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DETECTION AND IDENTIFICATION OF QUARANTINE BACTERIA AND FUNGI ASSOCIATED WITH IMPORTED AND LOCAL POTATO SEED TUBERS

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Abstract

Potato, one of the most important vegetable crops, is feeding the growing global population. It has an enormous economic outlet however; fungal and bacterial pathogens associated with seed tuber cause huge losses in its production. Planting healthy seed potatoes is a key factor in maximizing the production of potatoes for consumption or processing. In this study, the presence of different pathogenic fungi and two pathogenic bacteria (*Clavibacter michiganensis* subsp. *sepedonicus* that cause ring rot of potato and *Salmonella* sp., (a notorious human pathogenic bacterium) in imported seed potato varieties (Asterix, Ultra and Karuda) and four local varieties (Asterix, Lady Rusetta, Qasur and Mozika) were determined. Among highly plant pathogenic fungi, *Fusarium solani* (8-23%) and *Rhizoctonia solani* (9-13%) were associated with imported tubers, while *Fusarium oxysporum* (12-20%) and, *R. solani* (8-17%) were isolated from local seed tubers. *Clavibacter michiganensis* subsp. *sepedonicus* and *Salmonella* spp., were detected in all potato tubers examined. Association of highly pathogenic fungi and bacteria with seed tubers is a serious issue and needs further investigation.

Key words: Potato, *Salmonella, Clavibacter, Fusarium, Rhizoctonia*.

Introduction

Potato (*Solanum tuberosum* L.) is the fourth main food crop and number one among non-grain food commodities. More than 100 countries cultivate potatoes, primarily in Asia and Europe under different climatic conditions (Fier *et al*., 2012). Despite the ease in cultivation and fewer labour requirements, potato productivity in Pakistan is not yet promising as compared to neighbouring countries. Potato propagates vegetatively via underground tuber and harbors a vast range of pathogens that may lead to the development of various diseases and substantial yield loss and reduction in quality. An infestation of seed tubers with pests and pathogens are major factors of low potato yield (Thomas-Sharma, *et al*., 2016).

Many fungal diseases particularly early blight, late blight, black scurf, fusarium wilt/ dry rot, wart, powdery scab, and charcoal rot cause significant loss to potato production (Stevenson, 2001). Major tuber diseases, such as early blight and black scurf caused by *Alternaria solani* and *Rhizoctonia solani,* respectively are considered as a serious threat to potato cultivation and are responsible for the huge economic loss. These fungi can survive for long periods in the soil and infected potato debris, however, the dry rot caused by *Fusarium* sp., often occurs in storage, usually in hot or warm weather (Fier *et al*., 2012). Additionally, the two most important highly contagious bacterial pathogens associated with potatoes are *Clavibacter* and *Salmonella* sp. Ring rot of potato caused by *Clavibacter michiganensis* subsp. *sepedonicus* is a highly infectious potato disease and can cause huge losses to infected crops (Nelson, 1982). Potato seed is the main source of spreading this bacterium. Infection is rapidly spread by seed cutting, which is the common practice in many countries. Bacteria move from the tuber to the vascular region and lower leaves of the growing plant, wilting of leaves start as the disease progresses and finally, the whole potato plant may collapse (Van der Wolf *et al.,* 2005).

Salmonella, a highly notorious and generally known animal and the human pathogenic bacterium is an important economic and public health problem throughout the world (Wiedemann *et al*., 2015). Salmonella's primary habitat is human, and another intestinal tract of animals may lead to a wide variety of mild to serious infections called human Salmonellosis (Odumeru, & León-Velarde, 2012). Recent findings indicate that animal and human pathogenic bacteria can use plants as an alternative host; this bacterium adheres to the plant surface and actively infects the interior of the plant (Schikora *et al*., 2012). Plants or surface water used for irrigation and diluents pesticide or fertilizers can be directly contaminated by animal faces, the main reason for spreading this disease (Heaton & Jones, 2008). Both *Clavibacter* and *Salmonella* are quarantine pathogenic bacteria and have zero-tolerance by all regulatory agencies worldwide.

Important potato plant diseases that are caused by pathogenic fungi and bacteria are causing huge economic loss, posing the risk to food security and sustainable agriculture. Therefore, effective disease management is essential to overcome these risks. However, effective control measures cannot be taken in the dark. Correct and timely identification of disease-causing agents is necessary for preventing the entry of infected potato tubers in the country and also for taking control measures. The current study is investigating the pathogenic fungi and bacteria associated with potato tubers imported for seed purpose and compared with locally available potato tubers.

Materials and Methods

Sample collection: Varieties of potato (Asterix, Ultra, and Karuda) imported for seed purpose were obtained from the Department of Plant Protection, Government of Pakistan, Malir Halt Karachi. Ten tubers from each sample were randomly collected and kept at 4°C until isolation of fungi and bacteria was made within 24 hours. Whereas, local potato varieties (Asterix, Lady Rusetta, Qasur, and Mozika) were purchased from the local seed market.

Isolation of pathogenic fungi from potato tubers: Seed tubers were rinsed with tap water, cut into small pieces, and then surface sterilized by using 1% sodium hypochlorite, for 3 minutes; placed into potato dextrose agar plates supplemented with penicillin (100000 units/mL) and streptomycin (0.2 g/mL). Plates were incubated for 7 days at 28°C. Fungal colonies that emerged from each piece were purified on the PDA plate and identified with reference to Barnett and Hunter (1998), Ellis (1971), Booth, (1971), Domsch *et al*., (1980); Nelson *et al*., (1983), Dugan, (2006), Raper & Fennel (1965) and Raper & Thom (1949). Percent occurrence for each fungus was calculated using the formula:

$$\text{Occurrence \% of fungi} = \frac{\text{Total no. of occurrences of a particular fungus}}{\text{Total no. of potatoes examined}} \times 100$$

Isolation of *Clavibacter michiganensis* subsp. *sepedonicus* (Cms): Surface-sterilized (100 mg) basal portion of potato tuber (heel) was homogenized in mortar and pestle with 2 ml of 1% saline solution. Serial dilution was prepared up to 10^{-4} in saline. From the final dilution, 100 µL was spread over Petri plates containing NCP-88 medium (de la Cruz *et al*., 1992). Plates were incubated at 25°C for one week in the dark. Mucoid, white to cream-colored colonies with irregular margins of *Clavibacter michiganensis* subsp. *sepadonicum* was counted.

Identification of *Clavibacter michiganensis sepadonicus:* Identification of *C. michiganensis* subsp., *sepadonicus* was confirmed by using the biochemical tests as suggested by Schaad *et al*., (2001).

Isolation of *Salmonella* sp: Surface sterilized potato tubers were cut into small pieces (1 g long strip) and dip in trypticase soy broth (9 ml) and kept in the dark for overnight. A loopful of suspension was then streaked on Petri plates containing bismuth sulfite agar, and incubated at 35°C for 24 hours. Black shinny colonies of salmonella were purified. Identification of salmonella was further confirmed by Gram staining, catalase test, citrate utilization, phenol red mannitol agar test according to Tindall *et al*., (2005).

Result and Discussion

Fungi associated with potato tubers: All the samples of potatoes studied were found infested with different fungi. The most common fungi isolated from imported as well as local seed tubers were *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Curvularia lunata, Cladosporium* sp., *Drechslera australiensis, Fusarium oxysporum, Fusarium solani, Mucor* sp., *Penicillium* sp., *Phoma* sp., *Rhizoctonia solani Rhizopus stolonifer, Trichoderma harzianum* and *Trichoderma viride.* In both imported and local potato seed tubers, the highest percentage of plant pathogenic fungi was *Fusarium* spp., followed by *Rhizoctonia solani* (Tables 1 &2).

Fungi belonging to the genus *Fusarium* causes diseases like root rot, crown rot, and vascular wilts of more than 2000 plant species whereas *Fusarium oxysporum* is a more prevalent and destructive species than other species (Mandal *et al*., 2009). Whereas *Fusarium solani* is another important and very common species of Fusarium that causes serious loss of economic crops where higher abiotic stresses in developing the disease are more devastating (Hagerty *et al*., 2015; Schneider *et al*., 2001). In our observation, *Fusarium* spp is the most pathogenic fungi present in both local and imported seed tubers. The maximum percentage of *Fusarium solani* about 23% was present in the Asterix variety of imported seed tubers (Table 1). However, *Fusarium oxysporum* was found 21% in the Lady Rosetta variety of local seed tubers (Table 2).

Table 1. Fungi associated with different varieties of potato imported for seed purpose.

Name of fungi	% Occurrence		
	Potato varieties		
	Asterix	Ultra	Karuda
Alternaria alternata	12	12	11
Aspergillus flavus	25	20	19
Aspergillus fumigatus	4	5	3
Aspergillus niger	14	8	9
Cladosporium sp.	9	6	4
Curvularia lunata.	9	3	10
Fusarium solani	23	8	16
Penicillium sp.	9	15	7
Phoma sp.	8	4	4
Rhizoctonia solani	10	13	9
Rhizopus stolonifer	5	4	2
Trichoderma harzianum	7	4	0
Trichoderma viride	3	11	3

Table 2. Fungi associated with different local varieties of potato used for seed purposes.

Name of fungi	% Occurrence			
	Potato varieties			
	Asterix	Lady rosetta	Qasur	Mozika
Alternaria alternata	6	8	1	11
Aspergillus flavus	23	12	19	7
Aspergillus fumigatus	6	2	0	0
Aspergillus niger	18	8	20	5
Aspergillus terreus	1	8	3	2
Drechslera australiensis	1	5	1	3
Fusarium oxysporum	20	21	12	12
Mucor sp.	8	1	10	15
Penicillium sp.	1	5	2	7
Phoma sp	2	4	7	6
Rhizoctonia solani	8	8	12	17
Rhizopus stolonifer	4	1	1	1
Trichoderma viride	2	6	8	5

In this study, *Rhizoctonia solani* was found 17% in Mozika variety of local seed tubers while 13% in Ultra variety imported tubers (Tables 1-2). *Rhizoctonia solani* causes black scurf of potato, survives in warm, and humid weather, it can stay alive for a long period. It causes significant yield losses to several important food crops globally (Paulitz *et al*., 2006; Sneh *et al*., 1996). *Rhizoctonia solani* has a very high competitive saprophytic ability, colonizes dead organic matter, and attacks almost all-important crop plants, making it one of the most important pathogens worldwide, attacking plant roots (Tewoldemedhin *et al*., 2006). It is suggested that tuber-borne inoculum control is important for integral management *Rhizoctonia solani*. Losses caused by this pathogen can be minimized by using disease-free seed tubers (Al-Mughrabi, 2008).

Similarly, *Alternaria* spp., are filamentous fungi, considered as a serious threat to potato cultivation, causing early blight and leaf blight of potatoes. Highest percentage (12 %) of *A. alternata* was found in Asterix and Ultra varieties of imported seed tubers. *Alternaria alternata* can overwinter in the soil and infect debris of potatoes left in the soil for long periods in the absence of a host plant. *Alternaria* sp. has dark mycelium due to the pigment that retains resistance to lysis therefore it remains in the soil for many years.

In our study, it was observed that *Aspergillus flavus* was found in both imported and local samples. The highest percentage (25%) of *Aspergillus flavus* was found in the Asterix variety of imported seed tuber (Table 1), followed by Asterisk of local seeds in which it was at (23 %) (Table 2). On the other hand, the *Aspergillus niger* was 20% in the Qasur variety of local seeds. The presence of these fungi in tubers may result in a reduction of consumption materials, market value, and production of mycotoxin. Excess consumption of mycotoxins may cause allergies in susceptible individuals or can cause serious illness or death (Ji *et al.,* 2019).

In this analysis, both local and imported seed tubers were found contaminated with *Clavibacter michiganensis* subsp. *sepedonicus* (Tables 3 & 4) and *Salmonella* spp., (Tables 3 & 5). Asterix variety in both local and imported seeds found highly contaminated with both bacteria (Table 3). *Clavibacter* which is considered as a highly destructive and contagious plant pathogen of A2 Quarantine status affecting only potatoes, causing bacterial ring rot of potato (Schenk *et al*., 2019). Potato injury can occur at any point in potato growing and during the storage period. This bacterium is not thought to be soil-borne but is transmitted from tuber to tuber by wounds, injuries, and infected knives during seed cutting, picking, and seeding equipment, storage containers, and facilities (Inglis *et al*., 2013). A single contaminated tuber causes tremendous economic harm. Usually, this bacterium is present in cooler climatic areas of the world; however, it has been detected in warmer climate countries (Bragard *et al*., 2019). On the other hand, Salmonella, generally known as animal and human pathogenic bacteria can cause public health problems throughout the world. The presence of human pathogenic bacteria in food is considered a direct threat to human health. Several food materials are being rejected in international markets due to the presence of human pathogenic bacteria particularly *Salmonella* (Pigłowski, 2019).

Table 3. Presence of *Clavibacter michiganensis* and *Salmonella* sp., in potato seed tubers.

	Clavibacter michiganensis* subsp. *sepedonicus	***Salmonella* sp.**
Imported potato varieties		
Asterix	+	+
Ultra	+	+
Karuda	+	+
Local potato varieties		
Asterix	+	+
Lady rosetta	+	+
Qasur	+	+
Mozika	+	+

The ubiquitous distribution of *Salmonella* in the natural environment and its prevalence in the global food chain, physiological adaptability, and virulence of this important human bacterial pathogen, and its potentially serious economic impact on the food industry predicate the need for continued vigilance and stringent controls at all levels of food production. (D'Aoust, 1994). *Salmonella* adheres to the plant surface, before infecting the interior of the plant and colonizing plant tissues and suppressing the immune system of the plant (Schikora *et al.*, 2012). *Salmonella* which originates from the plants retains virulence toward animals (Schikora *et al.*, 2011). Microbiological pollution can account in large part for growing vegetables. Manures used to promote crop growth can contain substantial amounts of spoiling microorganisms. Pathogens linked to untreated manure are assumed to reach the food chain by crops. Consequently; crops grown from untreated fertilizers or water can play an important part in the dissemination of plant and human pathogenic microbes in food, and may risk public health (Malek *et al*., 2013).

Conclusions

Nowadays, when people are struggling with human health and food production problems, healthy and certified seeds for planting are vital for the management of these challenges. Total crop output will only be achieved if diseases impacting the crops are kept under the track. Good control of the disease is essential for effective potato development. Planting healthy seed potatoes for consumption or processing is a key factor in optimizing the yield of viable potatoes. Many diseases affect seed tubers and can reduce plant stand early in the season, additionally without the development of noticeable signs, microorganism infected plants and tuber yield (number and weight) are significantly lesser than healthy plants. Therefore, it is extremely important to carefully and immediately inspect all seed lots after seed procurement. Experiments that are explicitly and implicitly associated with food microbiology must also be carried out for the safety and health of consumers. Potato producers should limit interaction between plants and pathogens, for example by using healthy seeds. Without proper precautions, pathogenic fungi and bacteria will spread rapidly through seed tubers and cause significant loss of yield.

Table 4. Morphological and physiological characteristics used for the identification of *Clavibacter michiganensis. sepedonicus* isolates.

Tests	1	2	3	4	5	6
Shape of cell	club	club	club	club	club	club
Motility	-	-	-	-	-	-
Color of colonies	cream	cream	cream	cream	cream	cream
sporulation	-	-	-	-	-	-
Colony	mucoid	mucoid	mucoid	mucoid	mucoid	mucoid
Gram staining	+	+	+	+	+	+
Starch hydrolysis	- or weak	- or weak	- or weak	- or weak	- or weak	- or weak
Catalase test	+	+	+	+	+	+
Urease activity	-	-	-	-	-	-
Oxidase activity	-	-	-	-	-	-
Growth at 37°C	-	-	-	-	-	-
Urease activity	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-
Tolerance of 7% NaCl	-	-	-	-	-	-
Indole production	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-
Citrate utilization	-	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-	-
Acid glycerol	-	-	-	-	-	-
Acid from lactose	- or weak	- or weak	- or weak	- or weak	- or weak	- or weak
Acid from rhamnose	-	-	-	-	-	-
Acid from salicin	-	-	-	-	-	-
Aesculin hydrolysis	+	+	+	+	+	+

(+) sign shows positive, (-) sign shows negative test

Table 5. Morphological and physiological characteristics used for the identification of *Salmonella* sp.

Tests	1	2	3	4	5	6	7	8	9
Shape of cell	rod	rod	rod	rod	rod	rod	rod	rod	rod
Motility	+	+	+	+	+	+	+	+	+
Color of colonies	black shiny	black shiny	black shiny	black shiny	black shiny	black shiny	black shiny	black shiny	black shiny
Sporulation	-	-	-	-	-	-	-	-	-
Colony	mucoid	mucoid	mucoid	mucoid	mucoid	mucoid	mucoid	mucoid	mucoid
Gram staining	-	-	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+
Oxidase activity	-	-	-	-	-	-	-	-	-
Urease activity	-	-	-	-	-	-	-	-	-
Methyl Red	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+
Indole production	-	-	-	-	-	-	-	-	-
H_2S production	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+
Acid from lactose	-	-	-	-	-	-	-	-	-
Acid from rhamnose	+	+	+	+	+	+	+	+	+

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