Journal of Stored Products and Postharvest Research Vol. 3(6), pp. 67 – 74, 16 March, 2012
Available online at http://www.academicjournals.org/JSPPR
DOI: 10.5897/JSPPR11.047
ISSI 2141-6567 ©2012 Academic Journals

Full Length Research Paper

Identification and pathogenicity of phytopathogenic bacteria associated with soft rot disease of girasole tuber in Egypt

Ismail, M. E.[1]* and Moustafa Y.M.M[2]

[1]Department of Plant Pathology, Faculty of Agriculture, El-Minia University, Minia, Egypt.
[2]Department of Horticulture, Faculty of Agriculture, El-Minia University, Minia, Egypt.

Accepted 12 December, 2011

Six bacterial isolates were isolated from naturally infected tubers of girasole plants (*Helianthus tuberosus* L.) cv. Balady showing soft rot collected from experimental Farm of Department of Horticulture, Faculty of Agriculture, in El-Minia University during 2010 and 2011 growing seasons. Pathogenicity tests which showed various virulence for the isolated bacteria towards girasole tubers was found pathogenic and were characterized as rod-shaped, gram negative, α-methyl-d-glucoside medium, reducing substances from sucrose, phosphatase activity and deep cavities on pectate medium. Otherwise, diagnostic tests suggested that the pathogen was *Erwinia carotovora* sub sp*. carotovora.* The isolated bacterial caused soft rot of wounded tubers when inoculated into tissues. The bacterial isolates were compared for their degree of pathogenicity as well as for differences in the specific symptoms induced in the different hosts. The tested isolates could infect several hosts such as fruits of apricot, apple, olive, lemon, squash, eggplant and potato tubers and cloves of bulbs and garlic and onion, root of radish, carrot, sweet potato and rape were infected by the tested isolates. On the other hand, no symptoms were exhibited on pods of bean and cowpea, faba bean, fruits of pepper and tomato. The extracts of experimentally diseased girasole tubers were active in pectinase at pH 6 and also caboxymethyl cellulose on pH 6 compared to enzyme activities in healthy tissues. Also, the bacterial isolated increased the total and reducing sugars in infected than healthy tissues.

Key words: Girasole, *Erwinia carotovora* sub sp*. carotovora*, pectinase and caboxymethyl cellulose, total and reducing sugars.

INTRODUCTION

Jerusalem artichoke is grown primarily for tubers which can be eaten fresh or raw, cooked in appetizing ways similar to Irish potatoes, or pickled. Tubers are used to fatten cattle, sheep and hogs. Stems and leaves are rich in fats, protein and pectin, and make good forage and silage. The alcohol fermented from the tubers is said to be of better quality than that from sugar beets. It is good weed eradicator, as it makes so dense shade that few other plants can compete. It is good in ridding fields of quack grass (Margaritis and Bajpai, 1982). Post harvest diseases caused by bacteria, affects quality and availability of fruit and vegetable (Wells et al., 1993). Bacterial pathogens involved in this respect include the species of soft-rotting Erwinia, Pseudomonas, Xanthomonas, Cytophaga and Bacillus (Liao and Wells, 1987; Lund, 1983).

Plant diseases caused by plant pathogens are complicated process because a number of factors play a part. However, direct involvements of pectic and cellulitic enzymes produced by the pathogen in pathogensis were reported (Gaber et al., 1990; Walker et al., 1994). Bacteria soft rot caused by *Erwinia carotovora* sub sp*. carotovora* (Van Hall), Dye, is one of the most important and widespread bacterial disease of a wide verity plants either in the field or during storage (Hajhamed et al., 2007).

The objective of this investigation is to isolate and identify the pathogenic agent involved or associated soft

*Corresponding author. E-mail: ahmedismael91@yahoo.com.

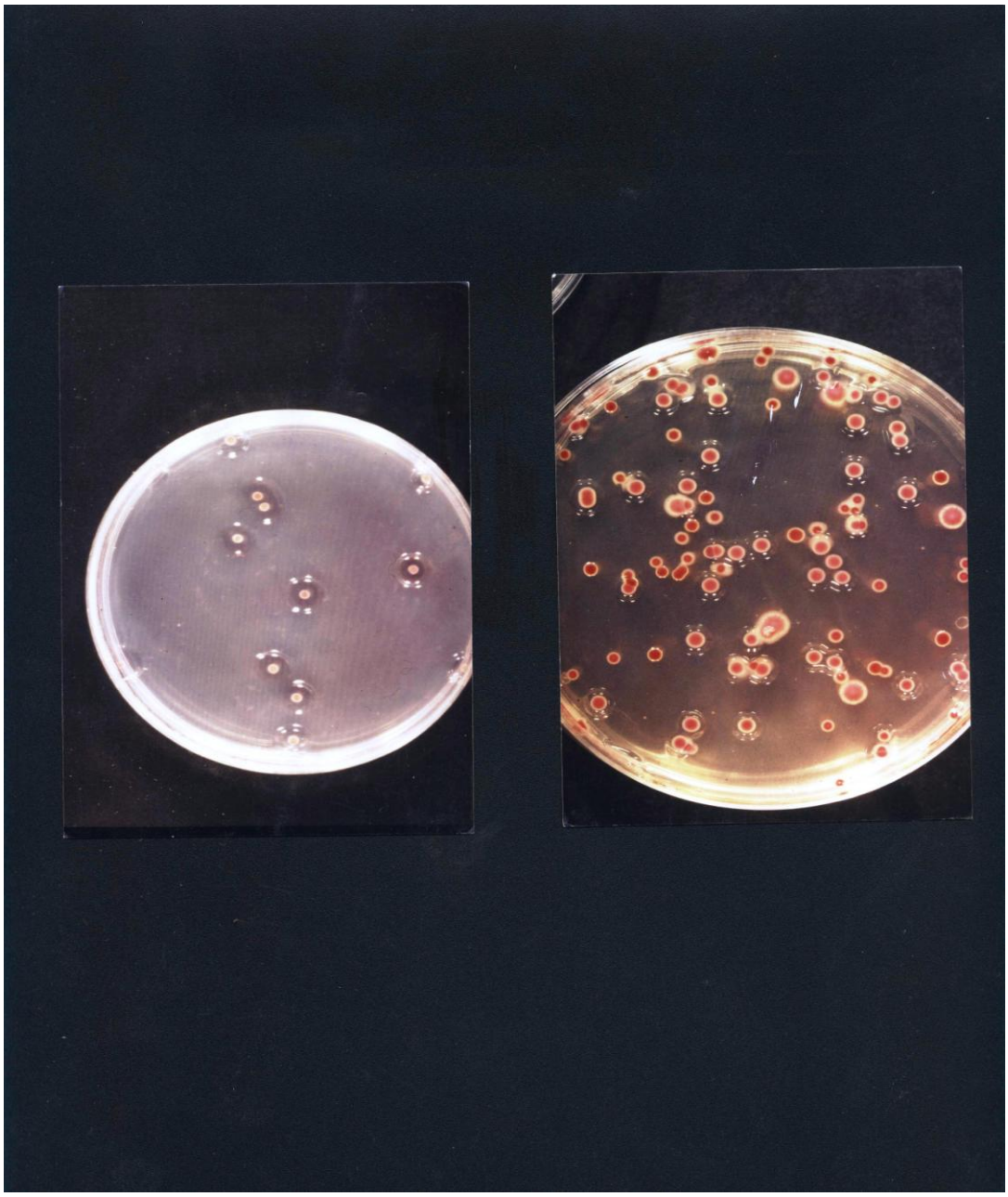

Figure 1. Cavity formation by soft-rot bacteria after incubation at 27°C for 24 h on the CVPM medium (left) and recovery of soft-rot bacteria from artificially inoculated tubers after 24 h (right).

rot disease of girasol tubers at El-Minia governorate. Furthermore, the cell wall degradation enzymes in pathogenesis were discussed.

MATERIALS AND METHODS

Isolation

Infected girasole tubers showing typically develop soft rotting (Figure 1) were subjected for isolation. Samples of girasole tubers rot (cv. Balady) were collected from experimental Farm of Faculty of Agriculture, Department of Horticulture, El-Minia University during 2010 growing season and isolation of the microorganism (s) associated with these symptoms was conducted. Diseased tubers were firstly washed with tap water then surface sterilized with 3% sodium hypochlorite solution (NaOCl) for 3 min then washed thoroughly 3 times with sterilized distilled water, the rotted tissues of tuber was put into sterilized mortar and homogenized then left to stand for 20 min then streaked into plates content crystal violet pectate modified (CVPM) medium (Ahmed et al., 2000). The plates were incubated at 27±1°C for 48 to 72 h. Only bacterial colonies in deep cavities (Figure 2) were subculture onto King's B medium and nutrient agar (NA) medium and stored on slants till they used.

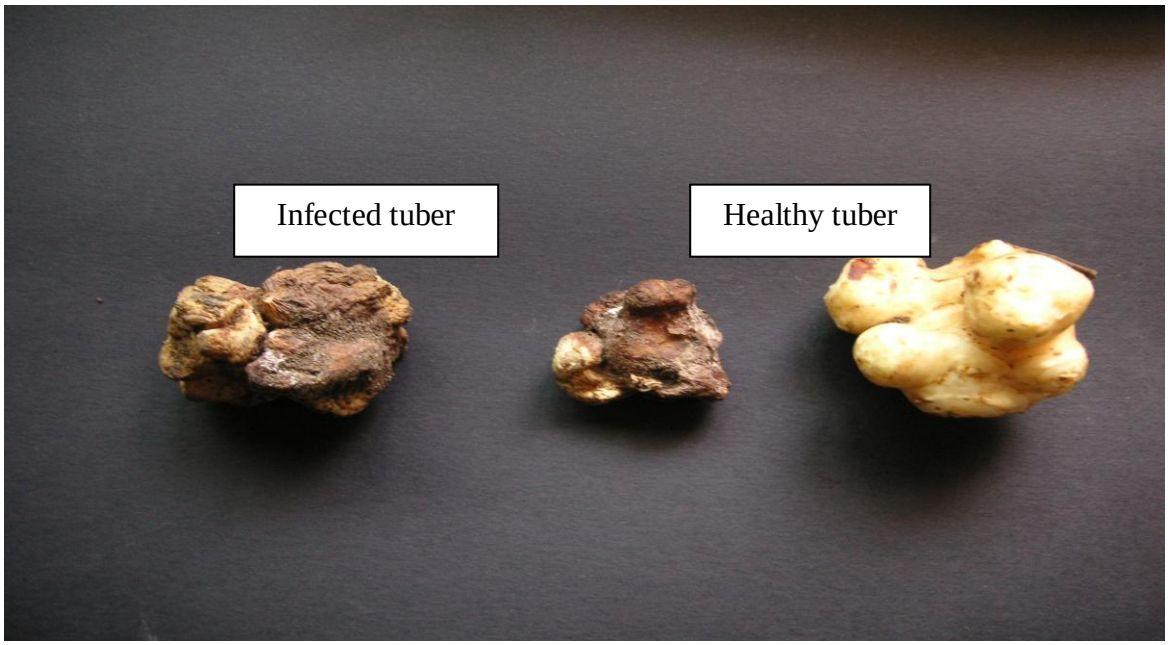

Figure 2. Naturally infected soft rot on girasole tubers cv. Balady infected by *Erwinia carotovora* sub sp. *carotovora* (left infected and right healthy tuber).

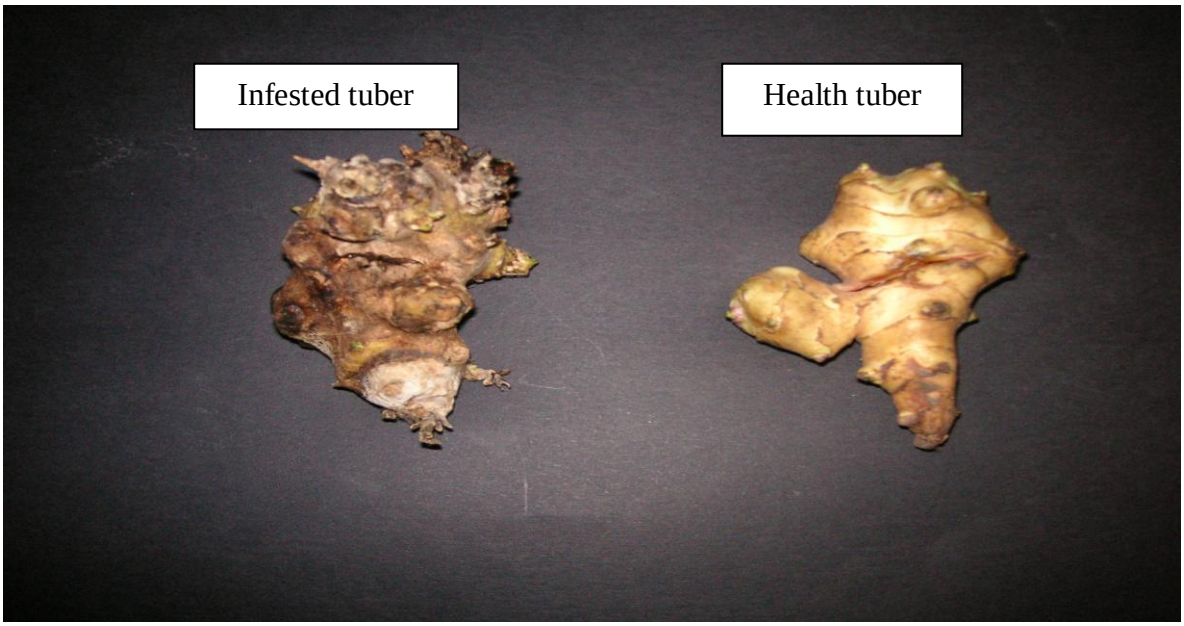

Figure 3. Artificially infested soft rot on girasole tubers cv. Balady infected by *Erwinia carotovora* sub sp. *carotovora* (left infected and right healthy tuber).

Identification of the causal pathogen

Six bacterial isolates, e.g. EC1, EC2, EC3, EC4, EC5 and EC6 were identified by studying their morphological, physiological and biochemical characters that recommended by Breed et al. (1974); Sneath et al. (1986); Lelliott and Stead (1987); Klement et al. (1990).

Pathogenicity tests on girasole tubers

The pathogenicity of the bacterial isolates was determined by inoculating intact unblemished healthy tubers. Each isolate was used to inoculate 5 tubers, 1 cm wound in the middle of tuber and inoculated by smearing the inside of the wound with an entomological needle filled with 48 h-old cultures of the bacterial

Table 1. List of plant species tested for their reaction to lupine foliar pathogens.

Hosts (common name)	Scientific name	Family name	Variety	Part organ
Apricot	*Prunus aremeniaca*	Rosaceae	Canino	Fruits
Bean	*Phaseolus vulgaris*	Leguminosae	Contender	Pods
Carrot	*Daucus carota*	Umbelliferae	Chantinay	Storage roots
Cowpea	*Vigna unguiculata*	Leguminosae	Black eye	Pods
Cucumber	*Cucumis sativus*	Cucurbitaceae	Balady	Fruits
Eggplant	*Solanum melogena*	Solanaceae	Black Beauty	Fruits
Lemon	*Citrus limon* (L.) Burm	Rutaceae	Balady	Fruits
Tobacco	*Nicotiana tabacum*	Solanaceae	Samsun	Leaves
Pepper	*Capsicum frutesences*	Solanaceae	Romy	Fruits
Radish	*Raphanus sativus*	Carucifera	Balady	Storage roots
Onion	*Allium cepa*	Amaryllidaceae	Giza 20	Storage onions
Squash	*Cucurbita pepo*	Cucurbitaceae	Eskandarani	Fruits
Sweet potato	*Ipomea batatas*	Convolvulaceae	Balady	Storage roots
Potato	*Solanm tuberosum*	Solanaceae	Diamant	Tubers
Tomato	*Lycopersicon esculentum*	Solanaceae	Super strain B	Fruits
Turnip	*Brassica rape*	Carucifera	White globe	Storage roots

isolates grown on NA medium individually. Inoculated tubers (cv. Balady) were kept in sterilized boxes containing piece of sterilized distilled water-saturated cotton to insure high humidity and incubated at 25±1°C. Seven days later, rot quantity and rot severity were assayed.

The amount of rotten tissue produced in each tuber was determined and the percentage of rotten tissue was calculated and taken as a criterion of the pathogenicity to each isolate every tuber was weighted before and after removing the rotten portion and calculation to the formula according to Kelman and Dickey (1980) as follows:

$$\text{Rot severity} = (W1-W2)/W1 \times 100 \quad (1)$$

Where, W1= weight of whole tuber and W2= weight of tuber after removal of the rotten tissue.

Host range

The highly pathogenic isolate EC1 of the causal pathogen was inoculated into 17 plant species as listed in Table 3. Five plants were used in each treatment. Control treatments were similarly tested with sterile water only and kept at the same conditions. Disease severity was recoded after 7 days as in Equation 1.

Assessment of some hydrolytic enzymes (cellulase and pectinase) in diseased and healthy girasole tubers

Assessment of pectinase and cellulase enzymes were assayed in tissue extracted from diseased and healthy tuber taken from the subjected plants during pathogenicity test. Half gram of either healthy and/or infected rot tissues were existed and separately macerated with sterilized mortar containing 5 ml of 0.05 M phosphate buffer (pH 6). The homogenated tissue extracts were filtered through two layers of cheese-cloth, cooled to temperature near zero then centrifuged at 5000 rpm for 20 min. The clarified enzyme preparation of healthy and infected tissues was directly subjected to the viscometrical assessment according to Mahadevan and Sridhar (1982).

Total carbohydrate and reducing sugars in healthy and artificially inoculated girasole tubers

Total carbohydrates: The phenol-sulphuric acid method was used for determine the total sugars in clarified tissue extract as described by Hodge and Horfreir (1962).

Determination of reducing sugars: This was performed according to the methods of Somogyi (1952).

Statistical analysis

All experiments were performed twice. Analyses of variance were carried out using MSTAT-C program version 2.10 (1991). Least significant difference (LSD) was employed to test for significant difference between treatments at $P \leq 0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Isolation and identification of the causal organisms:

Six isolates of creamy-white bacteria were isolated from girasole plants showing typical tuber rot symptom (Figure 1). Regardless of some slight differences in certain characteristics, all bacterial isolates appeared to be representative of *Erwinia carotovora* sub sp*. carotovora* (Table 1) according to description of Bergey's Manual of Determinative Bacteriology (1974), also in the Bergey's Manual of Systematic Bacteriology (Sneath et al., 1986). However, the tests were carried out as described by Lelliott and Stead (1987) and Klement et al. (1990). The results in the present work revealed that all the tested bacterial isolates are rod shaped, motile, gram negative, non spore forming, growth at 35°C, facultative anaerobic

Table 2. Morphological, biochemical and physiological characters of bacterial isolates.

Test	Bacterial isolates						Bradbury (1986)
	Ec1	Ec2	Ec3	Ec4	Ec5	Ec6	
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+	+	+
Gram reaction	+	+	+	+	+	+	-
Pigment on CaCO3 agar	-	-	-	-	-	-	-
Sporulation	+	+	+	+	+	+	-
Potato slices	-	-	-	-	-	-	+
Aerobiosis	+	+	+	+	+	+	F
Gelatin liquefication	+	+	+	+	+	+	+
Catalase production	+	+	+	+	+	+	-
Levan production	-	-	-	-	-	-	-
Indole formation	+	+	+	+	+	+	-
Tolerance 5, and 7% NaCl	+	+	+	+	+	+	+
Maximum temperature	35	35	35	35	35	35	30
Utilization of sugars from Arabinose	-	-	-	-	-	-	?
Galactose	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	?
Lactose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	D
Insitol	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	-
Mannitol	+	+	+	+	+	+	?
Mannose	+	+	+	+	+	+	?
Sorbitol	+	+	+	+	+	+	?
Trehalose	+	+	+	+	+	+	+
Celliobiose	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	?
Raffinose	+	+	+	+	+	+	+
Sucrose	-	-	-	-	-	-	-

+ = all isolates are positive, (+) = weakly reaction, - = negative reaction, D = isolates differed, F = facultative anaerobic and? = isolates not tested.

and negatively reacted with phosphatase production, indol formation, and H_2S production, while they positively reacted toward gelatine liquefaction, rot of potato and carrot slices, growth in presence of 5% NaCl, nitrate reduction was reduced. All the tested isolates produce deep cavities semi-selective (CVPM) medium. Otherwise, the bacterial isolates utilized glucose, galactose, fructose, cellubiose, lactose, mannitol, raffinose, trehalose, mannose and xylose but they did not utilize arabinose, maltose, sorbitol, sucrose and methy glucoside. Comparison of the characters of the isolated bacteria with those reported by Dye et al. (1980) and Dickey (1981) could be identified as *Erwinia carotovora* sub sp*. carotovora*.

Pathogenicity tests

Data presented in Table 2 indicate that all bacterial isolates under investigation were able to infect girasole tubers and induce soft rot although they varied in severity of rot they initiated. Inoculation with any of these isolates showed disease symptoms appearing soft rot at wounded sites, and eventually collapsed within two weeks. However, the control plants remained unaffected. Soft rot symptoms (Figure 2 and 3), sites of inoculation were obvious 5 to 10 days after inoculation, whereas from 10 to 15 days, the tubers were collapsed. Amount of rotting, also rated from 22.2 and 42.2% after 21 days from incubation. Also, the obtained results indicate that isolate

Table 3. Symptoms expression with 6 bacterial isolates of *Erwinia carotovora* sub sp. *carotovora* on different hosts.

Hosts bacterial isolates	Site of inoculation	*Erwinia carotovora* sub sp. *carotovora* isolates					
		Ec1	Ec2	Ec3	Ec4	Ec5	Ec6
Apricot	Fruits	+ [a]	+	+	+	+	+
Bean	Pods	-	-	-	-	-	-
Carrot	Storage root	+	+	+	+	+	+
Cowpea	Pods	-	-	-	-	-	-
Cucumber	Fruit	+	+	+	+	+	+
Eggplant	Fruits	-	-	-	-	-	-
Lemon	Fruits	+	+	+	+	+	+
Tobacco	Leaves [b]	+	+	+	+	+	+
Pepper	Fruits	-	-	-	-	-	-
Radish	Storage roots	+	+	+	+	+	+
Onion	Leaves	+	+	+	+	+	+
Squash	Fruits	+	+	+	+	+	+
Sweet potato	Storage roots	-	-	-	-	-	-
Potato	Tubers	+	+	+	+	+	+
Tomato	Fruits	-	-	-	-	-	-
Turnip	Storage root	+	+	+	+	+	+

[a]Data are means of 5 replicates per treatment; [b] Hypersensitive reaction (HR).

Table 4. Effect of extract of diseased girasole tubers on percentage loss of viscosity of 1% carboxymethyl cellulose (CMC) solution during incubation for 3 h at room temperature.

Time (min)	Percent loss in viscosity of 1% carboxymethyl cellulose (CMC) solution					
	Erwinia carotovora sub sp. *carotovora* isolates					
	Ec1	Ec 2	Ec 3	Ec 4	Ec 5	Ec 6
0.0	22.7 [a]	15.5	16.7	11.1	0.0	10.9
30	35.4	22.1	23.7	23.0	12.2	17.8
60	43.0	28.7	39.5	27.5	20.3	22.4
120	57.4	36.1	42.6	32.6	28.9	33.4
180	57.4	36.1	43.90	32.9	28.9	33.6

[a] Values are mean of 3 replicates.

Ec1 and Ec3 could be considered as highly pathogenic, whereas other isolates were weak virulent. Several authors reported that *Erwinia chrysanthemi* and *Erwinia carotovora* sub sp. *carotovora* were isolated from different plants and caused soft rot diseases (Liu et al., 2002; Scortichni and Ascenzo, 2003; Hajhamed et al., 2007).

Host range

Results in Table 3 show that all isolates produced soft rot on most different plant tested. On the other hand, the following plants are not affected by inoculated bacteria such as pods of cowpea, bean and fruits of eggplant, pepper, sweet potato and tomato.

Production of pectolytic and cellulolytic activity by *Erwinia chrysanthemi in vivo*

All tested isolates were active in secreting pectolytic and cellulolytic enzymes in tuber tissues of girasole plants after 10 days of inoculation (Tables 3 and 4), whereas the isolate Ec1 (more virulent) was higher after 180 min than their activities with the weakly virulent (isolate (Ec5). These results confirmed those reported by Saleh (1995), Ouf et al. (1997) and Galal et al. (2002). They reported that the pectolytic activity of the enzymes were higher in infected tissues than in the healthy ones.

Activity of these enzymes was higher in infected tissue than in healthy ones. Data indicated that the highest activity was shown after 2 h incubation at room temperature. These results are generally in line with those reported

Table 5. Total carbohydrate and reducing sugars in healthy and diseased tissue extracts of girasole plants inoculated with bacterial isolates.

Treatment	Total carbohydrate (mg/g fresh weight)	Reducing sugars(mg/g fresh weight)
Control (healthy tissue)	36.22 ± 1.4 [a]	20.42 ± 3.3
Isolate Ec1	95.44 ± 3.4	17.82 ± 3.0
Isolate Ec2	44.13 ± 3.2	10.19 ± 2.0
Isolate Ec3	82.85 ± 2.3	13.23 ± 2.4
Isolate Ec4	41.17 ± 3.0	11.22 ± 5.0
Isolate Ec5	35.27 ± 1.0	9.12 ± 4.1
Isolate Ec6	39.12 ± 3.0	7.33 ± 3.5

[a] Data are means of 3 replicates ± SD.

Table 5. Total carbohydrate and reducing sugars in healthy and diseased tissue extracts of girasole plants inoculated with bacterial isolates.

Treatment	Total carbohydrate (mg/g fresh weight)	Reducing sugars(mg/g fresh weight)
Control (healthy tissue)	36.22 ± 1.4 [a]	20.42 ± 3.3
Isolate Ec1	95.44 ± 3.4	17.82 ± 3.0
Isolate Ec2	44.13 ± 3.2	10.19 ± 2.0
Isolate Ec3	82.85 ± 2.3	13.23 ± 2.4
Isolate Ec4	41.17 ± 3.0	11.22 ± 5.0
Isolate Ec5	35.27 ± 1.0	9.12 ± 4.1
Isolate Ec6	39.12 ± 3.0	7.33 ± 3.5

[a] Data are means of 3 replicates ± SD.

Table 7. Effect of extract of diseased girasole tubers on percentage of viscosity of 1% citrus pectin solution during incubation for 3 h at room temperature.

Time (min)	Percent loss in viscosity of 1% citrus pectin					
	Erwinia carotovora sub sp. *carotovora* isolates					
	Ec1	Ec 2	Ec 3	Ec 4	Ec 5	Ec 6
0.0	6.8 [a]	5.4	5.4	2.4	2.4	2.0
30	19.7	8.2	13.2	14.5	6.5	7.1
60	33.2	11.8	20.8	17.8	15.8	17.7
120	38.0	21.4	30.4	21.3	17.3	23.6
180	38.0	21.4	30.4	21.3	17.3	23.9

[a] Values are mean of 3 replicates.

by previous investigators Ouf and El-Sadek (1997); Ouf et al. (1997). Similar results were reported for *Bacillus subtilis* and *Erwinia chrysanthemi* causing soft rot of carrot roots (Kararah et al., 1985; Saleh and Gabr, 1989; Saleh, 1995; Saleh and Stead, 2003). Severin *et al*. (1985) reported that *Erwinia carotovora* pv. *carotovora*, *E. c.* sub sp. *atroseptica*, *Erwinia chrysanthemi* sub sp. *chrysanthemi* and *Xanthomonas campestris* pv. *pelargonii* (the causal pathogens of soft rot of potato, dahlia and pelargonium, respectively) were able to produce pectinase (s) and cellulase (s) enzymes.

Generally, data in Tables 5 to 7 indicate that total carbohydrates were much higher in inoculated tissue extracts than in healthy ones particularly with isolate Ec1 (more virulent). Similar trends were obtained with reducing sugars. Data presented by Saleh (1995) indicate a similar effect of the pathogen (*Bacillus subtilis* and *B. pumilus*) on total carbohydrates in infected tissues.

REFERENCES

Ahmed ME, Mavridis A, Rudolph K (2000). Detection of latent contamination of potato tubers by *Erwinia spp*. using a semi-selective

agar medium. Phytomedizin, Mitteilung. Deutsch. Phytomedizin. Gesellsch., 30(1): 15-16.
Breed RS, Murray EGD, Smith NR (1974). Bergey's Manual of Determinative Bacteriology 8 th ed. 1268 p. The Williams and Williams Company, Baltimore.
Dickey DS (1981): *Erwinia chrysanthemi*: Reaction of eight plant species to strains from several hosts and to strains of other *Erwinia* species. Phytopathology, 71: 23-29.
Dye DW, Bradbury JF, Goto M, Hayward AC, Lelliott RA, Schroth MN (1980). International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Rev. Plant Pathol., 59: 163-168.
Gaber MR, Saleh OI, Abo El-Fotouh E (1990). Pectolytic, cellulolytic and proteolytic activities of two isolates of *Botryodiplodia theobramae.* Annals Agric. Sci., Fac. Agric., Ain Shams Univ., Cairo, Egypt, 35(1): 445-457.
Galal AA, Abdel-Gawaed, TI, El-Bana AA (2002). Post – harvest decay of garlic cloves caused by *Bacillus polymyxa* and *Fusarium moniliforme*. Egypt. J. Microbiol., 36: 71-88.
Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research. Wiley, Interscience Publication New–York, p. 678.
Hajhamed AA, Abd El-Sayed W. M, Abou El-Yazied A, Abd-El-Ghaffar NY (2007): Suppression of bacterial soft rot disease of potato. Egyptian J. Phytopath., 35(2): 69-80.
Hodge JE, Horfreir BT (1962). Determination of reducing sugars and carbohydrates (In: Methods in Carbohydrates Chemistry. Academic Press, New York, 1: 380-394.
Kararah MA, Barakat FM, Mikhail MS, Fouly HM (1985). Pathophysiology in garlic cloves inoculated with *Bacillus subtilis, Bacillus pumilus, and Erwinia carotovora*. Egyptian J. Phytopathol., 17(2):131-140.
Kelman A, Dickey RS (1980). Soft rot or "*carotovora*" group. In: Schaad. N.W. (ed) Laboratory "Guide gor Identification of Plant Pathogenic Bacteria APS St" Paul Mim. USA, pp. 31-35.
Klement Z, Rudolph K, Sands DC (1990). Methods in Phytobacteriology Akademiai, Kiado, Budapest, p. 568.
Lelliott RA, Stead DE (1987). Method in Plant Pathology. Method for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientfic Publication, Oxford, London, Edinburg, pp. 1-216.
Liao CB, Wells JM (1987). Diversity of pectolytic, fluorescence pseudomonas causing soft rot of fresh vegetables at produce market. Phytopathology, 77: 673-677.
Liu HL, Hsu ST, Tzeng KC (2002). Bacterial soft rot of *chrysanthemum* cuttings: characteristics of the pathogen and factors affecting its occurrence. Plant Pathol. Bull., 11 (3): 157-164.
Lund BM (1983). Bacterial spoilage. In*: Postharvest Pathology of Fruits and Vegetables.* (C. Demis, ED. Academic press, London), pp. 219-257.
Mahadevan A, Sridhar R (1982). Methods in Physiological Plant Pathology, Sivakami publications, India, p. 119.
Margaritis A, Bajpai P (1982). Continuous ethanol production from Jerusalem artichoke tubers. II. Use of immobilized cells of Kluyveromyces marxianus. Biotechnol. Bioeng., 24: 1483- 1493.
Ouf MF, El-Sadek SAM (1997). Bacterial stem rot of dieffenbachia in Egypt. Egypt J. Microbiol., 32 (2): 269-281.
Ouf MF, El-Sadek SAM, Abdel-Latif MR, Abd El-Aziz N (1997). Pectolytic and cellulolytic activity of enzymes by *Erwinia dieffenbachia*, causing stem soft rot on dieffenbachia plants. Egypt J. Microbiol., 32(1): 99-115.
Saleh OI (1995). Identification of phytopathogenic bacteria associated with post harvest disease of garlic cloves in relation to cell wall degrading enzymes. Egyptian J. Microbiol., 30: 177-202.
Saleh OI, Stead D (2003). Bacterial soft rot disease of pea in Egypt. Integrated control in protected crops, Mediterranean climate IOBC WPRS Bulletin, 26(10): 115-120.1-218. Editura Ceres, Bucharst, Romania.
Saleh OI, Gabr MR (1989): Studies on core rot of carrot in relation to cell wall- degrading. Minia J. Agric. Res. Dev., 11: 1713-1722.
Scortichni M, Ascenzo D (2003). New bacterial diseases striking ornamentals. Colture Protelle, 32(5): 81-84.
Severin V, Kupferberg S, Zurini I (1985). Plant Pathogenic Bacteria. Editura Ceres, Bucharst, Romania, pp. 1-218.
Sneath PHA, Mair NS, Sharpe ME, Holt JG (1986). Bergey's Manual of Systematic Bacteriology. Williams Wilkins Co., Baltimore, pp. 1123.
Somogyi M (1952). Notes on sugar determination. J. Biol. Chem., 195: 18-23.
Walker DS, Reeves PJ, Salmond GP (1994). The major secreted cellulose, CelV, of *Erwinia carotovora* sub sp. *carotovora* is an important soft rot virulence factor. MPMI, 7(3): 425-431.
Wells JE, Butterfield JE, Revear LG (1993). Identification of bacteria associated with postharvest diseases of fruits and vegetables by cellular fatty acid composition: An expert system for personal computers. Phytopathology, 83: 445-455.