

### PREVALENCE OF BACTERIAL AND FUNGAL PATHOGENS IN FRUITS AND PICKLES



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#### ABSTRACT

Fruits contain high levels of sugars, nutrients and they possess an ideal water activity for the microbial growth. The presence of nutrients and their low pH makes them to microbial spoilage particularly fungal spoilage. Some bacteria and fungi are plant pathogens and can start the spoilage from the field. Others can cause spoilage by various external sources such as air and water. In this present study, an attempt was made to isolate and identify the bacterial and fungal isolates from the fruit juices and pickle samples. The bacterial population was very high in fruit juices and low in pickle samples. Fungal population was high in pickles and low in fruit juices. Six different bacteria were isolated from the fruit juices and two bacteria were isolated from the pickle samples. Based on

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preliminary tests, plating on selective media and biochemical tests, the isolated bacterial colonies were identified as *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. Two different bacterial isolates were identified from the pickle samples viz., *Bacillus cereus* and *Proteus mirabilis*. Based on Lactophenol Cotton Blue Staining and Colony morphology on Sabouraud's Dextrose Agar, the fungi from fruit juice was identified as *Penicillium expansum*. Three different fungi viz., *Mucor racemosus*, *Fusarium oxysporum* and *Rhizopus stolonifer* were isolated from the pickles samples.

**KEY WORDS:** Fruit juice, Pickles, Spoilage, Bacteria and Fungi.

## 1. INTRODUCTION

Fruits are vital to our health and well being, as they are furnished with essential vitamins, minerals, fibers and other health - promoting phytochemicals. Fresh fruits have an external toughness, may be water proof, wax - coated protective covering or skin that functions as barrier for entry of most plant pathogenic microbes. The skin, however, harbours a variety of microbes and so the normal microflora of fruits is varied and includes both bacteria and fungi (Hanklin and Lacy, 1992). The microbes are restrained to remain outside on fruit surface as long as the skins are healthy and intact. Any cuts or bruises that appear during the postharvest processing operations allow their entry to the less protected internal soft tissue. Acidic fruit juices have also been implicated in outbreaks of gastroenteritis. Unpasteurized apple cider and apple juice were associated with outbreaks of *Salmonella typhimurium* and *Escherichia coli* (Parish, 2000).

The growth of filamentous fungi in foods and food products results in waste and is costly as well as sometimes hazardous. Many different fungal species can spoil food products or produce mycotoxins or both. Biocontrol, an alternative to synthetic fungicides, employs antagonistic microorganisms that protect fruits and vegetables from infection of the pathogens (Conway et al., 2000). In most preserved foods, microbial growth causes undesirable changes in the odor, colour, taste, texture, or appearance of the food. In most cases, ingredients of pickles act as carriers of microbial contaminants (Rhyall and Pentzer, 1974). The utilization of poor quality vegetables, fruits and spices also influences the spoilage intensity in the preserved food, particularly in the homemade pickles. Spices are often the source of high microbial numbers (Byran, 1974).

## 2. MATERIALS AND METHODS

### 2.1. Collection of samples

The fruit juices and pickles samples were collected from the local shops of Chidambaram, Cuddalore district, Tamil Nadu, India for the isolation and identification of bacterial and fungal species. The collected samples were stored in the refrigerator for further microbial isolation.

### 2.2. Isolation of bacterial and fungal species

The bacterial and fungal species were isolated by Serial dilution technique. One ml of fruit juices were aseptically transferred into 99 ml of sterile distilled water and one gram pickles were transferred into 100 ml of sterile distilled water and shaken well. The dilutions were made into  $10^{-1}$  and the samples were diluted upto  $10^{-6}$ . Pour plate technique was used to isolate bacterial and fungal isolates in Nutrient agar and Rose Bengal agar respectively. The inoculated plates were incubated at 37 °C for 24 hours (in Nutrient agar for bacteria) and room temperature for 3 days (in Rose Bengal agar for fungi). The bacterial and fungal species were enumerated by using Qubec colony counter and expressed as cfu/ml. The isolated colonies from Nutrient agar plates and Rose Bengal agar plates were sub-cultured into agar

slants and stored at 4 °C for further identification.

### 2.3. Identification of bacterial and fungal isolates

Identification of the different bacterial isolates was carried out by the routine bacteriological methods i.e., By the colony morphology, Preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Plating on selective media and by performing biochemical tests. Identification of purified fungal cultures were characterized by their morphology, hyphal characteristics, presence or absence of asexual spores, arrangement of conidia and reproductive structures (Alexopoulos and Mims, 1979, Beisher, 1991) and by performing Lactophenol cotton blue mount.

## 3. RESULT AND DISCUSSION

Microorganisms forms the part of the epiphytic flora of fruits and vegetables and many will be present at the time of consumption. The majority of bacteria found on the surface of plants is usually Gram negative and belong either to the *Pseudomonas* group or to the Enterobacteriaceae (Lund, 1992). Samples of spoiled mixed pickles in oil collected from several locations showed high water activity and pH, which is promising for fungal contamination (Khan et al., 2005). In this present study, the pathogenic bacteria and fungi were isolated from fruit juices and pickles collected from Chidambaram local shops. Six bacterial isolates and two fungal isolates were identified by Pour plate technique. The total bacterial and fungal population present in the fruit juices and pickles samples was estimated and the results were showed in Table-1. The bacterial population was very high in fruit juices and low in pickle samples. Fungal population was high in pickles and low in fruit juices.

*Staphylococcus aureus* is a dangerous pathogen and one of the most common causative agents of hospital infections (nosocomial infections) in human beings. Surface of vegetables may be contaminated by this organism through human handling and other environmental factors and can be able to survive for several weeks (Erkan *et al.*, 2008). Human skin and nasal cavity is the main reservoir of *Staphylococcus* sp. Contamination of food stuffs during distribution and handling may allow bacterial growth and subsequently production of toxins which may represent a potential risk to humans (Erkan *et al.*, 2008). In addition, vegetables and fruits have low buffering capacity, since the use of small amount of acid also lowers the pH significantly which provide promising growth condition for spoilage fungi. Similarly, all the samples have  $a_w$  0.81 to 0.938 while  $a_w$  should be less than 0.81 in order to preserve the oil pickles for a longer period (Bone, 1973). Six different bacteria were isolated from the fruit juices and two bacteria were isolated from the pickle samples. Based on preliminary tests, plating on selective media and biochemical tests, they were identified as *Pseudomonas aeruginosa* (Apple juice, Mango juice and Grape juice), *Salmonella typhi* (Apple juice, Pine apple juice and Orange juice), *Staphylococcus aureus* (Mango juice, Pine apple juice and Orange juice), *Escherichia coli* (Apple juice, Grape juice, Mango juice, Pine apple juice and Orange juice) and *Klebsiella pneumoniae* (Grape juice, Mango juice and Orange juice). Two different bacterial isolates were identified from the pickle samples viz., *Bacillus cereus* (Mango pickle and Lemon pickle) and *Proteus mirabilis* (Garlic pickle and Lemon pickle). The characteristics of the isolated bacterial isolates were given in the Table - 2 to Table - 8.

Samples of spoiled mixed pickles in oil collected from several locations showed high water activity and pH, which is promising for fungal contamination. Various filamentous fungi viz., *Aspergillus*, *Penicillium*, *Alternaria*, *Rhizopus* and *Mucor* were isolated. These isolations were able to produced lipolytic enzymes, which are responsible for the spoilage and rancidity of oil pickles. The lipolytic activity exhibited by spoilage fungal strains of pickles varied in the order of *Penicillium* > *Rhizopus* >

*Aspergillus* > *Mucor* (Khan et al., 2005). Generally, oil pickles containing fruits are spoiled by *Penicillium italicum*, *Penicillium digitatum* and *Penicillium expansum*. Berries are predominantly carriers of *Botrytis cinerea* and *Mucor mucedo*. When carrots, cabbage, cucumbers and peppers are in pickles, various species of *Aspergillus*, *Rhizopus* and *Alternaria* become dominant (Walbeck, 1973). In the present study, only one fungi was isolated from the fruit juices. Based on Lactophenol cotton blue staining and colony morphology on Sabouraud's dextrose agar, it was identified as *Penicillium expansum* (Apple juice, Grape juice, Mango juice, Pine apple juice and Orange juice). Three different fungi were isolated from the pickles samples. They are *Mucor racemosus* (Mango pickle), *Fusarium oxysporum* (Lemon pickle) and *Rhizopus stolonifer* (Garlic pickle) (Table - 9).

**Table - 1: Total bacterial and fungal population present in fruit juices and pickle samples**

Sample	Fungal population (cfu/g)		Bacterial population (cfu/g)	
	$10^{-3}$	$10^{-4}$	$10^{-5}$	$10^{-6}$
Apple juice	18	16	29	26
Grape juice	17	14	31	25
Mango juice	20	13	30	24
Pine apple juice	16	14	33	26
Orange juice	11	17	30	27
Mango pickles	25	22	18	15
Lemon pickles	23	19	15	12
Garlic pickles	21	17	14	10

**Table - 2: Characteristics of *Staphylococcus aureus***

Test	Results
Gram staining	Gram positive cocci, arranged in clusters.
Endospore	No spores present
Motility	Non-motile
Catalase	Negative
Oxidase	Negative
Nutrient agar	Colonies are smooth and golden yellow
MacConkey agar	Lactose fermenting colonies.
Glucose fermentation	Acid produced
Mannitol fermentation	Acid produced
Sucrose fermentation	Acid produced
Dextrose fermentation	Acid produced
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization	Positive
Coagulase	Positive
DNAase	Positive
Mannitol salt agar	Golden yellow colonies
TSI medium	No reaction
Urease	Negative

**Table - 3: Characteristics of *Pseudomonas aeruginosa***

Test	Results
Gram staining	Gram negative slender rods
Motility	Actively motile
Catalase	Positive
Oxidase	Positive
Nutrient agar	Blue coloured diffusible pigment producing colonies
MacConkey agar	Non-lactose fermenting colonies
Glucose fermentation	Not fermented
Mannitol fermentation	Not fermented
Dextrose fermentation	Not fermented
Sucrose fermentation	Not fermented
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Negative
Citrate utilization	Positive
Urease	Positive
TSI	Alkaline butt, alkaline slant. No H <sub>2</sub> S and No gas production
O-F test	Oxidative
Casein hydrolysis	Positive

**Table - 4: Characteristics of *Escherichia coli***

Test	Results
Gram staining	Gram negative straight rods
Motility	Motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Circular, smooth and colourless colonies
MacConkey agar	Smooth, gloosy and pink coloured lactose fermenting colonies
EMB agar	Small colonies with greenish metallic sheen
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid gas produced
Sucrose fermentation	Acid gas produced
Mannitol fermentation	Acid gas produced
Indole	Positive
Methyl Red Test	Positive
Voges Proskauer Test	Negative
Citrate utilization	Negative
Urease	Negative
TSI	Acid butt, alkaline slant, No H <sub>2</sub> S and gas produced



**Table - 5: Characteristics of *Salmonella typhi***

Test	Results
Gram staining	Gram negative rods
Motility	Motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Circular, moist, smooth, translucent colonies
MacConkey agar	Non lactose fermenting colonies
SSA agar	Small black colonies
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid and gas produced
Sucrose fermentation	Acid and gas produced
Mannitol fermentation	Acid and gas produced
Indole	Negative
Methyl Red Test	Positive
Voges Proskauer Test	Negative
Citrate utilization	Positive
Urease	Negative
TSI	Acid butt, alkaline slant, H <sub>2</sub> S and gas produced

**Table - 6: Characteristics of *Klebsiella pneumoniae***

Test	Results
Gram staining	Gram negative rods
Capsule staining	Capsules present
Motility	Non-motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Large, greyish white, dome, shaped and mucoid colonies of varying degrees of stickiness.
MacConkey agar	Pink coloured lactose fermenting colonies
Glucose fermentation	Acid and gas produced
Lactose fermentation	Acid produced
Sucrose fermentation	Acid produced
Mannitol fermentation	Acid produced
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization	Positive
Urease	Positive
TSI	Acid butt, alkaline slant, No H <sub>2</sub> S and gas produced

**Table - 7: Characteristics of *Proteus mirabilis***

Test	Results
Gram staining	Gram negative rods
Motility	Motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Swarming motility characterized by its fishy odour
MacConkey agar	Non-lactose fermenting colonies
Glucose fermentation	Acid and gas produced
Mannitol fermentation	Not fermented
Dextrose fermentation	Not fermented
Sucrose fermentation	Not fermented
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Negative
Citrate utilization	Positive
Urease	Positive
TSI	Acid butt, alkaline slant, H <sub>2</sub> S produced and gas producers
Phenylalanine deaminase test	Positive

**Table - 8: Characterization of *Bacillus cereus***

Test	Results
Gram staining	Gram positive, thick, short rods.
Endospore	Central spores present
Motility	Non-motile
Catalase	Positive
Oxidase	Negative
Nutrient agar	Large, circular, white, adherent, colonies, with membranous growth
MacConkey agar	Non-lactose fermenting colonies
Glucose fermentation	Acid produced
Mannitol fermentation	Acid produced
Sucrose fermentation	Not fermented
Dextrose fermentation	Not fermented
Indole	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization	Positive
O-F test	Positive
Nitrate reduction	Positive
Gelatin hydrolysis	Positive
Starch hydrolysis	Positive
Urease	Negative

**Table – 9: Colony morphology of fungi isolated from fruit juices and pickles**

Microscopic examination	Colony morphology on SDA plate
<b><i>Penicillium expansum</i></b>	
Septate hyphae with branched or unbranched conidiophores which have secondary branches known as medulla. On the medulla flask shaped sterigmata are arranged that bear unbranched chains of round conidia. Entire structure forms a brush border.	Colony surface at first appears white then becoming powdery bluish green with a white border. Some species differ in gross appearance. Reverse side was white.
<b><i>Mucor racemosus</i></b>	
Sporangiophores are long, often branched and bear terminal round sporangia filled with spores. Hyphae are non-septate and no rhizoids.	Colonies quickly covers the agar surface with white fluffy mycelia but later turns grey, reverse side is white.
<b><i>Fusarium oxysporum</i></b>	
Conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clusters. Macroconidia fusiform, slightly curved, pointed at the tip, basal cells pedicellate. Macroconidia abundant, never in chains, mostly non-septate, ellipsoidal to cylindrical, straight or often curved. Chlamyospores terminal or intercalary, haline, smooth or rough walled.	Colonies grow rapidly; aerial mycelium white, usually becoming purple; discrete, erumpent, orange sporodochia are present in some strains; reverse hyaline to dark blue or dark purple.
<b><i>Rhizopus stolonifer</i></b>	
Sporangiophores are long and usually branched. Sporangiohores originating just above rhizoids. Thick walled chlamyospores may form. Hyphae may appear distorted with swollen cell or compressed, twisted and folded-rhizoids are present.	Colonies are usually white to cream to gray – pigmented.

## 5. CONCLUSION

From this present study, it was concluded that the fruit juices and pickle samples collected from local shops harbor the pathogenic bacteria (*Pseudomonas aeruginosa*, *Pseudomonas fluorescence*, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Proteus mirabilis*) and fungi (*Penicillium chrysogenum*, *Mucor racemosus*, *Fusarium oxysporum* and *Rhizopus stolonifer*) which cause harmful diseases to human beings. It is must to prevent the spread of disease causing microorganisms from fruit juices and pickle samples.

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