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Inhibitory Effect and Morphological Changes by Organic Acids to Bacterial Strains Causing Sugar Beet Soft Root Rot *In Vitro*

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ABSTRACT

Sugar beet affected by soft rot bacteria and causes great loses in yield. Organic acids are known for their antibacterial and antifungal properties. Three strains of soft rot bacteria, *Erwinia carotovora* subsp. *carotovora* (Ecc), *Erwinia carotovora* subsp. *betavasculorum* (Ecb) and *Burkholderia cepacia* (Bc) were treated with acetic, ascorbic and citric acids with concentrations (1.0, 2.5 and 5%) and control was used SDW and antibiotic (Streptomycin 0.015%). The (MIC) of acetic acid for Ecc, Ecb and Bc was determined at concentrations ranging (0.25, 0.35, 0.50, 0.75, 0.80 and 0.90 % V/V); controls were maintained with sterilized water and antibiotic (Streptomycin 0.015%). Determination of inhibitory effect to acetic acid on Ecc by culturing it in NA broth medium to mid-log phase and bacterial suspension (10^8cfu/ml) was prepared. The cell suspension was incubated at 37^{o}C for 60 min with acetic acid at a concentration of MIC (0.90 %) then observed using a scanning electron microscope. The organic acid, acetic acid, had highest efficacy in inhibiting the growth of Ecc, Ecb and Bc at concentration 5% with inhibition zone 23 mm. The MIC of acetic acid on Ecc, Ecb and Bc was 0.90 %V/V showed the highest antibacterial activity against bacterial strains, while concentrate 0.25% revealed the lowest inhibitory effect. The cells treated with acetic acid became swell and enlarged in size then explode and smallest, surface roughening and corrugating compared with the control.

Keywords: sugar beet, soft rot bacteria, Acetic acid, ascorbic acid, citric acid, MIC.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the world's two major sugar crops (Alfaigey *et al*,. 2011, Draycott 2006 and Scalon *et al*., 2000). Sugar beet is an herbaceous dicotyledonous plant belonging to the *Chenopodiaceae* family. A soft rot decay of sugar beet was observed in multiple commercial fields in northern parts of Egypt and causes serious yield losses in the field and economic losses in the factory. The *Enterobacteriaceae* family include together different types of plant pathogenic bacteria that cause finely disinfection in a wide range of plants (Toth *et al*., 2003). Bacterial vascular necrosis and rot in sugar beet was discovered in 1972 (Thomson and Schroth, 1972). *P. carotovorum* divided into five subspecies: *atrosepticum*, *betavasculorum*, *carotovorum*, *odoriferum* and *wasabiae* (Hauben *et al*., 1998). The pathogen was later described as a strain of *E. carotovora*: subsp. *betavascculorum*, responsible for vascular necrosis of sugar beet (Thomson *et al*., 1981). The *Erwinia* genus produced a high levels of multiple exoenzymes, including pectinases, cellulases, and proteases, which interruption plant's cell walls (Barras *et al*., 1994; Thomson *et al*., 1999). Pectinases are the main enzymes involved in the development of the disease. These external enzymes disintegrate and pectin are used in the central plate and plant cell walls, causing tissue breakdown, cell damage, and cell leakage (Barras *et al*., 1994; Pérombelon, 2002; Barras *et al*., 1987). Pathogenic bacteria create infection by developing antibiotic resistance and modifying the host's immune system.. The use of chemicals to control mild rot is not generally recommended due to the high risk of residual impact of toxic chemicals that may be hazardous to the health of consumers (Agrios, 1997). Organic acids are known for their antibacterial and antifungal properties. Levin and Feller (1940) showed that acetic acid(AA) was more influential on microorganisms than lactic acid and concluded that the toxicity was due to the concentration of the hydrogen ion and the concentration of the acid separated. The inhibitory effect for AA on the micro-organisms due to pH alone, and can penetrate AA non-discrete microbial cell for the exercise of the toxic impact (Banwart, 1981). AA is generally recognized as a safe compound (GRAS) and can be compared to other GRAS compounds, such as hydrogen peroxide, bicarbonate salts, carbonates, chlorine and sugar isotopes, because they leave low or undiscovered residues rapidly degrade and degrade rapidly in plant tissues (Barkai, 2001). The antibacterial activity of ascorbic acid had been reported from the beginning of the twentieth century. The scientists found that inhibition of *Bacillus subtilis*, *Escherichia coli* and *Proteus vulgaris* by ascorbic acid was counteracted by the presence of reducing agents and by substances which catalyzed the breakdown of hydrogen peroxide. They concluded that inhibition was due to hydrogen peroxide formed during the oxidation of ascorbic acid automatic (Myrvik and Volk 1954). Citric acid as well as ascorbic acid can neutralize harmful oxygen radicals. The aim of this study is to investigate the minimum inhibition concentration (MIC) effect of three organic acids (acetic, ascorbic and citric acid) on growth of three bacterial soft rot strains under laboratory condition. Also, to observe the morphological changes of three bacterial isolates treated with acetic acid using scanning electron microscope (SEM).

MATERIALS AND METHODS

Source of bacterial soft root rot pathogens

The sugar beet roots of different cultivars namely (Megharbel, Farida, Debria, Glorius and Kawemira) collected from different fields of villages located in 3 governorates were used for isolated the bacterial soft rot (Table 1). Diseased roots showing the symptoms of soft rot were taken from field and storage area.

Isolation and Purification of soft rot bacteria

Soft rot bacteria were isolated from diseased sugar beet roots which were selected based on visible symptoms of soft rot disease. Infected root samples were surfaces sterilized with 5% sodium hypochlorite for 10 minutes then washed three successive times in distilled water. A small amount of tissue was removed from margins of the necrotic vascular bundles of diseased roots with a sterile scalpel and a sterile mortar and pestle were used to crow the diseased tissue then suspended in 5ml sterile water, macerated and allowed to settle 5 min then a serial dilution was made for each isolate. One loop full of resulting

suspension from dilution 10^{-5} was streaked on the solidified dry plates of modified nutrient agar (mNA) medium (Difco manual, 1953). The plates were incubated at 28° C for 24-hr.

Table 1. Source of infected roots collected from different sugar beet cultivars grown in different villages belongs to 3 governorates

Governorate	Location		Variety
	County	Village	
Dakahlia	Dekerns	Elmahroun Elrobia Manshit AbdElrahman Negeer	Megharbel, Farida Debria, Glorius and Kawemira
	Belquas	Belquas Qalabsho	
	Bany Abid Sherbin	Mit Adlan Yostin	
Kafer Elshekh	Kafer Elshekh	Sakha	Glorius, Kawemira
El Sharkia	EL-Hosinia	Elroade	Glorius, Kawemira

Identification of the bacterial isolates

Identification of the isolated bacteria as Erwinia's species and its strains was performed according to their morphological and cultural characteristics by the procedures as described in Bergey's Manual of Systematic Bacteriology. A series of physiological and biochemical tests were performed following standard methods for characterization of selected soft rot bacteria i.e., anaerobic growth, yellow colonies on YDC and NA media, fluorescent pigment on KB medium, growth at 37° C, reducing substances from sucrose, sensitivity to erythromycin, indole production, acid production from (lactose, inulin, cellobiose, glycerol and starch), oxidase test, urease activity and utilization of sucrose, maltose, D-tartrate, arginine. The common genera were subjected to further tests to identify their species according to Fahy and Persley (1983); Lelliott and Dickey (1984); Lelliott and stead (1987); Chun and Jones (2001); De Boer and Kelman (2001); Schaad (2001) and Sotokawa and Takikawa (2004).

Hypersensitivity Reaction (HR)

Hypersensitivity response of the isolates was measured on tobacco plant (*Nicotiana tabacum* L.). About 10^8-10^9 CFU/ml of bacterial culture were injected onto tobacco leaf at 5-6-leaf stage and kept in a damp chamber for a few hours. The inoculated plants were incubated in greenhouse for 24–48-hr at 20–28 °C. Controls were similarly inoculated with sterilized distilled water. Positive reaction was complete collapse of tissue after 24-hr, followed by necrosis was interpreted (Klement and Goodman, 1967; Dadaşoğlu 2007).

Pathogenicity test:

Pure cultures of isolated bacteria used for tested the ability to induced rot symptoms in healthy sugar beet plants. Plants were injected with bacterial suspensions into petioles of the first two true leaves. Controls were similarly inoculated with sterilized distilled water (SDW). Plants were then transferred to a 28ºC growth chamber and rated for disease development at 2 and 3 weeks post-inoculation using a 0 to 5 scale (0 = no disease, 1 = small black lesion, 2= wilted petiole, 3 = systemic movement of pathogen evident as black streaking in non-inoculated parts of plant, 4 = whole plant wilting, 5 = dead).

Screening of antibacterial organic acids effect against pathogenic bacterial strains

Three organic acids namely; acetic, ascorbic and citric acid produced by Sigma Chemical Company were used *in vitro* to test its inhibition effect on growth of the three bacterial strains of *Erwinia carotovora* subsp. *carotovora* (Ecc), *E. carotovora* subsp. *betavasculorum* (Ecb) and *Burkholderia cepacia* (Bc) that isolated from infected sugar beet roots.

Three concentrations were prepared of each acid (1, 2.5 and 5%). The disc diffusion method (Las llagas *et al*., 2014) was followed by dipping sterile filter paper discs (6mm diameter) in different organic acid concentrations. Bacterial suspension (10^8cfu/ml) of each fresh strain culture was spread over Petri's dishes contain sterilized NA medium. Acid treatments were prepared as imbibed paper discs of all acid concentrations that placed on the surface of the agar plates. Control plates were paper discs dipped in sterilized water (negative) and paper disc dipped in Streptomycin, 0.015% solution (positive). Three replicates for each concentrate were done. All plates were incubated for 24-hr at 28°C. Inhibition growth diameter were observed and determined. Percentage of inhibition calculated by dividing mean values of treatment inhibition zone / control growth zone X 100.

Determination of minimum inhibitory concentration (MIC) of acetic acid

The MIC of acetic acid for Ecc, Ecb and Bc was determined by NA dilution method. Preliminary screening tests were performed at concentrations ranging (0.25, 0.35, 0.50, 0.75, 0.80 and 0.90 %); then concentrations were added to NA medium immediately before it was poured into the Petri dishes at a temperature of 40–45°C. Parallel controls were maintained with sterilized distilled water and antibiotic (Streptomycin 0.015%) mixed with NA medium.

Bacterial suspension (10^8cfu/ml) was spread over the sterilized N.A medium then incubated at 28°C for 24 hours. The MIC was determined as the lowest concentration of the acetic acid required to completely inhibit a bacterial growth after incubation at 28°C for 24 hours.

Examination of *Erwinia* cells treated with acetic acid using scanning electron microscopy (SEM)

The best result obtained by acetic acid treatment resulted in highest bacterial growth inhibition was selected for further study by SEM examination.

Erwinia carotovora subsp. *carotovora* culture was grown in (NA) broth media to mid-log phase and bacterial suspension (10^8cfu/ml). The cell suspension was incubated at 37°C for 60 min at 120 rpm with acetic acid at a concentration of MIC (0.90 %) and harvested to obtain the bacterial cells. For the scanning electron microscopy analysis, harvested cells placed into eppendorf containing 2.5% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 4ºC and fixed for 24 h. The cells were pelleted, washed to remove the glutaraldehyde and suspended in the same buffer. A drop of bacterial suspension was transferred to poly-L-lysine to make the chips, which were kept in a hydration chamber for 30 min for cell adhesion. The samples were dehydrated through a graded ethanol series

(70 %, 80 %, 90%, 96% and 100%) for 15 min in each alcohol solution and dried, the chips were coated with gold-palladium membranes and observed in a Jeol JSM-6510 L.V SEM, The microscope was operated at 30 KV at EM Unit, Mansoura University, Egypt.

RESULTS AND DISCUSSION

RESULTS

Source of bacterial soft root rot pathogens

Diseased sugar beet roots samples were collected during 2015/2016. Internal symptoms of soft root rot were detected in infected roots samples collected in most cultivars grown in different field locations at three governorates. Roots symptoms wilting also occur below ground symptoms include both soft and dry root rot. Affected vascular bundles in roots become necrotic and brown, and tissue adjacent to necrosis becomes pink upon air contact. The plants that do not die completely may have rotted-out, cavernous roots (Fig 1).

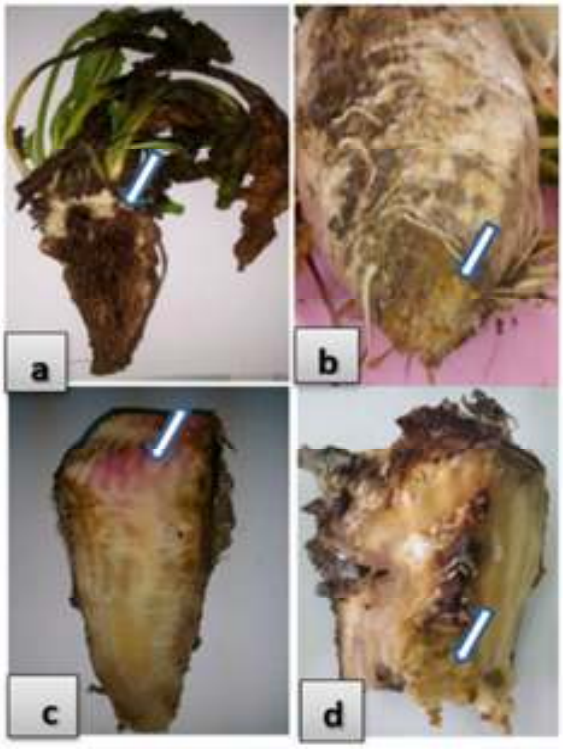

Fig.1. Natural infected sugar beet roots with bacterial soft rot symptoms: arrows in the figure indicate that a) dry root rot, b) external soft root rot, c) pinkish tissue and d) soft root rot.

Isolation, purification and characterization of soft rot bacteria

Data in Table (2) show that among several isolates recovered from sugar beet roots sixty-seven isolates were Gram negative. The following results of bacterial genera differentiation were based on the Schaad (2001) scheme identification chart.

Colony morphology of most of the isolates on mNA agar plates were white, creamy white, yellow and grayish creamy white, smooth, round, glistening, domed, slightly raised. Some colonies were flat to slightly raised, margins undulated to feathery and visible on isolation plates after about 24-hr. Colony size was within the limits of 0.5,1,3,4 and 5 mm.

Identification of the pathogenic bacterial isolates:

Hypersensitivity reaction (HR)

Soft rot bacterial isolates were infiltrated by injection into the mesophyll of tobacco leaves (*Nicotiana. tabacum*). Water soaked and collapse of inoculated tissues in 24-48-hr followed by a dry, light brown necrosis of water-soaked tissues within three days as positive result. All examined isolates elicits a strong HR in tobacco leaves indicating that they are phytopathogenic bacteria. The control and negative isolates failed to produce such response.

Pathogenicity test:

Sugar beet cultivar (Glorious) was inoculated with the 67 bacterial isolates. Only, fifty isolates produced positive results and showed soft rot symptoms. Twenty-four hours after inoculation bacterial strains brown water-soaked lesion was observed at the site of inoculation. After 48-hr bacteria had invaded the plant vascular system and maceration had spread throughout the leaf blade and the petiole. Maceration then occurred in other leaves and after 10-15 days the plants had completely collapsed (about 80% systemic response). Eighteen isolates were less virulent that maceration did not develop systemically in infected plants.

Physiological and biochemical properties

The differentiation of common species to subspecies. A series of physiological and biochemical tests were carried out following standard methods. Soft rot isolates were categorized to three groups based on previous differential characterization tests. The first group of 22 isolates was identified as *Erwinia* spp. *Erwinia* isolates were Gram-negative, motile with polar flagella, non-spore forming, facultative anaerobic and produce white colonies on YDC. Results of further physiological and biochemical tests to differentiate *Erwinia* isolates to subspecies show that isolates were positive in catalase test, negative in oxidase test, do not produce fluorescent pigment on KB agar, sensitive to erythromycin and arginine utilization and they found variable O/F test, growth on 37^0C and reducing substances from sucrose. Also, *Erwinia* isolates were tested for utilization and acid production from D-Lactose, Inulin, Cellobiose, Glycerol, Maltose, Xylose, Starch and Raffinose. Results of these tests show that 2 isolates were identified as *Erwinia carotovora* subsp. *atroseptica*, 9 isolates were identified as *Erwinia carotovora* subsp. *betavasculorum* and 11 isolates as *Erwinia carotovora* subsp. *carotovora*. The second group of bacterial (13) isolates were Gram-negative, motile with peritrichous flagella, non-spore forming, facultative anaerobic and produce yellow colonies on YDC. The isolates were identified as *Pantoea* spp. The third group of bacterial isolates (15) were Gram-negative, motile with multi-trichous flagella and non-spore forming. This third group belongs to *Burkholderia*, it was differentiated with properties as negative on YDC medium, no fluorescent pigment on KB, negative growth on DIM agar medium and negative result of arginine utilization test. Results of further physiological and biochemical tests to differentiate it to species and subspecies show that the isolates were positive in oxidase test, growth at different pH, growth at

40°C, growth in 3% NaCl, starch dehydrolysis, produce yellow colonies and utilization of arginine, D-tartrate Cellobiose, Sucrose and Maltose. Based on the previous results, the 9 isolates were identified as *Burkholderia cepacia* and 6 isolates were identified as *Burkholderia gladioli*.

Table 2. Characterization of soft root rot bacterial isolates recovered from sugar beet roots

	Isolates characterization Categories		
	Group I	**Group II**	**Group III**
Total number	**(22 isolates)**	**(15 isolates)**	**(13 isolates)**
Colony character	Positive growth on mNA agar plates. All colonies had the whitish to creamy or yellowish color and colony shapes of smooth, raised to convex or circular shape.	Colonies on mNA medium plates had circular, convex with entire margin and glistening shape; grown on YDC agar plates as non-yellow colonies, no fluorescent pigment on King's B agar plates.	Colonies on mNA medium plates its shape were smooth, round, glistening, slightly raised and some were flat to slightly raised, margins undulated to feathery and visible. Also; it had yellow color to pale beige.
Colony size (mm)	1, 4 and 5mm	1, 3mm	0.5 and 1mm
Pathogenicity test	+	+	+
Hypersensitivity Reaction	+	+	+
Pectolytic activity tests	+	+	-
Gram staining	G –ve rods	G –ve rods	G –ve rods
KOH test	+	+	+
The motility test	+	+	+
Spore form determination	-	-	-
Ref. strain I *	+	-	n.d.***
Ref. strain II**	-	+	n.d.

***, Ref. I = Reference strain of *Erwinia carotovora* subsp. *carotovora*.**
****, Ref. II =Reference strains of *Burkholderia cepacia*.**
*****, Reference strain not available (n.d. = not done).**

Screening of antibacterial organic acids effect against pathogenic bacterial strains

Among the organic acids tested against all bacterial soft rot bacterial species under study the acetic acid showed highest diameter zone of inhibition of 23 mm at concentration 5% followed by ascorbic acid, with a zone of 10 mm and citric acid with decreasing order of efficacy were showed low efficacy against the test bacteria. Standard check (streptomycin with concentration 0.015%) produce mean diameter zone of inhibition of 15mm. In negative control with sterilized distilled water there was any inhibition zone (Table.3). Based on the above the organic acids, acetic acid having highest efficacy in inhibiting the growth of (Ecc), (Ecb) and (Bc).

Table 3. Effect of three different organic acids concentrations on the growth of sugar beet soft root rot bacterial strains colonies *in vitro*.

Organic acids Concentration (%)	**Bacterial strains***					
	Ecc		**Ecb**		**Bc**	
	Inhibition zone (mm) **	**%Growth inhibition *****	**Inhibition zone (mm)**	**%Growth inhibition**	**Inhibition zone (mm)**	**%Growth inhibition**
Acetic acid						
1%	6	20	08	27	8	27
2.5%	11	36	13	43	11	36
5%	16	53	23	77	19	63
Ascorbic acid						
1%	0	0	0	0	6	20
2.5%	7	23	7	23	8	27
5%	10	33	10	33	10	33
Citric acid						
1%	0	0	0	0	0	0
2.5%	6	20	6.5	22	6	20
5%	7	23	7	23	7	23
Control with SDW	0	0	0	0	0	0
Control with Streptomycin	15	50	15	50	15	50

*** *Erwinia carotovora* subsp. *carotovora* (Ecc), *Erwinia carotovora* subsp. *betavasculorum* (Ecb) and *Burkholderia cepacia* (Bc).**The recorded value is the mean value of three replicates.; *** Percentage of inhibition calculated by dividing mean values of treatment inhibition zone / mean value of control growth zone X 100**

Determination of minimum inhibitory concentration (MIC) to acetic acid

Figure (2) shows the effect of different acetic acid concentrations (0.25, 0.35, 0.50, 0.75, 0.80 and 0.90 %) compared with controls to determine (MIC) of acetic acid on the growth of 3 different bacterial strains under study (Ecc), (Ecb) and (Bc). All concentrations of acetic acid had variable inhibitory effect on all bacteria strains. The MIC was reached at concentration of 0.8% that completely (100%) inhibited *E. c.* subsp. *betavasculorum* and *B. cepacia* while MIC was attained at 0.9% for *E. c.* subsp. *carotovora.*

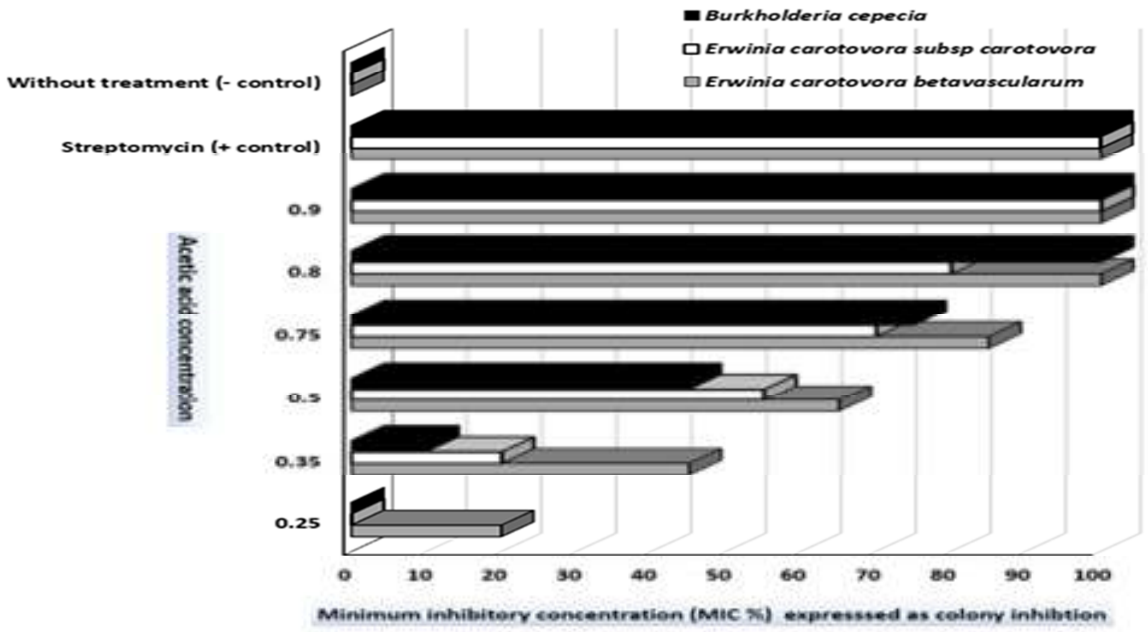

Fig. 2. *In vitro* efficacy of acetic acid against three different soft rot bacterial strains of sugar beet as minimum inhibitory concentration (MIC%) relative to standard antibiotics.

Morphological analysis of *Erwinia* cells treated with acetic acid using scanning electron microscopy (SEM)

Analyze the cell morphology of these microorganisms using scanning electron microscope (SEM) after treatment with acetic acid. Control cells were not treated with acetic acid (Fig.3) in which damage of the cells and deformations of cell wall were detected (Fig. 3d). The present study demonstrates the remarkable MIC conc. that cause alterations in the surface of the *E*. *carotovora* subsp. *carotovora* treated for 60 min. It is clear from the images that the treated bacterial cell forms showed significant structural changes compared to untreated bacterial cells (Fig. 3a). When cells were treated with MIC conc. of acetic acid, structural changes showed roughness on the cell surface, while the treated cells were short, elliptical rods. These bacteria showed deep surface cracks and complete degeneration of the organized structure (Fig. 3c).

The treated bacterial cells of *E. carotovora* subsp. *carotovora*, appeared swell and enlarged in size 4-5µm then explode and smallest in size 0.6-0.8 µm, surface roughening and corrugating, The surface of the membrane caused by the control, which showed a bright and smooth surface without any apparent irregularities (Fig. 3a).

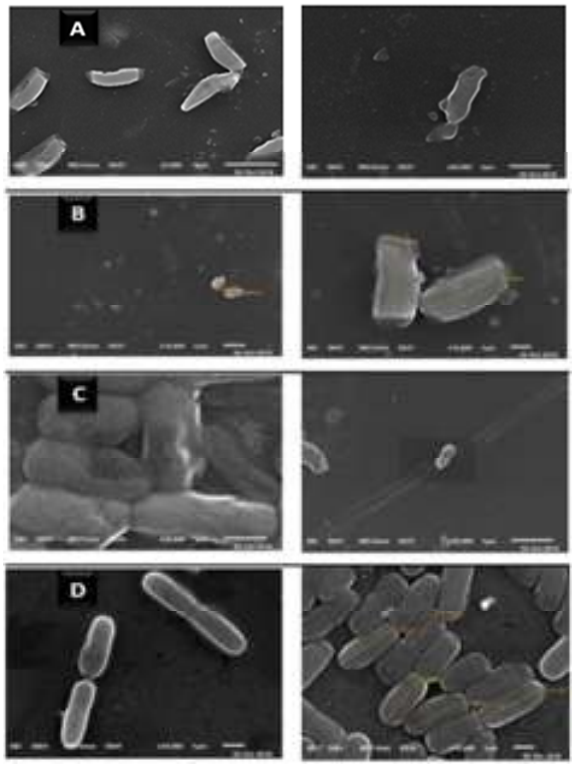

Fig. 3. Scanning electron microscopic micrographs of Ecc treated with acetic acid. (a) Bacteria treated with acetic acid at MIC for 60 min were swell and enlarged, (b) explode and smallest in size, (c) surface roughening and corrugating, (d) non treated standard cell morphology (control).

DISCUSSION

Sugar beet root are highly vulnerable to bacterial soft rot early in the field and after major transfer such as harvest, storage and sugar industry processing steps. The sustainable cultivation, harvesting and storage has several strategies and techniques to reduce environmental pollution impact. One of those strategies is the utilization of safe chemicals and alternative methods instead of pesticides to control of plant diseases.

A variety of organic chemical acids is non-toxic, inexpensive and very effective against strains of soft bacteria. It has been reported that in some cases of local use, chemical agents have advantages over antibiotics and pesticides. Also, many organic acids including acetic acid with their acid salts are incorporated into a large number of commercial and food products (Kang *et al*., 2003; Shemshura *et al*. 2016 and Morgunov *et al*., 2017)

In Egypt; pectolytic bacteria were isolated from roots and petioles of sugar beet exhibiting soft root rot and vascular necrosis symptoms by other researchers (Abdalla and Ismail, 1999, Abdalla *et al*., 2009)

In the present study, all soft rot bacteria (SRB) isolates were found to be inhibited in variable degrees by organic acids used. Acetic acid was found most effective followed by ascorbic, and citric acid. Susceptibility of bacteria to acetic acid, ascorbic and citric acid or their salts to control bacteria has been previously reported. (Carole *et al*., 2004; Huang *et al*., 2010, Kamzolova *et al*., 2014a; Wu *et al*., 2017). Based on our results; acid acetic acid led to highest efficacy in inhibiting the growth of sugar beet bacteria causing soft root rot disease (*E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *betavasculorum* and *Burkholderia cepacia*).

In this study, we analyzed the morphology of treated bacteria with acetic acid using SEM. The bacteria cells show structural changes as rough on the cell surface, deformation deep surface cracks and a complete decay of the organized structure (Fig. 3c). This result is in agreement with other researchers findings (Grower *et al*, 2004, Sin Mei *et al*, 2015)

In Egypt, sugar beet crop is harvested from March to June each year and the beet roots transported for processing in the factory during this period roots are subjected to soft rot bacteria and fungal pathogen attack, the result of which is increasing the loss of sugar in harvested beets. Our results indicate that acetic acid was shown to be an inexpensive and efficient agent for the elimination of multiple antibiotic resistant strains of SRB at low concentration. The findings of the present study are in agreement with the earlier reports. Therefore; organic acids are recommended in root treatment in small diluted solutions. Organic acids known for years for their antibacterial and antifungal properties which have been widely used in foodstuff industry and agriculture (Scholberg and Gaunce 1995, 1996; Laitila *et al*., 2002; Lavermicocca *et al*., 2003; De Muynck *et al*., 2004; Tripathi and Dubey 2004; Sathe *et al*. 2007 and Pao *et al*., 2008).

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التأثير المثبط والتغيرات المورفولوجية للأحماض العضوية على السلالات البكتيرية التي تسبب العفن الطرى فى جذور بنجر السكر معمليا

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يصيب مرض العفن الطرى البكتيرى محصول بنجر السكر ويسبب له خسائر كثيرة. ان للأحماض العضوية تأثير مضاد فطرى وبكتيرى. تم معاملة ثلاث سلالات بكتيرية (Bc) *Erwinia carotovora* subsp. *carotovora* (Ecc), *Erwinia carotovora* subsp. *betavasculorum* (Ecb) and *Burkholderia cepacia* , بثلاث أحماض عضوية مثل حمض الخليك، حمض ألاسكوربيك و حمض الستريك بتركيزات (1، 2.5 و 5%) مقارنة بالكنترول الذى تم معاملة البكتريا بالماء المقطر المعقم (كنترول سالب) ومعاملة البكتريا بالمضاد الحيوى أستربتومسين بتركيز 150جزء فى المليون (كنترول موجب). نسبة التركيز الأدنى من حمض الخليك المثبط للبكتريا Ecc, Ecb and Bc تم حسابه باختبار التركيزات (0.25، 0.35، 0.50، 0.75، 0.80، 0.90 % ح/ح) مقارنة بالكنترول. وتم دراسة التأثيرات المورفولوجية التى يحدثها حمض الخليك على البكتريا (*Ecc*) بزرع البكتريا فى بيئة (NA) سائلة وتم تحضير معلق بكتيرى فى مرحلة النمو اللوغارتيمى بتركيز 10^8خلية/مل، ثم معاملة هذا المعلق بحمض الخليك بتركيز 0.90% والتحضين على درجة 37^{o} م لمدة 60د وفحص العينات بواسطة الميكروسكوب الألكترونى الماسح. ومن أهم النتائج المتحصل عليها:أعلى نسبة تثبيط كانت لحض الخليك بقطر تثبيط 23مم لتركيز 5%، والحد الأدنى للتثبيط لحمض الخليك كان 0.90% ح/ح ضد البكتريا (Ecc), (Ecb) and (Bc). وبالفحص بالميكروسكوب الألكترونى وجد ان الخلايا البكتيرية المعاملة بحمض الخليك حدث لها انتفاخ وزيادة فى الحجم، انفجار فى الخلايا وصغر حجمها و حدوث تموجات ,تجعد فى الجدار الخلوى للخلية البكتيرية.