J. Agric. Sci. Mansoura Univ., 34 (9): 9747 - 9758, 2009

OCCURRENCE OF ONION BACTERIAL BULB ROT CAUSED BY *Burkholderia cepacia* AND THEIR PREVALENCE IN ONION RHIZOSPHERE, FIELD SOIL AND STORAGE DEBRIS IN EGYPT

Abdalla, M. E.
Plant Pathology Dept, Faculty of Agric., Mansoura Univ., Egypt.
e-mail: abdallah@mans.edu.eg

ABSTRACT

Burkholderia cepacia was identified in samples of onion bulbs, rhizosphere, field soil and storage debris from two different commercial onion production locations at Dakahlia and Gharbia governorates. Percentage of healthy onion bulbs with average of 85-89% was reduced gradually by the end of storage period after harvest to 59-64% in the end of storage period at the two storage locations, respectively. Data indicated that percentage of onion bulb scales with internal and external bacterial soft rot was minimum after harvest (0-2%) at early spring and increased gradually during summer to reach maximum infection (19 -22%) by the end of the summer at the two storage locations, respectively. Symptoms of bacterial rot include a breakdown of one or more inner bulb scales, while adjacent outer scales and inner portions of the bulb may remain intact. Rotten onion scales appear pale yellow, reddish-brown to a darker brown decay. Frequency of *B. cepacia* colonies detected in rhizosphere increased in rhizosphere of onion plants at mature bulbs stage (10^{-6} X 4.8 and 10^{-6} X 5.1 *cfu/g*) compared with rhizosphere of onion immature bulb stage (10^{-6} X 1.9 and 10^{-6} X 2.6 *cfu/ g*) in the two locations, respectively. The recovery of the *B. cepacia* colonies from onion storage debris was higher than any source of onion infection assayed in this study (10^{-7} X3.4 *cfu/ g*). Physiological and biochemical tests showed that onion isolates were gram-negative, rod-shaped with rounded ends, had a few polar flagella and non spore-forming. All the positive isolates accumulated poly-p-hydroxybutyrate, not synthesizing arginine dihydrolase, produced oxidase, not fluorescence, grew at a temperature of +41°C and caused the rot of potato tubers, but did not affect the tobacco leaves. Number of detected and confirmed as *B. cepacia* was 69 isolates from Trypan blue tetracycline (TB-T) medium and 43 isolates from Azelaic acid tryptamine (PCAT) medium. *B. cepacia* was recovered more efficiently on TB-T medium than PCAT medium. Pathogenicity of total 125, 75, 30 and 62 isolates from onion rhizosphere, field soil, bulb onion scales and storage debris, respectively showed that percent area of macerated scales tissue after inoculation and incubation at 30°C for 48h was estimated and a rating of 0 to 3 scale. The large majority of isolates tested were highly virulent, with pathogenicity ratings of 2- 3 indicating higher number of isolates maceration of the onion bulb tissue by 25 (83%) isolates from onion bulb scales followed by isolates from onion rhizosphere (32%) and 24% of isolates from onion storage debris.

Keywords: *Burkholderia cepacia*; Onion; bulb soft rot; Bacteria

INTRODUCTION

Burkholderia cepacia (synonym *Pseudomonas cepacia*; Yabauuchi *et al.* 1992) is a highly versatile bacterium that has adapted to a number of different environments. It is the causal agent of decay of onions or sour skin of onion bulb (Burkholder, 1950). *B. cepacia* emerged as an important

opportunistic human pathogen in the 1970s and 1980s following isolation from the respiratory tracts of patients with cystic fibrosis (CF) in patients with severe chronic lung infections. Therefore, it received intense interest in medical research. In recent years, a growing number of *Burkholderia* strains and species have also been reported as plant-associated bacteria. *Burkholderia* spp. can be free-living in the rhizosphere as well as epiphytic and endophytic, including obligate endosymbionts and phytopathogen (Coenye & Vandamme, 2003; Janssen, 2006). Several strains are known to enhance disease resistance in plants, contribute to better water management, and improve nitrogen fixation and overall host adaptation to environmental stresses (Coenye & Vandamme, 2003; Nowak & Shulaev, 2003; Compant *et al.*, 2005a; Sessitsch *et al*., 2005; Ait Barka *et al.*, 2006; Barrett & Parker, 2006; Janssen, 2006; Balandreau & Mavingui, 2007). These findings have stimulated a growing interest in using *Burkholderia* isolates in agriculture. Due to the fact that some species/isolates can be opportunistic or obligate pathogens causing human, animal or plant diseases, any development of agricultural and/or biotechnological applications using *Burkholderia* germplasm needs to include a stringent assessment of the potential risks (Coenye & Vandamme, 2003 and Compant *et al.*, 2008).

Advances in genetic techniques easily differentiated made *B. cepacia* species including plant pathogens, human pathogens, biological-control strains, and strains active in biodegradation. The results from these genetic analyses revealed that bacteria formerly identified as *B. cepacia* represent a complex of genetically related so far phenotypically diverse strains known as the *B. cepacia complex*. The *B. cepacia* complex currently includes at least nine distinct species. Strains from each of these species have been isolated from human CF patients, and all except *B. dolosa* have been isolated from the plant rhizosphere or soil. The species associated with natural occurrences of plant disease are *B. cepacia* and *B. cenocepacia* on onion and *B. cenocepacia* on banana. The environmental distribution of *B. cepacia* complex strains includes the rhizospheres of several agricultural-crop plants (Jacobs *et al.,* 2009). Plant pathogenic *B. cepacia* strains characteristically produce a polygalacturonase (Peh) that is responsible for the maceration of both bulb scale and leaf tissue and is implicated in disease development (Ulrich 1975 and Gonzalez *et al*., 1997).

Onion, (*Alluim cepa*, L.) is of an outstanding importance among the other crops in Egypt on account of its great value as an exportable commodity. In Egypt, Onion grown as winter, summer crop, as an inter-planted crop for mature dry bulbs and to some extent for the green bunch. More than 70 % of the winter crop is shipped all over the world especially to European countries, which are generally the main import countries (Abo El-Magd, 1973 and Leilah *et. al.,* 2003). Commercially in Egypt; postharvest decay of onion bulbs was examined by inspecting the commercial packages in the market or in storage. Bulb rot incidence was unexpectedly high, and onion bulbs with 1st quality grade were rotten most severely by 51%, followed by 32% for 2nd and 21% for 3rd grades. This indicates that larger bulbs had higher incidences of bulb rots. Moreover, other major pathogens associated with basal and neck rots were *Fusarium oxysporum* and *Aspergillus* sp. or *Botrytis allii*, respectively.

Quality of marketable yield of onion that placed in common burlap bags or kept under normal storage conditions was measured as percentage of total loss in weight of bulbs during a storage period of four months (summer). Total loss during storage onion bulbs within four months was determined by examining the yield, then rotted bulbs were discarded and the remaining yield was weighed. The aim of this work was to determine level of damage, detection frequency in onion bulbs and field soil (rhizosphere of onion). To isolate, identify, and determine the pathogenicity of *B. cepacia* isolates.

MATERIALS AND METHODS

Sample collection and onion bulb quality evaluation

Soil samples and onion rhizosphere were collected on two sampling dates during immature-bulb stage, and mature-bulb stage, respectively, from two commercial onion fields in Dakahlia and Gharbia governorates during the 2007–2008 onion-growing seasons. Moreover, samples of onion storage debris were taken from the two locations for bacterial assessment. The condition of the stored onion was studied in 2007/2008 harvest crop. For bacteriological investigations the onion bulbs were taken from private onion store yards of large farms at the previous two locations. All stored onions samples were evaluated for external and internal symptoms (rot, mold, shriveling). Onion bulbs were selected randomly from onion storage yards. Bulbs were cut in half to observe internal tissue of scales. Examination and evaluation was done on five replicates of average 20 onion bulbs for samples taken in **early spring, mid and late summer** at the two locations.

Pathogen isolation and identification.

The outer bulb scale of infected onion was removed prior to cut the bulbs with a sterile knife. One gram of onion scales were placed inside Petri dish with sterile water, macerated by sterile spoon and diluted with 90 ml of prechilled buffer (0.1 M potassium phosphate [pH 7.0], 0.1% peptone), shaken for 1 h., then transferred to test tubes for serial dilutions. Also, soil, root and onion storage debris samples were weighed (1-g fresh weight) and separately placed in 20 ml prechilled buffer, and bacterial cells were dislodged by shaker. For isolation of the *Pseudomonas* bacteria, samples (0.1 ml) from appropriate serial dilutions were plated on trypan blue tetracycline (TB-T) supplemented with crystal violet (5 mg/liter) and nystatin (50 mg/liter) (Hagedorn *et al.*, 1987) and *P. cepacia* azelaic acid tryptamine (PCAT) selective media (Burbage and Sasser. 1982). The plates were incubated at 29°C for 72 h, following which putative *B. cepacia* colonies were chosen (if present) for further analyses from each of the sampling substrates (soil, rhizosphere, onion scales and storage debris) and medium type (TB-T and PCAT) isolation combinations from the sampling dates. A single colony from each of was chosen randomly for further testing. The bacterial isolates were maintained as stock cultures in 20% glycerol at *5 °C.*

In addition; samples (0.1 ml) from appropriate serial dilutions were plated on the selective agar cetrimide medium (*Pseudomonas* (cetrimide) agar – sigma); the cetrimide, present in the medium, inhibit the growth of other Gram-positive and Gram-negative bacteria. In such medium only bacteria of the genus Pseudomonas grow only. Also, identification of bacterial isolates was performed basing on the analysis of their biological properties (Palleroni Norberto J. 1984, Choult *et al.* 1997).

Physiological, biochemical and Pathogenicity tests. Pathogenicity of strains was investigated employing the methods suggested by Lelliott & Stead (1987) and Hildelbrand *et. al.* (1988). Morphology of colonies was investigated by cultivating bacteria in three agar media: 5% of sucrose, King B, and dextrose. Pathogenic properties of bacterial strain isolated from the rotten onion and rhizosphere soil were tested and pathogenic properties of the isolates were tested artificially infecting tobacco leaves with bacterial suspension of one day (10^{-8} cfu·ml). The results were checked after 24 hours. Also, pathogenic properties of the isolates were also investigated using pieces of potato tubers. (Pasichnik, 1995 and El-Hendawy *et al.*, 2002).

Red onions cultivars (Giza 20 and Giza 6) mostly grown in governorates in Nile Delta of Egypt were used throughout this study in pathogenicity assays. The dry outer bulb scales, of each onion was removed prior to cut the bulbs with a sterile knife. The half's of onion bulbs were placed in a sterile aluminum pan (20 cm by 30 cm) containing two sheets of sterile Whatman no. 1 filter paper pre-moistened with 90 ml of sterile distilled water. The bacterial isolates used in the assays were grown overnight in KB broth. Onion half's were wounded on the inner surface with a sterile pipette tip, and 5 µl of bacterial culture (107 CFU/ml) was inoculated into the wound. The onion cuts were incubated at 30°C for 48 h. The degree of maceration was estimated by probing with a toothpick. The method described by Jacobs *et al.,* 2008 was followed to determine a rating scale of 0 to 3 used to indicate the degree of tissue maceration. A rating of 0 indicated no maceration, 1 indicated 1 to 33% macerated tissue area, 2 indicated 34% to 66% macerated tissue area, and 3 indicated 67% to 100% macerated tissue area. Inoculations with each isolate were replicated three times, and an average rating was tabulated. Negative controls consisted of no wounding and inoculation with King's B broth medium only.

RESULTS

Evaluation of onions quality during storage.

In the present study, stored onions samples from two different locations showed that external and internal symptoms (soft rot, mold, shriveling) were significantly varied during the storage period (5 months) in commercial storage yards. Storage of onion marketable yield was significantly affected by bulb quality. As shown in Fig.1, percentage of healthy onion bulbs was reduced gradually by the end of storage period with average of 85-89% after harvest and reduced to 59-64% in the end of storage period at the two storage locations, respectively. This reduction in quality and

healthy onion percentage was due to physical and biological damage caused mainly by fungi and bacteria. Data indicated that percentage of onion bulb scales with internal and external bacterial soft rot was minimum after harvest (0-2%) at early spring and increased gradually during summer to reach maximum infection (19 -22%) by the end of the summer at the two storage locations, respectively (Fig., 1). Symptoms of bacterial rot symptoms include a breakdown of one or more inner bulb scales, while adjacent outer scales and inner portions of the bulb may remain intact. Rotting scales appear pale yellow, reddish-brown to a darker brown decay. Other causes of onion quality loss were due to mold infection and shriveling symptoms reached to 7-10% reduction or loss of total stored onions.

Detection frequency of *B. cepacia* colonies

Samples assayed for presumptive colonies of *B. cepacia* from two different locations using *Pseudomonas* cetrimide Agar selective medium indicated that recovery of *B. cepacia* colony forming units (C.F.U.) was significantly different according to the source of samples assayed. Frequency of CFU detected in rhizosphere increased in rhizosphere of onion plants at mature bulbs stage (10^{-6} X 4.8 and 10^{-6} X 5.1 *cfu/g*) compared with rhizosphere of onion immature bulb stage (10^{-6} X 1.9 and 10^{-6} X 2.6 *cfu/ g*) in the two locations, respectively (Table 1). The recovery of the *B. cepacia* colonies from onion storage debris was higher than any source of onion infection assayed in this study (10^{-7} X3.4 *cfu/ g*) while the lower frequency of colonies detected was in samples assayed from onion rhizosphere immature (10^{-6} X 1.9 *cfu/g*) bulb stage followed by onion field soil (10^{-6} X2.5 *cfu/g*) as shown in Table 1.

Physiological, biochemical and Pathogenicity tests.

The bacteriological properties of the strains were investigated according to the methods described by Lelliott & Stead (1987) and Hildelbrand et. al. (1988). Examinations of colony morphology showed that the *Burkholderia* species could not be distinguished simply by colony or culture characters. All of the species formed convex colonies with smooth margins or flat colonies with pleated margins on King's B medium and the colonies were of the same colour, but excreted yellow pigment or no pigment into the medium. The properties of the onion isolates were characterized in Table 2. All the onion isolates were gram-negative, rod-shaped with rounded ends, had a few polar flagella and non spore-forming. On conventional YP agar medium (yeast extract 5 g, peptone 10g, agar 15 g, distilled water 1000 ml, pH 6.8) at 27°C., the colonies were circular and convex with entire margins. Moreover, all the positive isolates accumulated poly-p-hydroxybutyrate, not synthesizing arginine dihydrolase, produced oxidase, not fluorescence, grew at a temperature of +41°C and caused the rot of potato tubers, but did not affect the tobacco leaves. The results summarized in Table 2 indicated that 69 isolates from Trypan blue tetracycline (TB-T) medium and 43 isolates from Azelaic acid tryptamine (PCAT) medium were positive identified as *B. cepacia*. *B. cepacia* was recovered more efficiently on TB-T medium than PCAT medium. The pathogenicity of these isolates was further investigated for pathogenicity on onion.

Pathogenicity of *B. cepacia* isolates from different sources

Pathogenicity of total 125, 75, 30 and 62 isolates from onion rhizosphere, field soil, bulb scales and storage debris showed that percent area of macerated scale tissue after inoculation and incubation at 30°C for 48 h was estimated and a rating of 0 to 3 was given in Table 3. Positive pathogenic isolates caused water-soaking and maceration symptoms. The large majority of isolates tested were highly virulent, with pathogenicity ratings of 2- 3 indicating higher number of isolates maceration of the onion bulb tissue by 25 (83%) isolates from onion bulb scales followed by isolates from onion rhizosphere (32%) and 24% of isolates from onion storage debris (Table 3). However, the virulence of 50% of isolates with pathogenicity rating 2 were belong to isolates from rhizosphere and storage debris while isolates with lower pathogenicity ratings 0-1 was belong to isolates from field soil.

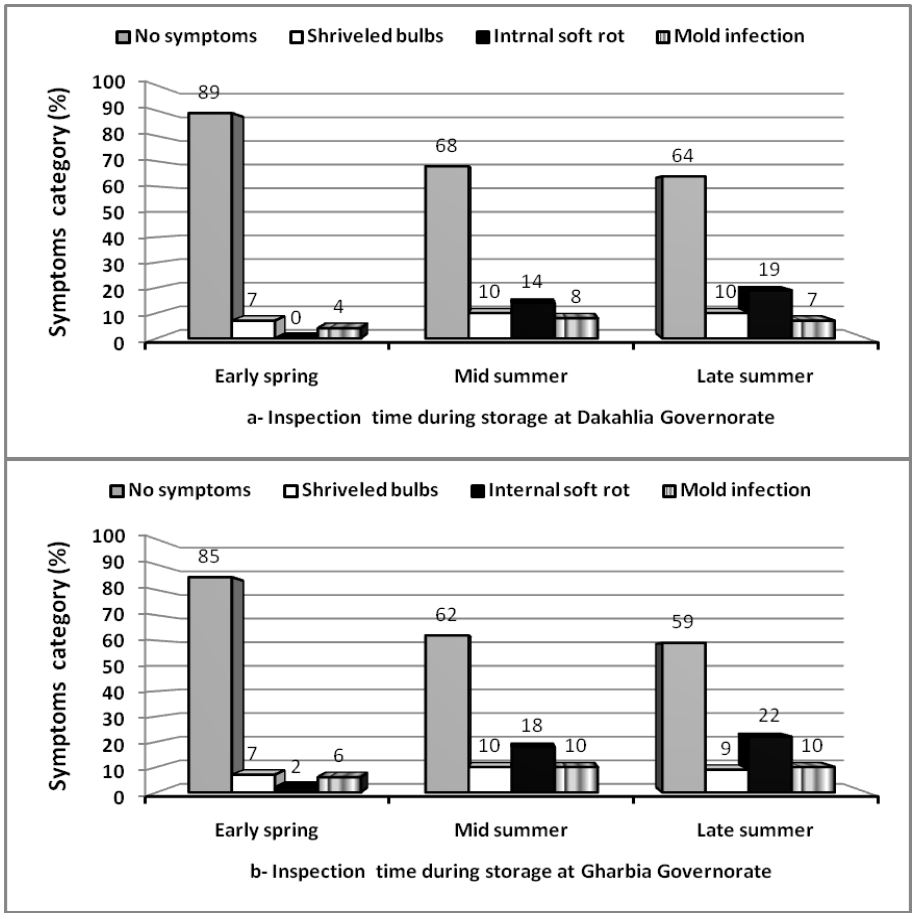

Fig.1: Evaluation of external and internal onion bulbs quality during 3 storage periods in commercial storage yards at two different locations (a) at Dakahlia Governorate and (b) Gharbia Governorate.

Table 1: Detection frequency of *B. cepacia* colonies on selective medium from different onion infection sources

Samples	No. of cells per 1 g of initially (10^{-6} - 10^{-7} X c.f.u ± SD)*	
	Location 1 **	Location 2
-Rhizosphere at immature-bulb stage	10^{-6} X 1.9 ± 0.23	10^{-6} X 2.6 ± 0.31
- Rhizosphere at mature-bulb stage	10^{-6} X 4.8 ± 0.38	10^{-6} X 5.1 ± 0.95
- Onion field soil	10^{-6} X 2.5 ± 0.43	10^{-6} X 3.3 ± 0.21
- Onion storage debris	10^{-7} X 3.2 ± 0.39	10^{-7} X3.4 ± 0.26

*** No. of bacteria (*c.f.u.)* detected on selective medium (*Pseudomonas* cetrimide Agar). Each value represents mean ± SD *(n* = 5).**

**** Isolation done for samples (*n* = 5) from two commercial onion fields and storage yards in Dakahlia and Gharbia governorates, respectively.**

Table 2: Physiological characteristics of *B. cepacia* isolates recovered from onion scales by using two selective media

Test	Trypan blue tetracycline (TB-T) Medium	Azelaic acid tryptamine (PCAT)Medium
	Positive isolates (%)	
- Fluorescence	25	45
- Oxidase	90	78
- Growth at 41 C	88	35
- Potato tuber	82	76
- Tobacco leaf	84	66
Utilization of:		
- L- Arginin	65	65
- Citrate	85	90
- P-B-H, Poly-p-hydroxybutyrate.	82	45
identified and confirmed as: *B. cepacia* (%)*	**69**	**43**

Table 3: Pathogenicity of *B. cepacia* isolates from different sources

Isolates sources[a]	No. of isolates	No. of isolates separated according to bulb maceration scale (0-3)[b]:			
		0	1	2	3
- Onion rhizosphere	125	5 (4%)[c]	18 (14%)	62 (50%)	40 (32%)
- Onion field soil	75	15 (20%)	35 (47%)	22 (29%)	3 (4%)
- Onion bulbs	30	0	2 (7%)	3 (10%)	25 (83%)
- Onion storage debris	62	3 (5%)	9 (14.5%)	35 (56.5%)	15 (24%)

[a] Inoculations with each isolate were replicated three times, and an average rating was tabulated. Negative controls consisted of no wounding and inoculation with KB broth only.

[b] The method described by Jacobs *et al.,* 2008 was followed and a rating scale of 0 to 3 was used to indicate the degree of tissue maceration. A rating of 0 indicated no maceration, 1 indicated 1 to 33% macerated tissue area, 2 indicated 34% to 66% macerated tissue area, and 3 indicated 67% to 100% macerated tissue area.

[C] values inside parenthesis are percentage of values without parenthesis.

DISCUSSION

Phytopathogenic strains of *B. cepacia* have been implicated in both field and postharvest disease problems (Cother and Dowling 1985; Kawamoto and Lorbeer 1972, 1974). In the present study, the majority of *B. cepacia* isolates from different isolation sources examined in an onion pathogenicity assay were incapable of causing water-soaking and maceration symptoms. Our results are similar with many studies shown that strains of *B. cepacia* are capable of causing water soaking and onion tissue maceration in laboratory inoculations (9, 18, 52, 53). All the isolates in this study were examined with appropriate physiological and biochemical tests as available of facilities in our laboratory strengthened with experience to ensure its identity as *B. cepacia*. The selective media used in other related research studies on *B. cepacia* revealed that TB-T medium was the most suitable medium developed for isolation of *B. cepacia* (Hagedorn *et al.*, 1987 and Jacobs *et al.,* 2008). *B. cepacia* isolates were differentially recovered on the PCAT and TB-T selective media. Our results are similar to those of Dalmastri *et al.* (2007) in this regard, with approximately two times more *B. cepacia* bacteria recovered on TB-T than on PCAT medium and an increased number of *B. cepacia* bacteria recovered on PCAT medium. Although neither of these media is sufficiently selective to eliminate other organisms, as shown in this study and in previous studies (Miller *et al*., 2002 and Jacobs *et al.,* 2008), It is recommend that both PCAT and TB-T media be used in combination in population analyses of *B. cepacia* from different environmental sources. However, the key tests for the confirmation of *B. cepacia* were a positive oxidase reaction, utilization of L-arginine and citrate, accumulation of poly-p-hydroxybutyrate, a negative arginine dihydrolase reaction, no fluorescence, growth at 41°C, and utilization of m-hydroxybenzoate (Kawamoto and Lorbeer 1972, 1974; Palleroni, 1984 and Hagedorn *et al*., 1987). Plant pathogenic *B. cepacia* strains characteristically produce a polygalacturonase that is responsible for the maceration of both bulb scale and leaf tissue and is implicated in disease development (Ulrich 1975).

Burkholderia cepacia can be separated from *P. marginalis* or *P. viridiflava* by its lack of production of a water-soluble, fluorescent pigment when grown on King's B medium. Since, many strains of *B. cepacia* will produce a nonfluorescent yellow-green, diffusible pigment in that medium, it is always wise to check for fluorescence using an ultraviolet light source. Other useful tests include checking for pectolytic activity on a pectin gel medium and for rot in onion slices. The most difficult organism to differentiate it from is the slippery skin pathogen, *B. gladioli* pv. *alliicola*. However, most strains of *B. cepacia* will utilize gluterate, putrescine, levulinate and tryptamine but not mesaconate, nicotinate or D(-) tartrate within 7 days, whereas *B. gladioli* pv. *alliicola* usually will test the opposite when exposed to those substrates. Recently developed methods to identify *B. cepacia* include whole cell fatty acid analysis, PCR (clode *et. al.,*1999 and Seo &Tsuchiya, 2004) and hybridization with species-specific rRNA gene probes (Leff *et al.,* 1995 and Sotokawa & Takikawa, 2004).

In this study, significantly more *B. cepacia* bacteria were recovered from onions storage debris and rhizosphere than from soil samples, and assays of the rhizosphere at two different onion bulb stages also indicated a significant association between the sampling time and recovery of *B. cepacia*. This virulent isolates were most commonly isolated after the complete development of the onion rhizosphere at mature bulb stage and was extremely low at the first sampling time, when onion roots were in an initial rhizosphere. The numbers of *B. cepacia* isolates from two different locations of large producing areas of onion fields and storage yards in north Delta of Egypt (Dakahlia and Gharbia governorates) were relatively similar.

In summary, it is determined that *B. cepacia* is present in the onion rhizosphere, infected onions, onion storage debris and onion field soil in Egypt. The vast majority of isolates were pathogenic in an onion disease assay, causing water soaking and tissue maceration. Further investigation are needed with more advanced techniques (PCR) to determine the genetic content, distribution, and mobility of gene sequences involved in virulence variable in other species within the genus of *Burkholderia and B. cepacia* complex species.

REFERENCES

Abo El-Magd, M.M. (1973): Comparative studies on some agricultural treatments and their effects on growth, yield and commercial quality of onion. M.Sc. Thesis Fac. of Agric., Univ. of Ain Shams.

Ait Barka E. ; J. Nowak and S. Clement (2006). Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain Ps JN. Appl Environ Microbiol 72: 7246–7252.

Balandreau, J. and P. Mavingui (2007). Beneficial interactions of *Burkholderia* spp. with plants. *Burkholderia*: Molecular Biology and Genomics (Vandamme P & Coenye T, eds), pp. 129–151. Horizon Scientific Press, Norwich, UK.

Barrett, C.F. and M.A. Parker (2006). Coexistence of *Burkholderia*, *Cupriavidus*, and *Rhizobium* sp. nodule bacteria on two Mimosa spp. in Costa Rica. Appl Environ Microbiol 72: 1198–1206.

Burbage, D. A., and M. Sasser. (1982). A medium selective for *Pseudomonas cepacia*. Phytopathology 72:706.

Burkholder, W. H. (1950). Sour skin, a bacterial rot of onion bulbs. Phytopathology 40:115-117.

Choult Dsz., N. Krieg; T. Snit, Dž. Steili, S. Wiljams (1997). Opredelitel' bakterii Berdszi. Moskva 1, 157–174.

Clode, F.E., M.E. Kaufman, H. Malnick and T.L. Pitt. (1999). Evaluation of three oligonucleotide primer sets in PCR for the identification of *Bukholderia cepacia* and their differentiation from *Burkholderia gladioli*. *J. Clin. Pathol. 52*: 173-176.

Coenye T and P. Vandamme (2003). Diversity and significance of *Burkholderia* species occupying diverse ecological niches. Environ Microbiol 5: 719–729.

Compant S, Duffy B, Nowak J, Cl´ement C & Ait Barka E (2005a) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future prospects. Appl Environ Microbiol 71: 4951–4959.

Compant S.; J. Nowak; T. Coenye; C. Clement and E. Ait Barka (2008). Diversity and occurrence of *Burkholderia* spp. in the natural environment. FEMS Microbiol Rev., 32: 607–626.

Dalmastri, C., A. Baldwin, S. Tabacchioni, A. Bevivino, E. Mahenthiralingam, L. Chiarini, and C. Dowson. (2007). Investigating *Burkholderia cepacia* complex populations recovered from Italian maize rhizosphere by multilocus sequence typing. Environ. Microbiol. 9:1632–1639.

El-Hendawy, Osman M. E. and H.A. Ramadan (2002). Pectic Enzymes Produced *In vitro* and *In vivo* by *Erwinia* spp. Isolated from Carrot and Pepper in Egypt. J. Phytopathology 150, 431–438.

Hagedorn, C., W. D. Gould, T. R. Bardinelli, and D. R. Gustavson. (1987). A selective medium for enumeration and recovery of *Pseudomonas cepacia* biotypes from soil. Appl. Environ. Microbiol. 53:2265–2268.

Hildelbrand D. C.; M.N. Schroth and D.C. Sands (1988). Laboratory Gide for Identification of Plant Pathogenic Bacteria. In.: Schaad N. W. (ed.) 60–80.

Jacobs, J. L.; A.C. Fasi; A. Ramette; J.J. Smith; R. Hammerschmidt and G.W. Sundin (2009). Identification and Onion Pathogenicity of *Burkholderia cepacia* Complex Isolates from the Onion Rhizosphere and Onion Field Soil. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 47(10) 3121–3129.

Janssen P.H. (2006). Identifying the dominant soil bacteria taxa in libraries of 16S rRNA and 16S rRNA genes. Appl Environ Microbiol 72: 1719–1728.

Leff, L.G., R.M. Kernan, J.V. McArthur, and L.J. Shimkets. (1995). Identification of aquatic *Burkholderia (Pseudomonas) cepacia* by hybridization with species-specific rRNA gene probes. *Applied and Environmental Microbiology 61*: 1634-1636.

Leilah, A.A.; S.A. El-Kalla; A.K. Mostafa and H.M.A. Afifi (2003). Performance Of Some Local Egyptian Onions Strains Under Different Planting Dates. Scientific Journal of King Faisal University, 4(1) 119-134.

Lelliott R. A.and D. A. Stead (1987). Methods for Diagnosis of Bacterial Diseases of Plant. Oxford, London.

Miller, S. C. M., J. J. LiPuma, and J. L. Parke. 2002. Culture-based and non-growth-dependent detection of the *Burkholderia cepacia* complex in soil environments. Appl. Environ. Microbiol. 68:3750–3758.

Nowak J and V. Shulaev (2003) Priming for transplant stress resistance in in vitro propagation. In Vitro Cell Dev Biol-Plant 39: 107–124.

Palleroni Norberto J., (1984). Bergey's Manual Systematic Bacteriology. Baltimore: Williams Palleroni, N. J. (1984). Family I. Pseudomonadaceae Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1917, 555AL, p. 141-219. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1., The Williams & Wilkins Co., Baltimore.

Pasichnik L. A., (1995). *Pseudomonas fluorescens* – novy vozbuditel' zabolevanii rži. Mikrobiol. Journ. 57(2), 3–7.

Seo, S.T. and K. Tsuchiya (2004). PCR-based identification and characterization of *Burkholderia cepacia* complex bacteria from clinical and environmental sources *Letters Appl. Microbiol. 39*: 413-419.

Sessitsch A.; T. Coenye; A.V. Sturz; P. Vandamme; E.A. Barka. D. Faure; B. Reiter; B.R. Glick; G. Wang-Pruski G and J.Nowak (2005). *Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant beneficial properties. Int J Syst Evol Microbiol 55: 1187–1192.

Sotokawa, N., and Y. Takikawa. (2004). Occurrence of bacterial rot of onion bulbs caused by *Burkholderia cepacia* in Japan. J. Gen. Plant Pathol. 70:348-352.

Yabauuchi, E., Y. Kosako, H. Oyaizu, I. Yano, H. Hotta, Y. Hashimoto, T. Ezaki and M. Arakawa (1992). Proposal of *Burkholderia* gen. nov.and transfer of seven species of the genus Pseudomonas homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. Microbiol. Immunol. 36:1251-1275.

تواجد مرض عفن البصل البكتيرى للمسبب المرضى *Burkholderia cepacia* وأنتشاره فى المحيط الجذرى لنباتات وتربة الحقل ومخلفات تخزين محصول البصل فى مصر.

محمد السيد عبد الله
قسم أمراض النبات ـ كلية الزراعة ـ جامعة المنصورة ـ المنصورة ـ مصر
e-mail: abdallah@mans.edu.eg

تم عزل وتعريف بكتيريا *Burkholderia cepacia* فى عينات مأخوذة من عدد ٢ موقع تجارى لأنتاج وزراعة وتخزين البصل فى محافظتى الدقهلية والغربية بمصر وأخذت العينات من مصادر مختلفة مثل البصل المصاب بالعفن الطرى ، المحيط الجذرى (*Rhizosphere*) لنباتات البصل ، تربة حقل البصل و مخلفات مخازن البصل المكشوفة. بدأت الدراسة بعمل حصر فى حقول مخازن البصل التجارية فى ٣ فترات لمحصول الموسم (بعد الحصاد فى الربيع وفى منتصف الصيف وفى نهاية الصيف) لمحصول بصل موسم ٢٠٠٨/٢٠٠٧ وأظهرت الدراسة ان نسبة الأبصال السليمة تراوحت من ٨٥-٩٠% ذات الجودة العالية فى بداية موسم التخزين ونقصت تدريجيا لتصبح ٥٩-٦٤% عند نهاية موسم التخزين فى كلا الموقعين الذين تم فيهم الحصر. وأظهرت النتائج ان نسبة الأبصال التى ظهرت عليه الأعراض الداخلية والخارجية للعفن الطرى البكتيرى ٠-٢% فى بداية موسم بعد الحصاد وقد زادت هذه النسبة لتصل إلى اعلى نسبة فى نهاية موسم التخزين فى نهاية الصيف لتصبح ١٩-٢٢% فى موقعين التخزين بالدقهلية والغربية على التوالى. وكانت الأعراض المميزة للعفن البكتيرى فى البصل عبارة عن انهيار فى عدد من الأوراق اللحمية الداخلية للأبصال المصابة وأخذت اللون الأصفر الشاحب إلى اللون البنى المحمر او البنى الداكن بينما الأوراق اللحمية المحيطة سليم ومتماسكة. وكانت تكرار عزلات البكتيريا التى عزلت من عينات المحيط الجذرى لنباتات البصل خلال الفترة الثانية للعزل عند مرحلة نضج الأبصال فى الحقل عالية (10^{-6} X 4.8 and 10^{-6} X 5.1 *cfu/g*) وذلك مقارنة بتكرار العزلات التى ظهرت فى العينات المأخوذة فى الفترة الأولى عند مرحلة الأبصال الغير ناضجة فى الحقل (10^{-6} X 1.9 and 10^{-6} X 2.6 *cfu/ g*) وذلك فى كلا الحقلين بالمحافظتين على التوالى. أجريت الأختبارات الفسيولوجية والبيوكيماوية على عزلات البكتيريا المأخوذة من الأبصال المصابة وأظهرت النتائج أن العزلات سالبة لجرام ، عصوية وذات عدد قليل من الأسواط القطبية ولاتكون جراثيم.

وأن العزلات التى عرفت بأنها *Burkholderia cepacia* تميزت بانها موجبة لأختبار *poly-p-Hydroxybutyrate* و*Oxidase* وسلبية لأختبار *Arginine dihydrolase* وتنمو عند ٤١ درجة مئوية(+41°C) وتحلل درنات البطاطس وسلبية لأختبار أوراق الدخان . وكان عدد العزلات التى تأكيد تعريفها ٦٩ عزلة مأخوذة بالعزل على بيئة *Trypan blue tetracycline (TB-T)* وعدد ٤٣ مأخوذة بالعزل من بيئة *Azelaic acid tryptamine (PCAT)* وكانت البيئة الأولى أكثر كفاءة وأختيارية فى عزل هذا النوع من البكتيريا من البيئة الثانية. كما أجريت أختبارات القدرة التطفلية لأجمالى عدد عزلات ١٢٥, ٧٥، ٣٠ و ٦٢ عزلة معزولة من المحيط الجذرى ، تربة حقل البصل، اوراق البصل المصابة ومخلفات مخازن البصل على التوالى وأظهرت النتائج ان نسبة المساحة المحلله من نسيج أوراق البصل التى تم عمل عدوى لها وتحضينها عند ٣٠° مئوية لمدة ٤٨ ساعة وبأتباع مقياس لتقدير نسبة الأصابة من ٠-٣ . أن معظم العزلات كانت لها قدرة تطفلية عالية عند المقياس ٢-٣ وكانت نسبة العزلات التى وصلت إلى درجة ٣ (١٠٠% تحلل للأنسجة) فى مقياس الأصابة هى ٨٣% من أجمالى العزلات المأخوذة من البصل المصاب بأعراض المرض يليها العزلات المأخوذة من المحيط الجذرى ٣٢% يليها العزلات المأخوذة من مخلفات مخازن البصل ٢٤%.