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Original Research Article

Analysis of gut bacterial flora from edible marine fishes of South east coast of India

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Keywords

Fishes; Parangipettai and Cuddalore landing centres; total bacterial count from gut; Seasonal variation.

A B S T R A C T

From the edible marine fishes collected from Parangipettai and Cuddalore landing centres total bacterial count from the gut microflora was estimated quantitatively and qualitatively. When compared with the two landing centres Cuddalore landing centre measured a highest range than Parangipettai in all the three marine fishes. The gut samples of *Rastrelliger kanagurta* from Parangipettai showed maximum count of $2.40x10^{-6}$ in summer and while minimum count of $8.3x10^{-4}$ at pre monsoon, whereas in Cuddalore gut bacterial count was found maximum ($2.66x10^{-6}$) in postmonsoon and minimum ($1.58x10^{-6}$) in premonsoon. The seasonal bacterial count of *Lates calcarifer* gut samples of Parangipettai showed minimum count of $2.4x10^{-4}$ in postmonsoon and maximum count of $1.60x10^{-6}$ in premonsoon, maximum ($2.35x10^{-6}$) in summer and minimum ($4.6x10^{-6}$) in post monsoon at Cuddalore centre. The results of total bacterial count found in the gut regions of *Lutjanus fulviflamma* in Parangipettai region showed maximum count of $1.24x10^{-6}$ and minimum count of $1.05x10^{-6}$ in summer and post monsoon seasons while gut bacterial count varied between $1.91x10^{-6}$ and $1.22x10^{-6}$ in summer and post monsoon at Cuddalore respectively. The microbial load of the fishes in this study may be due to mass pollution of the environments where the fish were caught and also by monitoring the bacterial contents of fish in gut samples, the quality of fish can be measured.

Introduction

Marine fishery resources are living natural resource which is self renewable with dynamic habitat. In India, the natural resources are highly rich where annual harvestable fishery potential to the country is estimated to increase in millions of tones day by day (Varadharajan *et al*., 2012). As the world's population increases inexorably at a current rate of almost 2% per year, the importance of sea food as a source of animal protein foodstuff gained more and more attention in recent years.

Even though, sea foods are nutritive, they act as a vehicle for the pathogenic bacteria naturally occurring in the aquatic environment referred to as indigenous or derived from the postharvest

contamination (Wallace *et al.,* 1999; Gillespie *et al.,* 2001). It is considered that the quality of seafood depends on the quality of water where the fishes are caught and the sanitary conditions of the landing centre. Microorganisms occur nearly everywhere in nature and occupy an important place in the life of human. The human activities had a great impact in coastal areas in the last two decades with the effects of industrialization, intensive agriculture and coastal engineering gave serious threat to marine life (His *et al*., 1999) that led to environmental pollution.

Freshly caught fish microbial flora is largely a reflection of microbial quality of the waters from where they are harvested. The microorganism present in the environment enables it to enter the food chain through raw materials and is a major problem in convenience foods and mass catering (Beattie and William, 2000; Guinebretiere *et al.,* 2006). Fish and fishery products are one of the major food components from ancient time (Noor *et al.,* 2013) which are highly perishable food, due to its quick perishability leads the main hurdle in its preservation (Okoro *et al.,* 2010; Musa *et al.,* 2010; Dewi *et al.,* 2011) easy digestibility and high nutritional value is an advantage of fish (Leisner *et al.,* 2001) this attributes makes the commodity readily susceptible to microbial attack particularly bacteria (Adam and Tobaias, 1999). Jassim *et al.* (1998) proved the fish extracts are the excellent sources of nutrients for bacterial growth. All living organisms including fish coexist with a wide range of pathogenic and non- pathogenic microorganisms which posses complex defence mechanisms which contribute for their survival. Fishes receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. From most of the fish digestive tracts bacterial species isolated have been reported to be aerobes or facultative anaerobes (Trust and Sparrow, 1974; Bairagi *et al.,* 2002; Saha *et al.,* 2006). Contamination of these edible portions could originate from digestive tract of the fish. Generally it was accepted that there is a possible symbiotic relationship between fish and gut microflora (Verschuere et al., 2000).

The bacteria found in the digestive tract of fish were highly variable and were a reflection of their aqueous environment, especially the food choice of the individual fish (Nieto et al. 1984). Bacteria appeared to vary with sampling season (Al-Harbi and Uddin, 2004) or geographical location (Skrodenyte-Arbaciauskiene, 2006) and often particularly with environmental conditions. Based on the above, this study was undertaken is to isolate and identify the gut microbial diversity of bacteria from the edible marine fishes of India.

Materials and Methods

Marine edible fishes (*Rastrelliger kanagurta, Lates calcarifer* and *Lutjanus fulviflammus*) were collected from landing centres of Parangipettai and Cuddalore. The identified samples were placed individually in the pre-sterilized polythene bags, sealed and kept in a portable ice chest and transported to the laboratory for further bacteriological analysis. The following fish species were chosen due to their worldwide availability in most tropical and subtropical waters throughout the year.

Sample Preparation

Bacterial isolates from each specimen were obtained, by removing the gut from the fishes. From each fishes 1gm was

taken, homogenate was made in 10ml distilled water. The solution was serially diluted ten folds. 0.1ml of (10^{-10}) dilution was spread on to Zobell marine agar in duplicate and incubated for 18-24 hrs at 37^0C.

Quantitative analysis of bacteria

To estimate bacterial numbers, the inoculated plates were incubated for 18-24 hrs at 37^0C and duplicates were prepared for each dilution. Following incubation, the total number of colony forming unit (CFU) was determined and representative colonies were sub-cultured for identification. Bacterial numbers were calculated as the average of each set of duplicates and expressed as CFU/ml of the homogenate. Bacteria were isolated by a random collection of colonies from the agar plates. The colonies were purified by repeated sub-culture.

Bacteria identification

Morphological identification of the bacteria present in all samples was carried out according to Bergey s Manual of Determinative Bacteriology (Holt *et al*., 1994).

Results and Discussion

The total bacterial load of gut sample was estimated after isolation and growth on marine zobell agar plates incubated at room temperature at 37℃. The numbers of cultivable bacteria present in fish gut of three edible marine fishes showing seasonal changes between stations are shown in fig. 1.

Lutjanus fulviflamma

The results of total bacterial count found in gut regions of Lutjanus fulviflamma were found to be maximum in Cuddalore and minimum in Parangipettai region. The seasonal bacterial counts in Parangipettai region of gut samples showed maximum count of 1.24x10-6 and minimum count of 1.05x10-6 in summer and post monsoon seasons. The bacterial count in gut bacterial count in Cuddalore varied between 1.91x10-6 and 1.22x10-6 in summer and post monsoon respectively.

Lates calcarifer

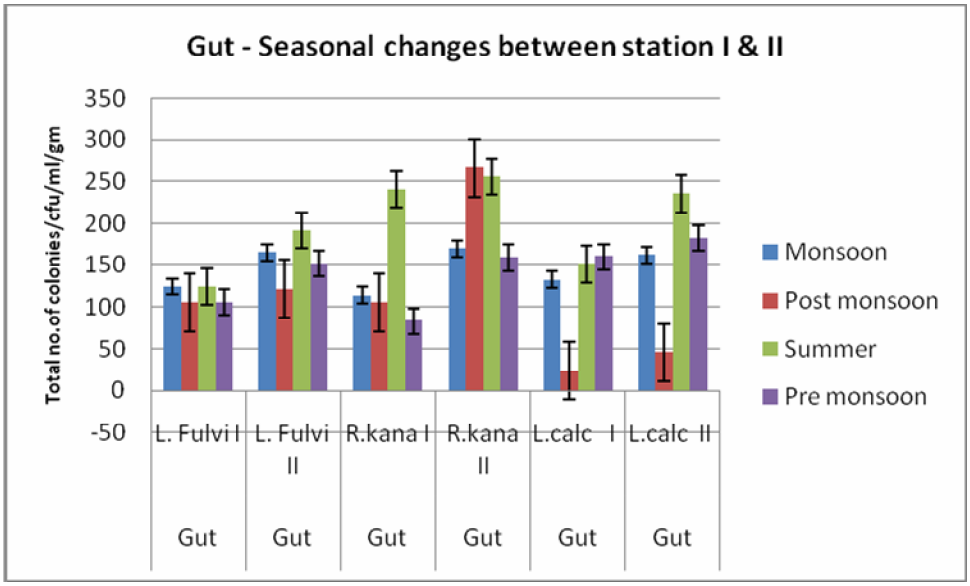
Lates calcarifer collected from both the stations had more bacterial counts in Cuddalore when compared with Parangipettai. The gut samples of Parangipettai showed minimum count of 2.4x10-4 in postmonsoon and maximum count of 1.60x10-6 in premonsoon. The gut bacterial count of Cuddalore was maximum (2.35x10-6) in summer and minimum (4.6x10-6) in post monsoon

Rastrelliger kanagurta

Gut samples of Rastrelliger kanagurta also showed maximum count of 2.40x10-6 in summer and while minimum count of 8.3x10-4 at pre monsoon at Parangipettai whereas gut bacterial count of Cuddalore was found maximum (2.66x10-6) in postmonsoon and minimum (1.58x10-6) in premonsoon. In Parangipettai and Cuddalore there was a definite trend of seasonal variation of bacteria observed.

The results showed that bacterial diversity in the gut of Rastrelliger kanagurta, Lates calcarifer and Lutjanus fulviflamma varied in Parangipettai and Cuddalore. High microbial load in the Cuddalore was occurred due to the pollution by means of untreated sewage disposed into the coastal waters.

Fig.1 Seasonal changes between stations I (Parangipettai) and II (Cuddalore)



The present results are very close to the study of Soundarapandian and Sowmiya (2013) and Thavasi *et al*. (2007) as they have reported coastal waters gets polluted by untreated sewage which has resulted in the spread of microbial pathogens. In the present study, the bacterial count was higher in the polluted site than in the unpolluted site which is a clear indication that the presence of antibiotic resistant bacteria can be related to polluted effluents.

In the present study, fishes collected from two stations, different season influences the differential bacterial count in all the three fish species which was similar to the study of Soundarapandian and Sowmiya (2013). Based on our results, Al- Bahry *et al.,* (2009) also found the highest bacterial counts in the colons from the polluted site are not surprising since the fish ingested antibiotic resistant. In Parangipettai and Cuddalore coasts, the gut of crab *P. pelagicus* and *P. sanguinolentus* (Soundarapandian and Sowmiya, 2013) showed a partial agreement of the present study bacteria which may consequently found their way to the colon.

The present study revealed 6 genera of bacteria in gut samples: *Micrococcus, Pseudomonas, Staphylococcus, Bacillus, Aeromonas, Vibrio* in different rate.

The bacterial diversity in the digestive tract of the fish generally, varies due the hydrobiological fluctuations occurring in the natural systems (Rheinheimer, 1985). It is considered that bacterial ecology of fishes are connected to environmental factors such as water pollution, hygienic procedures of slaughter, handling, transport, commercialization and storage conditions. Janina Syvokiene (2011) discussed that from the aquatic animals the microflora of digestive tract isolated is proved to be the first to be affected by any pollutants appearing in water. The abundance of bacteria in aquatic organisms was found to depend on fish species, nutrition habits, as well as seasonal and environmental effects, internal and external factors (Ringo et al., 2008; Spanggaard et al., 2000; Austin, 2002). The bacterial diversity in the fish might be increased with the increase of water temperature (Hossain et al. 1999). Hence, studies on fish gut microbiology

are needed for the management both in aquaculture and public health.

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