Jahangirnagar University J. Biol. Sci. **13**(1 & 2): 41-53, 2024 (June & December)

Isolation and identification of microbial load from the dried Chanda fish, *Chanda nama* (Hamilton, 1822)

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Abstract

The present study was conducted to determine the bacterial load, isolate, and identify the bacteria from the dried Chanda fish, *Chanda nama* (Hamilton, 1822). 20 dry fish samples were collected from two different districts: Mymensingh (Nowmohal fish market) and Dhaka (Kawran Bazar fish market); 10 raw fish samples were collected from the Dhaka Kawran Bazar fish market, dried at home (control) to compare them with the quality of commercially dried fish of different districts. In this study, the total bacterial counts (TBC) and total yeast mold counts (TYMC) were recorded in different dilutions. Bacterial isolation and identification were accomplished by analyzing the cultural, staining, and biochemical characteristics of each sample, which were separated into three sections (head, body, and tail). The results varied among fish samples from different districts. The highest bacterial load was found in the home-made dried fish samples which were collected alive from Dhaka (TBC was uncountable in 1^{st}, 2^{nd}, 3^{rd} dilutions and 4^{th} dilution it was 5.5×10^2 cfu/gm) followed by medium load in the sample of Dhaka (Uncountable in 1^{st} and 2^{nd} dilution but in 3^{rd} and 4^{th} dilution it was 7.8×10^3 cfu/gm and 5.4×10^2 cfu/gm respectively) and lowest load in the sample of Mymensingh (1^{st}, 2^{nd} dilution was uncountable and 3^{rd} and 4^{th} dilution containing 5.3×10^3 cfu/gm and 2.5×10^2 cfu/gm respectively). As the density of bacterial colonies was overlapped in the first two dilutions and uncountable, these two dilution related data were excluded from the analyses. The analysis shown here is based on those dilutions with countable separate colonies. Again, the highest load of yeast and mold was observed in the home-made fish sample (for 1^{st} and 2^{nd} dilutions it was uncountable), but medium in the fish sample of Mymensingh (for 1^{st} dilution uncountable but in the 2^{nd} dilution it was 2.2×10^3 cfu/gm) and lowest in the sample of Dhaka (for 1^{st} and 2^{nd} dilution 3.16×10^4 cfu/gm and 2.0×10^3 cfu/gm respectively). Pathogenic bacteria like *Salmonella spp*., *Pseudomonas spp.*, and *Staphylococcus spp*. were detected in all the fish samples of dried *C. nama*, which could indicate unhygienic drying processes. *Raoultella planticola*, a pathogenic bacterium, was identified from the home-made sample using the Biolog software. This study highlights that home-dried Chanda fish samples harbored higher bacterial loads than commercially dried ones, potentially due to a lack of preservatives such as pesticides, which are commonly used in commercial fish drying. These findings underscore the need for improved hygiene and safety measures in traditional fish drying methods to ensure public health safety.

Keywords: Total Bacterial Count, Total Yeast & Mold Count, Chanda, Food Safety

INTRODUCTION

Fish are essential to human diets because they are high in animal protein and other nutrients like lipids, vitamins including vitamins A, D and B, and minerals such as iron,

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calcium, zinc, and selenium to keep humans healthy (Dewi *et al.*, 2011; Ravichandran *et al*., 2012). Fish are high in omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been linked to a variety of health advantages, including cardiovascular health, brain development, and anti-inflammatory properties (Hassaan *et al.*, 2018). Fish and fishery products are Bangladesh's third-largest export and have been crucial to employment, foreign exchange earnings, and human nutrition (Hossain *et al*., 2015; Rana *et al*., 2020). Besides fresh fish, dried fish, or "shutki" as it is known in Bangladesh, is also quite popular among all social strata because of its unique flavor, texture, and taste (Rana *et al*., 2020). According to the Export Promotion Bureau (EPB), Bangladesh fetched US$ 2.55 million in the first six months of the 2023-2024 fiscal year by exporting dry fish to different countries including Malaysia, UK, USA and Middle Eastern countries. Various procedures have been used globally to extend the shelf life of fish, such as drying, salting, and smoking. Sun drying is a well-known method for preserving fish. Fish drying is a popular and cost-effective preservation method in tropical nations, such as Bangladesh. Additionally, dried fish are more nutritious than fresh fish on a dry weight basis. In Bangladesh and other Asian and African nations, drying fish is one of the most traditional, affordable, and successful fish preservation techniques ((Balachandran, 2001; Rasul *et al.*, 2020).

Chanda nama is well-known as a small indigenous fish of Bangladesh. It contains a high amount of protein, fat, mineral and vitamin A content. The chanda fish is common in the freshwater ecosystem of Bangladesh and it is cooked and consumed dried or not dried across the country (Gupta, 2015). Being nutritive and easily available, its microbial load need to be analyzed to ensure food safety. Dried fish contain different microbes, some are pathogenic. The pathogens occur due to improper handling and excessive harmful pesticides on the dried fish. Only a small percentage of the microorganisms from the pabda aquaculture system in Satkhira, Khulna, Bangladesh, were nonpathogenic, with the majority being pathogenic in some way (Haque, A. *et al.*, 2024). Fish deterioration due to pathogenic bacteria and fungal attacks harms Bangladesh's economic situation and public health and safety (Musa *et al*., 2010; Dewi *et al.*, 2011). Different bacterial flora were accessed from the fish *C. nama*; such as- *Salmonella* sp., *Pseudomonas fluorescens*, *Aeromonas hydrophila*, *Streptococcus iniae*, *Staphylococcus aureus* (Hassan and Qureshi, 2014). Due to irregularities at various stages of dry fish processing, such as using unapproved chemicals and insecticides at random, traditional drying methods, low-quality raw fish for drying, and unsanitary and unhygienic facilities, the market's demand for drying fish is currently declining (Yam *et al.*, 2015). According to research, fish infections in Bangladesh cause farmers to lose an average of Tk. 20,615/ha/year (US$344), or around 15% of produce. various districts and agricultural sizes had various losses. Comilla district had the highest average loss, Tk. 26,817/ha/year (US$447), followed by Dinajpur Tk. 23,412 (US$390), Mymensingh Tk. 19,685 (US$328), Jessore Tk. 18,177 (US$303), and Natore Tk. 15,037 (US$251) (MAR. Faruk. *et al.*, 2004).

Mycobacteriosis, Vibriosis, photobacteriosis, winter ulcer, furunculosis, streptococcosis flexibacteriosis, lactococcosis, piscirickettsiosis and winter disease are some of the most dangerous bacterial diseases that can affect fish cultured in marine waters around the

world (Toranzo *et al.*, 2005). Some species of *Staphylococcus* are feared due to their ability to cause overwhelming sepsis and death. It can cause different diseases both in humans and animals. Staphylococcus aureus is a normal inhabitant of many species of animals and they colonize the skin and mucosa (Somerville, 2016). *Pseudomonas* spp. are thin, rod-shaped, non-spore-forming gram-negative bacilli. *Pseudomonas* spp. are motile due to one or more polar flagella. (Albuquerque, W.F. *et al*., 2007). In sun-dried fish, *E. coli* produces histamine. *Salmonella* and *Staphylococcus* species occasionally create histamine residues. The infection caused by *Salmonella* is called salmonellosis. *Salmonella* has been considered the most important causal agent of foodborne illness worldwide. Hundreds of outbreaks of foodborne salmonellosis still occur in most countries every year (Kim *et al.,* 2003).

The biolog software system is one of the bacterial identification systems used to identify both gram positive and gram-negative bacteria. The Biolog Micro Plates was developed for the rapid identification of microbial isolates by sole-carbon-source utilization on a 96-well plate. There are four GEN III protocols: Protocol-A is the default protocol. Protocol-B is followed to identify strongly reducing and capsule-producing Gram-negative and Gram-positive microorganisms. Protocol-C1 is for microaerophilic, capnophilic Gram-positive microbial identification (B. Holmes *et al*., 1994).

The present study was carried out to isolate and characterize some pathogenic bacterial strains with Total Bacterial Count, Total Yeast & Mold Count per gm of dried *C. nama* fish sold at retail markets in Bangladesh compared with homemade dried fish used as a control. This study can help consumers, fishermen and all others involved in the trade to assess the food safety of fish dried under different conditions.

MATERIALS AND METHODS

Study areas and duration: In this investigation, common pathogenic bacterial agents were isolated from dried fish (*Chanda nama*) samples were collected from two different districts: the Dhaka Kawran Bazar fish market (23.7516° N, 90.3943° E) and the Nowmohal fish market of Mymensingh (24.7460° N, 90.4179° E). One dried sample was prepared at home. The whole research work was carried out in the Industrial Microbiology Laboratory, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka-1205; within the time frame from August 2018 to January 2019.

Sample collection and preservation: The experiment was conducted using 30 samples of the same species; raw fish (n = 10 samples, from the Dhaka Kawran Bazar fish market and dry fish (n = 20; 10 samples from the Dhaka Kawran Bazar fish market and another 10 samples from the Nowmohal fish market, Mymensingh. The samples were gathered aseptically in separate, sterile plastic zipper bags in different sizes. After that, the samples were taken to the laboratory and kept at 4°C for preservation.

Home-made dried fish processing: The raw fish samples were first cleaned and salted to prepare the homemade dried fish used as a control. After that, 50 g of fresh fish were mixed with 250 g of water and 5 g of salt. Then the fish were soaked in the solution for almost half an hour. The fish was removed and rinsed under running water to remove any remaining salt. After that, the fish were suspended, wrapped in a fresh net, and left to dry for 3 to 4 days in the sun.

Sample preparation: The fish samples were processed according to Sultana *et al*., 2010. In short, each fish sample was divided into three sections: the head, the body (the abdominal portion), and the tail. 10 g of each piece was taken, and the sample was carefully chopped and mashed using a sterile mortar and pestle. For each specimen, 30 g samples were taken in 225 ml Buffered Peptone Water (BPW) for 10 minutes, which was the stock solution.

Bacteriological analysis: Three distinct samples were subjected to bacterial investigation using the serial dilution technique with normal saline.

Total Bacterial Count (TBC): TBC was observed in four different dilutions by the pour plating method. After serial dilution, 1ml solution from every 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} test tube was pipetted into the four sterile petri dishes, and the plate count agar (PCA) medium was poured into the petri dishes up to two third portion (10cm) of the petri dish. Then, the petri dishes were rotated clockwise and anticlockwise to properly mix the medium with inoculation. The petri dishes were kept in an incubator at 37℃ and following the 18-24 hours of incubation period, the bacterial count was determined. TBC (cfu/gm) in dried *C. nama* was observed for different dilutions using the following formula: cfu/gm = (no. of colonies × dilution factor) / volume of culture plate

Statistical Analysis: Statistical research to determine whether the areas (Mymensingh, Dhaka, and Home-made) have a significant effect on the Total Bacterial Count (TBC) in dried C. names. To perform statistical analysis on this dataset, we considered only the countable values from the 3rd and 4th dilutions across the three sample sources: Mymensingh, Dhaka, and Home-made. The first and second dilutions, being uncountable, were excluded from the analysis since they do not have numerical values. Being the small sample size and the type of data, we used the Kruskal-Wallis test, a non-parametric method suitable for comparing medians across more than two groups when the assumption of normality may not hold. If we find a significant difference, we can further analyze pairwise differences using post-hoc tests such as Dunn's test.

Total Yeast & Mold Count (TYMC): TYMC was observed in the first and second dilutions by spread plate technique for each of the samples. For the first dilution, Rose Bengal Chloramphenicol Agar was poured into a petri dish and allowed to freeze the medium in a Laminar Airflow Cabinet. Then 1 ml sample from 10^{-1} tube was pipetted onto the agar plate and was spread using a sterile L-shaped glass spreader. The same procedure was repeated for the second dilution. Finally, all the petri dishes were kept in

an incubator for 18-24 hours. Following the incubation period, a colony counter was used to count the colonies.

Isolation of different pathogenic bacteria: Following each 10^{-3} dilution, 0.1 mL of suspension was streaked onto Xylose Lysine Deoxycholate (XLD) to isolate *Salmonella spp.*, *Pseudomonas* agar medium for detecting the presence of *Pseudomonas spp*., and Baird Parker Agar Base (BPAB) to isolate *Staphylococcus spp.* Characteristic colonies were observed following a 24-hour incubation period at 37°C. To verify the pathogenic identification, morphological and several biochemical assays were conducted following conventional procedures *viz*.; catalase test, indole test and methyl red (MR) test (Cappuccino and Sherman, 1996; Cheesbrough, 2006).

Bacterial identification using Biolog Software: Biolog software used for bacterial identification in dried *C. nama*. The samples were cultured on Mug MacConkey agar and subcultured on the nutrient agar by streak plate method. After culturing and isolating the bacterium, a liquid inoculum was prepared using a "gelling" inoculating fluid (IF) as per the manufacturer's instruction and incubated at 37°C for 20 minutes. The inoculator swab was touched to pick up the bacterial colony and released into the inoculating fluid to make a uniform bacterial cell suspension. The % transmittance was measured using a turbidimeter (BiOLOG, 21907, USA). Then the biolog plate was incubated at 37°C for 20 minutes before inoculating the liquid bacterial cell suspension. Then the inoculum was transferred into a reservoir and dispensed into each well of the biolog plate and incubated for 24 hours. After incubation, the color densities in wells of the GEN III MicroPlates containing bacterial isolates were read with the Biolog Microbial Identification System and MicroStation Reader.

RESULTS AND DISCUSSION

Assessment of Total Bacterial Counts (TBC): In August 2018**,** the highest bacterial load was present in the home-made sample in the 4^{th} dilution (7.88×10^2 cfu/gm), medium in the sample of Dhaka (7.53×10^2 cfu/gm) and lowest in the sample of Mymensingh (4.0×10^2 cfu/gm). In September 2018, the highest TBC was observed in the home-made sample, medium in Dhaka, and the lowest load was present in the sample of Mymensingh. In October 2018**,** the highest TBC was observed in the home-made sample, medium in the sample of Dhaka and the lowest load was present in the sample of Mymensingh. In November 2018, the highest bacterial load was present in Dhaka, medium in the home-made sample but lowest in the sample of Mymensingh. In December 2018, the highest bacterial load was present in the sample of Dhaka, lower in the home-made sample and lowest in the sample of Mymensingh. In January 2019, the highest bacterial load was present in the home-made sample, medium in Dhaka but lowest in the sample of Mymensingh. So, the average highest TBC was found in the home-made sample (5.5×10^2 cfu/gm), medium load was present in the sample of Dhaka (5.4×10^2 cfu/gm) and lowest load was observed in the sample of Mymensingh (2.5×10^2 cgu/gm) (Table 1, Figure 1).

Table 1. TBC (cfu/gm) of dried *C. nama* from August 2018 to January 2019

Samples sources	Dilution	August 2018	September 2018	October 2018	November 2018	December 2018	January 2019
Mymensingh	1^{st}	Unc	Unc	9.86×10^5	5.54×10^5	3.43×10^5	1.28×10^5
	2^{nd}	Unc	7.4×10^4	8.06×10^4	7.2×10^4	4.6×10^4	2.74×10^4
	3^{rd}	7.8×10^3	6.66×10^3	5.63×10^3	5.2×10^3	3.3×10^3	3.4×10^3
	4^{th}	4.0×10^2	3.8×10^2	3.02×10^2	2.2×10^2	1.5×10^2	0.8×10^2
Dhaka	1^{st}	Unc	Unc	Unc	Unc	7.90×10^5	2.54×10^5
	2^{nd}	Unc	Unc	9.2×10^4	9.88×10^4	6.28×10^4	4.75×10^4
	3^{rd}	8.86×10^3	9.30×10^3	7.70×10^3	8.83×10^3	8.5×10^3	3.56×10^3
	4^{th}	7.53×10^2	5.28×10^2	6.5×10^2	6.77×10^2	3.84×10^2	2.24×10^2
Home-made	1^{st}	Unc	Unc	Unc	Unc	3.64×10^5	6.49×10^5
	2^{nd}	Unc	Unc	Unc	8.41×10^4	5.92×10^4	4.80×10^4
	3^{rd}	Unc	Unc	Unc	5.60×10^3	5.6×10^3	6.80×10^3
	4^{th}	7.88×10^2	7.79×10^2	5.83×10^2	6.1×10^2	2.04×10^2	3.70×10^2

Legend: Unc =Uncountable

Results of Statistical Analysis

Step 1: Rank the Data
First, combine and rank all the data from all groups:
Data points: [5300, 250, 7800, 540, 0, 550]
Ordered: [0, 250, 540, 550, 5300, 7800]
Ranks: [1, 2, 3, 4, 5, 6]

Step 2: Assign Ranks to Each Data Point
Match ranks to the original data values:
Mymensingh: [5300, 250] → [5, 2]
Dhaka: [7800, 540] → [6, 3]
Home-made: [0, 550] → [1, 4]

Step 3: Calculate the Sum of Ranks for Each Group: Sum ranks within each group:
Mymensingh Sum of Ranks: R_1=5+2=7
Dhaka Sum of Ranks: R_2=6+3=9
Home-made Sum of Ranks: R_3=1+4=5

Step 4: Calculate the Kruskal-Wallis Statistic
The formula for the Kruskal-Wallis statistic

$$H = \left(\frac{12}{N(N+1)}\right)\sum_{i=1}^{k}\frac{R_i^2}{n_i} - 3(N+1)$$

N is the total number of observations= 6.
$R_1 = 7; R_2 = 9; R_3 = 5$
$n_{1=}\, n_2 = \; n_3 = 2$ [observations per group).
k=3; the number of groups.

$$H = \left(\frac{12}{6\times(6+1)}\right)\left(\frac{7^2}{2}+\frac{9^2}{2}+\frac{5^2}{2}\right) - 3(6+1)$$

⇨ H = 1.14

Step: 5: Hypothesis Testing
H_0= (Null Hypothesis): There is no significant difference in bacterial counts between samples from Mymensingh, Dhaka, and Home-made

$$H_0 : \text{Median}_{\text{Mymensingh}} = \text{Median}_{\text{Dhaka}} = \text{Median}_{\text{Home-made}}$$

H_a= (Alternative Hypothesis): There is a significant difference in bacterial counts among at least one of the sample locations compared to the others

$$: H_a : \text{At least one Median}_{\text{Group}} \neq \text{Others}$$

$$\text{p-value} = 1 - \text{CDF}_{\chi^2}(\text{test statistic} = 1.143, \text{df} = 2)$$

⇨ P-value = 0.565

p-value is greater than typical significance levels (e.g., α =0.05),
So do not reject the null hypothesis. In simpler terms, there is no statistical evidence to suggest that the locations (Mymensingh, Dhaka, and Home-made) differ significantly in terms of their median bacterial counts based on the available data.

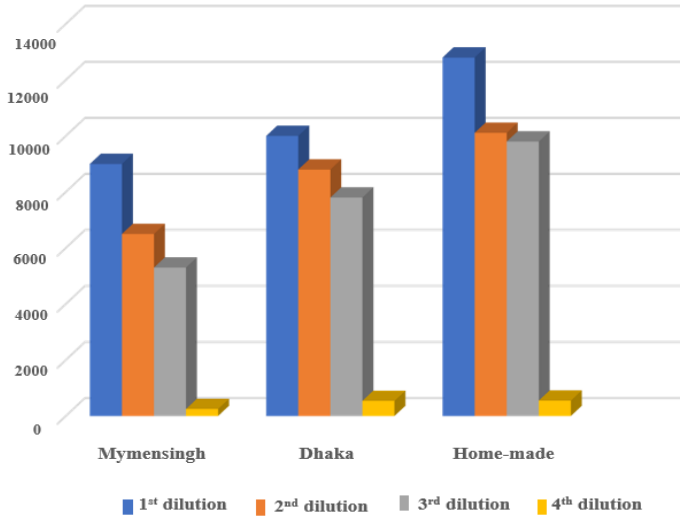

Fig. 1. Showing average TBC in dried *C. nama*

Estimation of Total Yeast and Mold Count (TYMC): In August 2018**,** no mold was present. But the highest yeast was present in the home-made sample, medium in the sample of Mymensingh and lowest in the sample of Dhaka. In September 2018, the highest TYMC was present in the home-made sample, lower in the Mymensingh sample and lowest in Dhaka. In October 2018**,** the highest TYMC was observed in the sample of Dhaka, medium in the home-made sample but lowest in the sample of Mymensingh. In November 2018, the TYMC load was highest in the sample of Mymensingh, medium in the home-made sample and lowest in Dhaka. In December 2018, the highest TYMC was observed in the home-made sample, medium in the sample of Mymensingh but lowest in the sample of Dhaka. In January 2019, the highest TYMC was observed in the home-

made sample, comparatively lower in the Mymensingh sample but lowest in Dhaka. So, the average highest yeast-mold was observed in the home-made sample, medium in the Mymensingh sample and lowest in Dhaka (Table 2, Figure 2).

Morphological and biochemical characteristics of the isolated bacterium: The Gram staining method was used to morphologically characterize distinct bacterial isolates, as shown in Table 3. All the microorganisms were catalase-positive bacteria and indole-negative bacteria. On the other hand, two were MR test positive bacteria and one MR test negative bacteria (Table 3).

Prevalence of pathogenic bacteria in different samples: Red colonies were present in the samples of Mymensingh and Dhaka and red colonies with black centers were observed in the home-made sample. So, *Salmonella* sp. was present in all the samples (Figure 3.1). Yellow colonies were seen in all the samples, and *Pseudomonas* was present in all the samples (Figure 3.2). Black colonies were observed. So, *Staphylococcus* was present in all the samples (Figure 3.3).

Identified bacteria by Biolog Software: *Raoultella planticola* was identified from home-made sample. No bacteria could be isolated from the remaining market samples.

Table 2. TYMC (cfu/gm) of dried *C. nama* from August 2018 to January 2019

Samples sources	Dilution	August 2018	September 2018	October 2018	November 2018	December 2018	January 2019
Mymensingh	1^{st}	Unc	7.68×10^4	2.06×10^4	8.7×10^4	5.06×10^3	3.8×10^2
	2^{nd}	1.24×10^3	3.51×10^3	2.8×10^3	5.08×10^3	4.9×10^2	1.1×10
Dhaka	1^{st}	4.5×10^4	4.8×10^4	5.87×10^4	3.6×10^4	2.12×10^3	1.57×10^2
	2^{nd}	5.0×10^3	1.32×10^3	3.90×10^3	1.4×10^3	1.17×10^2	0.6×10
Home-made	1^{st}	Unc	Unc	4.01×10^4	6.20×10^4	6.12×10^3	4.2×10^2
	2^{nd}	Unc	6.37×10^3	2.98×10^3	2.7×10^3	3.24×10^2	2.76×10

Legend: Unc =Uncountable

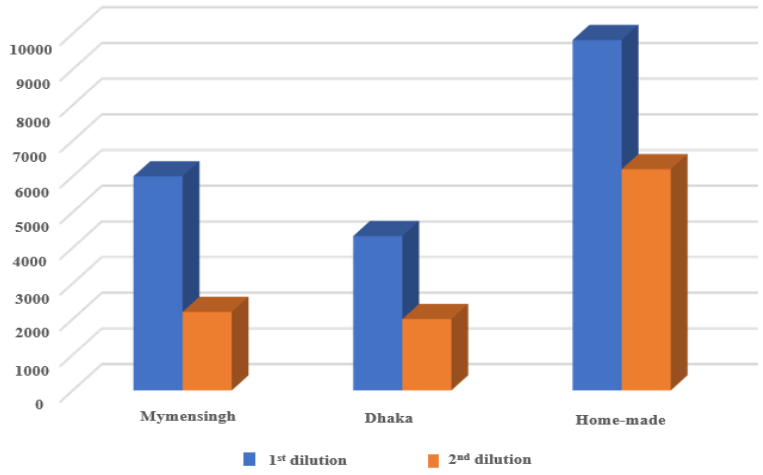

Fig. 2. Showing average TYMC in dried *C. nama*

Table 3. Morphological and biochemical properties of the isolated bacteria

Bacterial strains	Pigmentation	Shape	Size	Staining Properties	Category	Motility test	Catalase test	Ind test	MR test
Salmonella spp.	Reddish	Round	Small	Pink	Gram-negative	+	+	-	+
Pseudomonas spp.	Yellowish	Round	Small	Pink	Gram-negative	+	+	-	-
Staphylococcus spp.	Blackish	Round	Pinpoint	Violet	Gram-positive	-	+	-	+

Legends: MR: Methyl-Red; Ind: Indole; +: positive; -: negative.

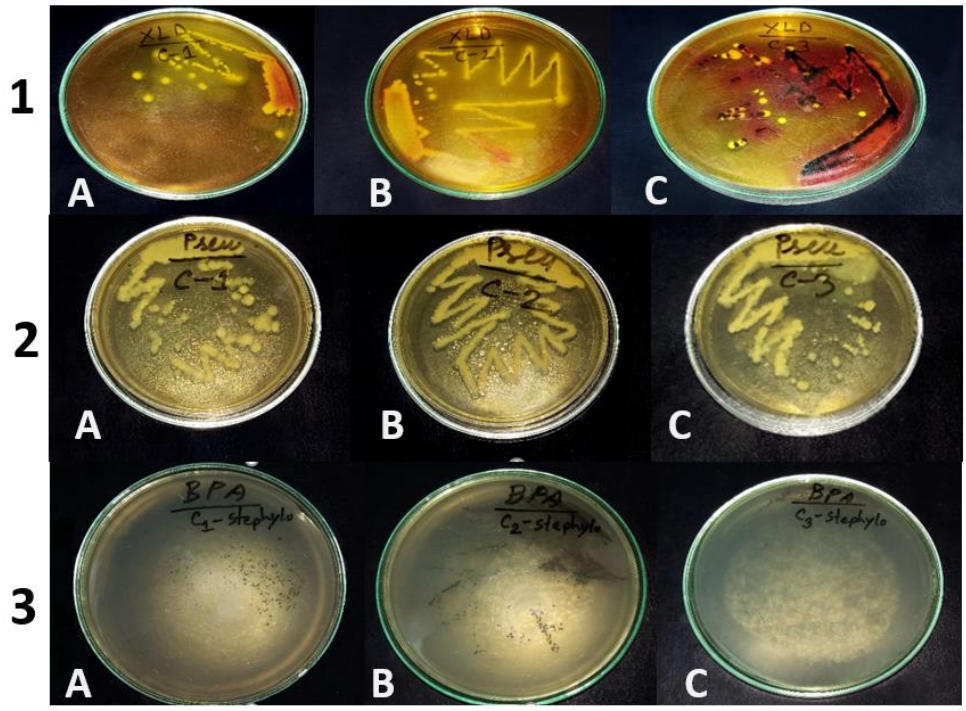

Fig. 3. Detection of pathogenic bacteria in different samples: 1. *Salmonella spp.* on X.L.D. medium in different samples; 2. *Pseudomonas spp.* on pseudomonas agar in different samples; 3. *Staphylococcus spp.* on BPAB agar in different samples; A= samples from Mymensingh; B= samples from Dhaka (Kawranbazar); C= Home-made dried fish samples

Fish is an important source of protein. Approximately 60% of the animal protein consumed by the world's population is derived from fish and over one billion people depend on fish as their main source of animal protein (Flowra, 2012). Dried fish has made a strong position in the economy of Bangladesh, as it has its appearance both on international and local markets. In Bangladesh, dried fish have gained popularity among

the consumer (Rana *et al*., 2020). The present study has revealed the presence of microorganisms in different samples of dried *C. nama* which is also one of the popular dried fishes to the commoners to consume across the four corners of Bangladesh. The study was carried out to determine and compare the total bacterial load, yeast mold load and iolate different pathogens and identify the bacteria from the samples of dried *C. nama*. The samples were collected from two different districts and one was home-made to be used as a control. The total viable counts (TVC) were observed in different dilutions. The highest bacterial load was present in the homemade sample, medium in the sample of Dhaka and lowest in the sample of Mymensingh. TYMC was also highest in the home-made sample, but medium in the Mymensingh sample and lowest in Dhaka.

ICMSF, (1986) suggested an acceptable limit of bacterial load in dry fish as < 5 log cfu/gm. A greater range of bacterial count than this study has obtained. This could be due to the differences in the manufacturing process/transport/ market's condition and locations. In this study, the bacterial load was almost double times higher in all the samples than this recommended value. *Salmonella* sp., *Pseudomona*s sp. and *Staphylococcus* sp. were also isolated from the samples. Three types of bacteria live in water and can infect fish: Gram-negative bacteria, the most common type of bacteria, acid-fast bacteria, which can be found in food or the environment and Gram-positive bacteria. Most diseases in tropical fish are caused by germs that are not gram-positive (Danba *et al*., 2015). Environmental sources like; soil, water and air can act as sources of contamination of these pathogens. Moreover, a lack of aseptic handling and hygiene can result in contamination with such bacteria. These pathogens are associated with food poisoning as they produce a toxin that makes the users sick, showing symptoms like nausea, vomiting and diarrhea after eating the staphylococci-infected food. Fish bacteria can cause a wide range of diseases, which makes them one of the biggest worries in the aquaculture industry. So far, many pathogenic microorganisms have been linked to fish diseases. These include *Pseudomonas spp., Aeromonas hydrophila, Streptococcus iniae, Citrobacter freundii, Edwardsiella tarda,* and *Flavobacterium columnare.* A septicemic illness called edwardsiellosis affects commercially significant fish for example eels, mullet, channel catfish, Chinook salmon, carp, flounder, striped bass and tilapia. It causes widespread lesions in the skin, muscles, and internal organs (Thune & Stanley, 1993; Yaliwal *et al.,* 2020).

Begum *et al*., 2014, studied the effect of lemon leaf extract treatment on the nutrient content and microbial properties of dried Chanda (*C. nama*) and found that the Standard Plate Count (340 cfu/g), Total coliform (absent), Total fungi (< 10 cfu/g) and E. coli (absent) that were in the acceptable range in case of control chanda but in market chanda the Standard Plate Count was too numerous to Count, Total coliform was > 240, E. coli was present and Total fungi were too numerous to Count. In contrast to this, the present findings show the highest bacterial loads found in the home-made dried fish sample than the market samples. The reason might be that the homemade dried fish were maintained hygienically and there were less or no pollutants, pesticides or competing pathogens. However, during drying microbes from air and wind are thronged into the fish and replicated exponentially which might have resulted in higher account in the study. The quality of dried fish is often adversely affected by the growth of fungi (Chakraborti &

Varma, 1999) and a substantial amount of fish are discarded during drying due to fungal growth (Gupta & Samuel, 1985). In the present study, dried fish samples were free from visible mold but yeast colonies were found when plated on Rose Bengal Chloramphenicol agar (RBCA).

R. planticola is a gram-negative bacterium of the genus *Raoultella* belonging to the Enterobacteriaceae family (Castanheira *et al*., 2009). It is a histamine-producing bacterium that is usually found in fish and water (Hajjar *et. al*., 2018). It was initially seen as an aquatic, botanic and soil bacterium. This organism has been found to cause a variety of infections, such as pancreatitis and soft tissue infections (Alves *et al*., 2007 and O'Connel *et. al*., 2010). The majority of the reports on *R. planticola* describe nosocomial infections in patients with significant systemic comorbidities such as chronic kidney disease, diabetes and cancer. It has even been suggested that infection with these organisms occurs mainly in patients with impaired defense mechanisms and weakened immune systems (Hajjar *et al*., 2018). Besides, a pathogenic bacterium *R. planticola* was found in the home-made sample but absent in the market samples. It may be due to the indigenous bacterial load of the fish *C. nama*. As no harmful chemicals were used in the home-made sample, it was found in the home-made sample. But the dried fish traders usually use many types of pesticides at an arbitrary rate that can kill or destroy other microorganisms and for this *R. planticola* might have been absent in the market samples. However, the dried fish in the markets showed the presence of certain harmful pathogens. Although published data on the use of pesticides in the dried fish are rare.

Conclusion: As fish is an extremely perishable food item, it requires preservation for future uses. Several preservation methods are followed all over the world for preserving fish. In Bangladesh, sun drying is the most widely used method of fish preservation. Demand for dried fish is high among Bangladeshis both at home and abroad, but the sector is shrinking now. One of the main reasons is the use of excessive amounts of harmful insecticides and pesticides. In Bangladesh, most of the market samples become slightly odorless and some lose their shelf life where rancid and bitter tastes develop. So, proper attention should be given during the processing and handling of the dried fish and maintaining a hygienic condition to ensure the better quality of the dried fish. The present study shows variations in microbial load in different sites of the country which deserve proper hygiene during handling, drying, preserving and selling. It is also suggested that the dried fish should be cooked thoroughly for a prolonged period at high temperatures to destroy the pathogen and their spores (if any) for the health and safety of the consumers. The findings are although bacteriological but the detection of certain pathogenic strains including *R. planticola* is very alarming. So, the data of the present study will raise awareness about the need for fish preservation among scientists, processors, and communities.

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