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ISOLATION AND IDENTIFICATION OF FUNGI FROM SOME SPOILED VEGETABLES

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Abstract

An investigation was carried to study the different fungal sp. present in various spoiled vegetables. Twelve fungi, which caused spoilage of vegetables were isolated from various samples of different spoiled vegetables by serial dilution agar plating method and enumerated. The isolated fungi were identified as *Rhizopus stolonifer*, *Aspergillus niger*, *Fusarium oxysporum*, *Saccharomyces* sp., *Aspergillus flavus*, *Penicillium chrysogenum*, *Aspergillus nidulans*, *Mucor* sp., *Alternaria solani*, *Aspergillus terreus*, *Penicillium oxalicum* and *Cladosporium oxysporum* on the basis of their macroscopic and microscopic characteristics. *Aspergillus* was the commonest genera found in all tested spoiled vegetable samples.

Key words: Spoiled vegetables, *Aspergillus* and Czapek Dox Agar (CDA).

Introduction

Food spoilage is a metabolic process that cause foods to be undesirable and unacceptable for human consumption due to changes in sensory characteristics such as visual, tactile, olfactory or flavour (Rawat, S. 2015). India is the second major producer of vegetables and ranks next to Brazil and China respectively, in the world. It contributes 14% of world vegetable production (Calvo *et al*., 2007). Vegetables are more prone to spoilage due to their nature and composition. The succulent nature of vegetables makes them easily invaded by microorganisms. These are excellent source of nutrients for spoilage organisms because of their neutral pH and high water activity (Tournas, 2005). In vegetables, naturally the source of microbial infection is from the field soil during harvesting and post harvest handling like water used for cleaning the surface, contact with equipments, containers, storage environment, transportation and can include some pathogens. In peak seasons due to improper handling practices, marketing, storage problems around 20-25% vegetable are spoilt in various stages. Bacterial spoilage first causes softening of tissues as pectins are degraded and the whole vegetables may eventually degenerate resulting in a slimy mass. In case of mould spoilage a furry growth covers the food and it becomes soft and often smells bad (Rawat, 2015). Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas and Katsoudas, 2005). Starches and sugars are metabolized next and unpleasant odours and flavours develop along with lactic acid and ethanol (Walker and Phillips, 2007).

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Materials and Methods

Seven types of various unwashed and unprocessed spoiled vegetables like, beans, cauliflower, lady's finger, parwal, peas, potato and tomato were collected in plastic zip bag from local market of Muzaffarnagar and brought in the laboratory for further analysis.

The fungi were isolated from spoiled vegetables by using serial dilution agar plate method (Aneja, 2009). The spoiled vegetables were crushed into mortar and pestle with distilled water to form suspension which was serially diluted from 10^{-1} to 10^{-10} dilutions. 100µL of food suspension from each higher dilution (10^{-6} to 10^{-10}) was spreaded over Czapek Dox Agar (CDA) plates. The CDA was supplemented with antibiotic chloramphenicol (100µg/mL) before pouring to prevent bacterial growth. The inoculated petriplates were incubated at 30^0C for 3 to 5 days for fungal growth. After incubation the

Table 1: Cultural and microscopic characteristics of fungi isolated fromdifferent spoiled vegetables.

Fungal isolate No.	Cultural characteristics	Reverse side of colony	Microscopic characteristics	Fungal species identified
VGf*1	Colonies light grey or dusty white in colour,fluffy, growing extreme rapidly filling the petriplate with and dense cottony mycelia and producing mass of sporangia with in 48 hrs of incubation	Hyaline	Aseptate,smooth walled,erect,columellate sporangiophores (15-30µm wide) arise in groups of 3-5 from stolons just opposite to the rhizoids, pale brown in colour;columella subglobose (70-120 µm in dia.) with some flattened base,pale brown in colour;sporangium (95-200 µm in dia.) colourless when young and black at maturity; sporangiospores subglobose (5.1-10×4.4-7.5 µm).	*Rhizopus stolonifer*
VGf*2	Colonies white becoming black on development of conidia, powdery	Hyaline	Septate and branched hyphae; conidiophores aseptate,smooth walled, long (2.5-3.0mm), thick (15-20 µm), hyaline or turning dark towards the vesicle,swollen at the apex in to a spherical vesicle; vesicle globose (15-50 µm in dia.), dark brown; conidial heads are biseriate with the phialides borne on brown, often septate metulae; phialids biseriate,flask shaped (8-10×3.0-3.5 µm); conidia catenate,rough walled,dry,globose (3.5-4.2µm dia),covering entire vesicle,blackish to brown in colour.	*Aspergillus niger*
VGf*3	Colonies initially white, becoming pink in colour on maturity, cottony, fast growing	Hyaline	Septate and branched hyphae;short, branched conidiophores bearing a whorl of phialids; phialids cylindrical;macroconidia hyaline, sickle shaped, multiseptate, fusiform (slightly curved and pointed at the tip);basal cells pedicellate (25-55×3.2-4.6µm). Microconidia hyaline, smaller than macroconidia,fusiform to ovoid.	*Fusarium oxysporum*
VGf*4	Colonies creamish in colour	Hyaline	Unicellular,multilateral budding,producing spherical spores (5-10 µm in dia.)in asci; cells globose to sub globose or ellipsoidal to cylindrical; ferment glucose and maltose while don't assimilate lactose.	*Saccharo-myces* sp.
VGf*5	Colonies yellowish green in colour, powdery	Yellow	Septate and branched hyphae;aseptate, highly branched,erect conidiophores arise separately from a foot cell,long (400-700µm) and thick (5-15µm);vesicle dome shaped (10-28µm in dia.) with primary and secondary phialids;phialids uniseriate (10-15×2-5µm);conidial heads radiating; conidia globose to subglobose (3.5-4.5µm in dia.), finely roughned to echinulate,typically radial, covering 3/4th vesicle.	*Aspergillus flavus*
VGf*6	Colonies bluish green in colour with a heap of white mycelium at center,fluffy, velvety	Reddish brown	Non septate hyphae,conidiophores smooth, hyaline, branched,relatively short (200-1000µm), brush like ending in phialids;phialids are aggre-gated in clusters of 5-7,ampuliform (7-11×2-3.2 µm) conidia smooth,ellipsoidal (3-5×3-5.5 µm), arranged in basipetal succession.	*Penicillium chrysogenum*
	Colonies dark green in		Septate hyphae,conidiophores smooth walled,short (50-60µm);vesicles hemispherical,	*Aspergillus*

Table 1 continued .....

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Fungal isolate No.	Cultural characteristics	Reverse side of colony	Microscopic characteristics	Fungal species identified
VGf*7	colour with brown tinch, powdery	Olive	small (7-10 µm in dia.)with metulae; conidial heads short, columnar (65-70×25-30µm in dia.) conidia globose (3.2-3.8 µm in dia.), rough, covering upper half of vesicle.	*nidulans*
VGf*8	Colonies white in colour	Whitish to brownish	Aseptate hyphae with right angle branching, columellate sporangiophores (5-20µm wide)arising singly from the aseptate mycelium,erect, without rhizoids;sporangia are spherical (60-250µm in dia.); columella of various shapes (20-82×15-50µm) branched, wall ruptured at maturity,; sporan-giospores smooth walled,globose to ellipsoidal (3-4.5µmin dia.) in shape.	*Mucor* sp.
VGf*9	Coloniesolivaceous black in colour	Hyaline	Conidiophores arising singly or in small groups up to 50-115µmlong,5-12µm thick;conidia usually singly,rarely in short chains,ellipsoidal (200-300 ×15-20µm),tapering to a beak (which may be longer than conidium),smooth,with 8-12 transverse and 2-5 longitudinal septa.	*Alternaria solani*
VGf*10	Colonies orange brown in colour,velvety	Yellow brown	Septate hyphae,conidiophores smooth, long (150-252µm),thin (3-6µm wide);vesicles subglobose (11-20µm in dia.)biseriate;conidia smooth,globose (1.4-2.7µm),covering upper 1/2-3/4 th vesicle.	*Aspergillus terreus*
VGf*11	Colonies ivy green in colour, velvety	Pale yellow	Conidiophores hyaline,rough walled,upto 150-200× 3.5-5.5µm;conidial fruitification consist of single, vertical of 2-5 branches (metulae)15-20×2.5-3.5µm; phialids cylindrical (12-15×2.5-4.0µm),tip tapering; conidia hyaline, elliptical (3.5-5.0×3-4µm),smooth walled and unicellular, arranged in basipetal succession.	*Penicillium oxalicum*
VGf*12	Colonies pale grey or greyish brown in colour,cottony	Olive green to brown	Conidiophores micronematous,straight(450-500 ×3.0-5.2 µm),with terminal and intercalary swellings, distinctly nodose,pale brown in colour,smooth;conidia smooth,subhyaline,cylindrical or ellipsoidal (10-32 ×2-5 µm), rounded at the apex, arise from terminal swellings.	*Clados-porium oxysporum*

VG-vegetable, f*-fungi.

morphologically different colonies of fungi were isolated and subcultured. The fungal isolates were maintained and stored on CDA slants at 4^0C for further use.

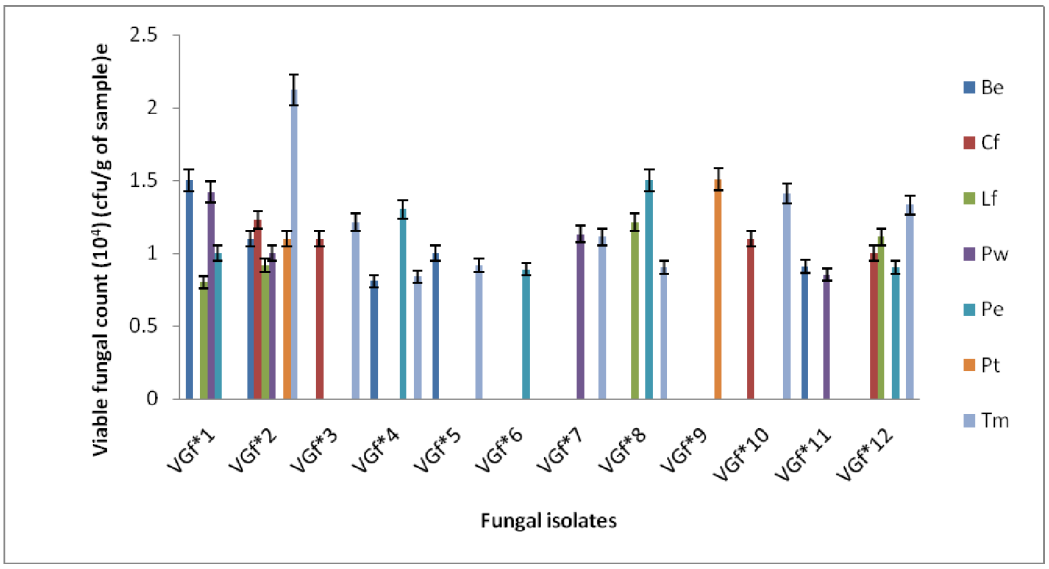
The fungal isolates were identified on the basis of colonial, cellular, morphological characteristics and micrometry. The fungal colonies identified on the basis of microscopic examination were purified. The cellular morphology of isolated fungi was studied by Lactophenol cotton blue staining (Cappuccino and Sherman, 2005).

Results and discussion

Twelve fungal species are isolated from various spoiled vegetables and enumerated. Figure 1 shows the viable count of fungal isolates (VGf*1-VGf*12) on CDA plates. Table 1 represents fungal species identified on the basis of reverse side of colony, cultural and microscopic characteristics. The fungal isolate VGf*12 was present in all tested spoiled vegetables and VGf*9 was isolated from spoiled potato only. The other dominating fungal isolate was VGf*2.

The food associated fungi isolated from various samples of different spoiled vegetables identified on the basis of their macroscopic and microscopic characteristics are *Rhizopus stolonifer* (VGf*1), *Aspergillus niger* (VGf*2), *Fusarium oxysporum* (VGf*3), *Saccharomyces* sp. (VGf*4), *Aspergillus flavus*

Fig. 1: Viable count (cfu/g) of fungi isolated from different spoiled vegetables.



VG-vegetable, f*-fungi,Be-beans, Cf-cauliflower, Lf-ladyfinger, Pw-parwal, Pe-peas, Pt-potato, Tm-tomato and cfu/g-colony forming unit per gram.

(VGf*5), *Penicillium chrysogenum* (VGf*6), *Aspergillus nidulans* (VGf*7), *Mucor* sp. (VGf*8), *Alternaria solani* (VGf*9), *Aspergillus terreus* (VGf*10), *Penicillium oxalicum* (VGf*11) and *Cladosporium oxysporum* (VGf*12). The dominant members of the fungi isolated from spoiled vegetables belong to the genera *Aspergillus*. Some of the above fungi were isolated by other workers also.

Dijksterhuis *et al.*, 2013 found that *Alternaria alternata, Aspergillus flavus, Aspergillus ochraceus, Aspergillus parasiticus, Fusarium proliferatum* and *Penicillium citrinum* are involved in spoilage of Beans and peas. Onuorah and Orji (2015) isolated *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Saccharomyces cerevisiae, Alternaria alternata, Penicillium digitatum* and *Geotrichum candidum* from spoiled tomatoes. Etebu *et al.*, 2013 reported that fungi affecting tomatoes include *Aspergillus phoenicis, Absidia* sp., *Trichoderma* sp., *Alternaria alternata, Fusarium oxysporum, Fusarium moniliformis, Aspergillius niger, Mucor* sp., *Rhizopus stolonifer, Penicillium* sp., *Geotrichum* sp. and *Phytophthora* sp. Wogu and Ofuase (2014) isolated *Aspegillius* sp., *Penicillum* sp., *Fusarium* sp. and *Saccharomyces* sp. from spoilt tomato fruits. Mbajiuka and Enya (2014) also isolated *Aspergillius* sp., *Penicillum* sp. and *Saccharomyces cerevisiae* from spoilt tomatoes. Oyemaechi *et al.* (2014) observed 6 species of fungi *Candidatropicalis, Penicillium notatum, Aspergillus niger, Fusarium oxysporum, Absidia corynbifera, Rhizopus stonolifer*in spoilt tomatoes.

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