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Prevalence of microorganisms in preserved yellow goat fish (*Sulphureus cuvier*)

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Keywords

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Abstract

Microorganisms are found on all surfaces and in the intestines of live fish or preserved fish. Microbial flora of fish on the fishing depends on the environment in which it was caught more than fish species. Fish fishing in clear water and very cold, carries a small number of microorganisms compared with fish from the warm water which has a number of microorganisms a little higher. In this study, an attempt was made to isolate and identify the bacteria and fungi present in the preserved Yellow goat fish (*Sulphureus cuvier*). The fish sample was collected from Kanchipuram fish market, Tamil Nadu, India. Isolation of bacteria and fungi was carried out by Pour plate method (Serial dilution technique). The bacterial isolates were identified based on the Staining techniques, Platting in Selective medium and Biochemical tests. The bacterial isolates were identified as *Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli*. The fungal isolates were identified based on the Lactophenol cottonblue staining techniques and Platting in Sabouraud's dextrose agar medium. The fungal isolates were identified as *Aspergillus niger* and *Aspergillus flavus.*

1. Introduction

Fishes are playing important role in aquaculture ecosystems and are an important part of the daily diet for human being. They are second only to meat and poultry as staple animal protein foods for most of the world. The global annual harvesting of form the wild in oceans and lake was 90.2 and 93 million tons in the last decades foods prepared by a broad spectrum of both traditional and modern food technology methods. In the last decades, there has been an extensive expansion in fish production primarily due to developments in aquaculture.

The microorganisms associated with fish may be beneficial or harmful nature and also they can present on the body surface or the internal organ like gut region. The initial bacterial flora of fish incensed for human consumption depends on the environmental conditions of its natural habitat (Ismail *et al.*, 2008). Bacterial floras isolated from intestines have been described for a limited number of fish species. Furthermore, it knows that the range of bacterial genera isolated changes by the aquatic habitat of the fish and the bacterial load in the water. The genera present in the gut generally seem to be those from the environment or diet (Cahill, 2010). Fish flesh

provides an excellent substrate for the growth of most heterotrophic bacteria with compositional attributes that affect bacterial growth and the related biochemical activities (Jageethadevi *et al*., 2012; Kelly *et al*., 2016).

Fish are susceptible to several bacterial infections, mainly when reared in high densities conditions. Diseases outbreaks are responsible for elevated mortality rates and decrease of the productivity efficiency, causing high economic losses to the fish farmers. The use of antibiotics is the main treatment applied to control bacterial illness in fish farms. Due to the use of a wide variety of antibiotics, aquaculture has been implicated as potential environment to the development and selection of resistant bacteria and a source of these pathogens to other animals and humans. The adoption of same antibiotics in different fields improves the emergence and occurrence of the resistance phenomenon. Some bacterial fish pathogens are also associated to diseases in humans, making the aquaculture products a potential risk to the customers (Choat and Clement, 2008; Saranraj and Geetha, 2012).

Many organisms were found in fish from polluted warm waters. Multiple differences of the bacterial species can be found on the body surface of fish (Kraft, 2012). Over 80 % of the microorganisms found in aquatic caught animals in temperate areas of the northern hemisphere are Gram negative bacilli which are belonging to the genera: *Pseudomonas*, *Aeromonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium* and *Vibrio*. Unlike marine animals, fresh water fish are often found bacteria family Enterobacteriaceae and the genus *Aeromonas*. Molluscs meat is contaminated with a large number of microorganisms (10^4 - 10^6/g), especially when it comes to animals caught in warm waters. Dominant microflora consists of Gram negative bacteria (*Vibrio* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Moraxella* sp., *Flavobacterium* sp. and *Cytophaga* sp.) (Daalgard, 2003). The most common fungal moulds isolated in fish samples were *Botrytis cinerea, Rhizopus stolonifer, Alterneria alternata, Penicillium chrysogenum*, *Cladosporium* sp., *Fusarium oxysporum* followed by the yeast isolates like *Candida* sp. The most common spoiling fungi were *Alternaria alternata* and *Cladosporium* sp. and less common fungal isolates were *Penicillium* sp., *Trichoderma* sp., *Geotrichum* sp. and *Rhizopus* sp. (Nishihara *et al.*, 2008). In this present study, an attempt was made to isolate and identify the spoilage causing bacteria and fungi present in preserved Yellow Goat Fish (*Sulphureus cuvier*).

2. Materials and Methods

Collection of samples

The preserved Yellow Goat Fish (*Sulphureus cuvier*) was collected from Kanchipuram fish market, Tamil Nadu, India. The collected fish was stored in refrigerator at 4 °C for further microbial isolation and identification.

Isolation of bacterial and fungal population

Pour plate method (Serial dilution technique) was used for the isolation of spoilage causing bacteria and fungi from the collected Indian mackerel fish (*Rastrelliger kanagurta*). In this method, one gram of muscle was obtained from the fish and homogenised with 100 ml of distilled water and it was serially diluted upto 10^{-6} by following the standard procedure. Then, one ml of serially diluted samples from each concentration of samples were transferred to sterile petridishes and evenly distributed. Sterile Nutrient agar and Sabouraud's dextrose agar was poured into the sample containing petridishes and allowed to solidify. The Nutrient agar plates were incubated at 37 °C for 24 hrs and Sabouraud's dextrose agar plates were incubated at room temperature for 3 days. After incubation, the bacterial colonies were isolated from the plates and microbial population was counted by using Quebec colony counter and the enumerated colonies were expressed as cfu/ml. Well grown bacterial and fungal colonies were maintained on Nutrient agar and Sabouraud's dextrose agar slants, respectively and stored at 4 °C.

Identification of bacterial and fungal isolates

Identification of the different bacterial isolates were carried out by the routine bacteriological methods i.e., Colony morphology, Staining techniques (Gram staining, Capsule staining & Endospore staining), Motility test, Plating on selective media and Biochemical tests. Identification of the fungal isolates was carried out by the routine mycological methods i.e., by Lactophenol cotton blue staining and plating on Sabouraud's dextrose agar.

Results and Discussion

Microorganisms are ubiquitous nature and it is present everywhere in the universe (Geetha *et al*., 2012; Darwina *et al*., 2012). Fish are continuously exposed to a wide range of microorganism present in the environment. The population of microorganism associated with living fish reflects the microflora of the environment at the time of capture or harvest, but is modified by the ability of different microorganisms (mainly bacteria and fungi) to multiply in the sub-environments provided by the skin/shell surfaces, gill areas and the alimentary canal. In this present study, the bacteria and fungi were isolated from preserved Yellow Goat Fish (*Sulphureus cuvier*) which was collected from the fish market in Kanchipuram, Tamil Nadu, India. The total bacterial and fungal population present in the fresh fish was estimated and the results were showed in Table - 1.

Table – 1: Microbial population present in the preserved Yellow Goat Fish (*Sulphureus cuvier*)

S. No	Microorganism	Microbial population (cfu/ml)
1	Bacteria ($\times 10^4$)	6.55
2	Fungi ($\times 10^3$)	5.10

The gut is sterile until hatching, but soon after hatching, the fish comes in contact with the environment and live food that leads to successive colonization by a variety of microbes (Ringo and Olsen, 2009; Saranraj *et al*., 2012; Kanchana *et al*., 2015). The balance of this microbiota was influenced by a variety of factors including food, animal physiology and immunological factors. The establishment of a normal gut flora may be regarded as complementary to the establishment of digestive enzymes, and under normal conditions, it serves as a barrier against invading pathogens. In this present study, five different bacteria were isolated from the preserved Yellow Goat Fish (*Sulphureus cuvier*). Based on the staining techniques, plating on selective media and biochemical tests, they were identified as *Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli*. The characteristics of the isolated bacterial isolates were given in the Table – 2 to Table – 6.

It was generally considered that the Gram positive bacteria including lactic acid bacteria are numerically dominant members of the normal microbiota in the gastrointestinal tract of endothermic animals at their early life stage. However, only three investigations have isolated lactic acid bacteria from the gastrointestinal tract kof larval and juvenile fish (Strom and Olafsen, 2010; Strom and Ringo, 2013). The important question arises as to why lactic acid bacteria are seldom isolated latval fish. The limiting factors may have been the incubation temperature, incubation time and the absence of glucose in the medium.

Fishes are prone to fungal contamination in the field, during harvest, transport, marking and with the consumer. Fish samples were surface disinfected incubated at room temperature for upto 14 days without supplement all media, and subsequently examined for mould and yeast growth. The most common moulds isolated were *Botrytis cinerea, Rhizopus stolonifer, Alterneria alternata, Penicillium chrysogenum, Cladosporium* sp., *Fusarium oxysporum* followed by the yeast isolates like *Candida* sp. The most common spoiling fungi were *Alternaria alternata* and *Cladosporium* sp. and less common fungal isolates were *Penicillium* sp., *Trichoderma* sp., *Geotrichum* sp. and *Rhizopus* sp. (Nishihara *et al.*, 2008). In this present study, two different fungi were isolated from the preserved fish. Based on Lactophenol cotton blue staining and colony morphology on Sabouraud's dextrose agar, they were identified as, *Aspergillus niger* and *Aspergillus flavus.* The characteristics of the fungi isolated from the preserved Yellow Goat Fish (*Sulphureus cuvier*) was tabulated in Table – 7.

Table – 2: Characteristics of *Bacillus cereus* isolated from preserved Yellow Goat Fish (*Sulphureus cuvier*)

Test	Results
Gram staining	Gram positive, thick, short rods.
Endospore	Central spores present
Motility	Non-motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Large, circular, white, adherent, colonies, with membraneous growth
MacConkey agar	Non-lactose fermenting colonies
Glucose fermentation	Acid produced
Mannitol fermentation	Acid produced
Sucrose fermentation	Not fermented
Dextrose fermentation	Not fermented
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization	Positive
O-F test	Positive
Nitrate reduction	Positive
Gelatin hydrolysis	Positive
Starch hydrolysis	Positive
Urease	Negative

Table – 3: Characteristics of *Pseudomonas aeruginosa* isolated from preserved Yellow Goat Fish (*Sulphureus cuvier*)

Test	Results
Gram staining	Gram negative slender rods
Motility	Actively motile
Catalase	Positive
Oxidase	Positive
Nutrient agar	Green coloured diffusible pigment producing colonies
MacConkey agar	Non-lactose fermenting colonies
Glucose fermentation	Not fermented
Mannitol fermentation	Not fermented
Dextrose fermentation	Not fermented
Sucrose fermentation	Not fermented
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Negative
Citrate utilization	Positive
Urease	Positive
TSI	Alkaline butt, alkaline slant. No H_2S and No gas production
O-F test	Oxidative
Casein hydrolysis	Positive

Table – 4: Characteristics of *Proteus mirabilis* isolated from preserved Yellow Goat Fish (*Sulphureus cuvier*)

Test	Results
Gram staining	Gram negative rods
Motility	Motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Swarming motility characterized by its fishy odour
MacConkey agar	Non - lactose fermenting colonies
Glucose fermentation	Acid and gas produced
Mannitol fermentation	Not fermented
Dextrose fermentation	Not fermented
Sucrose fermentation	Not fermented
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Negative
Citrate utilization	Positive
Urease	Positive
TSI	Acid butt, alkaline slant, H_2S produced and gas producers
Phenylalanine deaminase test	Positive

Table – 5: Characteristics of *Klebsiella pneumoniae* isolated from preserved Yellow Goat Fish (*Sulphureus cuvier*)

Test	Results
Gram staining	Gram negative rods
Capsule staining	Capsules present
Motility	Non-motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Large, greyish white, dome, shaped and mucoid colonies of varying degrees of stickiness.
MacConkey agar	Pink coloured lactose fermenting colonies
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid produced
Sucrose fermentation	Acid produced
Mannitol fermentation	Acid produced
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization	Positive
Urease	Positive
TSI	Acid butt, alkaline slant, No H_2S and gas produced

Table – 6: Characteristics of *Escherichia coli* isolated from preserved Yellow Goat Fish (*Sulphureus cuvier*)

Test	Results
Gram staining	Gram negative straight rods
Motility	Motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Circular, smooth and colouress colonies
MacConkey agar	Smooth, gloosy and pink coloured lactose fermenting colonies
EMB agar	Small colonies with greenish metallic sheen
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid gas produced
Sucrose fermentation	Acid gas produced
Mannitol fermentation	Acid gas produced
Indole	Positive
Methyl Red Test	Positive
Voges Proskauar Test	Negative
Citrate utilization	Negative
Urease	Negative
TSI	Acid butt, alkaline slant, No H_2S and gas produced

Kanchana *et al*. (2015) evaluated the detailed microbial status including food borne pathogen and spoilage bacteria. In the present investigation, yellow goat fishes were taken with regard to their microbial population in the isolates. The total heterotrophic bacterial load ranged from 155×10^4 to 140×10^4 CFU/ml of sample and it was found to be the maximum of 155×10^4 CFU/ml in Yellow goat fish (*Sulphureus cuvier*). The bacterial isolates were identified by Microscopic examination, Platting on Culture medium and Biochemical tests. The identified bacterial isolates were *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas fluorescens*.

Table – 7: Characteristics of fungi isolated from preserved Yellow Goat Fish (*Sulphureus cuvier*)

Microscopic examination	Colony morphology on SDA plate
Aspergillus niger	
Conidiophore stipes smooth-walled, hyaline or pigmented. Vesicles sub-spherical, conidial heads radiate. Conidiogenous cells biseriate. Medulla twice as long as the phialides. Conidia brown, ornamented with warts and ridges. Hyphae was septate.	Colonies are black, consisting of a dense felt of conidiophores.
Aspergillus flavus	
Conidiophore stipes rough walled, hyaline vesicles spherical, conidial heads radiate, unit and biseriate. Conidia echinulate, spherical or sub-spherical, sclerotic may be present. Hyphae was septate.	Colonies are yellowish – green, consisting of a dense felt of conidiophores.

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