens* was considered as fish spoilage organism by Otte (1963) as well as primary but poor pathogen.

Laycock and Regier (1970) have observed Pseudomonads and Achromobacters in the spoilage of Irradiated haddock. Hagler *et al*. (2001) reported *Pseudomonas* spp. in water, sediment fish and shellfish in coastal regions. Chai *et al*. (1968) reported about the presence of *Pseudomonas* sp. in ice containing fish vessel. Proteolytic strains of *Pseudomonas putrifaciens* and *Pseudomonas fluorescens* contributed significantly to spoilage because of rapid growth rates during low temperature storage. Shewan (1945b) observed *Pseudomonas* spp. in dehydrated fish. Characterization of *Pseudomonas* isolates harvested from marketed marine fishes was studied by Mariammal *et al*. (2003). Yagoub (2009) observed *Pseudomonas* spp. from retail fish markets of Sudan. Tryfinopuolou *et al*. (2002) studied the population dynamics of Pseudomonadas in gilthead seabream (*Sparus aurata*) stored under different temperatures (0˚C, 10˚C and 20˚C) and packaging condition (air and modified atmosphere of 40%, Carbon dioxide 30 %, Oxygen 30%), they found *Pseudomonas lundensis* was dominant species followed by *Pseudomonas fluorescens*, while *Pseudomonas fragi and Pseudomonas putida* were less frequent. They also found that *Pseudomonas fluorescens* dominates under air conditions while proteolytic and less lipolytic dominate under modified atmosphere packaging. Chai *et al*. (1968) reported about relative spoilage of fish (haddock) is due to *Pseudomonas putrifaciens* and *Pseudomonas fluorescens*. Babajide and Etanuoma (2004) studied the storage life of croaker (*pseudotolithus senegelensis*) in ice for 20 days and 12 hours in ambient temperature, findings were based on flavour and odour. Initially bacterial load was predominantly mesophilic in number and included *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Caryneforms* sp., *Flavobacterium* sp., *Alteromonas putrifaciens* and *Enteriobacteriae* sp., but main spoilage bacteria were *pseudomonas* sp. and *Alteromonas* sp.

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