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## ISOLATION AND IDENTIFICATION OF MICROORGANISMS FROM SOME SPOILED FRUITS AND VEGETABLES COLLECTED FROM NAGAON, ASSAM

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### ABSTRACT

Fruits and vegetables provide sufficient condition for the growth of several fungi and bacteria. Microorganisms spoil the fruits and vegetables and changes the texture and makes them uneatable. There is a heavy loss of fruits and vegetables due to spoilage by microorganisms; however during handling and transportation also, there is a greater risk to get damaged. Thus farmers suffered a great loss due to spoilage. In the present study a total of about 3 spoiled fruits and 3 spoiled vegetables were taken from some local markets of Nagaon, from which fungal and bacterial isolates were isolated and identified. Biochemical tests for the isolated bacteria were also done.

**Keywords:** Contaminate, spoilage, fungi, bacteria and surface sterilized

### INTRODUCTION

Fruits and Vegetables are rich source of vitamins, minerals and fibers and thus are essential to sustain a healthy lifestyle. They are also a rich source of antioxidants thus are essential to protect us from various diseases (Kalia and Gupta, 2006). Vegetables are consumed on a daily basis and their consumption is also key area for maintaining a balanced diet. One of the major risks for the preservation of fruits and vegetables is that they have a very short shelf life and they are exposed to the microbial contamination if contacted through soil, dust and water. Thus preservation of fruits and vegetables are very much important for consumption for a longer duration.

Many countries are engaged in the production of fruits and vegetables to a larger extent. India being an agricultural rich country produces fruits and vegetables in a huge scale. India is the second largest producer of fruits and vegetables after China. According to Kumar (2011), Food and agricultural organization (FAO) data shows that India has produced about 76424.2 tons of fruits, 156325.5 thousand tons of vegetables and 388269.2 tons of food grains in 2011. Even due to enormous production of fruits and vegetables, during transportation, handling, storage heavy losses of fruits and vegetables are reported (Chukwuka *et al.*, 2010, Zubbair, 2009, Barth *et al.*, 2013).

The differences in the presence or absence of microorganisms could be due to various factors which can be due to resident microflora in the soil, presence of any non-resident microflora through animal manures (Khatri and Sharma, 2018). Microorganisms spoil them and thus leads to a great loss to the Mankind. Spoilage

leads to change in texture, taste and which can't be eatable (Akinmusire, 2011). The present study aims to isolate and identify microorganisms from some spoiled fruits and vegetables collected from Nagaon, Assam.

### MATERIALS AND METHODS

Some unprocessed spoiled fruits like apple, banana and grapes and vegetables like potato, tomato and onion were collected from three different markets of Nagaon and are directly brought to the Botany laboratory for the isolation and identification of microorganisms which are responsible for their spoilage.

#### Isolation of fungi

Isolation of fungi from the spoiled fruits and vegetables were done following standard protocols in which they were firstly washed with sterile distilled water and ultimately surface sterilized with 1% Ca(OCl)<sub>2</sub> and with the help of sterilized sharp razor, tissues adjacent to the diseased portion were cut and placed into PDA media which was supplemented with penicillin (100000 units/L) and streptomycin (0.2 g/L). The plates with the sterilized tissues were incubated for a week at 26±2°C until the growth of the fungi was not observed. The fungi growing out from the adjacent tissues were identified following standard identification manuals (Barnett and Hunter, 1988; Gilman, 1957).

#### Isolation of bacteria

Isolation and identification of bacteria from the spoiled fruits and vegetables collected from three different sites of Nagaon were done following serial dilution method

**Table 1-** Table representing occurrence of different isolates of fungi from spoiled fruits and vegetable samples

Isolates	Colony morphology	Apple	Banana	Grapes	Potato	Tomato	Onion	Frequency
<i>Aspergillus</i> sp.1	Black mold like structure	+	-	+	-	+	-	3(20)
<i>Aspergillus</i> sp.2	Purplish colony	-	+	-	+	+	-	3(20)
<i>Penicillium</i> sp.1	White smooth colonies	+	+	-	+	+	-	4(26.6)
<i>Penicillium</i> sp.2	Green smooth colonies	+	+	+	-	-	-	5(33.3)
<i>Monilinia</i> sp.	Brown colony	-	+	-	+	+	+	4(26.6)
<i>Phytophthora</i> sp.	Greyish black colonies	+	-	-	+	+	-	3(20)
<i>Sclerotinia</i> sp.	Greyish white colonies	+	-	-	+	+	+	4(26.6)
<i>Rhizopus</i> sp.	Dark greyish brown colonies	-	+	+	+	+	+	5(33.3)
<i>Fusarium</i> sp.1	White violet colonies	-	+	-	+	-	-	2(13.3)
<i>Fusarium</i> sp.2	Peach colonies	+	-	-	-	-	-	1(7.69)
<i>Mucor</i> sp.	Cottony blackish colony	+	-	-	+	+	-	3(20)
<i>Plasmopara viticola</i>	White colonies	+	-	+	-	-	-	2(13.3)
<i>Botrytis</i> sp.	White cottony colonies	-	+	+	-	-	-	2(13.3)
<i>Phoma</i> sp.	White colony	-	-	-	+	-	-	1(6.66)
<i>Bipolaris</i> sp.	Light greyish colony	+	+	-	-	+	+	4(26.6)
Total	15							

\*+ represents presence of fungi and – represents absence of fungi

(Aneja, 2009). All the collected samples of fruits and vegetables were firstly crushed in mortar and pestle which was sterilized earlier and a suspension from them was prepared by adding distilled water upto 100 ml, which were diluted serially to  $10^{-1}$  to  $10^{-5}$  dilutions. From each of the dilution about 100 $\mu$ L were taken and streaked all over on nutrient agar medium (NAM) plates which were already supplemented with amphotericin B (10 $\mu$ g/mL) to prevent any contamination from fungi. The plates were then incubated at 37°C for about 24 hours until the growth of bacteria was not observed. The grown colonies were then subcultured on NAM slants at 4°C for further use (Chaudhary and Dhaka, 2016).

The bacterial isolates were identified following the Manual of Systematic Bacteriology (Claus and Berkeley, 1986). The morphology of the isolated bacteria were examined following Gram's staining and on the basis of some biochemical test viz., Methyl Red and Catalase test (Cappuccino and Sherman, 2005).

## RESULTS AND DISCUSSION

### Isolation of fungi

In the present study, a total of about 15 different fungal isolates were isolated from spoiled fruits and vegetables. All the fungal isolates were identified based on their colony characters and morphology. Table1 represents the fungal isolates isolated from the spoiled fruits and vegetable.

### Isolation of bacteria

Unlike fungal isolates, a total of about 6 isolates were isolated from spoiled fruits and vegetable samples.6 bacterial isolates were isolated (Table 2). The biochemical test results were also conducted (Table 3).

*Penicillium* sp.2 and *Rhizopus* sp. were found to be dominant among all the fungi. The occurrence of these

**Table 2-** Table representing occurrence of different isolates of Bacteria from spoiled fruits and vegetable

Bacterial isolates	Colony morphology	Apple	Banana	Grapes	Potato	Tomato	Onion	Frequency
<i>Bacillus</i> sp.1	White dry colony	+	-	+	+	-	+	4(66.6)
<i>Bacillus</i> sp.2	White smooth colony	-	+	+	+	+	+	5(83.3)
<i>Staphylococcus</i> sp.1	Yellowish golden colony	-	-	-	-	+	-	1(16.6)
<i>Staphylococcus aureus</i>	Shiny off white colony	-	+	-	+	-	-	2(33.3)
<i>Escherichia coli</i>	White colony	+	+	+	-	+	+	5(83.3)
<i>Klebsiella</i> sp.	Light pinkish colony	-	-	-	-	+	-	1(16.6)
<b>Total</b>	<b>6</b>							

\*+ represents presence of bacteria and – represents absence of bacteria

**Table 3-** Identification of bacteria using biochemical tests and Gram staining

Bacterial isolates	Gram reaction	Biochemical tests	
	Gram staining	Methyl red test	Catalase test
<i>Bacillus</i> sp.1	Gram +ve	+	+
<i>Bacillus</i> sp.2	Gram +ve	+	-
<i>Staphylococcus aureus</i>	Gram +ve	+	-
<i>Staphylococcus</i> sp.	Gram -ve	+	-
<i>Escherichia coli</i>	Gram +ve	+	+
<i>Klebsiella</i> sp.	Gram -ve	+	+

+ represents 'presence' and – represents 'absence'

two fungi is particularly common in most of the spoiled fruits and vegetables. *Phoma* sp. and *Fusarium* sp. 2 were found to be least occurred fungi, in which *Phoma* sp. was found to be occurred in Potato while *Fusarium* sp. 2 occurred only in Apple. The dominant bacterial isolates were *Bacillus* sp.2 and *Escherichia coli*.

*Pseudomonas* sp. and *Bacillus* sp. were dominant bacterial isolates collected from spoiled fruits and vegetable collected from local and supermarket (Raja *et al.*, 2012). According to Chaudhary and Dhaka (2016) *Bacillus* sp. was dominant in spoiled fruits and vegetable collected from local market. Occurrence of *Staphylococcus aureus*, a pathogenic organism in most of the spoiled fruits and vegetables is of major concern for public health. Similarly the occurrence of some bacteria like *Klebsiella* sp., in spoiled fruits and vegetables highlighted the need to safe the humankind (Eni *et al.*, 2010).

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