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ISOLATION AND CHARACTERIZATION OF BACTERIAL SPECIES ASSOCIATED WITH FISHES FROM LOCAL MARKET OF DEGLOOR IN NANDED DISTRICT, MAHARASHTRA, INDIA

A. A. Atnoorkar[1*], T. A. Kadam[2] and R. R. Rakh[3]
[1] Department of Microbiology, Vai. Dhunda Maharaj Degloorkar College, Degloor, District Nanded.
[2] School of Life Sciences, Sami Ramanand Teerth Marathwada University, Nanded.
[3] Department of Microbiology, Shri Guru Buddhiswami College, Purna (Jn), District Hingoli.
[*] Corresponding author
A. A. Atnoorkar
Department of Microbiology , Vai. Dhunda Maharaj Degloorkar College, Degloor.
E-mail: anand_atnoorkar@rediffmail.com

ABSTRACT :

Two fish samples viz. Catla catla and Labeo rohita were collected from local retail fish market of Degloor. The results of bacteriological quality of fishes showed variation in total bacterial counts. The highest total bacterial count 42 x 10^4 CFU/g was found in Catla catla fish whereas the total bacterial count in Labeo rohita was 32 x 10^3 CFU/g. A total 57 isolates from both fishes were identified by using standard bacteriological tests. Forty two isolates were from Catla catla fish and fifteen isolates were from Labeo rohita. The isolates identified were Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Aeromonas hydrophila from both the fishes. However, Salmonella typhi was identified only from Catla catla fish. The highest incidence of bacterial species was associated with Catla catla fish. The bacterial species associated with these fishes could pose serious health problems if consumed and causes major protein food loss due to spoilage of fishes.

KEYWORDS: *Catla catla, Labeo rohita, bacterial isolation, characterization, spoilage.*

INTRODUCTION:

The three Indian major carps viz. catla (Catla catla), rohu (Labeo rohita) and mrigal (Cirrhnius mrigala) contribute the bulk production of 70-75% of the total fresh water fish production (Hand book of fisheries and aqua culture, 2013).

Fish is the most important source of protein and is one of the main food components of humans. 30% of fish for human consumption comes from aquaculture (Hastein et al, 2006). The advantage of fish as a food is due to its easy digestibility and high nutritional value (Eze et al, 2011).

The type of microorganisms found associated with particular fish depends on the water it was found (Clucas and Ward, 1996). Fish contamination is due to inadequate processing, poor standards of hygiene and sanitation. The bacterial species associated with fish includes *Pseudomonas, Alcaligens, Aeromonas, Staphylococcus, Vibrio, Klebsiella, Bacillus, Proteus, E. coli* (Clucas and Ward, 1996, Rashid et al, 2013, Karthiga Rani, 2016). The hazards associated with handling fish during farming and capture may pose risk to human health (Yagoub, 2009). The fresh fish spoilage can be rapid after it is caught. Microbial growth and metabolism is major cause of fish spoilage. The quality of fresh fish in retail markets of Bombay was carried out by Iyer et al (1986), Madurai fish market by Karthiga Rani (2016). Therefore, this study was carried out to characterize bacterial species associated with raw fishes sold at local retail market in Degloor, District Nanded.

MATERIALS AND METHODS

Location of study

The study was carried out at Degloor, District Nanded, Maharashtra. Degloor is located at Latitude 18.533° N and Longitude 77.585° E. The study was carried out in the laboratory of Department of Microbiology, Vai. Dhunda Maharaj Degloorkar College, Degloor.

Collection of fish samples

Catla catla and *Labeo rohita* fishes were collected from a local retail fish market in Degloor. The collected samples were aseptically and immediately transported in a bag to the laboratory and processed within 3 hr of acquisition.

Preparation of sample

Sample preparation was made using the method described by Obi and Krakowiaka (1983). The 10 g of the fish sample was cut from skin surface with sterile knife. The cut samples were crushed into mall pieces in sterile mortar with 10 ml of sterile saline. 1 ml of aliquot was homogenized in 9 ml of sterile saline and was serially diluted up to 10^{-5}.

Total bacterial count

0.1ml of the diluted solutions (10^{-2} to 10^{-5}) was inoculated on sterile nutrient agar plates using spread plate technique. The plates were incubated at 37°C for 24 hr for colony enumeration and isolation.

Isolation and characterization of bacteria.

Serial dilutions were inoculated on selective media viz. MacConkey's agar, Mannitol salt agar, Wilson and Blair agar using spread plate technique. The plates were incubated at 37°C for 24 hr. After incubation pure cultures of bacterial species were obtained on nutrient agar slants. The bacterial isolates were characterized on the basis of morphological, physiological and biochemical characters. These cultures were subjected to Gram's staining, motility and various biochemical tests such as sugar fermentation, Indole, Methyl red, Voges Proskauer, citrate utilization, H_2S production, catalase, coagulase, caseinase and gelatinase for identification using Bergey's Manual of Systematic Bacteriology (Holt et al, 1994).

RESULTS

The results of total bacterial counts associated with two species of fresh water fish samples were expressed in colony forming unit per gram (CFU/g) is shown in table 1. The *Catla catla* fish samples obtained from Degloor market showed the highest bacterial contamination with bacterial count of 42×10^4 CFU/g. The *Labeo rohita* fish samples were showed least bacterial contamination with bacterial count of 32×10^3 CFU/g.

Table 1. Total bacterial counts associated with fishes.

S. No.	Fish species	Total bacterial count CFU/g
1	*Catla catla*	42×10^4
2	*Labeo rohita*	32×10^3

A total of 57 isolates from both fishes were characterized according to morphological, physiological and biochemical tests (Table 2). Forty two isolates were from *Catla catla* fish and fifteen isolates were from *Labeo rohita*.

Table 2. Morphological, Physiological and Biochemical characteristics of bacterial isolates from fishes.

S. No.	Characteristics	Bacterial species identified					
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. hydrophila*	*S. typhi*
1	Gram's staining	+	+	-	-	-	-
2	Morphology	cocci	rods	rods	rods	rods	rods
3	Motility	-	+	+	+	+	+
4	Sugar fermentation						
	Glucose	+	+	+	-	+	+
	Lactose	+	-	+	-	+	-
	Mannitol	+	+	+	+	+	-
	Sucrose	+	+	-	-	+	-
5	Indole	-	-	+	-	-	-
6	Methyl red	+	-	+	-	-	-
7	Voges Proskauer	+	+	-	-	+	+
8	Citrate utilization	+	+	-	+	–	+
9	H_2S production	-	-	-	-	+	+
10	Catalase	+	+	+	+	+	+
11	Coagulase	+	-	-	-	-	-
12	Caseinase	+	+	-	-	-	-
13	Gelatinase	+	+	-	+	-	-

- : Negative + : Positive

The number and percentage of incidence of bacterial species in the fish samples is shown in table 3.

Table 3. Incidence of bacterial species in the fishes.

S. No.	Bacterial species	Fish species	
		Catla catla	*Labeo rohita*
1	*Staphylococcus aureus*	16 (38.09%)	05 (33.33%)
2	*Bacillus subtilis*	04 (9.52%)	03 (20.0%)
3	*Escherichia coli*	11 (26.9%)	04 (26.0%)
4	*Pseudomonas aeruginosa*	05 (11.9%)	02 (13.33%)
5	*Aeromonas hydrophila*	03 (7.14%)	01 (6.66%)
6	*Salmonella typhi*	03 (7.14%)	--
Total isolates		42	15

The values in brackets indicate percentage of incidence of bacterial species.

The number and percentage of incidence of bacterial species *Staphylococcus aureus* (16, 38.09%) (05, 33.33%), *Bacillus subtilis* (04, 9.52%) (03, 20.0%), *Escherichia coli* (11, 26.9%) (04, 26.0%), *Pseudomonas aeruginosa* (05, 11.9%) (02, 13.33%), and *Aeromonas hydrophila* (03, 7.14%) (01, 6.66%) were from *Catla catla* and *Labeo rohita* respectively. However, *Salmonella typhi* was (03, 7.14%) recorded only from *Catla catla* fish. The highest incidence of bacterial species was associated with *Catla catla* fish. The percentage incidence of bacterial species in *Catla catla* and *Labeo rohita* is shown in figure 1 and 2.

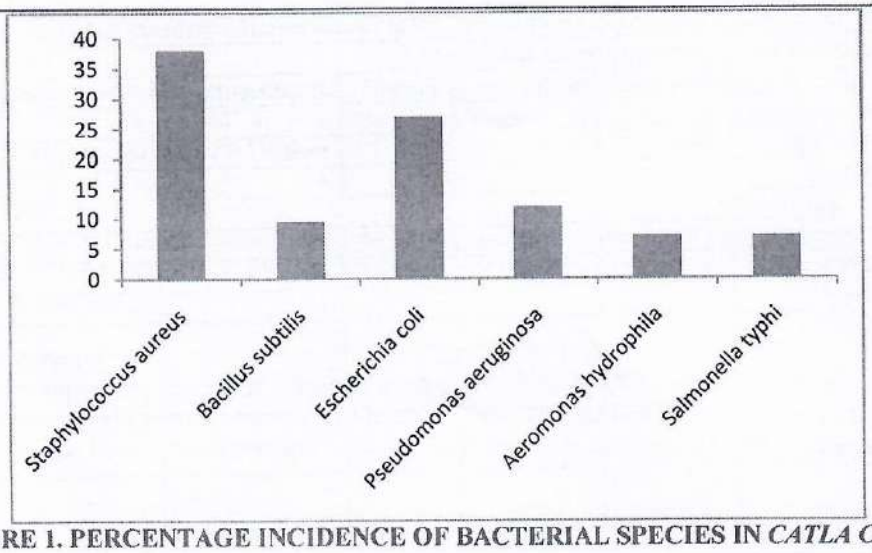

FIGURE 1. PERCENTAGE INCIDENCE OF BACTERIAL SPECIES IN *CATLA CATLA*

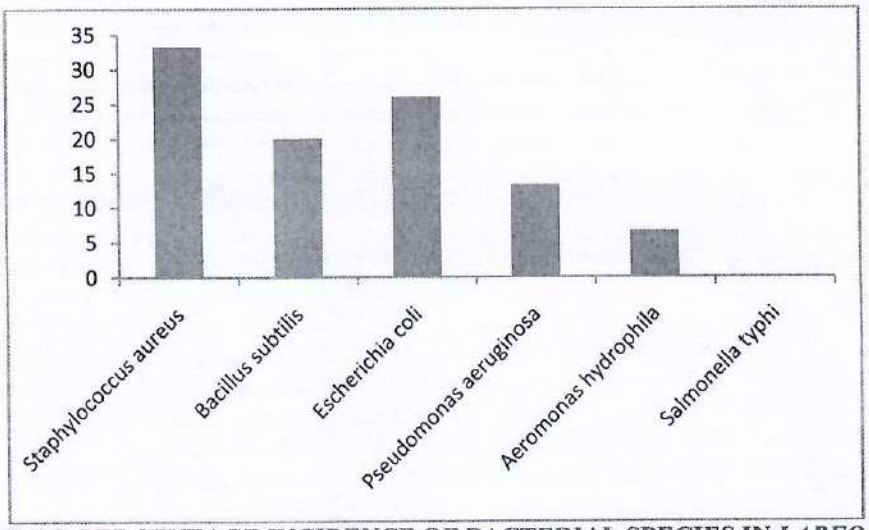

FIGURE 2. PERCENTAGE INCIDENCE OF BACTERIAL SPECIES IN *LABEO ROHITA*

DISCUSSION

In the present study, the total bacterial counts associated with *Catla catla* fish was 42 x 10^4 CFU/g whereas 32 x 10^3 was recorded in *Labeo rohita* fish. Ibrahim and Adetyi (2013) recorded total bacterial counts from cat fish 83 x 10^5 CFU/ml from gill and 53 x 10^5 CFU/ml from skin. Yusuf et al (2012) observed 2.4 x 10^4 CFU/g total bacterial counts in dried smoked fish where as 2.8 x 10^4 CFU/g in ice smoked fish within Bauchi metropolis. Eze et al (2011) recorded 1.135 x 10^8 mean bacterial load from mackerel fish. The fish is highly perishable commodity and prone to vast variation in bacterial quality due to difference in fish species, environmental habitats and feeding habit (Karthiga Rani et al, 2016).

The bacterial species *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Aeromonas hydrophila* were found associated with *Catla catla* and *Labeo rohita, Salmonella typhi* was associated only with *Catla catla* fish in the present study. These results agree with the reports of Yusuf et al (2012) and Karthiga Rani et al (2016). *Staphylococcus aureus, Salmonella typhi, Proteus mirabilis, Klebiella spp, Streptococcus spp* and *Bacillus cereus* were reported in retailed smoked fish within

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Coordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist. Parbhani - 431511 (M.S.)

Shri Guru Buddhiswami Shikshan Prasarak Mandal Purna (Jn.)
Shri Guru Buddhiswami Mahavidyalaya
Estd. 1983

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani

Bauchi Metropolis by Yusuf et al (2012). *Escherichia coli, Proteus vulgaris, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus* were reported from marine market fish *Scomberomorus guttatus* from Madurai district by Karthiga Rani et al (2016). Rashid et al (2013) identified *Aeromonas hydrophila* from a fish farm in Mymensingh. The high prevalence of *Staphylococcus* in fish samples indicates the unhygienic handling of fish as well as contamination during post harvest handling (Ames, 1992). The *E. coli* is particularly useful indicator of contamination and mishandling of fish (Silliker and Gabis, 1976). The *E. coli* bacteria isolated from the samples were most reliable indicator of fecal pollution. *Pseudomonas species* are responsible for spoilage of fish. Discoloration of fish flesh may occur during spoilage (Jeyasekaraaran et al, 2006 and Koutsoumanis Nychas, 2000). *S. aureus, B. subtilis* and *Pseudomonas aeruginosa* showed the presence of proteolytic enzyme activity. Microbial fish spoilage produce amines such as putrescine, histamines and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off flavors (Dalagaard et al, 2006).

CONCLUSION

From this study, it could be concluded that bacterial species gets associated with the fishes from water environment of the fish, post harvesting, marketing, fish handlers. This processing will result in microbiological activities leading to loss of fish meat quality and fish spoilage. However, potential pathogenic organisms was found associated with fishes which need for proper hygienic conditions for processing and distribution of fish product for prevention of food borne diseases and sanitation should be followed to protect the consumers against public health hazards.

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Co-ordinator IQAC

Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Dist.Parbhani Estd.1983

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Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani