

Isolation and Characterization of Microorganisms from Some Spoiled Yellow Goat Fish (*Sulphureus cuvier* L.)

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Abstract: The street foods play an important socio-economic role in meeting food and nutritional requirements of city consumers at affordable prices. This present study was designed to evaluate the detailed microbial status including food borne pathogen and spoilage bacteria. In the present investigation, yellow goat fishes were taken with regard to their microbial population in the isolates. The total heterotrophic bacterial load ranged from 155×10^4 to 140×10^4 CFU/ml of sample and it was found to be the maximum of 155×10^4 CFU/ml in Yellow goat fish (*Sulphureus cuvier*). The bacterial isolates were identified by Microscopic examination, Plating on Culture medium and Biochemical tests. The identified bacterial isolates were *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas fluorescens*. The bacterial isolates were subjected to quantitative estimation of pH and temperature. The optical density at 580 nm of five fish spoilage bacteria was increased in the temperature levels from 0°C to 35°C and more weight was recorded in 30°C. Thus, the present preliminary study showed the variation in the microflora of fish species and the microbes from marine environment.

Key words: Yellow Goat Fish • Bacteria • Spoilage • Temperature and pH

INTRODUCTION

Fish is an extremely perishable food item [1, 2]. Soon after death, fish begins to spoil. In the healthy live fish, all the complex biochemical reactions are balanced and the fish flesh is sterile [3]. After death however, irreversible change that results in fish spoilage begins to occur. The resultant effect is the decomposition of the fish [4]. Various factors are responsible for the fish spoilage. The quality of capture is important at determining the rate of spoilage. The caught fish quality depends on the handling and preservation, the fish received from the hands of the fishers after capture. The handling and the preservation practice after capture affects the degree of spoilage of the fish [5, 6]. The important constituents of fish are water (70-85%), protein (15-20%), lipid (1-10 %), ash (1-1.5%) and carbohydrate (0.3-1.0 %).

Spoilage of food products can be due to chemical, enzymatic or microbial activities. Fish spoilage is brought about mainly by, the enzymes present in the live fish.

The enzymes begin to break down fish tissues. Prior to death, the enzymes were involved in the digestion of ingested food and all enzymatic reactions are controlled. In the dead fish, the control system fails and the enzymes begin to act on the alimentary system and fish flesh, thereby resulting in soft destructive changes. This process is referred to as autolytic spoilage [7, 8]. Bacteria are present in the gut, gills and skin surfaces of live fish. The live fish defense mechanism is able to combat the action of these bacteria. However, some after death, this defense mechanism also fails. Consequently, the bacteria invade the gut, gills and skin and cause the decomposition from within and the exposed surfaces of the fish [9, 10].

During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavor and texture of the fish meat [11, 12]. This represents a major concern of the freshness of saleable products and the breakdown of proteins and lipids [13]. Higher energy demanding freeze-storage preservation can

be altered by synthetic or natural preservatives for control of lipid oxidation and microbial growth in fish during storage. Combination of these preservatives and refrigeration diminishes the process of spoilage [14, 15].

MATERIALS AND METHODS

Collection of Fish Sample: The spoiled fish (Yellow goat fish; *Sulphureus cuvier*) was collected from three different locations viz., Chidambaram fish market, Killai and Bhuvanagiri. The collected spoiled fish samples were preserved in refrigerator at 4°C.

Isolation of Bacteria from the Spoiled Fish (Yellow Goat Fish-Sulphureus Cuvier) Sample: Pour plate technique was used for the isolation of bacteria in spoiled fish samples. In this method, a piece of intestine and mid gut part of the fish 6.0 cm in area operated in with or without underlying muscle was excised from the fish and homogenized with 10 ml of distilled water were used for the dilutions and then, it was serially diluted by following standard procedure up to concentration of 10^{-6} . Then, 1 ml of serially diluted samples from each concentration of samples were transferred to sterile Petri plates and evenly distributed throughout the plates and sterile solidified Nutrient agar was poured and it was allowed to solidify. The Nutrient agar plates were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were isolated from the plates and microbial population was counted by using Quebec colony counter (CFU/ml).

Effect of Ph on the Growth of Bacteria Isolated from Spoiled Fish (Yellow Goat Fish-Sulphureus Cuvier): The sterilized Nutrient broth was prepared and distributed at 100 ml quantities in 250 ml Erlenmeyer flask and the pH was adjusted to various levels from 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 in each flask by adding 0.1N HCl or 0.1 KOH and pH in each broth was tested with the help of glass electrode pH meter. After sterilization, 1 ml of the standard inoculums of the bacterial cultures isolated from spoiled fish viz., *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas fluorescens* were added respectively and incubated for 48 hours at 30°C. After the incubation period, the residue in an oven 50°C until a constant weight was obtained.

Effect of Temperature on the Growth of Bacteria Isolated from Spoiled Fish (Yellow Goat Fish-Sulphureus Cuvier): The sterilized Nutrient broth was prepared and distributed at 100 ml quantities in 250 ml Erlenmeyer

flasks. After sterilization, 1 ml of the standard inoculums of bacterial culture viz., *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas fluorescens* and were added respectively and incubated for 48 hours at different temperature viz., 0°C, 5°C, 10°C, 15°C, 20°C, 30°C, 40°C, 45°C, 50°C and 60°C in an incubator. After the incubation period, the residue in an oven 50°C until constant weight was obtained.

RESULTS

Estimation of Total Bacterial Load in Spoiled Fish Samples: The population of pathogenic bacteria in spoiled Yellow goat fish (*Sulphureus cuvier*) samples which was collected from three different locations was determined and the results were given in Table 1. Maximum bacterial load was recorded in the spoiled fish collected from Chidambaram (155×10^4 CFU/ml) followed by Killai (146×10^4 CFU/ml) and Bhuvanagiri (140×10^4 CFU/ml).

Identification and Characterization of Bacteria Isolated from Spoiled Fish Samples: Five bacterial isolates were isolated and identified from spoiled Yellow goat fish (*Sulphureus cuvier*) samples. The bacterial isolates were examined for the morphological characters and the results were furnished in Table 2. Based on the morphological characters like cell shape and size, Gram staining, motility test, plating on selective medium and biochemical tests. The Spoilage Bacteria-I was identified as *Escherichia coli*, Spoilage Bacteria-II was identified as *Vibrio cholerae*, Spoilage Bacteria-III was identified as *Staphylococcus aureus*, Spoilage Bacteria-IV was identified as *Salmonella typhi* and the Spoilage Bacteria-V was identified as *Pseudomonas fluorescens*.

Effect of Ph on the Growth of Bacteria Isolated from Spoiled Fish Samples: The growth of five different fish spoilage bacteria viz., *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* was studied at different pH levels viz., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 for 48 hours and the results were given in Table 2. The optical density at 580 nm of five fish spoilage bacteria at 48 hours increased with increase in pH levels from 4.0 to 7.5 and later slightly decreased at pH 7.5 and 8.5. More optical density was recorded in *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* at pH 7.5.

Table 1: Estimation of total bacterial load in spoiled Yellow goat fish (*Sulphureus cuvier*) samples

S. No.	Locations	Total bacterial load ($\times 10^4$ CFU/ml)
1	Chidambaram	155
2	Killai	146
3	Bhuvanagiri	140

Values are mean of three replicates

Table 2: Effect of pH on the growth of bacterial isolates from spoiled fish samples at 580 nm

S. No	pH	<i>Vibrio cholerae</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i>	<i>Pseudomonas fluorescens</i>
1.	4.0	0.024	0.012	0.010	0.006	0.008
2.	4.5	0.027	0.019	0.015	0.010	0.013
3.	5.0	0.030	0.021	0.016	0.012	0.015
4.	5.5	0.032	0.026	0.018	0.015	0.017
5.	6.0	0.035	0.029	0.021	0.023	0.022
6.	7.0	0.037	0.031	0.025	0.024	0.025
7.	7.5	0.042	0.034	0.029	0.027	0.028
8.	8.0	0.030	0.030	0.023	0.024	0.025
9.	8.5	0.027	0.027	0.019	0.020	0.021
10.	9.0	0.025	0.014	0.015	0.026	0.020

Table 3: Effect on the temperature on the growth of bacterial isolates from spoiled fish samples at 580 nm

S. No	Temperature (°C)	<i>Vibrio cholerae</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i>	<i>Pseudomonas fluorescens</i>
1.	0	-	-	-	-	-
2.	5	0.207	0.203	0.204	0.202	0.200
3.	10	0.219	0.214	0.222	0.215	0.219
4.	15	0.238	0.220	0.235	0.223	0.028
5.	20	0.247	0.238	0.252	0.242	0.246
6.	30	0.272	0.253	0.295	0.270	0.282
7.	40	0.260	0.249	0.272	0.260	0.265
8.	45	0.251	0.240	0.280	0.242	0.260
9.	50	0.245	0.238	0.260	0.230	0.248
10.	60	0.232	0.227	0.251	0.215	0.235

Effect of Temperature on the Growth of Bacteria Isolated from Spoiled Fish Samples: The growth of five different fish spoilage bacteria viz., *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* was investigated at different temperatures viz., 0°C, 5°C, 10°C, 15°C, 20°C, 30°C, 40°C, 45°C, 50°C, 60°C for 48 hours and the results are furnished in Table 3. The optical density at 580 nm of five fish spoilage bacteria was increased in the temperature levels from 0°C to 35°C and more weight was recorded in 30°C.

DISCUSSION

The population of pathogenic bacteria in spoiled Yellow goat fish (*Sulphureus cuvier*) samples collected from three different locations was determined. Maximum bacterial load was recorded in the spoiled fish collected from Chidambaram (155×10^4 CFU/ml) followed by Killai (146×10^4 CFU/ml) and Bhuvanagiri (140×10^4 CFU/ml). The Total viable counts in this study are within the range reported by Adams and Moss [1] and Liston *et al.* [12].

The bacterial loads on the skin of fish from catch can range from hundreds up to millions/cm² (10^{-2} to 10^7 numbers/cm²) of skin. The slow increase in counts may be caused by effect of ice whereby it retards the growth of microorganisms to less than one-tenth of the rate at optimal growth rates [6, 11]. In the present study, five bacterial isolates were isolated and identified from spoiled Yellow goat fish. The Spoilage Bacteria-I was identified as *Escherichia coli*, Spoilage Bacteria-II was identified as *Vibrio cholerae*, Spoilage Bacteria-III was identified as *Staphylococcus aureus*, Spoilage Bacteria-IV was identified as *Salmonella typhi* and the Spoilage Bacteria-V was identified as *Pseudomonas fluorescens*.

The psychrotrophic Gram negative rod shaped bacteria belonging to genera *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrio*, *Aeromonas* and *Micrococcus* dominate microflora in temperate waters. While, the Gram positive bacteria such as *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus* and *Corynebacterium* are found to dominate in higher temperature waters in varying proportions. Fresh water

fish's micro flora is dominated by the *Aeromonas* spp. The microflora consisting of *Pseudomonas*, *Acinetobacter*, *Moraxella* and *Vibrio* was reported in newly caught fish in tropical Indian marine waters studies [10] Findings from studies suggested that fresh water fishes have micro flora loads similar to the temperate water fishes with slightly higher in Gram positive and enteric bacteria [8].

The growth of five different fish spoilage bacteria was studied at different pH levels viz., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 for 48 hours. The optical density at 580 nm of five fish spoilage bacteria at 48 hours increased with increase in pH levels from 4.0 to 7.5 and later slightly decreased at pH 7.5 and 8.5. More optical density was recorded at pH 7.5. The growth of five different fish spoilage bacteria was investigated at different temperatures viz., 0°C, 5°C, 10°C, 15°C, 20°C, 30°C, 40°C, 45°C, 50°C, 60°C for 48 hours. The optical density at 580 nm of five fish spoilage bacteria was increased in the temperature levels from 0°C to 35°C and more weight was recorded in 30°C. The results of this present study were matched with the findings of Jageethadevi *et al.* [11].

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