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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 mucus 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

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 carps 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 Sri Lanka [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 organisms, and incubated at 37 °C for 24 hours. 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 freundii*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Klebsiella ozanemae*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Serratia marcescens*, *Serratia odorifera*, *Hafnia alvei*, *Proteus penner* [1]. Also the human bacterial pathogens, *E. coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Salmonella typhi* and *Shigella dysenteniae* were isolated from two edible fishes *Pricanthurus hamrur* (Percidae) and *Megalopsis cordyla* (Carangidae) from

### 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 mucus 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-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. Mannit 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].

#### 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 freundii*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Klebsiella ozanemae*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Serratia marcescens*, *Serratia odorifera*, *Hafnia alvei*, *Proteus penner* [1]. Also the human bacterial pathogens, *E. coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Salmonella typhi* and *Shigella dysenteniae* were isolated from two edible fishes *Pricanthurus hamrur* (Percidae) and *Megalopsis 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 Vibrion, Bacillus, Pseudomonas, Photobacterium, 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, Actinomycobacter, 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 Corynebacteria 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, Actinobacter and Corneybacterium stored at 1°C 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


