Study on Microbial Contents and Storage Stability of Fish (Oreochromic nilotica)

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Introduction

Fish is an excellent source of nutrition due to the abundence in good quality protein and polyunsaturated fatty acids and minerals specially iodine and potassium. These easily available nutrients are also good sources for the growth of bacteria and for the bacterial spoilage and deterioration of storage stability and nutritional quality of fish. The fish carry a wide range of microllora most of them are harmless, but organisms of human health concerns may be present due to faulty transport, storage or preparations. Therefore, the contaminations responsible for potential health hazards in connection with foodborne diseases are of greatest concern to those, entrusted with the responsibilities of supplying and producing of quality fish and fishery products.

According to Kautter and Lilly (1) there are representatives of Alcaligenes, Bacillus, Clostridium, E. Coli and Pseudomonas in the intestinal tract of many fishes. Fish or fishery products those are produced or processed in a contaminated environment may be contaminated with lots of bactaria and be responsible for outbreaks of gastro-intestinalosis (2). Fish may be contaminated with nematodes, some invade the intestinal tract and the other invade the fless (3). Salmonellae may cause a serious problem in shelfish, when the shelfish is harvested from polluted waters. Vibrio Para haemolyticus occur in sea water and has been associated with sea food of Bay areas of Hawaii, Washington, Texas and Louisiana (4). Contamination of fish and fish products with Clostridium was studied by many workers. The incidence of bacterial load was very low or nearly absent on fresh and frozen fish but processed items were more frequently contaminated with the organisms (5). The presence, growth and survival of Varsinia, Enterscolitia in oysters, shrimps and crabs was studied by many researchers (6).

Tilapia is an exotic fish in Bangladesh. But due to its higher growth rate, the cultivation of this fish is very much in practice in Bangladesh. As such, this is abundantly available in the markets. Also, Tilapia fish maintains a good market demands in Bangladesh due to its cheaper price. However, while considering the consumer's risks of health hazards, the study of bacteriological susceptibility of this perishable fish possess immense nutritional importance in Bangladesh.

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Considerable studies have been made on bacterial flora of markets fish in many other countries of the world in different fishes, but this type of studies are still insufficient in Bangladesh. Considering the nutritional importance of the Tilapia fish in Bangladesh, the present study was performed on this fish by assessing the microbial susceptibility and microbial growth and spoilage of the fish.

Material & Methods

Fresh Tilapia *(Oreochrome nilotica)* were bought from New Market, Dhaka and brought to the laboratory under cool and hygienic condition. The average weight of the fish was 225 gm. The fish were stored in small sterile dishes at room temperature (27 ± 1^0C) and at 4^0C. A small dish full of water were placed by the side of the fish to prevent the drying of their skin during the storage. Skin and gill part ($8cm^2$ in area) of the fresh sample were removed aseptically. Each sample was cut into small pieces, homogenized and transferred into test tube diluted with 0.9% NaCl solution of 9 times the weight of the samples. Serial dilution was made with the diluent (0.9%NaCl) and 0.1 ml portion of the diluted sample was placed on a plate of nutrient agar medium (DIFCO) polypeptons 10.0 gm, DIFCO beef extract 3.0 gm, DIFCO yeast extract 3.0 gm, NaCl 1.6 gm, KCl 2.0 gm, $MgSO_4$ $2H_20$ 0.6 gm, $CaCl_2 2H_20$ 0.3 gm, NaCl 1.6 KCl 2.0 gm, glucose 5.0 gm and agar 15.0 gm, dissolved in 1000 ml distilled water and pH was adjusted to 7.2) and spread on the plate using a bent glass rod. The lots of plates each composed of duplicate plates from serial dilutions were set up. The growth of the microflora were studied at an incubated temperature of 35^0C for 24, 48 and 72 hr to provide maximum time for bacterial growth. At 7 and 14 days of storage duplicate fish samples were collected for examinations. Analysis of the floras were carried out by the examination of morphological and physiological characteristics according to standard scheme (7,8,9) and the final examination was based on the schemes of Bergey's manual.

Results

The viable counts of fresh and stored fish in different temperature of storage (room temperature and 4^0C) from two parts of the body are shown in Table 1. Number of bacteria isolated from the skin of the fresh fish was lower than the bacteria isolated from the skin of fresh fish. But the counts were increased when fish were left for 3 hr at room temperature ($27+1^0$C). At the beginning of storage, the number of bacterial growth at 4^0C were remarkably low compared with the growth at room temperature. But after the storage at 4^0C for 7 days the bacterial counts were almost double when the fish left at 27 ± 1^0C for 3 hr. Again the viable counts in gills at 7 days were relatively high and the fish showed clear sign of spoilage. At 14 days of storage, putrifying odour and softening of the muscle were noticed.

The morphological and biochemical studies such as colonial morphology,

Table 1: Total viable counts of fish from gill and skin samples

Storage period hr/day	Storage temperature ^0C	No. of sample	Viable count/ml	
			skin	gill
O hr	Room temperature	1	6.5×10^5	5.2×10^4
		2	7.0×10^5	6.9×10^4
3 hr	Room temperature	3	7.0×10^6	8.2×10^6
		4	4.9×10^6	8.9×10^6
7 days	4^0C	5	7.9×10^5	9.5×10^6
		6	8.2×10^5	9.8×10^6
14 days	14^0C	7	3.5×10^4	8.2×10^8
		8	8.7×10^4	9.3×10^8

Note: In each case duplicate samples were used.

Table 2 : The characteristic feature of isolated bacteria

Strain no	Shape	Gram	Motality	Spore	Oxidase	Catalase	Nitrate reduction	H_2S production	Indole production	Hugh & Leifson	Methyl red	Genus name
Skin	R	+	+	-	-	-	-	-	-	-		Bacillus
Skin	C	+	-	+	+	+	+	-	-	O	-	Micrococcus
Skin	R	-	+	-	+	+	-	-	-	O	-	Pseudomonas
gill	R	-	+	-	+	+	+	+	-	O	-	Pseudononas
gill-	R	-	+	-	+	+	+	-	-	F	-	Aeromonas
gill-	R	-	+	-	+	+	+	-	-	O	-	Achromobacter
gill-	R	+	-	-	+	+	-	-	-	No	+	Unidentified

R : Rod; C: coccus
0 : Oxidation; F : Fermentation
NC : Not clear

Table 3 : Incidence of bacterial isolates from fresh and storaged fish

Storage period hr/day	Storage tempe-rature	Part of fish	Total No.of strain	Bacillus	Micro-coccus	Pseudo-monas	Aero-monas	Achromo-bacter	Uniden tified
0 hr	Room tempe rature	Skin	12	6	6	0	0	0	0
		gill	18	0	0	3	3	2	2
	(27±1 ^{0}C)	Skin	10	6	4	0	0	0	0
		gill	7	0	0	3	2	2	0
3 hr	Room temperature	skin	16	7	9	0	0	0	0
		gill	9	0	0	5	2	1	1
	(17±1 ^{0}C)	Skin	10	5	5	0	0	0	0
		gill	8	0	0	2	3	3	0
7 days 4 ^{0}C		Skin	8	5	3	0	0	0	0
		gill	10	0	0	5	2	2	1
14 days 4 ^{0}C		Skin	5	3	2	0	0	0	0
		gill	15	0	0	7	5	2	1
		Skin	4	2	2	0	0	0	0
		gill	14	0	0	5	3	4	2

shape, motility, ability to produce oxidase and catalase, ability to reduce nitrate and to ferment lactose by microflora from fresh and stored fish (Table 2) indicated the clear difference between skin and gill flora. The generic composition of the strains is shown in Table 3. The results revealed distinct differences in generic composition between skin floras, of which main types were Bacillus, Micrococcus and few Pseudmonas Dominated flora of gill where Aeromonas pseudo-monas and Aehromobacter group. The differences were observed through out the storage period. On the other hand no decisive diffeareances in the generic composition was seen between the isolate derived at 4^{0}C and 27±1^{0}C storages. A striking feature is that an appreciable number of the isolates of the samples stored at 4^{0}C fail to grow at 27±1^{0}C even after 72 hr of incubation.

Discussion

Previous research (10,11,12) showed that Bacillus and Micrococcus were predominant along with other gram negative bacteria like Pseudomonas and Aeromonas when the fish (grey mullet) live in fresh water, Vibrio was the main bacterial flora in the intestine of gray mullet which was directly related to the quality of water they live in. Most works support the view that gram negative rods predominate on fresh as well as on stored fish. It is also known that there are differences in the composition of bacterial flora due to

regional variation (13,14). In the present study it seems that Bacillus, Micrococcus, Pseudomonas, Aeromonas and Achromobacter occur in remarkable amounts in Tilapia from fresh water environment. In this work the predominancy of Bacillus and Micrococcus occur in the skin of the fish may be partly due to the traditional way of handling the fish in Bangladesh where the evisceration of fish immediately after the catch is not a common practice. The bacterial flora on fish is a function of the environment in which they are caught and subsequently stored (15,16). A striking feature was observed that the bacterial count in the gill sample were smaller in fresh fish, increased in fish stored at 4^0C. It is assumed that the deterioration is may be due to rapid bacterial growth in gill than in skin, because of the dryness of the skin for long time preservation at 4^0C. However, further study is needed concerning the relationship and behaviour of the floras present in the skin, gills and intestine for both fresh and stored fish.

Summary

The incidences of bacterial flora of Oreochromic nilotica both fresh and stored fish differed clearly according to the parts of the fish. The flora from the skin of fresh fish were composed mainly of Bacillus and Micrococcus while the main flora from the gill sample were Pseudononas, Aeromonas and Achromobacter. The bacterial counts from the skin was larger than the gill counts in fresh fish but these counts were increased in number in gills in fish stored at 4^0C.

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