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Research Article

ISOLATION, CHARACTERIZATION AND BACTERIAL DIVERSITY IN SPOILED FOOD PRODUCTS

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A B S T R A C T

This study aims to the enumeration of the colony forming units was done for the quantitative population analysis of different spoiled food samples. Highest count of 1.21×10^2 cfu/ml was observed in the case of pineapple sample with lowest count observed at 0.28×10^2 cfu/ml for bread sample. The isolates obtained from food samples were then stained and also identified based on their biochemical properties. The media used for enumeration were Plate CountAgar, Nutrient Agar and MacConkey Agar media for the count of total aerobic and anaerobic microbes. The targeted species were *Listeria monocytogenes, Coliform bacteria, Escherichia coli, Yeast & Molds, Lactobacillus, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa,* as these species have their role in causing spoilage of food samples. The identification was done based on biochemical tests including catalase test, indole test, sugar fermentation test, urease test, oxidase test, citrate utilization test. A total of 23 bacterial, 3 yeast, and 7 mold isolates were isolated from the food samples. These isolates were then classified by categorizing them into 13 bacterial species, 3 yeast species, and 4 mold species based on their phenotypic characteristics.

INTRODUCTION

The potentiality of producing and consuming the safe food products is considered as one of the best achievement of the last century as the food borne intoxicants are an enduring risk for the public health. Among the human population over 200 diseases are transferred through food consumption (Bryan, 1982). The food borne pathogens causing diseases possesses a wide spectrum, which includes viruses, fungi, heavy metals, fungal toxins, parasites bacteria and its toxins, among these bacterial poisoning is most prevalent. Every year more than 90% of all the food poisoning diseases are related to known pathogens that are mainly found in the raw food materials. These bacteria include *Staphylococcus aureus, Salmonella, Clostridium perfringens, Campylobacter, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus, and entero-pathogenic Escherichia coli* (Mead *et al*., 1999).

The rapid progress in the field of chemical detection technology has led this technology to find application in the estimation of the remaining self-life of the food products as well as its early detection of spoilage. The quantitative knowledge in the field of predictive microbiology provides a basis for the development of some devices that can implement to monitor the loss of the self-life of the food products during storage, distribution and retail sale. The microbial ecology of the food system is necessarily considered for the accurate prediction of the self-life of the food sample (McMeekin *et al*., 1996).

The most important components of any integrated program used to ensure the safety of food throughout the food supply chain includes the dtection and enumeration of the pathogens in food and surface that comes into the contact with food products.

The use of microbial analysis to monitor the state of contamination at all time is applied by both government authorities and the food companies as well as it is used to analyze the emerging risks. The microbial analysis of food sample is an essential tool for carrying out the test according the criteria established for each food type. It is also essential for the evaluation of actions of different management strategies based on the Hazard Analysisand Critical Control Points (HACCP) system (Stannard 1997; Jasson *et al.* 2010). The implementation of preventive systems such as the HACCP has greatly improved food safety, but it will not be fully effective until better methods of analysis aredeveloped. These new detection methods are the necessary technologies that will substantially improve our food safety once integrated in the HACCP (Bhunia, 2008).

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MATERIALS AND METHODOLOGY

Sample Collection

The fruit samples used in the study were collected from three different branded grocery stores in sterile bags from Lucknow (U.P.) and brought to the lab for further testing. A total of ten different samples of food items were collected. The samples were rinsed with sterile water, peeled, cut and then stored at room temperature for 3 days (72 hours). The list of the samples collected and the type of treatment given is given in the table below:

Table no1 Table showing the collected samples and the type of treatment given to them:

S.No.	Sample	Sample No.	Type of treatment	Sample Code	Storage Temp (ºC)	Storage period (Days)
1	Apples	Sample 1	Peeled & Cut	S1	RT	5-March
2	Pineapple	Sample 2	Peeled	S2	RT	5-March
3	Grapes	Sample 3	Normal state	S3	RT	5-March
4	Mango	Sample 4	Peeled	S4	RT	5-March
5	Watermelon	Sample 5	Peeled & Cut	S5	RT	5-March
6	Pear	Sample 6	Peeled	S6	RT	5-March
7	Meat	Sample 7	Cut	S7	RT	5-March
8	Potato	Sample 8	Cut	S8	RT	5-March
9	Bread	Sample 9	Cut	S9	RT	5-March
10	Raw milk	Sample 10	Peeled	S10	RT	5-March

Isolation of Microbial Species

For the isolation of microbes from the samples, their spoiled part was collected and crushed in a sterile mortar and pestle. One gram of the crushed sample was mixed with 10 ml of peptone water in a McCartney bottle. This suspension was shaken gently to prepare a homogenous mixture and this mixture was diluted ten folds and used for plating. The mixture was plated on Plate CountAgar, Nutrient Agar and MacConkey Agar media for the count of total aerobic and anaerobic microbes. The plates were incubated at 37˚C for 24 hours. Pure culture of the isolated microbe was obtained by streaking method on selective media

Identification of Microbial Species

The isolated pure culture was used for morphological and biochemical identification of the isolated microbes. Morphological identification was achieved through Gram's staining and Endospores staining. For staining, a thin smear of the culture was prepared on the slide and stained with different dyes. For Gram's staining the slide was primarily stained crystal violet then decolorized with ethyl alcohol and counter stained with safranin followed by washing after each step. For Endospore staining the smear was flooded with malachite green under continuous steaming for 5 min. the counter stained with safranin. Both staining slides were observed under oil immersion compound microscope to analyze morphology. Further identification was done by several biochemical tests for species identification as stated by Bergey's manual. The results were compared with the manual for species identification. Different culture media were prepared and inoculated with the pure culture of isolated microbe for biochemical testing.

Enumeration of Colony Forming Units (CFU)

The quantitative population analysis of the isolated culture was done by the plating of serially diluted samples and with subsequent counting of the Colony Forming Units (CFU). This was achieved by following the standard protocol for CFU enumeration. It was done to assess the quantitative population of the microbes present on the samples. Using the formula did the calculation of CFU; $N= \sum C /[(1x\ N_1) + (0.1\ x\ N_2)\ x\ (D)]$

Where;

N = Number of colonies per ml or gm of the product
$\sum C$ = Sum of all the colonies on all the plates
N1 =Number of plates in first dilution counted
N2 = Number of plates in second dilution counted
D = Dilution of the first plate counted

RESULT AND DISCUSSION

The samples were collected and treated as mentioned earlier and stored for 3 days. Their characterization and morphological analysis was done immediately after bringing them to the lab. The samples started showing morphological and appearance changes after three days due to spoilage. The changes were compared with earlier morphology. The spoiled part was used for further process.

Isolation of Microbial Species

The isolation of the microbes was done by plating the serially diluted sample of the spoiled parts of the food sample on the different culture media. The plates were incubated for 24 hours at 37˚C in the incubator and they showed several microbial colonies after incubation period. From this pure culture of the selected colonies was prepared on selective media. A total of 23 bacterial, 3 yeast, and 7 mould isolates were isolated from the food samples. These isolates were then classified by carectorizing them into 13 bacterial species, 3 yeast species, and 4 mould species based on their phenotypic characteristics.

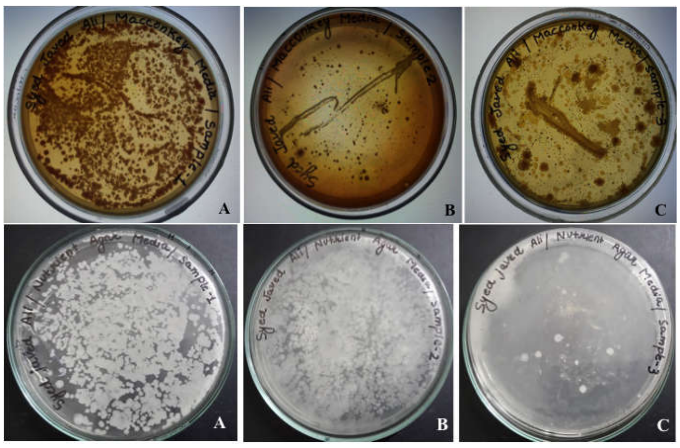

Figure 1 Spreading plate with grown colony after incubation of 24 hours at 37°C Media used: Row 1-MacConkey Agar Media; Sample S1[A], S2[B], S3[C]; Media used: Row 2- Nutrient Agar Media; Sample S1[A], S2[B], S3[C]

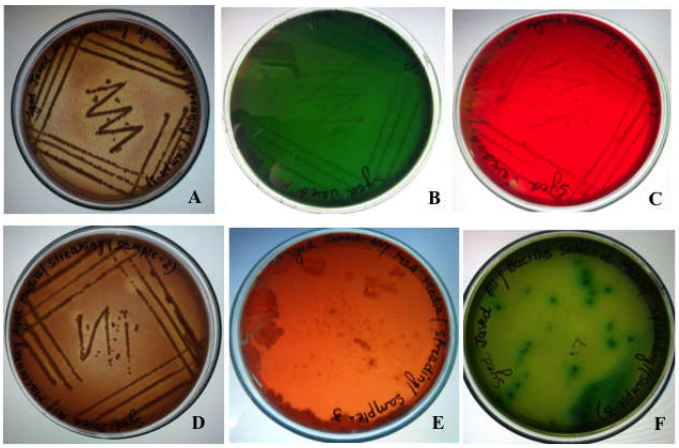

Figure 2 [A-D]Streaking plate with grown colony after incubation of 24 hours at 37°C;Media used: [A]MacConkey Agar Media [B]Bacillus selective agar media [C]Mannitol Salt agar [D]MacConkey Agar ; Spreading plate with grown colony after incubation of 24 hours at 37°C; [E] Mannitol Salt Agar [F] Bacillus selective agar media

Identification of Microbial Species

The morphological identification of the isolated species of the microbes was done through Gram's and Endospore staining and further by biochemical testing. The Biochemical test for identification of selected microbe was performed as per the Bergey's manual. The biochemical test was performed for the selected microbes as; *Listeria monocytogenus, Coliform bacteria, Escherichia coli, Yeast & Molds, Lactobacillus, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa.* Some of the biochemical tests performed the identification of the isolated microbes includes catalase test, indole test, sugar fermentation test, urease test, oxidase test, citrate utilization test etc. The result obtained from this biochemical test formed the basis characterization of the microbes.

Enumeration of Colony Forming Units (CFU)

The enumeration of the colony forming units was done for the quantitative population analysis of different samples. The counting for every sample was performed in triplicate form that is after 7 days, 14 days and 21 days. The total viable count of the cells was done on standard method agar and it gave highest count of 4.14×10^2 cfu/ml was observed in the case of potato sample on 21st day with lowest count observed at 1.04×10^2 in the case of bread samples. Several samples showed no growth of any organism even on the last day of incubation period. Cell forming unit count for *Salmonella typhi* was not detected at all in any sample till the last day. The highest and lowest cell-forming unit counts (cfu/ml) at 21st day for other microorganism is listed in the table below;

CONCLUSION

In the present study, 10 samples (six freshly prepared juices-Apples, Pineapple, Grapes, Mango, Watermelon and Pear, meat, potato, bread and raw milk) were examined for microbiological analysis, bacterial diversity and toxin protein detection in culture dependent and culture independent samples. The frequencies of occurrence of bacteria, molds andyeasts were expressed in terms of colony forming unit per ml of sample plated on selective media. In this study, the total viable bacterial count in the selected samples showed no visible growth on the first day of incubation in any of the samples. Highest count of 4.14×102 cfu/ml was observed in the case of potato sample on 21st day with lowest count observed at 1.04×102 in the case of bread samples. The *Listeria monocytogenes* count in the selected samples showed highest count of 0.33×102 cfu/ml in the case of raw milk sample with lowest count observed at 0.03×10^2 in the case of bread samples. The CFU count for staphylococcus in the selected sample showed no viable growth on the first day of incubation. Highest count of 6.4×10^1 cfu/ml was observed in case of meat on 21st day with lowest count observed at 1.0×101 cfu/ml for grape sample. CFU count on Bacillus Selective Agar Media in the selected samples showed no visible growth on the first day of incubation in any of the samples. Highest count of 1.21×10^2 cfu/ml was observed in the case of pineapple sample with lowest count observed at 0.28×10^2 cfu/ml for bread sample. A total of 23 bacterial, 3 yeast, and 7 mould isolates were isolated from the food samples. These isolates were then classified by categorizing them into 13 bacterial species, 3 yeast species, and 4 mould species based on their phenotypic characteristics. The isolates obtained from food samples were then stained and also identified based on their biochemical properties.

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Table showing the result of CFU count of various isolated species on the type of media used

Name	Media used	Sample	Highest CFU (cfu/ml)	Sample	Lowest CFU (cfu/ml)
Listeria monocytogenes	Listeria selective agar base	Raw milk	0.33×10^2	Bread	0.03×10^2
Coliform spp.	Eosin methylene blue agar	Meat	8.6×10^1	Mango	2.3×10^1
E. coli	MacConkey Agar	Meat	4.1×10^1	Watermelon	0.6×10^1
Yeast & molds	Dichloran rose Bengal chloramphenicol Agar	Pineapple	0.67×10^1	Meat	0.28×10^2
Lactobacilli	Man, Rogosa &Sharpe Agar	Grapes	7.3×10^1	Mango	2.3×10^1
Pseudomonas aeruginosa	Pseudomonas Isolation Agar	Watermelon	9.5×10^2	Pear	5.6×10^2
Staphylococcus spp.	Mannitol salt agar	Meat	6.4×10^1	Raw milk	0.8×10^1
Bacillus spp.	Bacillus selective agar base	Pineapple	1.21×10^2	Bread	0.42×10^2

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