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Isolation and identification of pathogenic bacteria from the spoiled yellow goat fish (*Sulphureus cuvier*)

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Abstract
The spoiled fish (Yellow goatfish – *Sulphureus cuvier*) was collected from three different locations *viz*., Chidambaram fish market, Killai, and Bhuvanagiri. The collected spoiled fish samples were preserved in the refrigerator at 4 ºC. 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 cholera*, 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 bacterial flora of fish incensed for human consumption depends on the environmental conditions of its natural habitat. The genera present in the gut generally seem to be those from the environment or diet.

Keywords: yellow goat fish (*Sulphureus cuvier*), *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas fluorescens*

Introduction
Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people as well as providing foreign exchange earnings to many countries (Al-Jufaili and Opara, 2006) [3]. Fish is also a very good source of vitamins and minerals (Edem, 2009) [4]. Most notice nutrient-content containing bio**c**hemical composition classified fish as highly perishable food. Various factors are responsible for fish spoilage. The quality of a capture is important at determining the rate of spoilage. Notably are the fish health status, the presence of parasites, bruises and wounds on, the skin, and the mode by which the fish was captured (Okonta and Ekelemu, 2005) [9]. The caught fish quality depends on the handling and preservation, of the fish received from the hands of the fishers after capture. The handling and the preservation practice after capture affect the degree of spoilage of the fish (Akinneye *et al*., 2007) [2]. The present study was conducted to isolate and identify the bacterial pathogens in the spoiled Yellow Goat Fish (*Sulphureus cuvier*).

Materials and Methods
1. Collection of Fish samples
The spoiled 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 the refrigerator at 4 ºC.

2. Isolation of pathogenic bacteria from the spoiled Yellow goat fish – *Sulphureus cuvier*
Pour plate technique was used for the isolation of bacteria in spoiled fish sample. 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 petriplates 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 the Quebec colony counter (CFU/ml). Well-grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar slants and stored at 4 °C.

3. Identification and characterization of bacterial isolates
Bacterial isolates were grown on nutrient agar plates and their morphological colony was recorded. The selected bacterial isolates were identified by Microscopic examination (Gram staining, Endospore staining and Motility test), Platting on Selective medium and Biochemical tests (Gram and Dalgaard, 2002) [5].

4. Effect of pH on the growth of bacteria isolated from Yellow goat fish
The sterilized Nutrient broth was prepared and distributed at 100 ml quantities in a 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 Optical density was measured at 580 nm in UV vis Spectrophotometer.

5. Effect of Temperature on the growth of bacteria isolated from spoiled Yellow goat fish

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 Optical density was measured at 580 nm in UV vis Spectrophotometer.

Result and Discussion

1. Estimation of Total bacterial load in the spoiled Yellow goat fish sample

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) followed by Killai (146×10^4) and Bhuvanagiri (140×10^4). The Total viable counts in this study are within the range reported by Adams and Moss (2008) [1]. They reported the bacterial loads on the skin of fish from the catch can range from hundreds up to millions per square centimeters ($10^2 - 10^7$ numbers/cm^2) of skin. The slow increase in counts may be caused by the effect of ice whereby it retards the growth of microorganisms to less than one-tenth of the rate at optimal growth rates.

2. Isolation of bacterial isolates in spoiled Yellow goat fish

Five different bacterial isolates were isolated from the spoiled Yellow goatfish samples which were collected from three different locations *viz*., Chidambaram, Killai, and Bhuvanagiri. In Chidambaram, Spoilage Bacteria - I and Spoilage Bacteria - II were observed. In Killai, Spoilage Bacteria – III and Spoilage Bacteria - IV were recorded. In Bhuvanagiri, Spoilage Bacteria - V was isolated.

3. Identification of bacteria isolated from spoiled Yellow goat fish

Five bacterial isolates were isolated and identified from spoiled Yellow goat fish (*Sulphureus cuvier*) samples. The bacterial isolates were examined for their morphological characteristics and the results were furnished in Table – 1. Based on the morphological characteristics 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*. The psychrotrophic Gram-negative rod-shaped bacteria belonging to genera *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shwewanella*, *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 (Hozbor, 2006) [7]. Freshwater fish's microflora 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. Findings from studies suggested that freshwater fishes have microflora loads similar to the temperate water fishes with slightly higher Gram-positive and enteric bacteria (Gram *et al*., 1990) [8].

Table 1: Morphology and Biochemical Characterization of bacterial isolates from fruit juice

Tentatively identified as Bacterial isolates						
S. No	**Test**	***E. coli***	***V. cholera***	***S. aureus***	***S. typhi***	***P. fluorescence***
1	Gram staining	Gram-negative straight rods	Gram-negative comma shaped rods	Gram-positive cocci, arranged in clusters.	Gram-negative rods	Gram-negative slender rods
2	Motility	Motile	Motile	Non-motile	Motile	Actively motile
3	Catalase	Positive	Positive	Negative	Positive	Positive
4	Oxidase	Negative	Positive	Negative	Negative	Positive
5	Nutrient agar	Circular, smooth and colourless colonies	Circular, moist, smooth, translucent and bluish tinge colonies	Colonies are smooth and golden yellow	Circular, moist, smooth, and translucent colonies	Green-colored diffusible pigment-producing colonies
6	MacConkey agar	Smooth, glossy, and pink-colored lactose fermenting colonies	Smooth, glossy, and late lactose fermenting colonies	Lactose fermenting colonies.	Non-lactose fermenting colonies	Non-lactose fermenting colonies
7	Glucose fermentation	Acid gas produced	Acid gas produced	Acid produced	Acid and gas produced	Not fermented
8	Mannitol fermentation	Acid gas produced	Acid gas produced	Acid produced	Acid and gas produced	Not fermented
9	Sucrose fermentation	Acid gas produced	Acid gas produced	Acid produced	Acid and gas produced	Not fermented
10	Indole	Positive	Positive	Negative	Negative	Negative
11	Methyl Red Test	Positive	Negative	Negative	Positive	Negative
12	Voges Proskauer Test	Negative	Positive	Positive	Negative	Negative
13	Citrate utilization	Negative	Negative	Positive	Positive	Positive
14	TSI	Acid butt, alkaline slant, No H_2S, and gas produced	Acid butt, alkaline slant, No H_2S, and	No reaction	Acid butt, alkaline slant,	Alkaline butt, alkaline slant. No

			gas produced		H_2S, and gas produced	H_2s and No gas production
15	Urease	Negative	Negative	Negative	Negative	Positive

4. 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 an 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. The results of this present study were matched with the findings of Jageethadevi *et al*. (2012) [8].

Table 2: Effect of pH on the growth of bacterial isolates from spoiled Yellow goat fish

pH	*V. cholera*	*E. coli*	*S. aureus*	*S. typhi*	*P. fluorescens*
4.0	0.024	0.012	0.010	0.006	0.008
4.5	0.027	0.019	0.015	0.010	0.013
5.0	0.030	0.021	0.016	0.012	0.015
5.5	0.032	0.026	0.018	0.015	0.017
6.0	0.035	0.029	0.021	0.023	0.022
7.0	0.037	0.031	0.025	0.024	0.025
7.5	0.042	0.034	0.029	0.027	0.028
8.0	0.030	0.030	0.023	0.024	0.025
8.5	0.027	0.027	0.019	0.020	0.021
9.0	0.025	0.014	0.015	0.026	0.020

5. 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 at 30°C. The results of this present study were matched with the findings of Jageethadevi *et al*. (2012) [8].

Table 3: Effect of the temperature on the growth of bacterial isolates from spoiled Yellow goat fish

Temperature (°C)	*V. cholera*	*E. coli*	*S. aureus*	*S. typhi*	*P. fluorescens*
0	-	-	-	-	-
5	0.207	0.203	0.204	0.202	0.200
10	0.219	0.214	0.222	0.215	0.219
15	0.238	0.220	0.235	0.223	0.028
20	0.247	0.238	0.252	0.242	0.246
30	0.272	0.253	0.295	0.270	0.282
40	0.260	0.249	0.272	0.260	0.265
45	0.251	0.240	0.280	0.242	0.260
50	0.245	0.238	0.260	0.230	0.248
60	0.232	27	0.251	0.215	0.235

Conclusion

The microorganisms associated with fish may be beneficial or harmful nature and also they can present on the body surface or the internal organ like gut region. The bacterial flora of fish incensed for human consumption depends on the environmental conditions of its natural habitat. Bacterial floras isolated from intestines have been described for a limited number of fish species. Furthermore, it knows that the range of bacterial genera isolated changes by the aquatic habitat of the fish and the bacterial load in the water. The genera present in the gut generally seem to be those from the environment or diet.

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