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Study of Susceptibilities of Saudi Isolated *Erwinia* and Standard *Erwinia* to some Antimicrobials Supplemented with Garlic Powder

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Since *Erwinia* play in the causation of soft rot disease in potato and spoilage of agricultural crops especially those with meat tissues, in their areas of outbreak, we sought to investigate the significant effect of some antibiotics and garlic powder (GP) on standard and locally isolated *Erwinia*. Diseased samples of three brand potato tubers: Hermes, Lady Rosetta and Carege were collected. *Erwinia* were isolated from soft rotted potato samples by using potato dextrose agar (PDA), tryptic soya agar (TSA), nutrient agar (NA) and crystal violet pectate (C.V.P) and were anaerobically cultured by incubating on TAS, NA in (Gas pak Jar) and were characterized by using API- 20E (Biomerieux) and BiologSoft ware, Inc., Haward, CA, USA. For further confirmation of the isolates, oxidase and catalase reactions were applied. The presence of two types of bacteria, *Erwinia carotovora* (E. C (N2) and *Erwinia chrysanthemi* (E. chr (Mn10) were observed and Pure single colony was obtained. The antibiotic susceptibility of the isolates was determined by using antibiotic disk with different dilution from (Mast Diagnostic GmH, D-23858 Reinfeld, and Germany). We have found fluctuations in the susceptibilities for antibiotics between the isolates and standards. These results provide the studies of the first locally isolated *Erwinia* from local potato tubers and their susceptibilities of sopresently used antimicrobials and simple methods for their assessment in the studies of disease and antibiotics.

Key words: *Erwinia*, Soft Rot Disease, Saudi isolate, Antimicrobials, Susceptibility,

Erwinia carotovora is a plant pathogen that causes cell death through plant cell wall destruction by creating an osmotic ally fragile cell. This is achieved through the production of extracellular pectic enzymes and cellulase that break down pectin and cellulose, respectively. The infection is transferred either by plant to plant or insect to plant, causing soft-rot diseases of many plants and vegetables that eventually become characterized as slimy and foul smelling. There has been increasing evidence for extensive post-transcriptional control of gene expression in bacterial pathogens. A prominent example of a global post-transcriptional regulator is Hfq[1]. The bacterial pathogens of *Erwinia carotovora* sub sp, *carotovora* and *Erwinia chrysanthemi* are responsible for soft rot disease. Antimicrobial activity against the crown gall and soft rot phytopathogens, *Agrobacterium tumefaciens* and Erwinia, respectively, has not been previously studied[2] in previous study of *E. chrysanthemi* strains isolates displayed resistance to bacitracin with two strains showing resistance to sulfamethoxazole. None of the *E. chrysanthemi* strains were resistant toward ampicillin, carbenicillin, cephalothin, ceftriaxone, cefuroxime,

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gentamicin, kanamycin, nalidixic acid, penicillin G, streptomycin and tetracycline and all the *E. chrysanthemi* strains studied were plasmid less[3].

Isolates of the genera *Acetobacter, Acinetobacter, Citrobacter, Enterobacter, Erwinia, Escherichia, Methylococcus, Xanthomonas, Vibrio, Bacillus, Micrococcus, Planococcus, Staphylococcus* and *Streptomyces,* were examined for plasmid DNA content and resistance to antibiotics. Resistance was most frequent to Ampicillin followed by Kanamycin and Tetracycline[4]. *Erwinia carotovora* sub sp. *atroseptica* strictly infects potatoes that may also produce a non ribosomal peptide phytotoxin that induces necrosis by electrolyte leakage through transmembrane pore formations. The first case of cervical lymphadenitis due to infection by a new *Erwinia*-like organism showing phenotypic characteristics that were different from other *Erwinia* species has been reported[5]. Two-component signal transduction systems (TCSTs), consisting of a histidine kinase (HK) response regulator played critical roles in sensing and responding to environmental conditions in bacterial pathogenesis. Most TCSTs in *Erwinia amylovora* have either not been identified or have not yet been studied[6]. Quorum sensing (QS), a population-density-sensing mechanism, controls the production of the main virulence determinants of the plant cell-wall-degrading enzymes (PCWDEs) of the soft-rot phytopathogen *Erwinia carotovora* sub sp. *Carotovora*[7]. *Erwinia chrysanthemi* 3937 (Ech3937) is a pathogenic bacterium with a wide host range. The pectinolytic enzymes secreted by the bacterium and the type III secretion system (T3SS) are essential for full virulence[8]. *Erwinia* causes also fire blight diseases of pears. It causes fire blight of different organs of the trees: blossoms, shoots, leaves, and fruits[9]. *Erwinia* Virulence Factor (Evf) has been identified in *Erwinia carotovora* 15 (Ecc15) as a virulence factor that promotes colonization of the Drosophila larval gut and provokes the triggering of a systemic immune response[10]. We have observed Bacteria living in the soil surrounding the area where Potato was growing antagonized the elimination of *Erwinia*. This study is intended to isolate and identify *Erwinia* from infected potato tubers showing symptoms of soft rot disease. Therefore, diseased samples of Hermes, Lady Rosetta and Carege belonging to *Solanum tuberosum* L were collected from the project of potato production in the area pertaining to the national company of agricultural development (NADEK), Saudi Arabia. *Erwinia chrysanthemi* and *E. carotovora* are the most commercially important soft rotting pathogens in Saudi Arabia and are economically important for the NADAK potato industry due to the potato cultivar susceptibility produced by this company in Saudi Arabia. Our purpose of this research was to investigate the effectiveness of some antibiotics sublimated with garlic powder (GP) on Saudi*Erwinia* isolates and standard.

MATERIALS AND METHODS

Diseased samples of three brand potato tubers: Hermes, Lady Rosetta and Carege Bio Merieux, API20E, nutrient medium, antibiotics Petri dishes , potato dextrose agar (PDA), tryptic soya agar (TSA) , Mueller-Hinton agar nutrient agar (NA), Standard Erwinia strains: *Erwinia carotovora* sub sp *carotovora* 3387 (E. c.c), *Erwinia carotovora* sub sp *atroseptica* 4344 (E.c.a), *Erwinia chrysanthemi* 3477 (E. chr), Biolog Soft Ware , Inc., Harward,CA, USA. Crystal Violet Pectate (C.V.P) medium, Oxidase, Catalase, sodium dodycelsulphate (SDS). The potato shell tubers were placed into tubes containing 10 ml of exterilized water with 70% isopropanol. After cleaning the surface of the samples, one gram of potato shell tuber reaction solution following the method of Omar[14] and have been shacked by Rotary shaker 180 rounds /minute for 24 hours at 3°C. After consecutive dilution of the supernatant, 0.1ml was taken from each tube and was placed on replicates of Petri dishes containing PDA, TSA, and NA medium and was incubated at different temperature: 25, 30,35 for 48 hours. Pure colonies were cultured in nutrient agar slant and were saved at 4oC in tilted solid nutrient agar medium with 1.5ml of 50% glycerol. Morphological characterization under light microscope observation after gram staining was carried. Following[11], oxidase test was carried to distinguish between Erwina strains which are oxidase negative and the Pseudomonas which is positive for this test. Bacteria from the pure culture have been cultured on selective medium, Crystal Violet Pectate (C.V.P) plus 0.1% of sodium dodycelsulphate,

Anaerobically cultured on TAS and NA, in Gas pak Jar. and were characterized by using API- 20E (Biomerieux) and BiologSoft ware, Inc., Haward, CA, USA. Antibiotic disk with different dilution from (Mast Diagnostic GmH, D-23858 Reinfeld, and Germany) were used to test bacterial susceptibility to 14 antibiotics with garlic powder.

The bacterial isolates were placed in sterile cotton swab and the bacterial suspension was removed by pressing the excess fluid and rotating the cotton against the inside of the tube above the fluid level. The swabs were streaked in at least three directions over the surface of the Mueller-Hinton agar mixed with garlic extract. The plates were allowed to dry for five minutes. Using sterile forceps, disks containing the antibiotics Ampicillin CephalothinCotrimoxazole Gentamicin, Nalidixic acid, Clindamycin, Oxacillin, Erythromycin, Oxytetracycline, Penicillin G, Amikacin, Chloramphenicole, Ciprofloxacin, Tetracycline, were placed on the plates and were incubated within 15 minutes soon after placing the disks. After overnight incubation, the diameter of the zone around each disk to the nearest whole mm was measured. The plates were examined carefully for well-developed colonies within the zone of inhibition and bacterial susceptibilities (sensitive (S) intermediate (I) and resistant (R) were determined.

RESULTS

Contamination

The working area was sterilized with 70% isopropanol. The surface of all the samples was cleaned with hypochlorite sodium so that other bacteria may not have chance to contaminate the sample and thus obscure the results of *Erwinia*. Isolation. *Erwinia* were isolated from the shells of potato tubers by using replicates of Petri dishes containing PDA, TSA, NA medium and selective medium (Fig 1.). We have used three different medium to know which one is capable of showing the specific characteristics of the bacteria in study, permitting to easily understand the bacteria. Culturing the suspected bacteria on the Petri dishes were repeated to gain single colony (Fig. 2.). *Erwinia* identification: The source of the standard Erwinia strains used in this study is detailed in (Table 2). Identification of Erwinia from the associated family of Enterobacteriaceae was performed through the use of enzymatic interaction system API 20E stripe specific and Biolog Soft Ware. The results of identification showed the presence of two types of bacteria, *Erwinia carotovora* E.c (N2), *Erwinia chrysanthemi* E.chr (Mn10).

Inducing infection

The Results of induced infection showed ability of the bacterial isolates and the standard *Erwinia* to cause soft rot disease to the three types

Table1. Ellustrates the used antibiotics, garlic powder and concentrations of each in microgram per milliliter

Antibiotics	Concentration µg/ml
Ampicillin	25 µg
CephalothinCotrimoxazole	30 µg
Gentamicin	25 µg
Nalidixicacid	10 µg
Clindamycin Oxacillin	30 µg
Erythromycin	2 µg
Oxytetracycline	5 µg
Penicillin G	15µg
Amikacin	30 µg
Chloramphenicole	10 µg
Ciprofloxacin	
Tetracycline	30units
Garlic extract	30 µg
	1 µg
	30 µg
	Capsule /430 mg (*Allium sativum*) 1.3% allicine. Arkopharma France

Table 2. Shows source and host of standard *Erwinia* strains, plant organ and origin.

Standard Strains	Source	Host	Plantorgan	Origin
Erwinia carotovora sub sp *carotovora* 3387(E.c.c)	NCPPB	Potato	Tuber	Egypt
Erwinia carotovora sub sp *atroseptica* 434(E.c.a)	NCPPB	Potato	Tuber	Netherlands
Erwinia chrysanthemi 3477(E.chr	NCPPB	Potato	Tuber	Tanzania

of potato. In spite of the differences and variation in ability to cause disease according to the estimations of rot ratio mathematically, the highest rot ratio resulted from the strain *Erwinia carotovora* sub sp *atroseptica* 4344 (E.c.a).

Antibiotic treatment

Erwinia were treated with antibiotics and with garlic powder and without garlic powder. Types of antibiotics and concentrations used in microgram per milliliter were Showed (Table 1). Using sterile forceps, antibiotics disks were placed on plates containing *Erwinia* and incubated within 15 minutes. After overnight incubation, antibiotic disks evaluated susceptibility of *Erwinia* to antibiotics showing a zone around each disk was observed (Fig3.) (Fig4.) and bacterial susceptibilities (sensitive (S) intermediate (I) and resistant (R) were determined. After 14 antibiotic

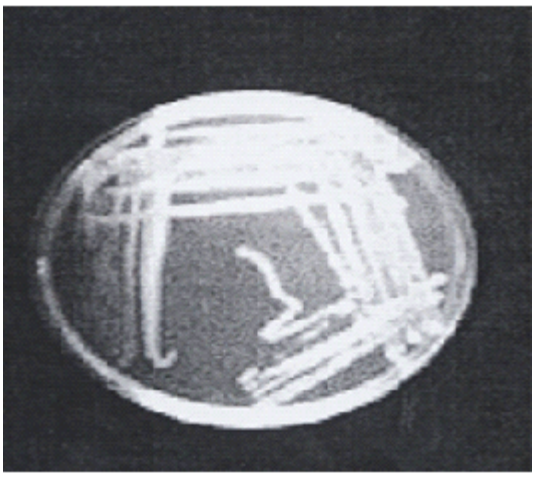

Fig. 1. Highlights the growth of *Erwinia* on Selective media (CVP)

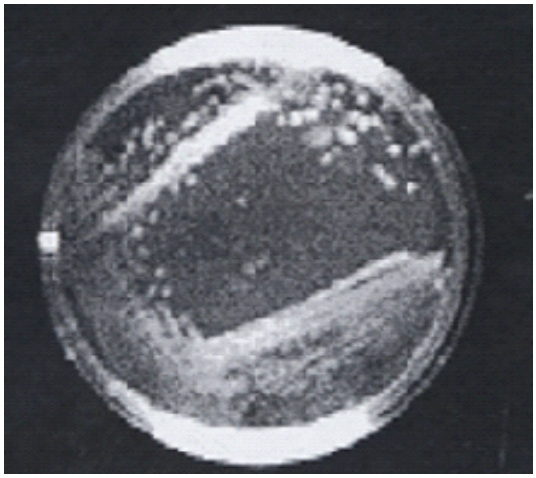

Fig. 2. Highlights single colony growth of *Erwinia* on Selective media (CVP)

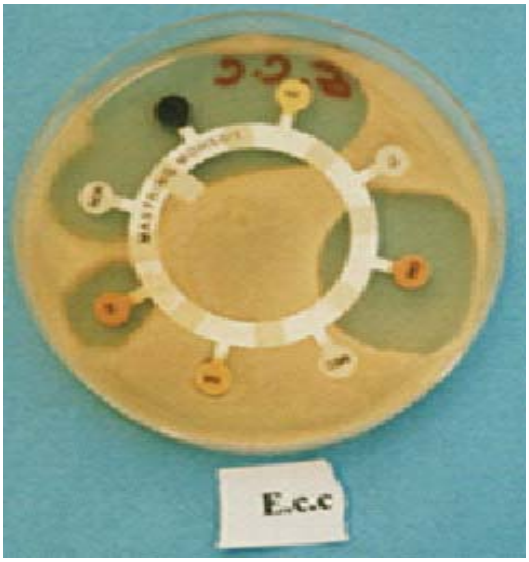

Fig. 3. *Erwinia carotovora* sub sp *carotovora* 3387 after overnight incubation with antibiotic disk show inhibition zone

Fig. 4. *Erwinia, Chrysanthemi* (E.chr (Mn10) after overnight incubation with antibiotic disk show inhibition zone

disk treatment on Muller Hinton with and without garlic powder standard strain E.c.c 3387 showed sensitivity to 8 antibiotics without garlic powder and 9 with garlic powder; (GM, AP, T, AK, PG, KF, CIP, OT) and (GM, AP, T, AK, PG, KF, CIP, OT, C) respectively and was resistant to 4; (E, OX, NA, TS) while it was intermediate to one; (CD) respectively. The standard strain E.c.a 434 was sensitive for 8 antibiotics (AP, CIP, PG, T, C, NA, OT, TS, and garlic powder). On the other hand this strain was intermediate to (E) but was resistant to (AK, OX, CD, GM, and KF). The standard strain E.chr 3477 was sensitive to (CIP, AK, CD, T, C, E, TS, KF, PG, OT, NA, GM, OX, and GP) while it was resistant to (AP). The local isolates, *Erwinia carotovora* E.c (N2), and *Erwinia chrysanthemi* E.chr (Mn10), were (S) for 8 antibiotics with GP (GM, AP, T, AK, PG, KF, CIP, C) and (R) to 5 antibiotics (E, OX, NA, TS, GM) and (I) for one antibiotic (CD). However, the E.chr 3477 scored the highest level of (S) to 13 antibiotics.

DISCUSSION

Diseased samples of Hermes, Lady Rosetta and Carege belonging to *Solanum tuberosum* L were collected from the project of potato production in the area pertaining to the national company of agricultural development (NADEK), Saudi Arabia. *E. chrysanthemi* and *E. carotovora* are the most commercially important soft rotting pathogens in Saudi Arabia and are economically important for the NADAK potato industry due to the susceptibility of potato cultivar produced by this company in Saudi Arabia. Our purpose of this research was to investigate the susceptibilityf 14 antibiotics sublimated with garlic powder (GP) in Saudi *Erwinia* isolates and standard *Erwinia*. The role of several multidrug resistance (MDR) systems in the pathogenicity of *Erwinia chrysanthemi* 3937 was analyzed. Mutants in genes encoding for two acridine resistance (Acr)-like systems have been generated and analyzed the virulence of the mutant strains in different hosts and their susceptibility to antibiotics, detergents, dyes, and plant compounds. It has been observed that the mutant strains are differentially affected in their virulence in different hosts and that the susceptibility to toxic substances is also differential[12]. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems has been investigated and reported[13]. In this study the susceptibility structure of Erwinia to some antibiotics sublimated with garlic powder, were adapted, validated and deployed to evaluate the effective antibiotic for controlling local soft rotting *Erwinia*. The results showed fluctuations in the capabilities of 14 antibiotic with the garlic powder to control the soft rotting *Erwinia* from 4 to 12 (S), from 4 to 8 (R) and from Zero to 2 (I) and were rapid and reproducible for accurate comparison between standard *Erwinia* susceptibility and of that isolates from local potato to antibiotics. Our data suggest that susceptibility to antibiotic is strain specific and differs from antibiotic to antibiotic and thus the need for annual checkup of the quality of antibiotics in Saudi Arabia. The results will help potato growers to improve control measures of soft rot disease. We conclude that the E. c (N2) and E.chr (Mn10) are the pathogen of potato in Saudi Arabia. These results provide the studies of the first locally isolated Erwinia from local potato tubers and their susceptibilities of some presently used antimicrobials and simple methods for their assessment in the studies of disease and antibiotics. Soft rot *Erwinia* epidemic disease occurrences need screening available methods necessary with respect of their sensitivity, specificity and improved performances. Benefits include that this will provide reliable screening methods and source of tracking would allow accurate epidemiological plant protection services, since results are quickly available and discussions on sanitary measures would be reached earlier.

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