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Isolation and identification of spoilage causing microorganisms in an Indian mackerel fish (*Rastrelliger kanagurta*)

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Abstract

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. In this present study, an attempt was made to study isolate and identify the spoilage causing microorganisms in an Indian mackerel fish (*Rastrelliger kanagurta*). The fish sample was collected from Mamallapuram fishery centre, Tamil Nadu, India. Isolation was carried out using Pour plate method (Serial dilution technique). The bacterial isolates were identified based on the Staining techniques, Platting in Selective medium and Biochemical tests. The identified bacterial isolates are *Staphylococcus aureus, Pseudomonas florescence, Escherichia coli, Vibrio cholerae* and *Salmonella typhi*. 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*, *Aspergillus flavus* and *Fusarium oxysporum.*

Keywords: Indian mackerel fish (*Rastrelliger kanagurta*), Spoilage, Bacteria and Fungi.

1. Introduction

Fish stock structure is an important component of successful and sustainable long term management and has attracted considerable interest because of a fundamental interest in biotic evolution (Stevens and Hume, 2015). Determination of population genetic structure provides essential information to underpin resource recovery and to aid in delineating and monitoring populations for fishery management. Molecular genetic techniques offer the ability to identify and delineate fish stock structure where it may not be apparent from phenotypic or behavioural characteristics (Storm and Ringo, 2013). Such techniques have been used successfully to understand the structure of marine fish species (Mountfort *et al*., 2002; Nelson *et al*., 2003; Ismail *et al*., 2008; Jageethadevi *et al*., 2012).

Aquaculture has emerged as one of the important branches of food production and has evolved as the fastest growing food processing sector and developed as an important component in food security (Saha *et al*., 2006). Aquaculture was estimated currently to account for 25 % of the total world seafood supply, including a wide range of aquatic, Esturiene and Asia presently contributes about 90 % to the global production and has become an important economic

activity in many countries (Sahu *et al*., 2008; Saranraj and Geetha, 2012).

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 (veterinary and human medicine) 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 (Castro *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. 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 (Bremner and Statham, 1983; Geetha *et al*., 2012; Darwina *et al*., 2012).

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, an attempt was made to isolate and identify the spoilage causing microorganisms present in an Indian mackerel fish (*Rastrelliger kanagurta*).

2. Materials and Methods

Collection of samples

The Indian mackerel fish (*Rastrelliger kanagurta*) was collected from Mamallapuram fishery centre, 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 found on all surfaces (skin and gills) and in the intestines of live fish or fresh fish. The total number of microorganisms vary greatly, Lindsay and Harrish (2010) establishing the normal 10^2 - 10^7 germs/cm^2 on the skin surface.

The gills and intestines together, contain between 10^3 and 10^9 germs/g. 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, 1992; Saranraj *et al*., 2012; Kanchana *et al*., 2015). 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.) (Lassuy, 2014). In this present study, the pathogenic bacteria and fungi were isolated from fresh fish collected from Mamallapuram fishery centre. The total bacterial and fungal population present in the Indian mackerel fish (*Rastrelliger kanagurta*) was estimated and the results were showed in Table - 1.

Table – 1: Microbial population present in Indian mackerel fish (*Rastrelliger kanagurta*)

S. No	Microorganism	Microbial population (cfu/ml)
1	Bacteria (× 10^4)	10.36
2	Fungi (× 10^3)	7.94

Bacteria floras isolated from fishes changes by the aquatic habitat of the fish and vary with factors such as the salinity of the habitat and the bacterial load in the water (Nishihara *et al*., 2008). Reports in actinomycetes population were also reported by Sahu *et al*. (2008) and Vanaja Kumar (2015). Five different bacteria were isolated from the Indian mackerel fish (*Rastrelliger kanagurta*) by Pour plate method. Based on the staining techniques, plating on selective medium and biochemical tests, bacterial isolates were identified as *Vibrio cholerae, Pseudomonas fluorescence, Salmonella typhi, Staphylococcus aureus* and *Escherichia coli*. The characteristics of the isolated bacterial isolates were given in the Table - 2 to Table - 6.

Table – 2: Characteristics of *Staphylococcus aureus* isolated from Indian mackerel fish (*Rastrelliger kanagurta*)

Test	Results
Gram staining	Gram positive cocci, arranged in clusters.
Endospore	No spores present
Motility	Non-motile
Catalase	Negative
Oxidase	Negative
Nutrient agar	Colonies are smooth and golden yellow
MacConkey agar	Lactose fermenting colonies.
Glucose fermentation	Acid produced
Mannitol fermentation	Acid produced
Sucrose fermentation	Acid produced
Dextrose fermentation	Acid produced
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization	Positive
Coagulase	Positive
DNAase	Positive
Mannitol salt agar	Golden yellow colonies
TSI medium	No reaction
Urease	Negative

Table – 3: Characteristics of *Pseudomonas fluorescence* isolated from Indian mackerel fish (*Rastrelliger kanagurta*)

Test	Results
Gram staining	Gram negative slender rods
Motility	Actively motile
Catalase	Positive
Oxidase	Positive
Nutrient agar	Green coloured diffusible pigment producing colonies
Mac Conkey 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 *Escherichia coli* isolated from Indian mackerel fish (*Rastrelliger kanagurta*)

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

Table – 5: Characteristics of *Vibrio cholerae* isolated from Indian mackerel fish (*Rastrelliger kanagurta*)

Test	Results
Gram staining	Gram negative, comma shaped rods
Motility	Motile
Catalase	Positive
Oxidase	Positive
Nutrient agar	Circular, moist, smooth, translucent and bluish tinge colonies
MacConkey agar	Smooth, gloosy and late lactose fermenting colonies
TCBS agar	Small yellow colonies
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid and gas produced
Sucrose fermentation	Acid and gas produced
Mannitol fermentation	Acid and gas produced
Indole	Positive
Methyl Red Test	Negative
Voges Proskauar Test	Positive
Citrate utilization	Negative
Urease	Negative
TSI	Acid butt, alkaline slant, No H_2S and no gas produced

Table – 6: Characteristics of *Salmonella typhi* isolated from Indian mackerel fish (*Rastrelliger kanagurta*)

Test	Results
Gram staining	Gram negative, comma shaped rods
Motility	Motile
Catalase	Positive
Oxidase	Positive
Nutrient agar	Circular, moist, smooth, translucent and bluish tinge colonies
MacConkey agar	Smooth, gloosy and late lactose fermenting colonies
TCBS agar	Small yellow colonies
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid and gas produced
Sucrose fermentation	Acid and gas produced
Mannitol fermentation	Acid and gas produced
Indole	Positive
Methyl Red Test	Negative
Voges Proskauar Test	Positive
Citrate utilization	Negative
Urease	Negative
TSI	Acid butt, alkaline slant, No H_2S and no 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*.

Three different fungi were isolated from the Indian mackerel fish (*Rastrelliger kanagurta*). Based on Lactophenol cotton blue staining and colony morphology on Sabourauds dextrose agar, they were identified as, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum.* The characteristics of the fungi isolated from the Indian mackerel fish (*Rastrelliger kanagurta*) was tabulated in Table – 7. In the study of Nishihara *et al*. (2008), 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.

Table – 7: Characteristics of fungi isolated from Indian mackerel fish (*Rastrelliger kanagurta*)

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.
Fusarium oxysporum	
Conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clusters. Macroconidia fusiform, slightly curved, pointed at the tip, basal cells pedicellate. Macroconidia abundant, never in chains, mostly non-septate, ellipsoidal to cylindrical, straight or often curved. Chlamydospores terminal or interacalary, haline, smooth or rough walled.	Colonies grow rapidly; aerial mycelium white, usually becoming purple; discrete, erumpent, orange sporodochia are present in some strains; reverse hyaline to dark blue or dark purple.

References

1. Bremner, H. A and J. A. Statham. 1983. Spoilage of vaccum packed chill stored sea lops with added *Lactobacilli. Food Technology*, 35: 284 - 287.
2. Castro, J., J. Cleveland and K. Montgomery. 2008. Gut characteristics and assimilation efficiencies in two species of herbivorous dam shellfishes. *Marine Biology*, 142: 35 - 44.
3. Darwina, M., D. Kanchana and P. Saranraj. 2012. Biocontrol efficacy of various preservatives against food borne pathogens in poultry chicken. *Novus International Journal of Biotechnology and Biosciences*, 1 (1): 1 - 13.
4. Geetha, M., P. Saranraj, S. Magalakshmi and D. Reetha. 2012. Screening of Pectinase producing bacteria and fungi for its pectinolytic activity using fruit wastes. *International Journal of Biochemistry and Biotech Sciences*, 1(1): 30 - 42.

5. Ismail, M. A., L. Bulushi, S. Poole, C. H. C. Deeth and G. A. Dukes. 2008. 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*, 24: 1867 - 1875
6. Jageethadevi, A., P. Saranraj and N. Ramya. 2012. Inhibitory effect of chemical preservatives and organic acids on the growth and organic acids on the growth of bacterial pathogens in poultry chicken. *Asian Journal of Biochemical and Pharmaceutical Research*, 1 (2): 1 – 9.
7. Kanchana, D., R. Kavitha and P. Saranraj. 2015. Microbial Spoilage of Modified Atmosphere Packaging on Fruits and Vegetables. *Advances in Biological Research*, 9(4): 253 – 256.
8. Kanchana, D., P. Saranraj and R. Kavitha. 2015. Isolation and characterization of some Spoiled Yellow Goat Fish (*Sulphureus cuvier* L.). *World Journal of Fish and Marine Sciences*, 7 (4): 243 – 246.
9. Lassuy, D. R. 2014. Diet, intestinal morphology and nitrogen assimilation efficiency in the dam shelfish, tegastes lividus, in Guam. *Environmental Biology and Toxicology*, 10: 181 - 193.
10. Lindsay, G. J. H and J. E. Harrish. 2010. Carboxy methyl cellulose activity in the digestive tracts of fish. *Journal of Fish Biology*, 16: 219 - 233.
11. Mountfort, D. O., J. Campbell and K. D. Clements. 2002. Hindgut fermentation in three species of marine herbivores. *Applied Environmental Microbiology*, 68: 1374 - 1380.
12. Nelson, K. E., S. H. Zinder, I. Hance, P. Burr, D. Odongo, D. Wasawo, A. Odenyo and R. Bishop. 2003. Phylogenetic analysis of the microbial populations in the wild herbivore gastrointestinal tract insights into an unexplored niche. *Environmental Microbiology and Toxicology*, 5: 1212 - 1220.
13. Nishihara, M., M. Kamata, T. Koyama and K. Yazawa. 2008. New phospholipase A1-producing bacteria from a Marine Fish. *Marine Biotechnology*, 10: 382 - 387.
14. Saha, S., R. Roy, N. Sen and A. K. Ray. 2006. Characterization of cellulose producing bacteria from the digestive tract of tilapia, *Oreochromis mossambicas* (peters) and grass carp, *Ctenopharyngodon idella* (Valenciennes). *Aquaculture Research*, 37: 380 - 388.
15. Sahu, M. K., N. S. Swarnakumar, K. Sivakumar, T. Thangaradjou and L. Kannan. 2008. Probiotics in aquaculture: importance and future perspectives. *Indian Journal of Microbiology*, 3 (6): 1 - 10.
16. Saranraj, P and M. Geetha. 2012. Microbial spoilage of Bakery products and its control by preservatives. *International Journal of Pharmaceutical and Biological Archives*, 3 (1): 204 - 214.
17. Saranraj, P., D. Stella and D. Reetha. 2012. Microbial spoilage of vegetables and its control measures: A Review. *International Journal of Natural Product Science*, 2 (2): 1 -12.
18. Stevens, C. E and I. D. Hume. 2015. Comparative physiology of the vertebrate digestive system, 2nd Ed. Cambridge University Press, Cambridge, United Kingdom.
19. Storm, E and E. Ringo. 2013. Changes in bacterial flora in developing cod, *Gadus morhua* L. larvae after inoculation of *Lactobacillus plantarum* and Biochemical Aspects of fish larval development. University of Bergen, PP. 226 - 228.
20. Vanaja Kumar, K. 2015. Studies on Actinomycetes associated with Mollusks from Porto Novo coastal waters. Ph. D. thesis, Annamalai University, India.

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