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Capability of *Erwinia* Isolates in Saudi Arabia in Inducing Soft Rot

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Bacteria attack all agricultural crops which are considered a source of human food in all regions of the world and causes many economic loses. *Erwinia carotovora* subsp attack vegetables and specially those with meat tissues such as potato tuber and cause soft Rot disease. Soft Rot in all regions of the world and especially in hot regions is one of the serious diseases that attack crops in the field, during storage and transport leading to great loss in production more than any other bacterial disease. Diseased samples of three brands of potato tubers were collected: *Hermes, Lady Rosetta* and *Carege* in addition to soil samples were also taken from the rhizosphere region surrounding the tubers. Chemo-biological tests and bacterial development on Crystal Violet Pectate (C.V.P) medium and enzymatic interaction system API 20 E stripe for identification of the bacteria associated with the family *Enterobactericeae* were also carried. Pure colonies of *Erwinia* were obtained as well as pure single colonies. The result of identification showed the presence of two types of bacteria, *Erwinia carotovora* (E.c) and *Erwinia chrysanthemi* (E.chr).

Key words: *Erwinia* sp., Soft Rot Disease, Saudi isolate.

The plant diseases caused by bacteria worldwide attack all agricultural crops which are considered a source of human food in all regions of the world causing many economic losses. Plant diseases caused by bacteria are considered as an important factor in limiting production in same agricultural crops in their areas of outbreak. The bacterial soft Rot which attacks vegetables and specially those with meat tissues such as potato tuber and which are causes by the bacteria *Erwinia carotovora* sub sp *carotovora*. There has been an increased evidence for extensive post-transcriptional control of gene expression in bacterial pathogens[1]. The activity against the crown gall and soft rot phytopathogens, *Agrobacterium tumefaciens* and *Erwinia* has not been studied well[2]. In all regions of the world and especially in hot regions is one of the serious diseases that attack crops in the field and during storage and transport leading to great loss in production more than any other bacterial disease. According to the importance and causes of this disease, innovative diagnostic tools such as RFLP Analysis of validated screening tests for *Erwinia* in asymptomatic plants have been developed[3]. Little is known about the bacterial communities with the plant inhabiting desert ecosystem[4]. The first case of cervical lymphadenitis due to infection by *Erwina* has been identified by 16s rRNA gene sequence analysis[5]. Two component signal transduction systems (TCST), containing of a histidene kinase (HK) and a response regulatory have been studied and it plays critical role in sensing and responding to

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the environmental conditions and in bacterial pathogenesis[6]. Quorum sensing (QS) regulatory of *E.carotovora* subsp have recently been described more extensively[7].The bacterial pathogens of *Erwinia carotovora* subsp, *carotovora* and *Erwinia chrysanthemi* are responsible for soft rot disease[8]. *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[9]. *Erwinia* causes also fire blight diseases of pears. It causes fire blight of different organs of the tree: blossoms, shoots, leaves, and fruits[10]. This study is intended to isolate and identify *Erwina* from infected potato tubers showing symptoms of soft rot disease. Therefore, continuous visits were performed to the project of potato production in the area pertaining to the national company of agricultural development (NADEK) for the collection of diseased samples of three brands of potato tubers: Hermes, Lady Rosetta and Carege in addition soil samples were also taken from the rhizosphere region surrounding the tubers. The genus *Erwinia* is a member of the family *Enterobacteriaceae* consisting of 18 species that fall into two main groups that are the necrogenic or Amylovora group and the soft rot or *carotovora* group, *Erwinia chrysanthemi* and *E. carotovora* are the most commercially important soft rotting pathogens in Saudi Arabia. *E. chrysanthemi is* of economic importance for the potato industry since the potato cultivar released by NADAK was susceptible to this plant disease.

MATERIAL AND METHODS

Materials used in this study include Potato tubers of three types: *Hermes, Lady Rosta* and *Carege*, belonging to the genus *Solanum tubersum* L. Soil samples, Nutrient Agar (NA), Tryptic Soya Agar (TSA), Crystal Violet Pectate, Potato Dextrose Agar. API20 E Stripe, Standard strains of *Erwinia*, *Erwinia carotovora* subsp *Carotovora* 3387 from National Collection of plant pathogenic Bacteria Egypt, *Erwinia carotovora* subsp astroseptica 434 obtained from Netherlands and *Erwina chrysanthemi* 3477 obtained from Tanzania, acetone, iodine safranin, bunsen flame, staining rack, slide, water, vaseline. Potato sample tubers were collected from potato project in Hail, Saudi Arabia. Tubers were obtained from depth 30cm and 80cm periphery and storied at room temperature. Potato tubers were sterilized with hypochlorite sodium. One gram from the infected part of tubers were obtained and injected into tubes containing 10ml of distilled water. By shaking and vortaxing and centrifugation a supernatant were save and plated on three different medium, nutrient agar medium for isolation of the bacteria from the infected tubers, Tryptic soya agar medium, for the isolation of the bacteria from the soil samples and Crystal Violet Pectate medium for the identification of bacteria. The isolated bacteria were saved in nutrient agar slant added 1.5ml (50%) glycerol. After isolation, gram staining and oxidase test was performed to distinguish between *Erwina* and *Pseudomonas* strains. Gram staining was performed by using slide with bacterial smear placed on a staining rack. The slide was stained with crystal violet for 2 -3 minutes. The stain was poured off. The slide with gram was flooded with iodine for 2-3 minutes and iodine was poured off, decolorized by acetone and immediately it was counterstained with safranin for 2 minutes. Washed with water, blotted excess water and dried over Bunsen flame. Oxidase test was also performed by using a sterile swab, a small amount of organism from an agar slant was obtained and drop by drop dispensing of the reagent was placed onto the culture on the swab. Positive reaction turned the bacteria violet to purple within 15 seconds. Hang drop test was also performed to discover the characteristic movement of the bacteria by using small drop of liquid bacterial culture in a center of cover slip and small drop of water at each corner of the cover slip. A slide with central depression of ring vaseline has been inverted over the cove slip.

The cover slip sticked on the slide, when it was inverted the drop of bacteria was suspended in the ring vaseline. To get pure culture of *Erwinia* the selective medium crystal violet pectate added 1.0% of Sodiumdodycel sulphate (SDS) was used and incubated in Gaspar anaerobic jar for 48 hours. Using TSA and NA medium, the bacteria have been sub cultured and identified by using API-20E stripe (Biomerieux).

Pseudomonas and *Erwinia* grew in TAS and NA culture medium after incubation period 48 hours at 35, 30, and 25°C. From the three types of potato tubers 5 mm thick of slices were taken and sterilized with 1.0% of hypochlorite sodium for 2 min and washed with deionized water and dried in an oven at 70°C. Wounds of 2mm deep were made in the slices and by using micropipette, 5µl containing 10 c.f.u / ml of bacterial filtrates were injected into the parenchyma of the tubers. The injected tubers were placed in sterile plastic bags and incubated at 100% humidity and 35, 30, 2 for 120 hours. By measuring the weight of the soft rot portion and the uninfected portion, the percent soft rot to uninfected ratio were calculated.

RESULTS AND DISCUSSION

Erwinia carotovora is a plant pathogen that causes cell death through plant cell wall destruction by creating an osmotically 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, this organism causes soft-rot diseases of many plants and vegetables that eventually become characterized as slimy and foul smelling. *Erwinia carotovora* subsp. *atroseptica* strictly infects potatoes that may also produce a nonribosomal peptide phytotoxin that induces necrosis by electrolyte leakage through transmembrane pore formations. In addition, Eca1043 is predicted to synthesize large hemagglutinin-like proteins, pili fimbrial proteins for host adhesion. Finally the possible horizontal genetic transfer of may contribute to pathogen city because the mutation of these genes negatively affected virulence[11].

The results will help fruit growers and nurseries to improve control measures of soft rot disease. Soft rot *Erwinia* epidemic disease

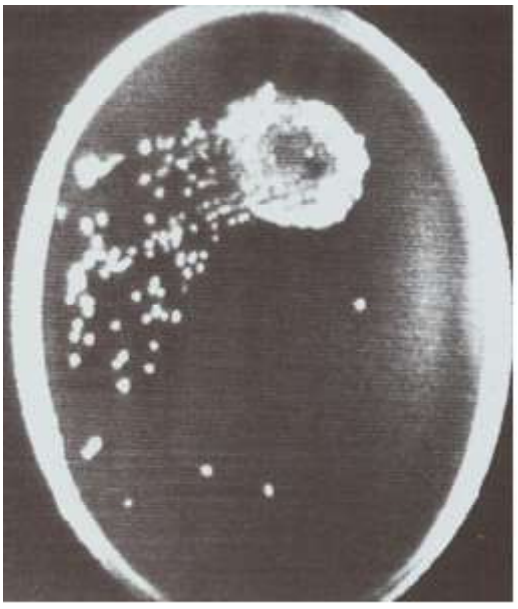

Fig. 1: Growing bacteria *Erwinia* on Cystal Violet Pectate medium

E.c.c. Herms

Fig. 2: Pathogenic capability of E.c. (N2) to potato

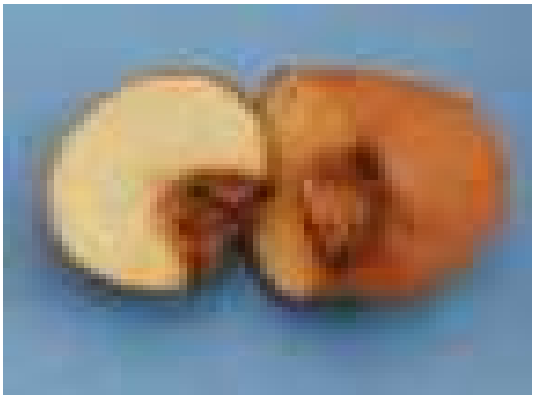

Fig. 3: Pathogenic capability of E.c.c (3387) to potato

Table 1. Comparison between *Erwinia* species capabilities in inducing soft rot

Potato variety	Days	Rot (%)				
		Ecc. 3387	E.c.a 434	E.chr 3477	E.c (N2)	E.chr (Mn 10)
	3	00	00	00	00	00
Control	5	00	00	00	00	00
Hermes	3	11.21	10.77	8.5	13.48	5.5
	5	19.72	14.35	17	18.37	7.95
Carge	3	5.33	3.55	4.3	8.13	5.53
	5	9.11	8.35	11.5	12.23	16.16
Lady rosette	3	7.43	4.74	6	17.6	2.25
	5	15.91	11.37	13.3	21.23	8.97
Mean	4	11.5	8.9	10.1	15.1	7.7

occurrences need screening available methods necessary with respect of their sensitivity, specificity and improved performances. Benefits include the availability of reliable screening methods and source of tracking would allow accurate epidemiological plant protection services, since results are quickly available and discussions on phytosanitary measures would be reached earlier. We conclude that the (E. c) and (E. chr) are the *pathogen* of potato in Saudi Arabia. The result of this current study showed the presence of two types of *Erwinia carotovora* (E.c) and *Erwinia chrysanthemi* (E.chr), causing soft rot disease.

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REFERENCES

1. Yanjie Chao, Jorg Vogel. The role of Hfq in bacterial pathogens. *Current Opinion in Microbiology.* 2010; **13:** 24-33.
2. Ismail Saadoun, Khalid M, Hameed, Fouad AL-MomaniI, Qotaiba Ababneh. Effect of three *Orobanche* spp. Extracts on Some Local Phytopathogens, *Agrobacterium* and *Erwinia. Turk J Biology.* 2008; **32:** 113-117.
3. Sahilah AM, Rozeita L, Umi KalsumM S, Son R. Typing of *Erwinia Chrysanthemi* Isolated from Josapine Pineapple in Malaysia using Antimicrobial Susceptibility, Plasmid Profiles, ERIC-PCR and RFLP Analysis. *International Food Research Journal.* 2008; **15:** 1-8.
4. Abdulla D, Altalhi. Plasmid profiles antibiotic and heavy metal resistance incidence of endophytic bacteria isolates from grapevine (*Vitis vinifera* L.). *African Journal of Biotechnology.* 2009; **8:** 5873-5882.
5. Sang Yop Shin, Mi Young Lee, Jae-Hoon Son, Kwan Soo Ko. New *Erwinia* Like organism causing cervical lymphadenitis. *J of Clinical Microbiology.* 2008; **46:** 3156-3158
6. Youfu Zhao, Dongping Wang, Sridevi Nakka, George W Sundin, Schuyler S Korban. Systems level analysis of two-component signal transduction Systems in *Erwinia amylovora*: Role in virulence, regulation of amylovoran biosynthesis and swarming motility. *BMC Genomics.* 2009; **10:** 1471-2164.
7. Solveig Sjoblom, Heidi harjunpaa, Gunter brader, Tapio Palva E. A novel Plant Ferredoxin-Like Protein and the Regulator Hor Are Qourum-Sensing Targets in the Plant

pathogen *Erwinia carotovora. The American Phytopathological Society.* 2008; **21:** 967-978.

8. Costa AB, Eloy M L, Cruz JD, Janse H, Oliveira. Studies on Tudies on pectolytic *Erwinia* sp in Portugal reveal unusual Strains of *E. carotovora* subsp. *Astroseptica*. *Journal of Plant Pathology.* 2006; **88:** 161-169.
9. Quan peng, Shihui Yang, Amy o, charkowsk, Mee-Ngan Yap Douglas A.Steeber. Noel T, keen, Ching-Hong Yang. Population Behavior Analysis of dspE and pelD Regulation in *Erwinia chrysanthemi* 3937. *The Amer Phytop Society.* 2006; **19:** 451-457.
10. Niknejab Kazempour M, Kamran E, Ali B. *Erwinia amyolovora* causing fire blight of pear in the Guilan province of Iran. *J of Plant Pathol.* 2006; **88:** 113-116.
11. Acosta Muniz C, Jaillard D, Lemaitre B, Boccard F. "*Erwinia carotovora* Evf antagonizes the elimination of bacteria in the gut of *Drosophila* larvae." *Cellular Microbiology***.** 2007; **9:** 106-19.