

BACTERIAL STEM ROT DISEASE OF *Yucca aloifolia* IN EGYPT

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Abstract: A devastating stem rot disease of *Yucca aloifolia*, an ornamental plant, was observed in the greenhouse of the Faculty of Agric., Minia University. Naturally infected plants showed typical symptoms of soft rot on the stems. Four isolates of motile, rod shaped, gram negative bacteria were isolated from soft rotted areas. All isolates were pathogenic to yucca plants and induce soft rot symptoms, however, bacterial isolates differed in their virulence. Through different biochemical tests, bacterial isolates differed in their characteristics. The isolated pathogenic bacteria was identified as *Erwinia chrysanthemi*.

Host range studies revealed that the bacterium was able to produce soft rot on fruits of eggplant, tomato, squash, pepper, potato tubers, carrot, turnip, onion, chrysanthemum and sunflower. Stems of maize, cowpea, broad bean show no symptoms and remain unaffected.

The extracts of experimentally diseased yucca stems were active in pectinase and cellulase. On the other hand, no enzymes activities were detected in healthy tissues.

A correlation between the total and reducing sugars and the pathogenicity of the tested bacterial isolates were detected.

Keywords: *Yucca aloifolia*, Bacterial stem rot, *Erwinia chrysanthemi*, sugars and enzyme activities.

Introduction

Yucca aloifolia (Spanish bayonet) is an ornamental plant, which is commonly grown in gardens, room and house in Egypt. These plants are tree-like succulents of the lily family (Liliaceae) with stemless stiff, pointed leaves that end in a sharp needle. Extracts from the plant roots are used in alternative medicine as a soap and as an herbal dietary supplement (Chase and Osborne, 2004). The yucca has at least 40 species including

only two species growing in Egypt (e.g. *Yucca aloifolia* and *Yucca gloriosa* (Spanish dagger). A number of commerical uses for yucca extract has been found, including adding it to root beer, alcoholic beer and cocktail mixtures as a foaming agent. Yucca plants can also tolerate nearly full shade and makes a wounderful accent at entryways or in a shrub border (Gilman,1999).

Leaf spot can be a problem in areas with poor air circulation

(Gilman,1999). Bacteria stem rot disease is considered an important disease of yucca plants. Dickey (1981) also described a stem rot of *Diffenbachia* caused by *Erwinia chrysanthemi*. Several authors reported that yucca plants infected by different fungi such as *Fusarium* stem rot and *Nectria spp.*, southern blight caused by *Sclerotium rolfsii* which were isolated from stem rot and all portions of the plants (Chase and Osborne, 2004). Furthermore, Simone and Chase (1989) reported that four pathogens were isolated from yucca plants (e.g.*Coniothyrium concentricum* a pathogen of brown leaf spot, gray leaf spot caused by *Cytosporina sp.*, *Fusarium* stem rot caused by *Fusarium spp.* and southern blight caused by *Sclerotium rolfsii* and according to available literature unless otherwise indicated, no previous studies indicated that this disease was previously reported in the world.

The present work was carried out to isolate and identify the causal organism, studies including also the host range. Furthermore, the effect of the pathogen on the role of the cell wall degrading enzymes as well as on carbohydrate content in healthy and inoculated plants during pathogenesis was discussed.

Materials And Methods

Isolation of the causal pathogen:

The causal organism was isolated from naturally infected rot of *Yucca aloifolia* showing typical stem rot

symptoms grown in the greenhouse of Faculty of Agric., Minia University. Infected stems were washed twice and cut into small pieces which were dipped in ethyl alcohol and flamed. Sterilized pieces were then macerated in a few amount of sterile distilled water and left for 20 min then loopfuls were streaked into Nutrient Glucose Agar (NGA) and incubated plates at 25°C. The inoculated plates were daily observed for 7 days .Single colonies from the developing growth were transferred by loop exhaustion in three successive tubes with slanted NGA to obtain pure cultures. Four isolates of a rod shaped bacteria were secured from four different yucca plants showing typical stem rot symptoms. They were designated as Y1,Y2,Y3 and Y4. The isolated bacteria were kept at 5°C until used.

Identification of the pathogen:

Four infective bacterial isolates, e.g. Y1,Y2,Y3 and Y4 were identified by studying their morphological, physiological and biochemical characters (listed in Table 1) that recommended by King *et al.* (1954), Kovac's (1956), Stapp (1961), Breed *et al.*, 1974 and also in the Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986 and Bradbury 1986). However the tests were carried out as described by Dye (1968), Lelliott and Stead (1987) and Klement *et al.* (1990).

Pathogenicity tests:

Pathogenicity trials were performed on healthy plants grown in 25 cm pots containing a sterilized loamy soil (one plant/pot) under field conditions in the Experimental Farm of Department of Plant Pathol., Fac. Agric. Minia Univ. Four months after planting, each isolate was inoculated into stems by puncturing. Puncture method was carried out by puncturing the bacterial isolates with sterile teeth picks bearing small portions of 24 h old bacterial growth of the tested

isolates through these punctures where the teeth picks left. Five plants were used for each isolate. Check stems were similarly treated as described by using bacterial free sterile teeth picks. The inoculated stems were covered with plastic bags to keep high moisture for 24 h. Three weeks later, the inoculated stems were examined for the development of disease symptoms.

Bacteria were again isolated from artificially infected plants and compared with the original inocula.

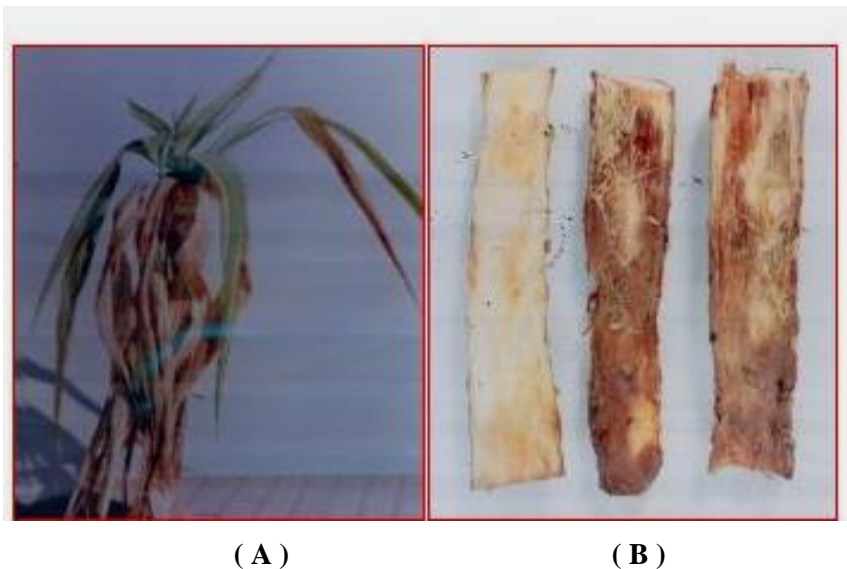


Fig.(1): Symptoms on *Yucca aloifolia* inoculated with *Erwinia chrysanthemi* showig stem rot A) on the whole plant. B) Left control right symptoms on infected stem of the plant.

Preparation of bacterial suspension:

The bacterial isolates of 24h old cultures were washed from agar surface with 0.1 % methylcellulose

and the concentration was adjusted to 10^9 colony forming unit (CFU/ml) by using Milton Roy spectrophotometer at 600 nm at OD .01 (Goth and Webb, 1981).

Reaction of some economic plant species to bacterial isolates:

Fourteen plant species were subsequently tested against other hosts were inoculated at different stage by *Erwinia chrysanthemi* isolates.

Surface sterilized eggplant, tomato, squash and pepper and lemon fruits were tested. Also, potato tubers and roots of carrot, turnip and onion stem were inoculated by forcing a sharp flamed needle contaminated with 48 h-old bacterial growth and kept in a clean, semi-sterile plastic containers, each supplemented with a sterile moist cotton pad at room temperature. Stems of maize, cowpea, broad bean, chrysanthemum and sunflower were inoculated in the same manner, as described in pathogenicity test. The inoculated plants were kept at 30°C in a greenhouse. The inoculated stems were covered with plastic bags to keep high moisture for 24 h. Five samples or plants were used for each isolate. Control samples or plants were similarly tested with sterile water only and kept in the same conditions.

Determination of enzyme activity:

The rate of stem rot was weightly determined, three weeks after incubation at 30°C, where the rotted stem tissue was removed with a scalpel and weighed. The percent of weight of rotted tissues was calculated as follows:

$$\% \text{ rot} = \frac{\text{weight of rotted tissues}}{\text{Whole weight of stem}} \times 100$$

The pectolytic and cellulolytic enzymes were determined in tissues of healthy and artificially inoculated *Yucca aloifolia* stems. Plants were inoculated as described in pathogenicity tests. For crude enzyme extraction, healthy or inoculated stems were mixed with 0.2 M phosphate buffer pH 7.0 for pectolytic enzyme or acetate buffer pH 4.5 for cellulase activity (30g tissue/100ml buffer) in a waring blender for 30 seconds. The mixture was filtered through double layers of cheese-cloth and centrifuged at 3000 rpm for 20 min. The supernatants were used either directly for analysis or stored in a freezer until used.

Pectolytic assay:

To detect the activity of pectic enzyme in yucca stem tissues artificially inoculated with bacterial isolates, the reduction in viscosity of 1.0% citrus pectin solution was measured using the method described by Barker and Walker (1962). The reaction mixtures consisted of the 2 ml of tissue extracts and mixed thoroughly with 5 ml of 1.0% (w/w) citrus pectin solution at pH 5.2, 4.2 ml of 0.05M sodium acetate buffer pH 5.2 and 1.0 ml NaCl (0.01M). The reaction mixtures were incubated at 30°C for one and half hour and also after 3 hr. The loss in viscosity was calculated using the formula adapted by Martekov *et al* (1963):

$$\% \text{ loss in viscosity} = \frac{T_1 - T_0}{T_0} \times 100$$

whereas, T1 : viscosity of inoculated tissue extract.

T0: viscosity of control (heated enzyme).

Cellulase assay:

Cellulase activity was measured using the viscometric method described by Barker and Walker (1962), by measuring the reduction in viscosity of 1% carboxy methyl cellulose (CMC) at 30°C for 3hr incubation. The reaction mixture consisted of 2 ml CMC (1%,w/w) dissolved in 0.1 M acetate buffer at pH 4.5 and 2 ml of tissue extract. Data were figured out in the same way described above.

Total carbohydrate and reducing sugars in healthy and artificially inoculated yucca stems:

A) Total carbohydrates:

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

B) Determination of reducing sugars:

This was performed according to the methods of Nelson (1944) and Somogyi (1952).

Statistical analysis:

Standard deviation (SD) was calculated according to the methods described by Gomez and Gomez (1984) to compare the variances between treatments.

Results and Discussion

Isolation and identification of the causal pathogen:

Four isolates of a creamy-white bacteria were isolated from *Yucca aloifolia* plants showing typical stem rot symptom. Regardless of some slight differences in certain characteristics, all bacterial isolates appeared to be representative of *Erwinia chrysanthemi* (Table 1) according to description of Bergeys Manual of Determinative Bacteriology (1974), also in the Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986 and Bradbury 1986). However, the tests were carried out as described by Dye (1968), 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 and negatively reacted with ammonia production, starch hydrolysis, H₂S production, urease activity and ice nucleation, while they positively reacted toward gelatin liquefaction, indol formation, rot of potato and carrot slices, nitrate reduction was reduced. All the tested isolates produce of diffusible green fluorescent pigment on nutrient agar medium. Otherwise, the bacterial isolates utilized glucose, galactose, fructose, arabinose, cellubiose, mannitol, mannose and xylose but they did not utilize maltose, sorbitol, and starch. Comparison of the

Table(1): Morphological, biochemical and physiological characters of bacterial isolates compared with descriptions of reference isolates (previously identified)

Test	The isolated bacteria	<i>Erwinia chrysanthemi</i> Goto (1979)	<i>Erwinia dieffenbachii</i> Ouf and El-Sadek (1997)
Shape of cell	Rod	Rod	Rod
Motility	+	+	+
Gram reaction	-	-	-
Pigment on CaCO ₃ agar	DGFP	DGFP	-
Sporulation	-	-	-
Rot potato slices	+	?	+
Aerobiosis	aerobic	aerobic	aerobic
Gelatin liquefaction	+	+	+
Starch hydrolysis	-	-	-
Lecithinase production	+	?	?
Levan production	+	ND	+
Indole formation	+	V	-
Ammonia production	-	ND	+
H ₂ S production	-	-	-
Urease activity	-	-	+
Nitrate reduction to nitrite	+	+	+
V.P.test	V	V	-
M.R. test	+	+	+
Minimum temperature	10	ND	5-10
Maximum temperature	37	38-43	40
Optimum temperature	32 °C	30-36 °C	30 °C
Utilize of sugars from Arabinose	A+	A+	A+
Galactose	A+	A+	A+
Glucose	A+	A+	A+
Fructose	A+	A+	A+
Inositol	+	?	?
Lactose	A+	A+	A+
Maltose	-	-	A+
Mannitol	+	A+	A+
Mannose	+	ND	ND
Sorbitol	+	ND	ND
Trehalose	+	ND	ND
Starch soluble	-	-	-
Xylose	A+	A+	A+
Hepersensitive reaction	+	?	?
Maltose	-	-	A+

+ = positive reaction, - = negative reaction, ND = isolates not detected and ? = isolates not tested, A+ = Acid but not gas and DGFP = Diffusible green fluorescent pigment and v = variable reaction.

characters of the isolated bacteria with those reported by Goto (1979) for *Erwinia chrysanthemi*, Dye (1968), Dye *et al.* (1980), Young *et al.* (1978) and Dickey (1981) could be identified as *Erwinia chrysanthemi*.

pathogenicity test

Data presented in Table 2 indicate that all bacterial isolates under investigation were able to infect *Yucca* plants and induce stem 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 three weeks. However, the control plants remained unaffected. Soft rot symptoms (Fig.1) sites of inoculation were obvious 5 to 10 days after stem inoculation,

whereas from 10 to 21 days, the plants were collapsed. Amount of rotting, also rated from 22.2 and 42.2% after 21 days incubation. Results indicate that isolate Y1 and Y1 could be considered as highly pathogenic, whereas isolate Y3 and Y4 were weak virulent. Choi and Han (1992) reported that *Erwinia carotovora subsp. carotovora*, *Erwinia rhapontici* and *Erwinia chrysanthemi* are the causal pathogens of soft rot on garlic cloves. Also, Abdalia (2001) recorded a new bacterial disease on date palm trees caused by *Erwinia chrysanthemi*. Several authors reported that *Erwinia chrysanthemi* were isolated from different plants and caused soft rot diseases [Aysan and Yildiz (2000); Lee, *et al.*, (2002); Liu, *et al.*, (2002); Gray, *et al.* (2000) and Scortichni and Ascenzo (2003)].

Table(2): Pathogenicity of four isolates of *Erwinia chrysanthemi* on *Yucca aloifolia* plants.

Isolates	Time of apperance Symptoms (days) tissue (g)	Weight of rotted	% rot after 21days from inoculation
Y1	5	105.5 ±0.64	42.2 ±*0.22
Y2	7	83.3 ±0.56	33.3 ±0.16
Y3	10	55.5 ±0.23	22.2 ±0.11
Y4	10	71.5 ±0.42	28.6 ±0.25

* Mean of five replicates; calculated as percentage of rotted tissues.

Data are means of 3 replicates ± SD

Host range:

The reaction of some plants to bacterial isolates were determined in the present work. Isolates

produced soft rot on eggplant, tomato, pepper, squash fruits and potato tubers, carrot, lemon, onion and turnip within 5-7 days.

Inoculated stems of chrysanthemum and sunflower developed water soaked areas at the site of infection (4-5 days) which became soft. When the pathogen inoculated into the stems of maize, broad bean and cowpea, they showed no symptoms.

According the host range tested, data assumed that the obtained bacteria have a wide range toward different hosts

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

All tested isolates were active in secreting pectolytic and cellulolytic enzymes in stem tissues of yucca plants after 10 days of inoculation (Table 3 and 4), whereas the isolate Y1 (more virulent) was higher after 180 min than their activities with the weakly virulent (isolate (Y4). These results confirmed those reported by Ouf *et al.*, (1997). They reported that the pectolytic activity of the enzymes were higher in infected tissues than in the healthy ones.

Table(3): Effect of extract of diseased Yucca stem plants on percentage of viscosity of 1% citrus pectin solution during incubation for 3 h at room temperature.

Time (min)	% loss in viscosity of 1% citrus pectin				
	Isolate Y1	Isolate Y 2	Isolate Y 3	Isolate Y 4	Mean
0.0	5.6	3.4	0.0	0.0	2.25
30	19.7	14.5	3.2	7.1	11.12
60 `	33.2	25.8	11.8	17.7	22.12
120	38.0	31.3	21.4	26.9	29.40
180	38.0	31.3	21.4	27.0	29.42
Mean	26.9	21.26	11.56	15.74	18.86

L.S.D 0.05 = 2.85

Table(4): Effect of extract of diseased Yucca stem plants on percentage loss of viscosity of 1% caboxy methyl cellulose (CMC) solution during during incubation for 3 h at room temperature.

Time (min)	% loss in viscosity of 1% citrus pectin				
	Isolate Y1	Isolate Y 2	Isolate Y 3	Isolate Y 4	Mean
0.0	26.9	15.59	0.0	16.71	14.60
30	35.14	22.10	12.21	23.70	23.28
60 `	43.20	28.71	20.34	39.50	32.93
120	52.42	36.12	28.93	42.60	40.01
180	52.42	36.12	28.93	43.90	40.34
Mean	41.8	27.72	18.08	33.28	30.23

L.S.D 0.05 = 1.90

Activity of these enzymes were higher in infected tissue than in healthy ones. Data indicated that the highest activity was shown after 2 h incubation at room temperature and showed significant differences between the virulence and these enzymes. These results are generally in line with those reported by previous investigators Kelman and Cowling, (1965) and Ouf and El-Sadek (1997) and 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 and Saleh, 1995 and Saleh and Stead, 2003). Severin *et al* (1985) reported that *Erwinia carotovora pv. carotovora*, *E.c.subsp. atroseptica*, *Erwinia chrysanthemi subsp.*

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. Hinton *et al.* (1987) reported that rotting of fleshy tissue is due to production of cell wall degrading enzymes and protease

Generally data in Table 5 indicate that total carbohydrates were much higher in inoculated tissue extracts than in healthy ones particularly with isolate Y1 (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.

Table(5): Total carbohydrate and reducing sugars in healthy and diseased tissue extracts of yucca 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	20.42 ± 3.3
Isolate Y1	85.44 ± 3.4	17.82 ± 3.0
Isolate Y2	79.85 ± 2.3	13.23 ± 2.4
Isolate Y3	44.13 ± 3.0	10.19 ± 2.0
Isolate Y4	40.17 ± 3.0	11.22 ± 5.0

Data are means of 3 replicates ± SD

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مرض عفن ساق اليوكا البكتيري في مصر

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شوه مرض خطير يصيب سيقان نباتات اليوكا المنزرعه في صوبه كلية الزراعة بجامعه المنيا علي هيئه عفن طري وأدت شدة الأصابه الي موت النباتات المصابه. تم عزل اربع عزلات بكتيرييه من السيقان المصابه وكانت البكتيريا عصويه قصيره سالبه لجرام ومفرزه لصبغه فلورسنتيه في البيئه الناميه عليها.

بدراسه الصفات المورفولوجية والفسيلوجية لهذه العزلات وجد انها جميعا مشابهة للبكتيريا ايرونيا كرازانثيمم *Erwinia chrysanthemi* وذلك طبقا لمراجع التقسيم المعتمدة. وطبقا للمراجع المتاحه يعتبر هذا البحث اول تسجيل لهذه البكتيريا علي نباتات اليوكا في مصر.

أظهرت العزلات جميعا قدرتها المرضيه علي أحداث العفن الطري عند حقنها صناعيا في سيقان النباتات السليمه ولكن أختلفت العزلات في قدرتها المرضيه وأدت شدة الأصابة الي ذبول وموت النباتات المحقونة.

بدراسه المدى العوائلي للبكتيريا المعزوله وجد انها تسبب عفن طري لثمار الباذ نجان والطماطم والفلفل والكوسه ودرنات البطاطس وجذور كل من الجزر واللفت والبصل كما احدثت العزلات عفن طري ايضا في سوق الكريزانثيم وعباد الشمس و فشلت العزلات المختبره في أصابه كل من الذرة الشاميه واللوبيأ تحت ظروف العدوى الصناعيه.

أوضحت بعض الدراسات الفسيلوجية لسيقان النباتات المصابة صناعيا أن هناك علاقه بين نشاط كل من انزيم البكتينيز والسيليلوز وبين القدره المرضيه للبكتيريا المعزوله مقارنة بالنباتات السليمه.

أدت الأصابة ايضا الي زياده محتوى السكريات الكلية والمحتزلة في سوق النباتات المصابة مقارنة بالنباتات السليمه..