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Characterization of the causal organisms of soft rot on tomatoes grown in Lesotho

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In order to study the causal agents of tomato rot in tunnels/greenhouse, the tomato fruits (*Solanum lycopersicum* L) showing symptoms of soft rot was collected and their juices were used for the identification of microbes. Microbial isolates were obtained from lesions following surface sterilization and rinsing with distilled water. Bacteria cultures were prepared on nutrient agar while fungal cultures were prepared on PDA (Potato Dextrose Agar). The morphological characteristics of cultured bacteria revealed a negative gram, a white shiny colony with mucoid growth, and characteristics of *Erwinia corotovora* causing soft rot. Pathogenicity test on ripe tomatoes in an experiment designed as complete randomized design (CRD) with four replicates revealed water-soaked lesions that gradually expanded. Following f-test and separation of means using least significant difference at 5%, the three different strains of *E. carotovora* were identified based on the diameter of the infection lesions. Fungal strains were identified as *Rhyzopus stolonifer* based on colony morphology and pathogenicity test on ripe tomato. Colonies on potato dextrose agar at 25°C was white-cottony at first, the brownish-black sporangia with many hanging black spores grew later. The Sporangia were globose or sub-globose. The two different strains of *R. stolonifer* were identified based on their infection lesion size.

Key words: *Erwinia Carotovora, Rhizopus stolonifer*, tomato (*Solanum lycopersicum* L), soft rot.

INTRODUCTION

Post-harvest loss in yield is the most common cause of food insecurity in Sub-Saharan Africa. *Solanum lycopersicum* L (tomato) is an important vegetable providing essential minerals and vitamins for human diet. It is also a universally acceptable vegetable making it an important component of human diet. Tomatoes are attacked by many kinds of pathogens such as fungi and bacteria (Huang et al., 2017). Also tomato crop faces huge challenges of post-harvest losses due to diseases. Such losses range from 20 to 30%, while losses due to soft rot remaining the most economically important reaching up to 100%. As such these losses have a huge negative impact on the tomato markets (Adam et al., 2017).

Tomato soft rot is caused by bacterial and fungal pathogens. Most bacterial soft rot is a disease complex caused by multiple genera of Gram-negative and Gram-positive bacterial, the most common of these species of Gram-negative bacteria are *Erwinia*, *Pactobacteria* and *Pseudomona* (Khan et al., 2015; Ahmen et al., 2017).

The soft rot bacterium enters plant tissue primarily through wounds that are often created by insect feeding.

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During wet weather, due to high humidity, bacterial soft rot tends to be more of a problem. This can be worsened by a calcium deficiency in tomatoes as it causes cracks in tomato fruits (Zhoukun et al., 2018).

On the growing plants, bacterial soft rot causes softening and browning of the plant's stems (Ahmed et al., 2018). On the tomato fruits *Erwinia carotovora* causes water soaking of tissues, with or without brown discoloration beginning at wounds. This is then followed by rapid softening and liquefaction of the affected tissues. Juices from damaged tissues will spread disease to adjacent or nearby fruit causing an infection to spread (M'hamed et al., 2018). The softening of tissues is caused by *E. carotovora* secreting enzymes that decompose cell wall structure. The plant tissue eventually becomes soft and watery, soft rots commonly occur on fleshly vegetables such as tomato (Thabet and Abdelhafidh, 2018). There are different strains *E. carotovora* affecting tomatoes and they differ in aggressiveness.

Fungal soft rot is commonly caused by *Fusarium acumuninatum* and *Rhizopus stolonifer*. It infects fresh wounds occurring during packing and shipping (Bartz et al., 2016). These fungi secrete cellulase from their hyphae and break the cellulose in tomato tissues. *Rhizopus* soft rot occurs in the succulent tissues on tomatoes. The lesions caused by fungi on tomatoes start water-soaked and are rapidly softened expanding gradually (Athayde et al., 2016). This is accompanied by gray hyphae growing from the sites where the fungus primarily invaded. The hyphae then grow to cover the affected portions by producing tuft whisker like gray sporangiophores and sporangia. The infected tissue finally breaks down and disintegrated in watery rot (Kwon et al., 2001). This watery rot is infectious and has a characteristics fermentation odor (Khokhar et al., 2019). Generally, the control of fungal pathogen such as *R. stolonifer* soft rot in tomatoes involves the application of synthetic fungicides in the field and during the postharvest period (Andrade et al., 2017).

Soft rots are common in greenhouse vegetables production and are also known for affecting tomato. The disease can rapidly colonize wounds that may have occurred during handling and shipping eventually causing a rot (Charkowski, 2018). The tunnels and greenhouses of Lesotho are dominated with tomato *(S. lycopersicum)* cultivation; however, the causal agent of soft rot of tomatoes harvested from these farms had not been identified and characterized yet. Thus, this work focused on the identification of the causal organism of soft rot in tomatoes and the evaluation of their aggressiveness.

MATERIAL AND METHODS

Isolation of bacterial and fungal pathogens

Tomatoes showing symptoms of soft rot were collected from local tunnels and greenhouses from January (2020) for isolation of the bacterial and fungal pathogen. The tomato fruits were cleaned with running water, surface sterilized with 0.5% sodium hypochlorite solution (for a few seconds) and washed with sterile distilled water. The bacterial pathogen and fungal pathogens were then isolated by taking juices from the wounds in water suspension (Kwon et al., 2001). The bacterial cultures were made on nutrient agar, and the fungal cultures were done on PDA (Potato Dextrose Agar) plates supplemented with NaCl for selective growth of fungi.

Bacteria from the fruit juice were identified as per (Akbar et al., 2015) method with modifications. The bacteria suspension and microbial suspension was collected. The resulting bacteria suspension was streaked on the surface of plates containing nutrient agar for bacterial growth. The colonies showing clear morphology were picked for pure culture growth at 27°C incubator for 48 h. The colonies were then characterized by *E. carotovora* traits.

For identification of fungal pathogens, PDA was supplemented with antibiotics to inhibit bacterial growth. The plates were incubated at 30°C for 24 h. Individual colonies growing on nutrient agar and potato dextrose agar were picked up and streaked on nutrient agar and potato dextrose agar plates again, and then incubated at 30°C for another 24 h. This was repeated several times to obtain pure cultures (Kwon et al., 2001).

Identification and characterization

The isolates were identified based on disease pathogenicity tests, colony morphology, and biochemical tests. The colony morphology of *Erwinia* was based on the Mucoid growth from individual colonies on agar plates supplemented with NaCl (Rashid et al., 2016). The fungus was identified based on the morphology of the colony; the colour of sporangia and the shape (Kwon et al., 2001).

The pathogenicity test was performed as per Lee et al. (2019). The bacterial and fungal colonies with clear morphology were picked with a toothpick form the plates and used to inoculate by piercing the tomato fruits in triplicate per colony. The fruits were incubated at 30 degrees for 48 h. The lesion sizes caused by inoculation with different isolates were recorded as diameters of the lesion in mm. The isolates that caused rotting of tomato fruits were then taken for further tests (Lee et al., 2019).

Identification of *Erwinia* was carried out using biochemical tests specific for genus *Erwinia* subsp. *corotovora*. 3% potassium hydroxide (KOH) solution was freshly prepared and then a drop of this solution was placed on regular microscopic slide. A 24 h old bacterial culture was placed in this drop and mixed for 10 sec. Bacterial suspension making strands when lifted up by toothpick were considered gram-negative (Khan et al., 2015). Production of a watery suspension on viscous strands when lifted up indicates gram positive result.

The bacterial colony was also evaluated for tolerance to NaCl. For this test, nutrient agar medium was prepared with 5% NaCl. The medium was inoculated with bacterial culture. The colonies that grew in this medium were considered salt tolerant.

RESULTS

Identification and characterization of the bacterial isolates

The isolates were identified based on disease symptoms, pathogenicity tests, colony morphology and biochemical tests. The colonies obtained on nutrient agar were

Table 1. Characterization of *Erwinia carotovora* subsp. *carotovora* isolates obtained from tomatoes from different green houses.

Isolate	KOH test	Mucoid growth	NaCl test
Farm 1	-	+	+
Farm 2	-	+	+
Farm 3	-	+	+
Farm 4	-	+	+
Farm 5	-	+	+
Farm 6	-	+	+
Farm 7	-	+	+
Farm 8	-	+	+
Farm 9	-	+	+
Farm 10	-	+	+
Farm 11	-	+	+
Farm 12	-	+	+

+= Positive, - = negative

morphologically transparent, circular, raised, shiny and creamy white after 28 h incubation at 27˚C. All of the three isolates were tolerant to 5% NaCl: (sodium chloride) and gram negative based on KOH test (Table 1).

In the studied samples of *Erwinia*, the isolates differed in aggressiveness based on the lesion mean (Table 2). Three strains of a negative gram bacteria that cause soft rot on tomatoes were identified and grouped based on their aggressiveness.

Identification and characterization of the fungal isolates

Fungal mycelium was collected from the cracked rot lesion of tomato fruits. The colonies growing on PDA were white colonies at first, becoming heavily speckled by black, brown sporangia. The shapes of the sporangia were globose and sub-globose with a flattened base. The colour of the sporangia was white at first then turned blackish with spores hanging. Based on this description the causal organism was identified as *R. stolonifer* (Table 3). Upon inoculation of ripe tomato fruits by piercing with toothpicks, the colonies caused rot lesions on tomato fruits. The lesions were water-soaked at first, and rapidly softened. The lesions expanded gradually and came in different sizes.

Four fungal isolates were cultured from the rotten fruits. After culturing them on PDA plates, the colonies were inoculated onto ripe fruits with the toothpick. The isolates caused different lesion sizes indicating varying aggressiveness of the isolates (Table 4). The two different strains of *R. stolonifer* were identified based on aggressiveness.

DISCUSSION

Soft rot is the common disease of tomato causing drastic post-harvest fruit losses worldwide. This disease highly affects the shelve-life of tomato (Bhat et al., 2010) and can affect the retail price of tomatoes. The cause of the rot by *E. carotovora* on tomatoes is well documented by various researchers (Khan et al., 2015). Morphological characteristic of the Erwinia isolates found in this study are in line with the findings of Akbar et al., (2015). Morphological characteristic of the Erwinia isolates found in this study are in line with the findings of Akbar et al., (2015).

There are reported strains of *E. carotovora* that differ in aggressiveness in tomato and other susceptible crops such as lettuce and pepper (Khan et al., 2015). It is confirmed by the studies in green paper that there are different strains of *E. carotovora* causing soft rot within the crop spices itself (M'hamed et al., 2018). Other previous work on potato has reported the degree in variation of aggressiveness of the soft rot causing *E. carotovora* (Smith and Bartz, 1990). Moreover, isolates from different sources have varying aggressiveness on other plant species. isolates from different sources have varying aggressiveness on other plant species. The *E. carotovora* species have been observed not to exhibit host specificity, rather they have a wide host range (Akbar et al., 2015). have been observed not to exhibit host specificity, rather they have a wide host range (Akbar et al., 2015).

Although this pathogen has been observed in tomatoes grown in Lesotho, it had not attracted any scientific research. This is the first report in Lesotho addressing the existence of probably 3 strains of *E. carotovora* differing in aggressiveness causing rots. However, the molecular

Table 2. *In vitro* aggressiveness (mm) of *Erwinia carotovora* subsp. *carotovora* isolates on ripe tomato fruits.

Isolate number	Mean soft-rot lesion (cm)
1	1.350[c]
2	1.525[b]
3	1.275[c]
4	0.950[c]
5	1.300[c]
6	1.150c
7	1.775[a]
8	1.150[c]
9	1.650[a]
10	1.800[a]
11	1.625[a]
12	1.475[b]
S.e	0.1071
L.s.d	0.219

Fpr <0.001, CV 10%, L.s.d (least significant difference) = 0.219. Means followed by same letter(s) do not significantly (P ≤ 0.05) differ from each other. S.e= Standard error of the difference.

Table 3. Morphological characteristics of pathogenic fungus isolated from soft rot of tomato comparison with Kwon et al (2001)'s description of *Rhizopus*.

Fungal isolate	Colony color	Sporangia shape	Sporangia colour
1	White to brown	Globuse, sub-globuse	Blackish
2	White to brown	Globuse, sub-globuse	Blackish
3	White to brown	Globuse, sub-globuse	Blackish
4	White to brown	Globuse, sub globuse	bLackish

Table 4. *In vitro* aggressiveness (mm) of *Rhizopus stolonfer* isolates on ripe tomato fruits.

Fungal isolate	Soft-rot diameter means
1	3.125[b]
2	4.600[a]
3	4.500[a]
4	3.325[b]
S.E.D	0.3241
L.S.D	0.7473

Fpr <0.001, CV 13%, LSD = 0.7473. Means followed by same letter(s) do not significantly (P ≤ 0.05) differ from each other.

characterization of this strain is required for accurate characterization.

The second most important soft rot causing organism is *R. stolonifer* (Akinro et al., 2015). In agreement with the previous studies, the fungus isolates from collected tomato samples were identified as *R. stolonifer* based on its morphology and its pathogenicity test results. Previous studies indicated differences in aggressiveness of fungal strains causing soft rot as observed *in vitro* (Bautista-Baños et al., 2008, 2014). This existing variation in

aggressiveness of *R. stolonifer* isolates is also observed in this study reported in this paper. Moreover, the bread-mold is a common around the house holds and had not attracted much of scientific research in Lesotho.

The economic significance of these two pathogens in Lesotho is not quantified in this study. However, the knowledge regarding its economic relevance in tomato value chains of Lesotho is very important to advice investment into tomato soft-rot research.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adam SH, Lai AA, Simon S (2017). In vitro efficiency of certain botanicals against bacterial soft rot of tomato (Solanum lycopersicum L.). African Journal of Agricultural Research 12(23):20.

Ahmed FA, Larrea-Sarmiento A, Alvarez AM, Arif M (2018). Genome-informed diagnostics for specific and rapid detection of Pectobacterium species using recombinase polymerase amplification coupled with a lateral flow device. Scientific Reports 8(1):15972.

Ahmen FA, Arif M, Alvares M (2017). Antibacterial effect of potassium tetraborate against soft rot disease agent Pectobacterium Carotovora in tomato. Frontiers in Microbiology 8:1728.

Akbar A, Ahmad M, Neelam A, Khan SZ, Ahmad Z (2015). Characterization of the causal organism of soft Rot of tomatoes and other vegetables and evaluation of its most aggressive isolates. American Journal of Plant Sciences 6:511-517.

Akinro EB, Adetuberu IA, Efunwole OO, Olakunle TP (2015). Isolation and identification of fungal species associated with the spoilage of some selected edible fruits in Iree town of Boripe Local Government, Osun State, Nigeria. Journal of Research in Pharmaceutical Science 2(7): 2347-2995.

Athayde A, Oliveira DP, Guerra L, Conceicao D, Lima DM (2016). A coating composed of chitosan and cymbopogan citrating (DC.Ex nees) essential oil to control Rhizopus soft and quantity in tomato fruit stored at room. The Journal of Horticultural Science and Biotechnology 91(6):582-591.

Bartz JA, Huber DJ, Stahl Sl, Lee JH, Spiceland D, Elkahky MT (2016). Susceptibility of lentils within the stem depression of tomato fruit bacterial soft rot. Journal of Plant Diseases 100(9):1906-1909.

Bautista-Baños S, Bosquez-Molina E, Barrera-Necha L (2014). *Rhizopus stolonifer* (Soft Rot). Postharvest Decay: Control Strategies. 1-44. 10.1016/B978-0-12-411552-1.00001-6.

Bautista-Baños S, Velaquez-del M, Hernandez-Lauzardoa A, Ait-Barka E (2008). The *Rhizopus stolonifer*-tomato interaction. Plant Microbe Interactions, Edition: Research Signpost, Editors: Essaid Aït Barka, Christophe Clément, pp. 269-289.

Bhat KA, Masood SD, Bhat NA, Ashraf-Bhat M, Razvi SM, Mir MR, Akhtar S, Wani N, Habib M (2010). Current status of post-harvest soft rot in vegetables: a review. Asian Journal of Plant Sciences 9:200-208.

Charkowski OA (2018). The Changing face of bacterial Soft-Rot Diseases. Annual Review of Phytopathology 56(1):269-288.

Khan A, Ahmad A, Nadeem M, Neelam A, Sana K, Zahoor A (2015). Characterization of the causal organism of soft rot of tomatoes and other vegetables and evaluation of its most aggressive isolates. American Journal of Plant Sciences 6(4):511-517.

Khokhar I, Jorihuon W, Yang J, Yanchum Y (2019). First report of Rhizopus soft rot on tomato (*Lycopersian esculentum*) caused by Rhizopus oryzae. Plant Disease 45:321-330.

Kwon JH, Kang SW, Kim LS, Park CS (2001). *Rhizopus* Soft Rot on cherry tomato caused by *Rhizopus stolonifer* in Korea. Mycobiology 29(3):176-178.

Lee YE, Lee JJ, Volynchikova E, Kim KD (2019). Isolation and Identification of a Bacterial Agent Causing Soft Rot in Chinese Cabbage. Life Science and Natural Resources Research 27:41-45.

M'hamed B, Boualem B, Sofiane B, Omar K, Btteche G (2018). Variability of aggressiveness and virulence of erwinia carotovora subsp. Carotovorum causing the soft rot on potato tubers in the western of Algeria. International Journal of Plant Biology 9(1):7568.

Huang NC, Huynh T, Cong MN, Nguyen VH, Pham TD, Phuong T, Trang ST (2017). Antibacterial activity of chitosan from pens (loligo chenisis) aganist *Erwinia Carotovora* from soft rot postharvest tomato fruit. Journal of Polymer Materials 34:319-330.

Rashid TS, Sijam K, Awla HK, Saud HM, Kadir J (2016). Pathogenicity assay and molecular identification of fungi and bacteria associated with diseases of tomato in Malaysia. American Journal of Plant Sciences 7:949-957.

Smith C, Bartz JA (1990). Variation in pathogenicity and aggressiveness of strains of *Erwinia carotovora Sub. Carotovora* isolated from different hosts. Plant Disease 74:505-509.

Thabet Y, Abdelhafidh D (2018). Sensitivity of Pectobacterium carotovora to hydroxytryoso inch extra and the effects on the development of soft rot in potato tuber during storage. Journal of Crop Protection 53:52-54.

Zhoukun L, Ting W, Xue L, Xiangmin L, Chengyao X, Yuqiang Z, Xianfeng Y, Yan H, Xiangyang G, Hui C, Zhongli C, Jiaqin F (2018). Biocontrol potential of Myxococcus sp. strain BS against bacterial soft rot of calla lily caused by *Pectobacterium carotovorum*. Journal of Biological Control 126(10):1016.