MICROFLORA OF ICE IN FISH CARRYING CRATES FROM CERTAIN FISH MARKETS OF BHOPAL

SUMER HASSAN, T. A. QU'RESHI, BILAL AHMAD* AND SUSAN MANOHAR
Department of Zoology and Applied Aquaculture
Barkatullah University, Bhopal - 462 026, (M. P.)
E-mail: bilalaqua@gmail.com

INTRODUCTION
Proper preservation begins the moment the fish is harvested from the water. Fish should be chilled quickly to the temperature of melting ice soon after capturing and maintained at that temperature, but micro-organisms found in fish from cold water can easily adapt to the low temperature of ice (Shewan, 1977). Ice storage has been the most widely used method for the preservation of fresh fish on sea and on land. After being caught, fishes are placed in fish boxes or mixed with crushed ice and kept in fish carrying crates during trips between the fishing grounds and fish piers or markets. In cold regions, thick layers of slime containing a high bacterial load of diverse organisms often accumulate on the bottom of fish holds. In warm weather or in tropical areas, fish holds are usually filled with an ice water mixture that also accumulates fish secretions. The bacterial flora in ice melt from fish containers is important for economic reasons and for fish quality control. (Chen and Chai, 1982) Observed very high microbial load in ice-melt drainage collected from fish holds of fishing vessels, ranging from $2.1 \times 10^7$ to $2.2 \times 10^9$/mL for bacteria and $6.3 \times 10^3$ to $7.2 \times 10^4$/mL for yeasts and molds.

Human infections that may be caused by fish bacteria include food poisoning and gastroenteritis. Consumption of fish is responsible for 5-8% of food-borne disease outbreaks. The risk to public health arises if toxigenic strains multiply to high number during improper handling and storage leading to auto-enzymatic action and bacterial degradation. The microbial population of the ice obviously has a bearing on public health. The investigation of the microorganisms in the drainage of fish holds can provide clues for the improvement of sanitation in fishing boats, the optimization of fish processing, and the extension of fish shelf life (Chal and Levin, 1975). The objective of the present study was to evaluate the hygienic handling of fish and various materials used for fish preservation and transportation including, ice, crates, water etc.

MATERIALS AND METHODS
Ice samples from fish carrying crates from four fish markets of Bhopal viz. Itwara, Bittan, Piplani and Govindpura were selected for the study. The samples of ice water melt were collected. For each sample two ice-covered boxes of fish (each containing 20 to 25 kg of fish) were removed from fish holds and placed tilted against the deck so that the ice-water melt could drain into sterile sample bottles. The drainage samples were kept in ice and transported to the laboratory. Immediately on arrival at the laboratory, pH, aerobic counts and sanitary indicator organisms, were determined. Organisms of health concern, sanitary indicator and pathologically significant organisms, including fecal coliforms, Salmonella cultures and Staphylococcus aureus, were determined.

Samples in peptone water or broths at $10^0$, $10^1$, $10^2$, and $10^3$ dilutions were analyzed for these indicator organisms. Fecal coliforms were confirmed by the

ABSTRACT
The present study was carries out to investigate microflora of ice in fish carrying crates from fish markets of Bhopal. Microbial load from fish carrying crates was very high, ranging from $2.1 \times 10^7$ to $2.2 \times 10^9$/mL for bacteria and $6.3 \times 10^3$ to $7.2 \times 10^4$/mL for yeasts and molds. Analysis of 100 colonies each randomly isolated from fish carrying crates showed that the occurrence of bacterial genera as a percentage of the total was Aeromonas, 61 to 62%, Pseudomonas, 19 to 21%, Salmonella sp., 5 to 10%, Shigella, 1 to 4%, Vibrio 0 to 2%. The organisms demonstrated versatile hydrolytic activities to a wide range of biological substrates including casein, gelatin and starch.

KEY WORDS
Fish crates
Microbial load

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*Corresponding author
positive reactions in EC broth at 44.5°C and on eosin methylene blue agar after the test of positive reactions in lauryl sulfate tryptose broth and brilliant green lactose bile broth. For enrichment of Salmonella cultures, samples were inoculated into lactose broth. The organisms were grown on bismuth sulfite agar and Deoxycholate agar. Colonies from each plate were screened biochemically with routine procedures and identified. Staphylococcus aureus was confirmed with Baird-Parker agar and examined for the coagulase reaction. Bacterial counts were determined by spreading the appropriate sample dilutions on peptone beef extract agar containing 1% proteose peptone, 0.2% beef extract, 0.5% NaCl, and 1.5% agar.

All plates were incubated at 20°C and observed after 3 days of incubation. It was observed that proteose peptone beef extract agar containing 0.5% NaCl yielded 20 to 30% higher counts than did nutrient agar, each Surface-spread plates gave 40 to 50% higher counts than pour plates in each medium. Plates which contain 30 to 300 colonies per plate were selected for the isolation and identification of bacteria. A total number of 100 colonies from each drainage sample were picked after single colony purification. Identification and characterization of pure culture was done by applying Morphological and biological tests (Bullock et al., 1971; Austin and Austin, 1999).

Biochemical analysis comprised of oxidation / fermentation test, Oxidase test, Catalase test, Indole production test, Hydrogen sulphide production test, Methyl red and Voges Proskauer (MR-VP) test, Decarboxylase test, Urease production test, Coagulase test and Starch hydrolysis test.

**RESULTS AND DISCUSSION**

Ice samples from fish carrying crates containing different species of fish were studied. The pH of these ice samples was about 7.0. Bacterial count from two ice samples collected from Itwara and Bittan fish markets was very high (Table 1). Samples of summer give more bacterial counts than winter season. A. hydrophila count ranged from 1.5x10³ to 5.5x10³ in different locations. The highest count 10.8x10³ CFU/g was found for Staphylococcus aureus in Itwara while its lowest count was in Piplani(Table 1).

The quality of fish continuously changes during different stages from harvest to marketing. After death, the fish has to cross rigor mortis and the body of fish acts as a suitable medium for the growth and multiplication of bacteria. Even the crates, ice, water used for preservation and transportation have high

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Itwara</th>
<th>Bittan</th>
<th>Piplani</th>
<th>Govindpura</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas hydrophila</td>
<td>4.5x10³</td>
<td>5.5x10³</td>
<td>2.5x10³</td>
<td>1.5x10³</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>2.3x10³</td>
<td>1.5x10³</td>
<td>0.5x10³</td>
<td>1.4x10³</td>
</tr>
<tr>
<td>Streptococcus iniae</td>
<td>4.1x10³</td>
<td>3.5x10³</td>
<td>1.0x10³</td>
<td>1.0x10³</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10.8x10³</td>
<td>10.0x10³</td>
<td>1.0x10³</td>
<td>3.5x10³</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>10.0x10³</td>
<td>9.1x10³</td>
<td>Nil</td>
<td>1.0x10³</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>10.0x10³</td>
<td>2.0x10³</td>
<td>Nil</td>
<td>1.5x10³</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>10.1x10³</td>
<td>8.1x10³</td>
<td>3.5x10³</td>
<td>4.5x10³</td>
</tr>
<tr>
<td>Escherichia coli MPN/g</td>
<td>360</td>
<td>73</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Fecal coliforms MPN/g</td>
<td>220</td>
<td>60</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
bacterial load due to the unhygienic condition prevailing in the fish preservation and processing duration. Jayasinghe (2006) investigated the quality of tap water, sea water, ice, fish surface and landing centre and found them contaminated by fecal bacteria like E. coli and Salmonella sp. Spencer (1959) has emphasized on the sanitation of fish boxes and has made investigations on their quantitative and qualitative bacteriological studies.

Hess (1934), Kiser (1944), Ingram (1958) and Redfort (1932) who have reported that bacterial multiplication occurred in frozen fish at -11°C, although the speed of development might be strikingly altered. Some bacteria suffer death while freezing however, freezing and storage under frozen conditions have virtually no action on bacterial spores and yeasts. Ingram (1951), Lochhead and Jones (1938), Lund and Halvorson (1951), Gunderson and Rose (1948) and Hartsell (1951) investigators have stated that freezing did not appear to have practically any effect on the cultural or other characteristics of most psychrophilic bacteria after thawing. They have also reported that frozen fish spoil quickly than slow frozen fish. Chistyakov and Noskova (1955) have observed that some micro-organisms have adapted to freezing temperature. Castell (1956) and Reay et al. (1943) have mentioned that ice has a bacterial count of only 10^7 to 10^8/mm at the time of delivery from ice plant which may rise to 10^9 to 10^10/mm with the lapse of time. Tejada and Huidobr (2002) reported about the influence of slaughter method and post-mortem treatment on the quality of iced storage of cultured gilthead sea bream collected under market conditions. The results of the present investigation and the conformity lend by various workers stress towards the hygienic handling of fish and various materials used for fish preservation and transportation including, ice, crates, water etc. if effective preservation and sanitation measures are opted various outbreak of food-borne diseases can be checked.

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REFERENCES


