Isolation and identification of bacteria from marine market fish *Scomberomorus guttatus* (Bloch and Schneider, 1801) from Madurai district, Tamil Nadu, India

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Abstract The present study was conducted to evaluate the hygienic quality and freshness of fish Indo-pacific King Mackerel “*Scomberomorus guttatus*” through the investigation of the occurrence of bacteria which is an indicator for fish quality. Fishes were collected every fortnight from Madurai fish market on monthly twice of January 2014 to March 2014. Skin surface of the fish was examined. *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were identified by Biochemical tests (IMViC Tests). Among the six bacterial species *E. coli* and *K. pneumonia* were found in all the collected samples where as other bacterial species were not found. The result of this study revealed that raw fish sold in Madurai fish market has high contamination so the presence of the bacterial species has strongly suggested the urgent need to improve the quality control systems in Madurai fish market.

Keywords *S. guttatus* · Bacterial isolation · Madurai fish market · Tamil Nadu

Introduction

Fish and fish products are the most important source of protein and it is estimated that more than 30 % of fish for human consumption comes from aquaculture (Hastein et al. 2006). Consumption of fish and shell fish may cause diseases due to infection or intoxication. Some of these diseases are caused by the microorganisms present on the external surfaces including slime, gills and the gut of the fish. These microorganisms are kept away from invading the sterile flesh by the normal defense mechanism of fish, when they are alive. On death, the microorganisms or the enzymes they secrete are free to invade or diffuse into the flesh where they react with complex mixture of natural substances present resulting in a well-defined sequence of changes in odoriferous and flavourous compounds. The mackerel (*S. guttatus*) is commercially important fatty fishes that lives in epipelagic-neritic zone, it occurs all through the year in the landings on Rameswaram. The summer months (March to September) witness high landings with the peak of the season in March and the catches are poor during the winter months (October to February), reaching the lowest in February (Jones 1961). The bacteria present on the fish are normally associated with those found in their natural environment and influenced by the season and the harvesting conditions (ICMSF 1998). The outlined and discussed the hazards and challenges associated with handling fish during farming, capture and the environmental contaminants in seafood that may pose a risk to human health (Yagoub 2009). The quality of fresh fish in retail markets of Bombay was carried out by (Iyer et al. 1986). The quality of the raw fish (fresh or iced) at markets varies so widely that there is an obvious need for developing quality standards. The characteristic of mackerel tissue is the amount of dark muscle which is soft and

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has desirable flavor in raw and cooked form. There is no study on Microbial identification of these fishes. Therefore this study was carried out to identify certain microbial analysis to assess the quality and freshness of raw fish (*S. guttatus*) sold at a market in Madurai, Tamil Nadu, India.

Materials and methods

Laboratory analysis

Fish samples

S. guttatus were collected from a local fish market in Madurai, where the fishes were regularly obtained from Rameswaram landings. Totally 20 numbers of samples were collected monthly twice of January 2014 to March 2014. The collected samples were aseptically and immediately transported in a thermal bag to the laboratory and processed within 3 h of acquisition, and samples were kept in the refrigerator (4–8 °C).

Sample preparation

The 10 g of the fish sample was cut from skin surface with a sterile knife. The cut samples were crushed into small pieces in a sterile mortar with about 10 ml sterile water. From the crushed sample, 1 ml aliquot volume was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of distilled water giving a 1:10 dilution. This was done for the 20 fish samples (Obi and Krakowiaka 1983).

Isolation and identification bacteria form fish sample of *S. guttatus*

The collected samples were processed in the research laboratory, Department of Zoology, Yadava College, Madurai, Tamil Nadu, and India according to the standard microbiological methods under complete aseptic conditions. The swabs were inoculated on, mannitol salt agar (Oxoid, England) and nutrient agar (Oxoid, England) and

incubated at 37 °C under aerobic condition. The isolated bacterial colonies were identified on the basis of their morphological, physiological and biochemical characters. These culture were subjected to various biochemical tests such as gram staining, spore staining, motility, indole, methyl red, voges proskauer, citrate, Triple sugar ion, oxidase, carbohydrate fermentation, hydrogen sulfide production tests for identification of phosphate solubilizing bacteria using Bergey's manual of systematic bacteriology (Holt et al. 1994). The bacteria such as *E. coli*, *K. pneumonia*, *P. vulgaris*, *B. subtilis*, *P. aeruginosa* and *S. aureus* were isolated from the fish samples.

Results and discussion

In bacteria, bio-oxidation reactions are very important. These reactions help bacteria to provide energy by oxidation of organic substances or by fermentation. Based on these reactions, the bacteria were identified and the results were presented in (Tables 1, 2, 3, 4, 5, and 6). In this study, *E. coli*, *K. pneumonia*, *P. vulgaris*, *B. subtilis*, *P. aeruginosa* and *S. aureus* were identified from the fish. *E. coli* and *K. pneumonia* were found in all the fortnight samples (Tables 1, 2, 3, 4, 5 and 6). Whereas, *B. subtilis* (Tables 1, 5 and 6) and *P. aeruginosa* (Tables 2, 4 and 6) were identified three times respectively. *P. vulgaris* (Tables 1, 3) and *S. aureus* (Tables 4, 5) were identified two times respectively. The fish and shellfish are highly perishable and prone to vast variations in quality due to differences in species, environmental habitats, and feeding habits. They can also function as carriers of several microbial and other health hazards. Therefore maintenance of quality is most important in production and trade of fishery products. Although only a few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities. However, the greatest risk to human health due to the consumption of raw or insufficiently processed fish and fish products. In this study, *Pseudomonas* sp was isolated from 63 % of collected fish samples is of highly importance because this bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator

Table 1 Bacteria isolated from the *S. guttatus* purchased on first week of January 2014

Microorganisms	Gram staining	Motility	Indole production	Methyl red	Voges-Proskauer	Citrates	Triple sugar iron	Oxidase	Carbohydrate fermentation	Hydrogen sulfide production
<i>Escherichia coli</i>	–	+	+	+	–	–	–	–	+	–
<i>Klebsiella pneumonia</i>	–	–	–	–	+	+	–	–	D	–
<i>Proteus vulgaris</i>	–	+	+	+	–	+	+	D	D	–
<i>Bacillus subtilis</i>	+	–	+	–	–	+	–	D	D	–

+: Positive, –: negative, D: different

Table 2 Bacteria isolated from the *S. guttatus* purchased on last week of January 2014

Microorganisms	Gram staining	Motility	Indole production	Methyl red	Voges-Proskauer	Citrates	Triple sugar iron	Oxidase	Carbohydrate fermentation	Hydrogen sulfide production
<i>Escherichia coli</i>	–	+	+	+	–	–	–	–	+	–
<i>Klebsiella pneumonia</i>	–	–	–	–	+	+	–	–	D	–
<i>Pseudomonas aeruginosa</i>	+	–	+	–	–	+	–	D	D	–

+: Positive, –: negative, D: different

Table 3 Bacteria isolated from the *S. guttatus* purchased on first week of February 2014

Microorganisms	Gram staining	Motility	Indole production	Methyl red	Voges-Proskauer	Citrates	Triple sugar iron	Oxidase	Carbohydrate fermentation	Hydrogen sulfide production
<i>Escherichia coli</i>	–	+	+	+	–	–	–	–	+	–
<i>Klebsiella pneumonia</i>	–	–	–	–	+	+	–	–	D	–
<i>Proteus vulgaris</i>	–	+	+	+	–	+	+	D	D	–

+: Positive, –: negative, D: different

Table 4 Bacteria isolated from the *S. guttatus* purchased on last week of February 2014

Microorganisms	Gram staining	Motility	Indole production	Methyl red	Voges-Proskauer	Citrates	Triple sugar iron	Oxidase	Carbohydrate fermentation	Hydrogen sulfide production
<i>Escherichia coli</i>	–	+	+	+	–	–	–	–	+	–
<i>Klebsiella pneumonia</i>	–	–	–	–	+	+	–	–	D	–
<i>Pseudomonas aeruginosa</i>	+	–	+	–	–	+	–	–	D	–
<i>Staphylococcus aureus</i>	+	–	–	–	–	+	–	–	D	–

+: Positive, –: negative, D: different

Table 5 Bacteria isolated from the *S. guttatus* purchased on first week of March 2014

Microorganisms	Gram staining	Motility	Indole production	Methyl red	Voges-Proskauer	Citrates	Triple sugar iron	Oxidase	Carbohydrate fermentation	Hydrogen sulfide production
<i>Escherichia coli</i>	–	+	+	+	–	–	–	–	+	–
<i>Klebsiella pneumonia</i>	–	–	–	–	+	+	–	–	D	–
<i>Bacillus subtilis</i>	+	–	+	–	–	+	–	D	D	–
<i>Staphylococcus aureus</i>	+	–	–	–	–	+	–	–	D	–

+: Positive, –: negative, D: different

of food quality as spoilage organism. This is in accord with previously mentioned by (Jeyasekaran et al. 2006; and Koutsoumanis and Nychas 2000). In this study six bacterial species were isolated from *S. guttatus* on six fortnights of January to March 2014. Among the six bacterial species *E. coli* and *K. pneumonia* were found in all the fortnights and others were not found. Apart from the enteric-organisms, *S. aureus* encountered in this study are known enterotoxin producing agent and a microorganism which is poisonous. This is in agreement with the previous study by

some authors in Nigeria and outside Nigeria (Okonko et al. 2008, 2009). The results from this study and according to published microbiological guidelines as cited by (Gilbert et al. 1996) suggest that the microbiological quality of the fish examined is unacceptable and pose a potential risk to public health. The diversity of potential pathogens from the samples of fish is of concern particularly at a time when many in our communities are immunologically compromised as a result of various illnesses (Mhango et al. 2010). The result of this study revealed that raw fish sold in

Table 6 Bacteria isolated from the *S. guttatus* purchased on last week of March 2014

Microorganisms	Gram staining	Motility	Indole production	Methyl red	Voges-Proskauer	Citrates	Triple sugar iron	Oxidase	Carbohydrate fermentation	Hydrogen sulfide production
<i>Escherichia coli</i>	–	+	+	+	–	–	–	–	+	–
<i>Klebsiella pneumonia</i>	–	–	–	–	+	+	–	–	D	–
<i>Bacillus subtilis</i>	+		+	–	–	+	–	D	D	–
<i>Pseudomonas aeruginosa</i>	+		–	+	–	+	–	+	D	–

+: Positive, –: negative, D: different

Madurai fish market has high contamination, may be as a result of certain factors like temperature which favours some organisms and the character of fish handler, by not maintaining personal hygiene, contaminated water taken in by the fishes which may contain faecal matter in their ecosystem which resulted in the isolation of enteric organism like bacteria's as well as other microorganisms which causes contamination like that of *Aspergillus niger* which may produce toxic material, when fish is consumed by man. So the present conclusion of the study on the bacterial species has suggested that the urgent need to improve the quality control systems in Madurai fish market.

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