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ISOLATION AND CHARACTERIZATION OFMICROORGANISM ASSOCIATED WITH SPOILED FRUITSOBTAINED FROM DIFFERENT MARKET PLACES IN AND AROUND PUNE REGION

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ABSTRACT

Foods, by their chemical nature are powerhouse of numerous nutrients, easilyget metabolize and therefore offer appropriate substrates for the growth of microorganisms. Improper handling and temperature abuse during transit and storage of these foods may favor growth of microflora initially present in the raw materials. Production of extracellular enzymes by these microorganisms at different temperature indicates their spoilage potentiality which further can cause food borne diseases. This paper deals with the enumeration and characterization of bacteria with spoilage potential from some selected foods articles like *Manilkarazapota*, *Musa paradisiaca*fruit, and *Solanumlycopersicum* vegetable which are collected from local market of Pune region. An investigation was carried to study the different bacterial and fungal sp. present in various collected food articles. Various bacteria species, which caused spoilage of fruits, were isolated by serial dilution agar plating method by used of Nutrient agar media and enumerated.The bacterial and fungal contaminants were isolated and identified using specific culture techniques. The predominant bacterial pathogen isolated was *Escherichia coli, Salmonella spp., Staphylococcus aureus*and *Pseudomonas spp.* The predominant fungal pathogen isolated was *Mucor spp., Aspergillusniger*, were identifiedon the basis of cultural characteristics, colony morphologicalcharacteristics , Gram staining , motility and biochemical characteristics.

INTRODUCTION

Foods, by their chemical nature are powerhouse of numerous nutrients (vitamin, minerals), easily get metabolize and therefore offer appropriate substrates for the growth of [1]microorganisms Improper handling and temperature abuse during transit and storage of these foods may favor growth of microflora initially present in the raw materials Production of extracellular enzymes by these microorganisms at different temperature indicates their spoilage potentiality which further can cause food borne diseases due to produced toxins by pathogenic bacteria[2].Spoilage refer to any change in the condition of food in which the food becomes undesirable or unacceptable for human consumption [3].



Microbial spoilage of food articles is a part of global concern,causing serious foodborne intoxications and resulting inhigh economic fatalities for the food manufacturing and processing industries. This work examined selected foods and vegetables sold by vendors in Pune region market for microbial contamination. Isolated and identified bacterial and fungal species associated with food contamination and their microbial loads were determined in the present study.

MATERIALS AND METHODS

Manilkarazapota(Chiku),Musa paradisiaca fruit (Banana), and *Solanumlycopersicum (Tomato)*fruits/ vegetable were selected as per survey conducted in Pimpri- Chinchwad, NigadiPradikaran , Akurdi and Ravetarea which revealed that these fruits are commonly used in huge amount. Selected fruits collected in plasticzip bag from local vegetable market of Pune regionand brought in the laboratory for further analysis as shown in below flow diagram.

Enumeration of coliform

Lactose broth was prepared and then autoclaved at 121^{o}C for 15 minutes. The media was allowed to cool before dispensing into the test tubes. Lactose broth was dispensed into 9 test tubes containing Durham's tube; each Durham's tube was inverted in each of the 9 test tubes. From serially diluted 9 test tubes 1ml from each was transferred to correspondent test tubes containing the Lactose broth and Durham's tube . The 9 test tubes containing the Durham's tube and lactose broth were incubated at 37oC for 24 hours.

RESULT AND DISCUSSION

The bacterial isolates were identified based on morphological and biochemical characteristics. Six bacterial and two fungal species were isolated from selected spoiled fruits and enumerated. The viable count of bacterial and fungal isolates on Nutrient agar plates and Potato dextrose agar media plate which explained in below figure No.06.

The food associated bacteria isolated from various samples of different spoiled fruits identified on the basis of cultural, morphological and biochemical characteristics are mentioned in Table No.02. In this study, six type of bacteria and two types of fungal species were successfully isolated from selected *Manilkarazapota* (Chiku), *Musa paradisiaca* fruit(Banana) spoiled fruits. According to classical bacteriology, most species of bacterial isolate can be differentiating based on simple Gram staining technique[10]. Three Gram positive bacteria were isolate from *Manilkarazapota*(Chiku),*Musa paradisiaca* fruit(Banana) while three Gram negative bacteria were found in banana spoiled fruit as shown in table no.01. Morphological identification of bacteria and fungi based on morphological characteristics like shape, texture and color of bacterial, fungal isolated colony were further analyzed. The bacterial isolates were considered as belonging to the same group or genus if their morphological characteristics matched the morphological descriptions previously described or reported. Morphological characteristics of the isolated bacteria are given listed in Table 1. Bacteria labeled as b1, b2,b3, b4,b5,b6 and fungi f1, f2 were isolated from selected fruits , details were tabulated in figure no.04 which elaborates color of colony, cell shape, arrangement, gram staining and Motility of bacteria and fungi. IMViC test are generally employed in the identification or differentiation of the Enterobacteriaceae members of family Enterobacteriaceae. Based on biochemical characteristic, isolated bacteria from three types of spoiled fruits were identified as Bacillus spp.,Strept spp., Staphylococcus spp., Klebsiella spp. Escherichia, Aspergillus spp., Rhizoctonia spp. Etc. Findings in this study suggested support the requirement of safety and hygienic handling process of fruits / vegetables as well as good efficient preservation method to reduce the growth pathogenic microorganisms. Besides, street vended fruits must be educated about food safety and hygiene practices to ensure the quality and safety of the fruits to consumers and also furture plan for development of effective procedure for detection and prevention of such spoilage.

Fig.No.01: Bruised spot on *Solanumlycopersicum, Musa paradisiacal fruit ,Manilkarazapota*

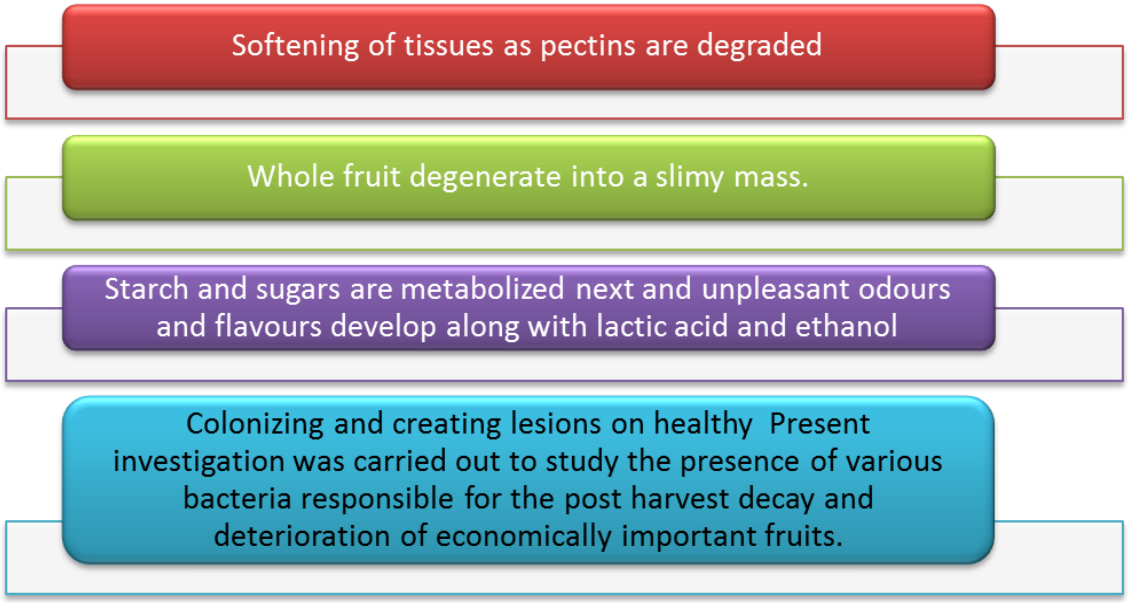

Fig.No.02: Bacterial spoilage characteristics

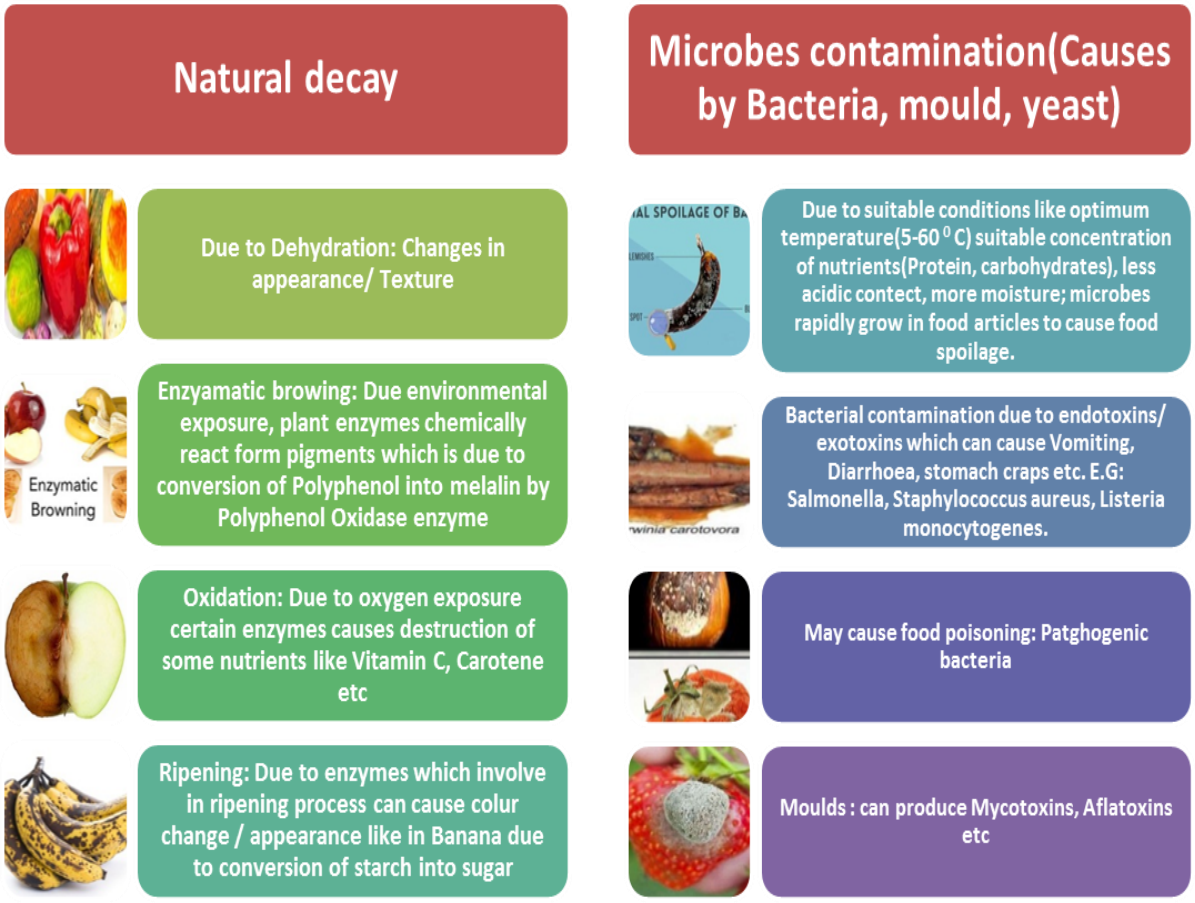

Fig.No.03: Divers spoilage process and their cause

1.Study area and sampling:	• Collection of unwashed and unprocessed Fruits and vegetables from local market
2. Sample Preparation:	• 10g of each sample was taken, then blended by sterile mortal and pestle with 90 ml normal saline (pH 7.8) to form suspension
3. Dilutions:	• Suspension was diluted up to 10-5
4. Total viable bacterial count , total fungal load and total coliform count :	• The enumeration was performed by spreading 0.1 ml sample from the 10-5 dilution onto nutrient agar (NA) and 0.1 ml from the 10-3 dilution onto Sabouraud Dextrose Agar (SDA) for the determination of total viable bacterial count (TVBC) and total fungal load, respectively were prepared in triplicates. • Plates were incubated at 37oC for 24 hours for bacteria and at 25oC for 48 hours for fungal load respectively.
5. Morphologically Identification and isolation:	• Different colonies of bacteria were isolated and subcultured and were maintained ,stored on NA slants at 4^0C.Isolated bacteria and fungi details were mention in Fig No:04
6. Detection of total coliform count:	• 0.1 ml sample from the dilution 10-3 was spread onto Mac-Conkey agar media and incubated at 37 oC for 24 hours.
7. Identification:	• On the basis of morphological and biochemical characteristics

Fig.No.04: Methodology of study[6,7,8,9]

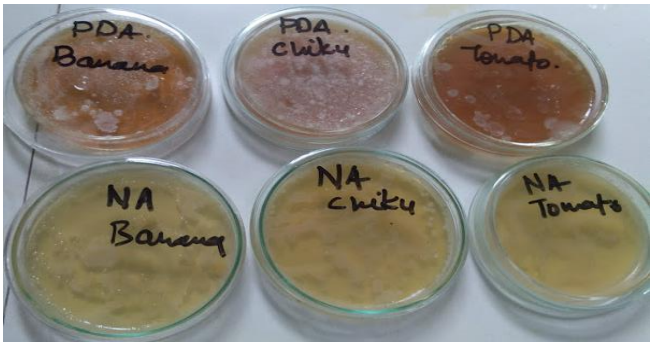

Fig.No.05: Initial Isolation of Bacterial and Fungal species from selected fruits Enumeration of coliform

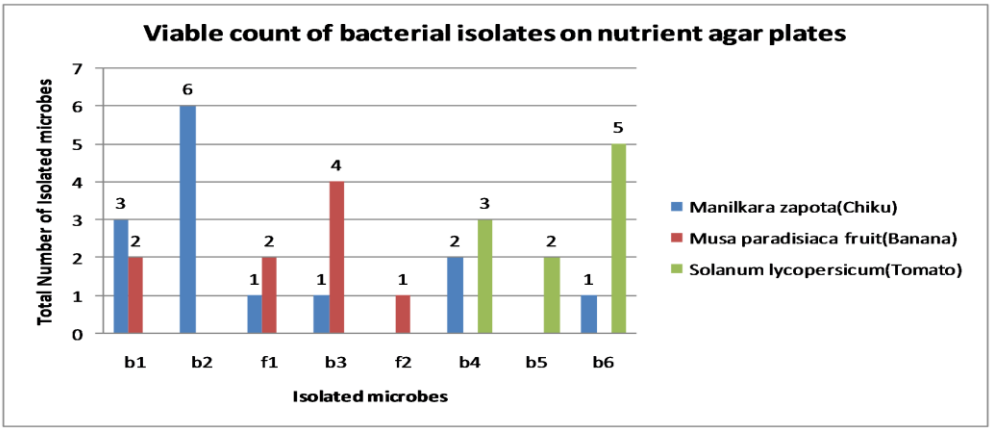

Fig No.06: Viable count of bacterial isolates on nutrient agar plates

Table No1: Cultural and morphological characteristics of bacteria/ fungi isolated from different spoiled fruits.

[*b= bacteria, f=fungi, += positive result, - = negative result]

Sr. No	Isolation Code No	Colony characteristics	Observed shape by staining techniques	Gram reaction	Motility test
1	b1	Large, opaque, raised, irregular surface, margined, non-pigmented	Rod	Gram positive	Non motile/motile
2	b2	Gray , mucoid, while	Round, chain arrangement	Gram Positive	Non motile
3	b3	Dome shaped , opaque, white colony	Round	Gram positive	Non motile
4	b4	Round, white translucent, large, unbonate, undulate, muciod		Gram negative	Non motile
5	b5	Off-white or beige in color with a shiny texture	Rod	Gram Negative	Motile
6	b6	Smooth, shiny,convex	Round	Gram Negative	Motile
7	f1	Dark, brown conidia(Reverse colour: colorless to light yellow) 53-69 mm,	Globus vesicles	-	Non motile
8	f2	White and cream color isolate turns to brown	Round	-	Non motile

The food associated bacteria isolated from various samples of different spoiled fruits identified on the basis of cultural, morphological and biochemical characteristics are mentioned in Table No.02.

Table No.02: Biochemical Tests / IMViC test of isolated Bacteria and Fungi

Biochemical Tests	Isolation Code No							
	b1	b2	b3	b4	b5	b6	f1	f2
Starch hydrolysis	√	√	-		-	-	√	√
Casine test	√	√	√	√	√	-	√	√
Gelatin lequification test	√		-		-	-	√	
Methyl red test	-	√	-	-	√	√		
Citrate utilization test	-		√	-	-	√		√
Voges–Proskauer test	-	-	-	√	-	-		
Sugar fermentation test	-	√	√	√	√	√		√
Name of bacteria/Fungi	Bacillus spp.	*Streptspp*	Staphylococcus spp	Klebsiellaspp	Escherichia spp	Proteus spp	*Aspergillus spp*	*Rhizoctoniaspp*

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