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An Investigation of the Bacterial Flora Causing Spoilage of Fishes at Board Fish Market, Peshawar, Pakistan

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Abstract.- Bacterial flora from the epidermis of different species of carp fishes were isolated and identified as potential causes of spoilage of fish at Board fish market, Peshawar, Pakistan from November to December, 2013. Nutrient agar medium was used for mixed culture of bacteria. Other selective media, such as MacConkey agar, Blood agar medium, EMB medium, Pseudomonas medium and Mannitole salt agar medium were used for culture and identification of specific bacteria. Five bacterial groups that are pathogenic to humans *(Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas* spp, *Klebsiella* spp. and *Escherichia coli*) were reported to reside on the fish epidermis. *Staphylococcus aureus* was isolated from *Labeo rohita, Tor putitora, Hypophthalmichthys molitrix, Wallago attu, Ctenopharyngodon idella* and *Cyprinus carpio. Staphylococcus epidermidis* was also found on the epidermis of *Labeo rohita* and *Wallago attu.* Bacillus species like *E. coli, Pseudomonas* and *Klebsiella* spp. were isolated from the epidermis of Maha Sher (*Tor putitora),* Silver carp *(Hypohthalmichthys molitrix),* Rohu (*Labeo rohita),* Malli *(Wallago attu),* Gulfam *(Cyprinus carpio)* and Grass carp (*Ctenopharyngodon idella).* Harvesting, handling, transportation and storage of local market fish should be performed hygienically to reduce the risk of spoilage.

Key Words: Carp fishes, spoilage, bacteria, Nutrient agar medium, EMB (Eosin Methylene Blue) medium.

INTRODUCTION

Microbial degradation of fish often results in spoilage, which is the quality deterioration due to changes in sensory properties rendering the fish unusable for human consumption. Along with chemical changes and physical damage, microbial growth and metabolism lead to the formation of aldehydes, ketones, alcohols, sulfides, organic acids and amines which make the fish off-flavor (Gram and Dalgaard, 2002). Spoilage is the deterioration of food, which make its taste and smell bad (*e.g.*, when it is sour, rotten or mouldy) and/or makes it a carrier of disease organisms (Brigitte *et al*., 2004). Spoilage of fish not only results in a loss of protein for human consumption, but may also lead to great economic loss due to food borne illness (Abbas, 2014). Microbial contamination was reported to be dependent on water, fishing conditions and unsuitable processing, distribution and storage applications, following the capture of fishes. Expansion of culture fishery enterprises has also increased the consumption of fish. Concurrent with the increase of aquaculture there has been an increase in bacterial diseases of fish. Nearly 70 bacterial species have been reported pathogenic to humans (Yagoub, 2009). However bacteria recovered from the skin and gills of fish may be transient rather than resident on those surfaces (Cahill, 1990). The micro flora of fish is influenced by water in which the fish are grown as well as the water that is sprawed over fishes to keep them fresh as in some fish markets (Liston, 1980).

The pollution of fresh water resources in densely populated areas by various anthropogenic activities is a matter of grave concern (Mehboob *et al*, 2014). Each year a considerable amount of fish is lost due to spoilage. According to Eyo (1997) during post harvesting of fishes approximately 1 out of 14 Kg of fish are spoiled and discarded due to improper handling. This is a significant economic loss and there is a great need to identify the species of bacteria responsible and to develop effective methods of fish handling that will minimize the loss of this important food product due to spoilage.

MATERIALS AND METHODS

Six species of fishes namely Rohu *(Labeo rohita),* Mahasheer (*Tor putitora),* Silver carp

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(*Hypophthalmichthys molitrix),* Malli *(Wallago attu),* Grass carp *(Ctenopharyngodon idella)* and Gulfam *(Cyprinus carpio)* were sampled from Board Fish Market which is located at University Road, in the west of Peshawar City of Pakistan. The study area is located approximately 3 km from the laboratory of PCSIR (Pakistan Council of Scientific and Industrial Research). The temperature of market fluctuated between 14°C to 28°C during the study period. Samples *i.e.* pieces of muscles with skin on were brought in ice packed polythene bags to the PCSIR laboratory within 15 minutes of its collection. Fishes were taken and brought to the laboratory for culturing and identification of bacterial flora on their epidermis.

Preparation of sample

The fish samples were brought to the laboratory and kept in a petri dish. The sample was directly brought to the laboratory and streaking was done through the use of a sterilized stick swab (Bie and Berntsen, Copenhagen) on the prepared media under aseptic conditions. The stick swab had been soaked in peptone water and was then used to collect the bacterial sample by rubbing the stick against the fish tissue (Kumari and Ichhpujani, 2000). The stick was then streaked on the prepared media.

Then a small piece (2-3cm) of fish epidermis was removed from various regions of the body and was kept in another sterilized petri plate under the aseptic environment of a laminar air flow hood (Technical Scientific Supply Lahore, Standard: US Federal Standard 209 E) in an effort to avoid post-collection bacterial contamination.

Culturing bacteria from the sample

Nutrient agar medium was used to culture the suspect spoilage bacteria. Other selective media such as MacConkey agar medium, Pseudomonas agar medium, Minitol salt agar, EMB medium, Blood agar and Simmon citrate agar medium were also used for identification of specific bacteria isolated individually on the epidermis of the fish.

Incubation

The medium plates were then sealed with parafilm and kept in an incubator (Banstead lab, Model number 4951) at 35°C for about 24 (±2) h for the growth of bacteria (Kumari and Ichhpujani, 2000). This resulted in the initial mixed bacterial culture.

Pure/sub culture for identification of bacteria

Media used for identification of bacterial pathogens were: Mannitol salt agar medium, Pseudomonas agar medium, Blood agar medium, EMB and MacConkey agar.

Confirmation of bacterial species by different tests

Biochemical tests, including catalase, coagulase, oxidase, indole, haemolysis and citrate utilization tests were performed for bacterial species identification as recommended in Prescott (2002). In addition, morphological characteristics such as Gram staining was performed to properly confirm identification of microbial isolates.

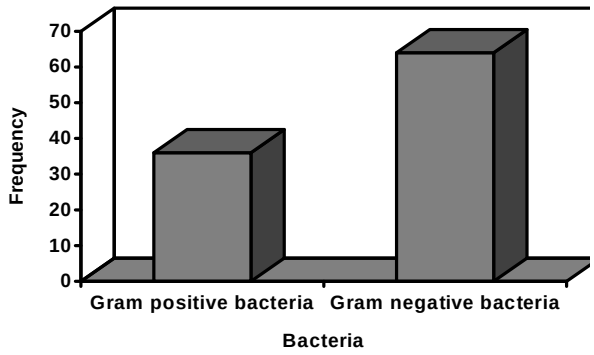

Fig. 1. Percentages of Gram positive and gram negative bacteria.
Total number of Gram positive bacterial colonies = 15 (36%)
Total number of Gram negative bacterial colonies = 27 (64%)

RESULTS

In present study 65 samples of fishes were collected from the study site (Board Fish Market) for the identification or prevalence of bacteria on the epidermis of fishes

Table I reveals the morphology (colour, form and elevation) of the pathogen and the type of media used to culture it and finally identified. Table II shows the details of the media used for the culture

Table I.- Morphology of bacterial colonies on different media.

S.No.	Media	Colony morphology			Bacterial species
		Colour	Form	Elevation	
1	Mannitole	Pale yellow, milky white	Circular and rod shaped	Convex	*Staphy. aureus/epidermidis*
2	EMB	Metallic green	Circular	Convex	*Escherichia coli*
3	MacConkey	Creamy white	Circular	Convex	*Klebsiella spp*
4	Pseudomoneas	Creamy white	Circular and fringed	Convex	*Pseudomonas spp*
5	Nutrient agar	Creamy/Pale yellow	Circular and rod	Convex	*For all above bacteria*

Table II. - Different media used for identification of different bacterial species

S.No.	Bacterial species	Media used				Fish names
		Mannitol agar	Pseudomonas agar	MacConkey agar	EMB	
1	*S. aureus*	+	--	--	--	*Wallago attu, Hypophthalmichthys molitrix, Labeo rohita, Ctenopharyngodon idella, Tor putitora* and *Cyprinus carpio.*
2	*S. epidermidis*	+	--	--	--	*Wallago attu* and *Labeo rohita.*
3	*Pseudomonas* spp	--	+	--	--	*Wallago attu, Hypophthalmichthys molitrix, Labeo rohita and Tor putitora.*
4	*Klebsiellas* sp	--	--	+	--	*Wallago attu, Hypophthalmichthys molitrix, Labeo rohita, Tor putitora* and *Cyprinus carpio.*
5	*E.coli*	--	--	+	+	*Wallago attu, Hypophthalmichthys molitrix, Labeo rohita, Ctenopharyngodon idella, Tor putitora* and *Cyprinus carpio.*

The + sign in each column under the specific medium shows that the particular type of bacterial colony has cultured on that medium. The – sign means the opposite.

and identification of all the types of bacterial colonies from the epidermis of all the different types of fishes. The + sign in each column under the specific medium shows that the particular type of bacterial colony has cultured on that medium. The – sign means the opposite. Table III shows the bacterial flora extracted from each fish species individually. Each fish species were found to carry more than one species of bacteria that may be capable of causing spoilage.

Gram's staining

Grams staining of the samples further indicated that Gram positive bacteria were *Staphylococcus aureus* and *Staphylococcus epidermidis.* Gram negative species were *E. coli*, *Pseudomonas* spp. and *Klebsiella* sp. *S. aureus* and *E. coli* were found to be with high percentages followed by *Klibsiella* sp, *Pseudomonas* sp. and *S. epidermis* (Table IV).

Table III.- List of various bacterial species from each procured specimen of fishes.

S.No.	Specimen	Bacterial species
1	*Wallago attu*	*S.epidermidis, E. coli* and *Pseudomonas* spp.
2	*Tor putitora*	*S.aureus, E. coli* and *Klebsiella* spp.
3	*Hypophthalmichthys molitrix*	*S.aureus, E. coli* and *Klebsiella* spp.
4	*Labeo rohita*	*S.aureus, E. coli* and *Pseudomonas* spp.
5	*Labeo rohita*	*S.epidermidis* and *E. coli*
6	*C. idella*	*S. aureus* and *E. coli*
7	*Tor putitora*	*S.aureus, E.coli* and *Klebsiella spp*
8	*Hypophthalmichthys molitrix*	*S.aureus, E.coli* and *Pseudomonas* spp.
9	*Labeo rohita*	*S. aureus, E. coli, Pseudomonas* and *Klebsiella*
10	*Labeo rohita*	*S. aureus, E. coli* and *Klebsiella* spp.
11	*Labeo rohita*	*S. aureus, E. coli* and *Klebsiella* spp.
12	*Hypophthalmichthys molitrix*	*S. aureus, E. coli* and *Pseudomonas* spp.
13	*Tor putitora*	*S. aureus* and *Pseudomonas* spp.
14	*Cyprinus carpio*	*S. aureus, E.coli* and *Klebsiella* spp.
15	*Wallago attu*	*S. aureus* and *Klebsiella* spp.

Table IV.- The frequencies of occurrence of various bacterial pathogens

S.No.	Bacterial isolates	Number of bacterial colonies	Percentage of occurrence
1	*S. aureus*	13	30.95
2	*S. epidermidis*	2	04.76
3	*Pseudomonas* spp.	6	14.29
4	*E. coli*	13	30.95
5	*Klebsiella* spp.	8	19.05

DISCUSSION

The analysis of present work indicated that the bacteria that may play a role in fish spoilage were: *S. aureus, S. epidermidis, Pseudomonas* spp, *E. coli* and *Klebsiella* spp. Our findings are consistent with the findings of Sinha et al., 1991 which also indicated the presence of *Salmonella, Staphylococcus, Aeromonas, Pseudomonas, E. coli, Micrococcus, Streptococcus, Proteus, Klebsiella* and molds in the marketed *Labeo rohita.* This study indicated that at Board Bazaar, Peshawar, Pakistan, the conditions for the prolong time of storage and selling of fishes are not ideal and may lead to spoilage.

It is recommended that fish should not be kept beyond six hours at ambient temperature until it is iced if its shelf life is to be maintained to meet the market's quality demands. This recommendation is in agreement with the findings of Amos (2007). It is therefore recommended that good hygienic conditions and use of clean water during fish processing should be strictly adhered to. After harvest, fresh fish should be properly stored at low temperatures to inhibit survival and growth of bacteria.

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REFERENCES

ABBAS, M. S., 2014. Isolation of bacteria from fish. *Int. J. Adv. Res*., **2**: 274-279.

AMOS, B., 2007. *Analysis of quality deterioration at critical steps/points in fish handling in Uganda and Iceland and suggestions for improvement*. Project. pp 5-23. The United Nations University, Fisheries Training Programme.

BRIGITTE, M.B., BOOGAARD, B. V. AND HEIJNEN, C., 2004. Preservation of fish and meat. Agromisa Foundation, Wageningen. 3 Edn. Print: Digigrafi, Wageningen, the Netherlands. ISBN: 90-72746-01-9

CAHILL, M., 1990. Bacterial flora of fishes. *Microbial. Ecol*., **19**:21-41.

EYO, A. A., 1997. *Post-harvest losses in the fisheries of Kainji Lake.* Nigerian-German (GTZ) Kainji Lake Fisheries Promotion Project. New Bussa, Nigeria. (ISBN: 978-037-004-8; ISSN:1119-1449). Retrieved online on 29 March 2014.

GRAM, L. AND DAGLARD, P., 2002. Fish spoilage bacteria – problems and solutions. *Curr. Opin. Biotech*., **13**:262–266

KUMARI, S. AND ICHHPUJANI, R. L., 2000. *Guidelines on standard operating procedures for microbiology*. World Health Organization Regional Office for South-East

Asia New Delhi.

LISTON, J., 1980. Microbiology in fishery science. In: *Advances in fish science and technology* Connell (ed. Connell, J.J), (Ed. 1st). Fishery News (Books) Ltd., Surrey, U.K. pp. 138–157.

MAHBOOB, S., GHAZALA., AL-GHANIM, K.A., SULTANA, S., AL-MISNED, F. AND AHMAD, Z., 2014. Fish cholinesterases as biomarkers of sublethal effects of organophosphorus and carbamates in tissues of *Labeo rohita*. *Pakistan J. Zool*., vol. **46**. 121-127, 2014.

PRESCOTT, L. M., 2002. *Microbiology* 5th edition. retreived online from (www.mhhe.com/prescott5) on 12 March, 2014. ISBN: 0-07-282905-2

SINHA, D. K., CHOUDHARY, S. B. AND NARAYAN, K.G., 1991. Microbiological characteristics of marketed rohu *(Labeo rohita). Indian J. Fish.*, **38**: 69-71.

YAGOUB, S. O., 2009. Isolation of *Enterobacteriaceae* and *Pseudomonas* spp. from raw fish sold in fish market in Khartoum state. *J. Bact. Res.*, **1**: 085-088.

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