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OCCURRENCE AND PREVALENCE OF THE BACTERIAL ONION BULB ROT PATHOGENS IN EGYPT.

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ABSTRACT

Different bacterial strains were recorded as onion rot pathogens. The candidate strains in one region may differ from the other and it may be only one pathogen responsible of the infection in an area or a combination from different pathogens. The present research aimed at detection of the main bacterial pathogen (s) causing onion rot in Egypt throughout storage. Five bacterial pathogens were detected with different occurrence percentage; *Erwinia carotovora* subspecies *carotovora* (48.14 %), *Erwinia cacticida* (18.51 %), *Erwinia carotovora* subspecies *atroseptica* (14.81 %), *Burkholderia cepacia* (14.81 %) and *Pantoea* sp. (3.7 %).

Keywords: onion, bacterial rot, soft rot, sour, slippery skin, *Erwinia*, *Burkholderia*,

INTRODUCTION

Onion is considered one of the most important economic crops in Egypt, not only for local consumption but also for export. Onion bulbs are constantly confronted with a wide variety of potential bacterial pathogens that develop bulb rot during onion storage leading to economic damage in yield. Several bacteria cause a range of disease syndrome known as soft rot, slippery skin, and sour skin (Chaput, 2006).

Onions infected by the bulb rot bacteria often appear healthy on the outside but when cut open some of the inner scales are showed brown, water-soaked and have a cooked appearance with a characteristic foul smell. Soft rot symptoms can range from a spongy, water-soaked scale to complete bulb breakdown. Slippery skin symptoms appear as pale yellow to light brown decay of the inner scales of the bulb and when pressure applied to the base of the infected bulb, the rotten inner portion slip out through the neck. Sour skin symptoms appear a decay of one or few outer scales, but adjacent outer and center scales remain firm, sour skin is not as watery as soft rot and slippery skin and the diseased scales separate from healthy ones (Agblor and Waterer, 2001 and Chaput, 2006).

Onion bulb rot bacteria live in the soil and disseminated by irrigation water; insects and agricultural practices. Bacteria enter the plant through natural openings or through wounds; on the leaves or the necks; caused by mechanical injuries, or cut necks through harvest or the feeding wounds

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caused by onion insects that enter the bulb and carrying the bacteria from one plant to another. Generally; Bacterial bullb rot symptoms appear at time of the harvest or in storage (McNab, 2004 and Chaput, 2006).

—The symptoms of these diseases occasionally start in the field on the leaves where leaves wilt, turn pale yellow and the plant die, but often not detected until the bulbs have been in storage for some time, depending on the environmental conditions. In general these bacterial diseases occur during wet periods and warm temperatures (Chaput, 2006).

—Many bacterial strains were recorded as onion rot pathogens and strains in one region may differ from the other. The most commonly recorded organisms; *Erwinia carotovora* subsp. *carotovora* as soft rot pathogen, *Burkholderia cepacia* as sour skin pathogen and *Burkholderia gladioli* subsp. *allicola* as slippery skin pathogen **(**Dainello, 2000; Davis, 2000 and Schwartz and Gent, 2004a and b). Other bacteria also rot onion including; *Erwinia chrysanthemi*, *Enterobacter cloacae* and *Erwinia herbicola*, the later is often associated with storage rots of onion especially when curing temperature is high (Lelliot and Stead, 1987 and Beer *et al*., 2010).

—The main objective of this research study is the detection and identification of the bacterial pathogens responsible for rotting of onion bulbs during storage in Egypt.

MATERIALS AND METHODS

~~1.~~ Sampling and symptoms description:

Bulbs of red onion cultivar (Giza red cultivar) infected with bacterial rot were collected from storage places at different villages (Aga, Nawasa, Salaca, Mit-Ghorab, and Bany-Ebed) in Dakahlia Governorate, Egypt. Collected samples were transferred to the laboratory for symptoms description and pathogen(s) isolation. Vertical cut was carried out in each bulb to expose the internal scales in order to describe the rot symptoms. The samples were taken for this study in two consecutive seasons of 2007 and 2008.

~~2.~~ Isolation of the rot bacteria from rotted bulbs:

Infected onion bulbs with different symptoms were used for pathogen(s) isolation. A small piece from a rotted scale tissue was removed and suspended in 3 ml sterile water, macerated and allowed to settle for 15 min. A loopful of the suspension was streaked onto dry plates of nutrient agar media. Plates were incubated at 28°C for 24 hours. Single colonies of growing bacteria on nutrient agar were picked up and each colony was sub-cultured on nutrient agar plate for 24 hours at 28°C to verify purity. Pure isolates were transferred to nutrient agar slants for maintenance till use in subsequent tests.

~~3.~~ Pathogenicity test:

Pure cultures isolated from infected onion bulbs were tested for the ability to induce rot symptoms in healthy onion bulbs. Bulbs of red cultivar onion (Giza red cultivar) with approximate uniform of shape and size were used in this experiments. To prepare the onion bulbs, outer dry scales were removed; bulbs were washed with tap water then, sterilized by wiping with

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95% ethyl alcohol. Bacterial suspension of each isolate was prepared from fresh culture (24 h old); suspended in sterilized water and adjusted to approximately 10^8 colony forming unit (cfu)/ml.

Two techniques were applied in this experiment; in the first, the whole bulb was used as follow: the outer fleshy scale was carefully removed and the newly exposed scale was surface sterilized by wiping it with 95% ethyl alcohol. Sterilized toothpick was dipped into the bacterial suspension and used to inoculate onion bulb (Lelliot and Stead, 1987) by pricking the bulb 4-5 times with the toothpick, this was repeated 2-3 times in the same area of the bulb. The removed outer fleshy scale was placed back into its original position to protect the injected area from drying and contamination. Three replicates were done for each bacterial isolate. Inoculated bulbs were placed in a sterilized plastic containers (sterilized with 95% ethyl alcohol) lined with two sterilized filter paper moistened with 5 ml sterile water, then containers were covered by their sterilized lids. The containers were incubated at 28° C for 7 days or more.

In the second technique (Aguilar *et al.*, 2003), onion slices were used as follow: onion bulbs were cut aseptically into slices of about 1cm thick; a slight well was made in the center of each slice before being placed in a sterilized Petri dish lined with two sterilized filter paper moistened with 5 ml sterile water. 100 µl of each bacterial culture was placed in this center wells in onion slices. Plates were covered and sealed with parafilm, then incubated at 28° C for 7 days or more. Three replicates were prepared for each bacterial isolate. Controls of this experiment were made using sterile water instead of bacterial cultures. Onion bulbs and onion slices were tested for rot symptoms after the incubation period.

~~4.~~ Identification of the pathogenic bacterial isolates:

All pathogenic bacterial isolates; showed bacterial rot symptoms on onion were subjected to complete identification. Morphological, cultural and biochemical characters according to Schaad (2001) were used to differentiate isolated genera. These characters include: Gram reaction, anaerobic growth, yellow colonies on yeast dextrose calcium carbonate (YDC) and nutrient agar (NA) media, fluorescent pigment on King's medium B (KB medium), growth on DIM agar medium and utilization of arginine and betaine. Then the common genera were subjected to further tests to identify their species according to Fahy and Persley (1983); Lelliott and Dickey (1984); Lelliot and Stead (1987); De Boer and Kelman (2001); Chun and Jones (2001) and Sotokawa and Takikawa (2004). These tests include: growth at 37°C, reducing substances from sucrose, sensitivity to erythromycin, indole production, acid production from: lactose, inulin, cellobiose, trehalose, glycerol and starch, oxidase, urease activity, and utilization of sucrose, maltose and D-tartrate.

RESULTS AND DISCUSSION

~~1.~~ Symptoms description:

The collected samples of onion bulbs, when examined by vertical cutting, showed the typical symptoms of the onion bacterial rot diseases with

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substantial differences in the habit and degree of rotting, varying from complete bulb rotting, rotting of only outer few scales or rotting of the inner scales. They all had a light brown cooked appearance with a foul smell due to the accompanying bacterial ooze.

2. Isolation of the rot bacteria from rotted bulbs:

The isolation process from onion samples resulted in 50 isolates denoted with numerical symbols from 1- 50 which in turn exposed to the pathogenicity test.

3. Pathogenicity test:

Results of pathogenicity test demonstrated that only 30 isolates (Table 1) were pathogenic; produced symptoms of typical bacterial bulb rot in onion while controls; of inoculated bulbs with sterilize water showed no symptoms.

4- Identification of the pathogenic bacterial isolates:

The generic identification of the pathogenic isolates, based on their morphological, cultural and biochemical characteristics and according to Schaad (2001), resulted in: 1 isolate was identified as *Pantoea* (isolate no. 13), 3 isolates (no. 5, 26 and 28) were identified as yeasts, 4 isolates (no. 11, 17, 20 and 48) were identified as *Burkholderia* and the rest of isolates (22 isolates) were identified as *Erwinia* (Table 1).

Based on the biochemical and physiological tests used to differentiate species and subspecies of the 22 isolates of the genus *Erwinia* in Tables 2 and 3; it was found that: 5 isolates (no. 1, 6, 14, 25 and 39) were identified as *Erwinia cacticida*, 4 isolates (no. 7, 18, 34 and 35) as *Erwinia carotovora* subspecies *atroseptica* and 13 isolates as *Erwinia carotovora* subspecies *carotovora*.

Biochemical tests used to differentiate species of 4 isolates belong to the genus *Burkholderia*, In Table 4, confirmed that all 4 isolates are *Burkholderia cepacia* .

Based on the aforementioned results; data were graphically presented in Fig 1 for all pathogenic bacterial isolates except yeast isolates; it could be concluded that number of 27 pathogenic bacterial isolates caused onion bulb rot can be categorized into 5 different bacterial pathogens namely; *Erwinia carotovora* subspecies *carotovora* (the highest ratio of occurrence, 48.14 %), *Erwinia cacticida* (18.51 %), *Erwinia carotovora* subspecies *atroseptica* (14.81 %), *Burkholderia cepacia* (14.81 %) and *Pantoea* spp. (3.7 %) (Fig. 3).

The detection of *Erwinia carotovora* subspecies *carotovora* and *Burkholderia cepacia* as onion rot pathogens is in agreement with many other investigations that recorded them as the main causes of onion bacterial rot (Fahy and Persley, 1983; Lelliot and Stead, 1987; Davis, 2000; Chun and Jones, 2001; Schwartz and Gent, 2004a and Sotokawa and Takikawa, 2004)

Erwinia carotovora subspecies *atroseptica* has been recorded as soft rot pathogen for few vegetables hence it is almost restricted to the cool and temperate regions and has a host range limited almost exclusively to potato (Fahy and Persley, 1983; Lelliot and Stead, 1987; Toth *et al*., 2003 and Badr, 2006), so this is from the few reports of bacterial rot of onion caused by *Erwinia carotovora* subspecies *atroseptica*. This finding is in harmony with McDonald *et al*. (2003) who reported *Erwinia carotovora* subspecies *atroseptica* as onion rot pathogen in Colorado

Erwinia cacticida was recorded as soft rot pathogen for some plants (De Boer and Kelman, 2001 and Jiménez Hidalgo *et al*., 2004; on *Agave tequilana*, but this is the first report to record as onion rot pathogen in Egypt).

The detection of a *Pantoea* sp. as onion rot pathogen is in agreement with many recent investigations which reported *Pantoea* spp. to rot onion (Citaitic *et al*., 2002; Goszczynska, 2007; Morohoshi *et al*., 2007; Beer *et al*., 2010 and Brady *et al*., 2010).

Identification of the pathogenic bacterial isolates:

The generic identification of the pathogenic isolates, based on their morphological, cultural and biochemical characteristics and according to Schaad (2001), resulted in: 1 isolate was identified as *Pantoea* (isolate no. 13), 3 isolates (no. 5, 26 and 28) were identified as yeasts, 4 isolates (no. 11, 17, 20 and 48) were identified as *Burkholderia* and the rest of isolates (22 isolates) were identified as *Erwinia* (Table 1).

Table 1: Cultural and biochemical tests used for identifying the pathogenic.

Isolate no.	Gram reaction	Anaerobic growth	Yellow colonies on YDC/ NA	Fluorescent pigment on KB	Growth on DIM agar	Utilization of arginine and betaine
1	G –ve rods	+	-			
5	Yeast					
6	G –ve rods	+	-			
7	G –ve rods	+	-			
8	G –ve rods	+	-			
11	G –ve rods	-	-	-	-	+
13	G –ve rods	+	+			
14	G –ve rods	+	-			
17	G –ve rods	-	-	-	-	+
18	G –ve rods	+	-			
20	G –ve rods	-	-	-	-	+
25	G –ve rods	+	-			

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26	Yeast					
28	Yeast					
30	G –ve rods	+	-			
33	G –ve rods	+	-			
34	G –ve rods	+	-			
35	G –ve rods	+	-			
38	G –ve rods	+	-			
39	G –ve rods	+	-			
40	G –ve rods	+	-			
41	G –ve rods	+	-			
42	G –ve rods	+	-			
43	G –ve rods	+	-			
45	G –ve rods	+	-			
46	G –ve rods	+	-			
47	G –ve rods	+	-			
48	G –ve rods	-	-	-	-	+
49	G –ve rods	+	-			
50	G –ve rods	+	-			

Isolate no.	Gram reaction	Anaerobic growth	Yellow colonies on YDC/ NA	Fluorescent pigment on KB	Growth on DIM agar	Utilization of arginine and betaine
1	G –ve rods	+	-			
5	Yeast					
6	G –ve rods	+	-			
7	G –ve rods	+	-			
8	G –ve rods	+	-			
11	G –ve rods	-	-	-	-	+
13	G –ve rods	+	+			
14	G –ve rods	+	-			
17	G –ve rods	-	-	-	-	+
18	G –ve rods	+	-			
20	G –ve rods	-	-	-	-	+
25	G –ve rods	+	-			
26	Yeast					
28	Yeast					
30	G –ve rods	+	-			
33	G –ve rods	+	-			
34	G –ve rods	+	-			
35	G –ve rods	+	-			
38	G –ve rods	+	-			
39	G –ve rods	+	-			
40	G –ve rods	+	-			
41	G –ve rods	+	-			
42	G –ve rods	+	-			
43	G –ve rods	+	-			
45	G –ve rods	+	-			
46	G –ve rods	+	-			
47	G –ve rods	+	-			
48	G –ve rods	-	-	-	-	+
49	G –ve rods	+	-			
50	G –ve rods	+	-			

Based on the biochemical and physiological tests used to differentiate species and subspecies of the 22 isolates of the genus *Erwinia* in Tables 2 and 3; it was found that: 5 isolates (no. 1, 6, 14, 25 and 39) were identified as *Erwinia cacticida*, 4 isolates (no. 7, 18, 34 and 35) as *Erwinia carotovora* subspecies *atroseptica* and 13 isolates as *Erwinia carotovora* subspecies *carotovora*.

Table 2: Biochemical and physiological tests used to differentiate species and subspecies of *Erwinia*.

Isolate no.	Growth at 37° C	Reducing substances from sucrose	Sensitivity to erythromycin	Indole production
1	+	-	-	-
6	+	-	-	-
7	-	+	-	-
8	+	-	-	-
14	+	-	-	-
18	-	+	-	-
25	+	-	-	-
30	+	-	-	-
33	+	-	-	-
34	-	+	-	-
35	-	+	-	-
38	+	-	-	-
39	+	-	-	-
40	+	-	-	-
41	+	-	-	-
42	+	-	-	-
43	+	-	-	-
45	+	-	-	-
46	+	-	-	-
47	+	-	-	-
49	+	-	-	-
50	+	-	-	-

Isolate no.	Growth at 37° C	Reducing substances from sucrose	Sensitivity to erythromycin	Indole production
1	+	-	-	-
6	+	-	-	-
7	-	+	-	-
8	+	-	-	-
14	+	-	-	-
18	-	+	-	-
25	+	-	-	-
30	+	-	-	-

33	+	-	-	-
34	-	+	-	-
35	-	+	-	-
38	+	-	-	-
39	+	-	-	-
40	+	-	-	-
41	+	-	-	-
42	+	-	-	-
43	+	-	-	-
45	+	-	-	-
46	+	-	-	-
47	+	-	-	-
49	+	-	-	-
50	+	-	-	-

Table 3: Acid production from some organic compounds by *Erwinia* isolates.

Isolate no.	Lactose	Inulin	Cellobiose	Trehalose	Glycerol	Starch
1	-	-	+	+	+	-
6	-	-	+	+	+	-
8	+	+	+	+	+	-
14	-	-	+	+	+	-
25	-	-	+	+	+	-
30	+	-	+	+	-	-
33	+	-	+	+	+	-
38	+	-	+	+	-	-
39	-	-	+	-	-	-
40	+	-	+	+	+	-
41	+	-	+	+	+	-
42	+	+	+	+	+	-
43	+	-	+	+	+	-
45	+	-	+	+	+	-
46	+	-	+	+	+	-
47	+	-	+	+	+	-
49	+	-	+	+	+	-
50	+	+	+	+	+	-

Isolate no.	Lactose	Inulin	Cellobiose	Trehalose	Glycerol	Starch
1	-	-	+	+	+	-
6	-	-	+	+	+	-
8	+	+	+	+	+	-
14	-	-	+	+	+	-

25	-	-	+	+	+	-
30	+	-	+	+	-	-
33	+	-	+	+	+	-
38	+	-	+	+	-	-
39	-	-	+	-	-	-
40	+	-	+	+	+	-
41	+	-	+	+	+	-
42	+	+	+	+	+	-
43	+	-	+	+	+	-
45	+	-	+	+	+	-
46	+	-	+	+	+	-
47	+	-	+	+	+	-
49	+	-	+	+	+	-
50	+	+	+	+	+	-

Biochemical tests used to differentiate species of 4 isolates belong to the genus *Burkholderia*, In Table 4, confirmed that all 4 isolates are *Burkholderia cepacia*..

Based on the aforementioned results; data were graphically presented in Fig 1 for all pathogenic bacterial isolates except yeast isolates; it could be concluded that number of 27 pathogenic bacterial isolates caused onion bulb rot can be categorized into 5 different bacterial pathogens namely; *Erwinia carotovora* subspecies *carotovora* (the highest ratio of occurrence, 48.14 %), *Erwinia cacticida* (18.51 %), *Erwinia carotovora* subspecies *atroseptica* (14.81 %); *Burkholderia cepacia* (14.81 %) and *Pantoea* spp. (3.7 %) (Fig. 1).

The detection of *Erwinia carotovora* subspecies *carotovora* and *Burkholderia cepacia* as onion rot pathogens is in agreement with many other investigations that recorded them as the main causes of onion bacterial rot (Fahy and Persley, 1983; Lelliot and Stead, 1987; Davis, 2000; Chun and Jones, 2001; Schwartz and Gent, 2004a and Sotokawa and Takikawa, 2004)

Table 4: Biochemical tests used to differentiate species of *Burkholderia*.

Isolate no.	Oxidase	Urease activity	Utilization of		
			Sucrose	Maltose	D- Tartrate
11	+	+	+	+	-
17	+	+	+	+	-
20	+	+	+	+	-
48	+	+	+	+	-

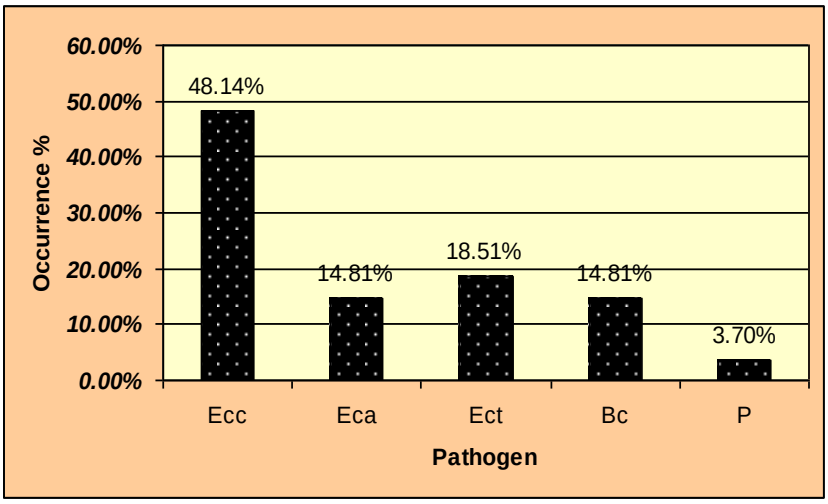

Fig. 1: Occurrence percentage of different bacterial species isolated from onion bulbs samples.

Ecc= *Erwinia carotovora* subspecies *carotovora*, Eca= *Erwinia carotovora* subspecies *atroseptica*, Ect= *Erwinia cacticida*, Bc= *Burkholderia cepacia*, P= *Pantoea*

Erwinia carotovora subspecies *atroseptica* has been recorded as soft rot pathogen for few vegetables hence it is almost restricted to the cool and temperate regions and has a host range limited almost exclusively to potato (Fahy and Persley, 1983; Lelliot and Stead, 1987, Toth *et al*., 2003 and Badr, 2006), so this is from the few reports of bacterial rot of onion caused by *Erwinia carotovora* subspecies *atroseptica*. This finding is in harmony with McDonald *et al*. (2003) who reported *Erwinia carotovora* subspecies *atroseptica* as onion rot pathogen in Colorado *Erwinia cacticida* was recorded as soft rot pathogen for some plants (De Boer and Kelman, 2001 and Jiménez-Hidalgo *et al*., 2004; on *Agave tequilana*, but this is the first report to record as onion rot pathogen (in Egypt).

The detection of a *Pantoea* sp. as onion rot pathogen is in agreement with many recent investigations which reported *Pantoea* spp. to rot onion (Gitaitis *et al*., 2002; Goszczynska, 2007; Morohoshi *et al*., 2007; Beer *et al*., 2010 and Brady *et al*., 2010).

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تواجد وانتشار البكتيريا المسببة للأعفان البكتيرية للبصل في مصر

فتحي عواد منصور*، محمد السيد عبد الله، سامية علي هارون*، أحمد أحمد جمعة*** و هدى حسين بدر*****

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تم تسجيل العديد من السلالات البكتيرية كمسببات للأعفان البكتيرية في البصل و التي تختلف كما و نوعا من منطقة إلى أخرى. أجري هذا البحث كمحاولة لتحديد البكتيريا المسئولة بشكل رئيسي عن احداث الأعفان البكتيرية للبصل أثناء

التخزين في مصر. وجدت خمس سلالات بكتيرية بنسب متفاوتة ممرضة للبصل تم تصنيفها كالآتي:

Erwinia carotovora subspecies *carotovora* (48.14 %),
Erwinia cacticida (18.51 %),
Erwinia carotovora subspecies *atroseptica* (14.81 %),
Burkholderia cepacia (14.81 %),
Pantoea sp. (3.7 %).

قام بتحكيم البحث

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