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ISOLATION AND IDENTIFICATION OF FUNGI AND BACTERIA FROM SOME SPOILED FRUITS AND VEGETABLES

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ABSTRACT

Fruits and vegetables provide an ideal condition for the growth of microorganisms. They contaminate fruits and vegetable and made them insufficient for consumption. Many fungal and bacterial species are responsible for the spoilage of fruits and vegetables. In our present study, an investigation has been carried out to isolate and identify the fungal and bacterial species which are responsible for the spoilage of some fruits and vegetable particularly apple, grapes and tomato. Samples of contaminated fruits and vegetables were collected from three different markets (Tezpur Bazaar, Mission Chariali Bazaar and Poruwa Bazaar) and were immediately surface sterilized and studied.10 fungal isolates were found to be most commonly occurring in all of the samples, Similarly many isolates of bacteria were found to be common contaminants from the three samples. Biochemical tests and viable bacterial counts (10^{-2})/g of sample was also calculated.

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

INTRODUCTION

Fresh fruits and vegetables are rich in vitamins, minerals and fibers and thus are very much important to sustain a healthy life. It has been reported that, a well balanced diet, rich in fresh fruits and vegetables helps us to protect from several diseases (Kalia and Gupta, 2006). India is the second largest producer of fruits and vegetables in the world after China. Food and agricultural organization (FAO) data shows that India has produced about 76424.2 tones of fruits, 156325.5 thousand tones of vegetables and 388269.2 tons of food grains in 2011(Kumar, 2011). However due to heavy losses in the field ,during handling ,storage ,transportation, it has led to great loss to the farmers (Chukwuka *et al*., 2010, Zubbair, 2009, Barth *et al*., 2013).

Fruits and vegetables provide ideal condition for the growth of microorganisms. Fruits and vegetables are widely exposed to microbial contamination when contact with soil, dust and water or during handling processes. Microorganisms particularly lead to the spoilage of fruits and vegetables. Spoilage is defined as the change in the texture, which are undesirable for consumption by the mankind (Akinmusire, 2011). These microorganisms colonize and create lesions on healthy and undamaged tissue (Tournas and Katsoudas, 2005). The present study was undertaken to investigate the presence of some bacteria and fungi responsible for the post harvest decay and deterioration of economically important fruits and vegetables.

MATERIALS AND METHODS

Unwashed and unprocessed spoiled fruits viz , apple and grapes and one vegetable tomato were collected from different local markets of Tezpur (Mahahairab Bazaar,Poruwa Bazaar and Mission Chariali Bazaar) and brought to the laboratory for further analysis.

Isolation of fungi

The spoiled fruits and vegetables were surface sterilized with the help of 1% $Ca(OCl)_2$ and tissues adjacent to the diseased tissues were cut with the help of sterilized sharp razor and inoculated in PDA media which were supplemented with penicillin (100000 units/L) and streptomycin (0.2 g/L).The plates were incubated for a week at 28°C (Fatima *et al.,* 2009).Fungi growing out from the tissues were identified following standard identification manuals.(Barnett and Hunter,1988;Gilmann,1957)

Isolation of bacteria

The isolation of bacteria from the spoiled fruits and vegetable was done using serial dilution agar plate method (Aneja, 2009). Fruits and vegetables samples were crushed in presterilised mortar and pestle to form a suspension by adding distilled water, which was serially diluted to 10^{-1} to 10^{-5} dilutions. 100µL from all the samples from each dilution was streaked all over on nutrient agar medium (NAM) plates which were already supplemented with amphotericin B (10µg/mL) to prevent fungal contamination. The plates with the inoculated samples were incubated at 37°C for about 24 hours after the completion of subsequent period of incubation, different colonies of bacteria were isolated and subcultured on NAM slants at 4°C for further use (Chaudhary and Dhaka,2016).

The isolates were identified referring morphological and biochemical characteristics on the basis of Manual of Systematic Bacteriology(Clauss and Berkeley, 1986).The morphology was examined following Gram's staining and bacterial isolates were identified on the basis of biochemical test viz., Methyl Red and

Table 1: Occurrence of fungi from fruits and vegetable samples

Fungal isolate	Media	Colony morphology	Frequency (%)	Samples		
				Apple	Grapes	Tomato
Mucor spp.	PDA	Blue-black mold, thread-like mycelium	3(21.4)	+	+	+
Aspergillus spp.	PDA	Black mold like appearance	2(14.2)	+	+	-
Penicillium spp.	PDA	Bluish green appearance	2(14.2)	+	+	-
Botrytis spp.	PDA	Grey mold like appearance	1(7.1)	+	-	-
Plasmopara viticola	PDA	Greyish powdery appearance	1(7.1)	+	-	-
Monilia spp.	PDA	Beads like appearance	1(7.1)	-	+	-
Eurotium spp.	PDA	Whitish colony	1(7.1)	-	+	-
Rhizopus spp.	PDA	Black mold like appearance	1(7.1)	-	-	+
Sclerotiana spp.	PDA	White mold like appearance	1(7.1)	-	-	+
Fusarium spp.	PDA	White cottony type appearance	1(7.1)	-	-	+
Total(10)			14(100)	5	5	4

*PDA-Potato Dextrose Agar, + represents 'presence of fungi',- represents 'absence of fungi'.

Table: 2-Occurrence of bacterial isolates from spoiled fruits and vegetable

Bacterial isolates	Media	Colony morphology	Frequency (%)	Samples		
				Apple	Grapes	Tomato
Bacillus sp.	NAM	White dry attached	3(33.3)	+	+	+
Escherichia coli	NAM	White flat smooth opaque	2(22.2)	+	+	-
Staphylococcus aureus	NAM	Golden yellow, large circular	1(11.1)	-	+	-
Staphylococcus sp.	NAM	Off white shiny and irregular	2(22.2)	+	-	+
Proteus vulgaris	NAM	Blue grey, spreading	1(11.1)	-	-	+
Total			5	9(100)		

*NAM-Nutrient Agar Media'+' -represent presence and '– 'represents absence

Table 3: Identification of bacteria using biochemical tests and Gram staining

Bacterial isolates	Gram reaction	Biochemical tests	
	Gram staining	Methyl red test	Catalase test
Bacillus sp.	+	+	+
Escherichia coli	+	+	+
Staphylococcus aureus	-	+	-
Staphylococcus sp.	-	+	-
Proteus vulgaris	+	+	+

+ represents 'presence' and – represents 'absence'

Catalase test (Cappuccino and Sherman, 2005).

RESULTS AND DISCUSSION

Isolation of fungi

From the present study, 10 fungal isolates were isolated from the spoiled fruits and vegetable. The fungal isolates were identified on the basis of morphology and colony characters. Table1 represents the fungal isolates isolated from the spoiled fruits and vegetable.

Isolation of bacteria

Bacterial isolates were isolated from the samples of spoiled fruits and vegetable. A total of about 5 isolates were found to be prominent in the samples (Table 2) .The biochemical test results were also conducted (Table 3)**.** Viable bacterial counts (10^{-2})/g of sample was also calculated (Fig 1).

Fungal isolate *Mucor* sp. was found to be dominant in all the samples. Other dominating fungal isolates *Aspergillus* sp. and *Penicillium* sp. were also found to be common in both Apples and Grapes. The dominant bacterial isolate was *Bacillus* spp.The other dominating bacterial isolates were *E.coli* and *Staphylococcus* sp.

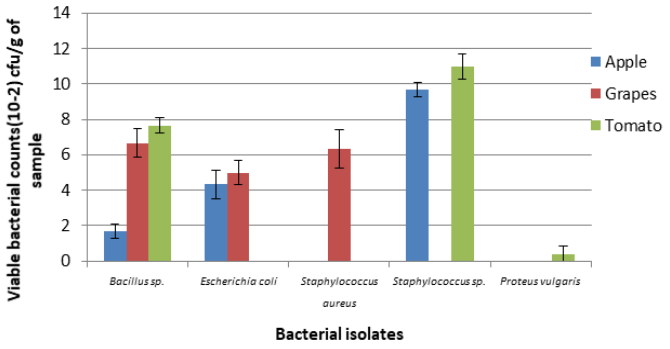

Fig 1: Viable count of bacteria from different spoiled fruits and vegetable (Data mean of three replicates±SE(n=3). Means were significantly different ($p<0.05$).

Raja *et al.,* (2012)reported *Pseudomonas* sp. and *Bacillus* sp. as dominant bacteria isolated from spoiled fruits and vegetable collected from local and supermarket. Chaudhary and Dhaka (2016) reported that Bacillus sp. was found to be dominant in spoiled fruits and vegetable. Tafuinta *et al.,* (2013) reported that *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* and *Rhizopus stonifer* were dominant fungi isolated from Sweet orange (Citrus sinensis) in Sokoto state.

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