International Journal of Fisheries and Aquatic Studies

ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 3(1): 169-172

www.fisheriesjournal.com
Received: 14-07-2015
Accepted: 17-08-2015

Subhash Walke
School of Life sciences, S.R.T.M. University, Nanded 431606. MS, India.

Shivaji Kashte
Department of Biosciences and Technology, Defence Institute of Advanced Technology, Girinagar, Pune 411025. MS, India.

Navnath Parwe
School of Life sciences, S.R.T.M. University, Nanded 431606. MS, India.

Shivaji Chavan
School of Life sciences, S.R.T.M. University, Nanded 431606. MS, India.

Correspondence
Shivaji Kashte
Department of Biosciences and Technology, Defence Institute of Advanced Technology, Girinagar, Pune 411025. MS, India.

Opportunistic Pathogens in the Mucous Of Skin, Gills, Fins and Mouth of Labeo Rohita

Subhash Walke, Shivaji Kashte, Navnath Parwe, Shivaji Chavan

Abstract
Fishes could be cradle of infection for human beings as many fishes found to be amalgamated with infectious pathogens. In accordance with human health safety fishes should be free of infectious pathogens with long lasting nutritional value. The unique attempt has been carried out to study mucous membrane micro flora of nutritious *Labeo rohita,* a widely preferred fish. The study confirmed the presence of bacterial species of *Moraxella* in mucous membrane of skin while species of *Corynebacterium* in the gills, fins and mouth mucous membranes. These species are commensal micro flora of the fish however acts as opportunistic pathogen and encompassed in diminishing food quality of fish.

Keywords: Food borne infections, Food spoilage, opportunistic pathogens, Moraxella, Corynebacterium, Labeo rohita.

1. Introduction

Fig: *Labeo rohita*

Classification of Labeo rohita

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii (Ray finned fishes)
Order:	Cypriniformes
Genus:	*Labeo*
Species:	*Labeo rohita* (Hamilton, 1822)

Food spoilage and food borne infections are the foremost roots for deteriorating human health. Microbial species ordinarily involved in fish food spoilage and poisoning are of *Salmonella, Staphylococcus, Aeromonas, Listeria, Vibrio, Plesiomonas, Aeromonas, Shigella and Escherichia coli, Clostridium perfringens, Clostridium botulinum* [1-4]. The microbes associated with fishes rely on their niche environment, usually are psychrophilic and mesophilic microbes like *Acinetobacter, Aeromonas, Alcaligenes, Flavobacterium, Moraxella and Corynebacterium* [1-5]. Researchers reported that many food fishes like *Labeo rohita, Catla catla, Cirrhinus mrigala, Cyprinus carpio* are spoiled by these microbes [6-9].

The *Labeo rohita,* being an important source of proteins, vitamins and minerals is usually preferred major carp by humans. It contains vitamins like A, D, E, K and C as well as essential fatty acids like PUFA. Omega -3 fatty acids like alpha linolenic acid, eicosapentaenoic acid, docosahexaenoic acid are mostly present in fat content of Labeo fish. It also contains amino acids, minerals like calcium, zinc, iron and thallium [10, 11].

The *Labeo rohita* frequently present in fresh water reservoirs, lakes, rivers, and is reported from all over India and neighbouring countries like Bangladesh, Nepal, Pakistan and some regions of Myanmar and Srilanka [12-15]. Average weight of *Labeo rohita* is 740 gm and average length is 34 cm. It is fast growing omnivorous fish, mostly feeds on the plank tonic, filamentous algae, aquatic plant leaves, phytoplankton, zooplanktons and minor on small insects [16]. The *Labeo rohita* found only in south Asia and has huge demand of import from other countries like Japan due to its great nutritional value. For export fish should be of with long

Lasting nutrional value and should not harbour pathogenic microbes with it. Therefore it is impervious to study the microbes associated with fish.

In the present study bacteria isolated and identified from mucous of skin, fin, gills and mouth of *Labeo rohita* and analysed the consequences of those bacterial association with *Labeo rohita* fish mucus membrane and its impact on human beings.

2. Materials and Methods

2.1 Collection of fish

The aseptic collection of *Labeo rohita* carried out from fresh water pond located in S.T.R.M. University campus, Nanded, MS, India. Micro flora of mucous skin, gills, fins and mouth were collected by sterile cotton buds. These buds used for preparation of suspension in saline. The 1ml of this suspension is inoculated in Luria Bertani broth (LB) for enrichment of organisms, and incubated at 37 °C for 24 hours.

2.2 Isolation of bacteria

The content from the LB broth were streaked on Mac Conkey's agar and Nutrient agar plates, the plates were incubated at 37 °C for 24 to 48 hours. After appearance of growth the morphological and cultural characteristics were recorded, and the selected individual colonies after gram's staining are transferred on the respective agar slants.

2.3 Identification of bacteria

These isolates were then identified by different biochemical test like

1) Catalase test: A loopful of suspension dipped in hydrogen peroxide gas formation in the form of bubbles shows positive test whereas absence of gas formation shows negative test [17].
2) Oxidase test: N, N, N, N-tetramethyl-P-phenylenediamine reagent containing discs touched to bacterial colony, change in color from dark blue to maroon shows positive test [17].
3) Indol test: A sample grown on tryptone broth, after addition of Kovac's reagent red layer observed on top layer shows positive whereas lack of red ring shows negative test [18].
4) Methyl red test: A bacterial sample grown on Glucose phosphate broth, after addition of methyl red, change in color shows positive test [18].
5) Vogous proskaur test: A sample grown on glucose phosphate broth, shows change in color from red to brown after addition of Barritt's reagents:A and B, confirms positive test [18].
6) Citrate test: A sample grown on citrate medium shows growth, confirms positive test [18].
7) Mannitol utilization test: Growth observed on ammonia free mannitol medium shows positive test [19]
8) Lactose utilization test: Growth observed on Mac Conkeys agar with formation of pink colonies show positive test [19].
9) Starch hydrolysis test: Growth observed on starch agar shows positive test [19].
10) With these test the isolates were identified by using Bergey's manual of Determinative Bacteriology [20].

Table 1: Identification of Bacterial types by Gram's staining

Type of Microflora	Type of Bacteria
Skin micro flora	Gram negative rods
Gills micro flora	Gram positive rods
Fin micro flora	Gram positive rods
Mouth micro flora	Gram positive rods

Table 2: Biochemical tests of different micro flora: + = Positive test, - =Negative test

Test	Skin micro flora	Gills micro flora	Fin micro flora	Mouth micro flora
Mannitol test	+	+	+	+
Lactose test	-	+	+	+
Indole test	+	-	-	-
VP test	-	-	-	-
Citrate test	-	+	+	+
Methyl red test	+	-	-	-
Catalase test	+	+	+	+
Oxidase test	-	-	-	-

3. Result

The microbial species found on skin, gills, fin and mouth mucous membrane skin mucous membrane isolated, enumerated and performed Gram's staining and different biochemical tests. The Gram's staining (Table 1) confirms Gram positive rods associated with gills fin and mouth mucous membrane skin mucous membrane while Gram negative rods associated with mucous skin of Labeo rohita. Further biochemical tests (Table 2) and their comparison with Bergey's manual confirmed the presence of bacterial species of *Moraxella* in mucous membrane of skin while species of *Corynebacterium* in the gills, fins and mouth mucous membranes.

4. Discussion

The quality of fishes and their spoilage is always been the severe concern for human health. Therefore many researchers studied the micro flora associated with various fishes. Human pathogen from three anatomic sites (skin, gill, intestine) of fifty different fishes were isolated and reported eleven species including *E. coli* Type 1, *Citrobacter fruendii, Proteus vulgaris, Klebsiella pneumonia, Klebsiella ozaenae, Enterobacter cloacae, Klebsiella oxytoca, Serratia marcescens, Serratia odorifera, Hafnia alvei, Proteus penner* [1]. Also the human bacterial pathogens, *E. coli, Pseudomonas aeroginosa, Vibrio cholera, Salmonella typhi* and *Shigella dysentriae* were isolated from two edible fishes *Priacanthus hamrur* (Percidae) and *Megalapsis cordyla* (Carangidae) from

the waters of royapurum coast, Chennai, Tamilnadu, India to highlight the quality of these two edible fishes [21]. Similarly the gut micro flora of farmed Tiger shrimp (*Penaeus monodon*) was reported with species of *Vibrio, Bacillus, Pseudomonas, Photo bacterium, Laseomonas* [22].

Since *Labeo rohita* is widely preferred fish, its study of micro flora becomes impervious. Some researchers showed that strains of *Bacilli, Pseudomonas, Aeromonas, Enterobacter* [23], *Flavobacterium, Micrococcus, Achromobacter, and Vibrio* [24] found in the gastrointestinal tract of *Labeo rohita*. The present study confirmed the incidence of bacterial species of *Moraxella* in the mucous skin and *Corynebacterium* in the mucous of gills, fins and mouth mucous membrane. The species of *Moraxell* and *Corynebacterium* also found in other types of fishes like Atlantic salmon (*Salmo salar*) [25, 26].

Most of the storage techniques kills or resist the growth of the microbes but bacterial species of *Moraxella, Flavobacterium, Pseudomonas* still survive freezing temperature and resume growth on fishes when thawed [27].The refrigerated fresh haddock fillet with low load of bacterial species of *Moraxella, Acinobacter* and *Corneybacterium* stored at 1^0C for 14 days, there were increased load of bacterial species and occurred spoilage of fish [28]. Species of *Moraxella* metabolizes amino acids and produces undesirable odours [29]. Tetrodotoxin, one of the most deadly toxins accumulated in the fishes is produced by bacterial genera like *Moraxella, Flavobacterium, Bacillus, Aeromonas, and Micrococcus* [30]. Therefore it is important to improve the storage techniques for complete killing of these bacterial species which can act as opportunistic pathogens and cause spoilage of food and human infections.

5. Conclusion

The fish Labeo rohita is found to be associated with commensal bacterial species of '*Moraxella*' in skin mucous and '*Corynebacterium*' in gills, fin and mouth mucous membranes respectively. Both are opportunistic pathogens and may cause fish spoilage and human infections in improperly stored or cooked fish consumptions. The better methods of storage and cooking the fishes should be adapted and precaution should be taken before consumption of such fishes.

6. References

1. Akoachere JF, Rhoda N, Bughe Benedicta O. Oben, Lucy M Ndip, Roland N. Ndip. Phenotypic characterization of human pathogenic bacteria in fish from the coastal waters of South West Cameroon: Public health implications. Reviews on environmental health 2009; 24(2):147-156.
2. Nieto Teresa P, Alicia E, Toranzo, Juan Barja L. Comparison between the bacterial floras associated with fingerling rainbow trout cultured in two different hatcheries in the north-west of Spain. Aquaculture 1984; 42(3):193-206.
3. Kraft Allen A. Psychotropic Bacteria in Foods Disease and Spoilage. CRC Press, 1992.
4. Al Bulushi, Ismail M, Susan Poole, Hilton C Deeth, Gary A. Dykes. Quantitative assessment of total and Gram-positive aerobic bacteria in fresh and ambient-temperature-stored sub-tropical marine fish. World Journal of Microbiology and Biotechnology. 2008; 24(9):1867-1875.
5. Emikpe BO, Adebisi T, Adedeji OB. Bacteria load on the skin and stomach of Clarias gariepinus and Oreochromis niloticus from Ibadan, South West Nigeria: public health implications. Journal of Microbiology and Biotechnology Research. 2011; 1(1):52-59.
6. Ježek František, Hana Buchtová. Shelf-life of Chilled Muscle Tissue of the Common Carp (Cyprinus carpio L) Packaged in Carbon Monoxide Enriched Modified Atmosphere. Acta Veterinaria Brno 2010; 79(9):117-125.
7. Ghosh Shubhadeep, Archana Sinha, Chittaranjan Sahu. Isolation of putative probionts from the intestines of Indian major carps. 2007.
8. Wahab MA, Rahman MM, Milstein A. The effect of common carp, Cyprinus carpio (L.) mrigal, Cirrhinus mrigala (Hamilton) as bottom feeders in major Indian carp polycultures. Aquaculture Research 2002; 33(8):547-556.
9. Nair KKS, Nair RB. Bacteriological quality of freshwater fish from Krishnarajendra Sagar reservoir (India). Fishery Technology 1988.
10. Kaneko S. Microbiological study of fresh fish. New food industry 1971; 13(6):83-86.
11. De Silva, Sena S, Trevor A Anderson. Fish nutrition in aquaculture. Springer Science and Business Media, 1995, 1.
12. Kanwate VS, Kulkarni AN. Fish and Fisheries of Kandhar Tank, Dist. Nanded (MS). J Aqua Biol. 2006; 21(3):10-13.
13. Bhat Anuradha. Diversity and composition of freshwater fishes in river systems of Central Western Ghats, India. Environmental Biology of Fishes 2003; 68(1):25-38.
14. Menon Ambat Gopalan Kutty. The Fauna of India and the Adjacent Countries: Pisces. Teleostei-Cobitoidea. Zoological Survey of India, 1987.
15. Jayaram KC. Freshwater fishes of the Indian region. Narendra Pub. House, 2010.
16. Bairagi, Abhinanda, Keka Sarkar Ghosh, Sukanta Kumar Sen, Arun Kumar Ray. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International 2002; 10(2):109-121.
17. O'Leary, William M. Practical handbook of microbiology. CRC press, 1989.
18. Ananthanarayan R, Ananthanarayan, Paniker's Textbook of Microbiology. Orient Blackswan, 2005.
19. Kim, Byung Hong, Geoffrey Michael Gadd. Bacterial physiology and metabolism. Cambridge university press, 2008.
20. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manual of determinative microbiology. Williams and Wilkins, Maryland, 1994.
21. Sujatha K, Senthil kumaar P, Sangeetha S, Gopalakrishnan MD. Isolation of human pathogenic bacteria in two edible fishes, Priacanthus hamrur and Megalaspis cordyla at Royapuram waters of Chennai, India. Indian Journal of Science and Technology. 2011; 4(5):539-541.
22. Shakila RJ, Saravana kumar R, SAP Vyla, Jeyase karan G, Jasmine GI. Antagonistic activity of the gut microflora isolated from farmed tiger shrimp (Penaeus monodon). Asian Fisheries Science 2006; 19(3-4):247.
23. Ghosh, Koushik, Moitreyee Roy, Nibedita Kar, Einar Ringo. Gastrointestinal bacteria in rohu, Labeo rohita (Actinopterygii, Cypriniformes, Cyprinidae) scanning electron microscopy and bacteriological study. Acta Ichthyologica et Piscatoria 2010; 40(2):129-135.
24. Hossain MM, Uddin MN, Islam MN, Chakraborty SC, Kamal M. Study on the intestinal bacteria of Labeo rohita

(Ham.). Bangladesh Journal of Fishery Research. 1999; 3:63-66.
25. Colwell, Rita R. The bacterial flora of Puget Sound fish. Journal of Applied Bacteriology. 1962; 25(2):147-158.
26. Cahill, Marian M. Bacterial flora of fishes: a review. Microbial ecology 1990; 19(1):21-41.
27. Eze EI, Echezona BC, Uzodinma EC. Isolation and identification of pathogenic bacteria associated with frozen mackerel fish (Scomber scombrus) in a humid tropical environment. African Journal of Agricultural Research. 2011; 6(8):1947-1951.
28. Fraser, Owen P, Sam Sumar. Compositional changes and spoilage in fish (part II)-microbiological induced deterioration. Nutrition & Food Science 1998; 98(6):325-329.
29. Neeraj D, Sharma S. Food spoilage, food infections and intoxications caused by microorganisms and methods of their detection. Food and Industrial Microbiology, 2007.
30. Wysok Beata, Jan Uradzinski, Malgorzata Gomolka-Pawlicka. Toxins occurring in fish, crustacea and shellfish-a review. Polish Journal of Food and Nutrition Sciences. 2007, 57:1.