Studies on bacterial soft-rot of onion in the western parts of Libya *

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ABSTRACT

The disease incidence of bacterial soft-rot of onion was the highest in Tripoli followed by El-Zawia, Ameria, Benghashir and Azizia, respectively. The onion cultivar Texas Yellow Grano 502 was more susceptible to infection than Red D-amposta. Two pathogenic bacteria were isolated from diseased onion bulbs, and were identified as *Pseudomonas cepacia* (Burkh.) and *Erwinia cartovora* (Jones). Each bacterium caused rotting of onion cultivars. Inoculation with a mixture of both bacteria showed an increased pathogenic effect.

INTRODUCTION

Onion production in Libya is concentrated in certain areas. Tripoli produces 49% of the total production, El Zawia 33%, Misurata 4%, Sebha and El-Jofra 10% and the remaining percentage is distributed among other areas (1, 14). In 1961 the cultivated area was 4000 hectares producing 24,000 tons, and in 1977 the cultivated area increased to 8000 hectares producing 41,000 tons (13). The average production of onion in Libya is 6 tons/hectare (13).

Disease problems are a threat to inion production wherever onion is grown (1, 14). In Libya, few studies were conducted on onion diseases. Pucci (10), and Martin (9) observed a few fungal diseases, but they did not record most of the important ones or their distribution. Kranz (8) made an assessment of the damage caused by diseases in the major producing areas. Abughnia and Adam reported more than eight fungal species in Libya causing onion diseases (1). These diseases were considered endemic in El-Zawia, Bengashir and Tripoli. They also reported that bacterial damage constituted more than 50% of onion losses.

In Libya, the bacterial rot disease causes a great damage to onion in transit and in storage. These bacteria cause a decay to onion bulbs leading other microorganism to cause greater damage to onion.

Few attempts have been made to investigate the distribution of bacterial diseases in Libya. However, there is no information available on the causative microorganisms and their response to temperature and medium pH. Therefore, this study was conducted to provide the following information:

(a) - Distribution of bacterial diseases in the western parts of Libya.
(b) - Isolation and identification of the casual agents.
(c) - Pathogenitiy tests.
(d) - Effect of pH and temperature on growth of the pathogens in vitro.

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MATERIAL AND METHODS

Onion samples used in this study were collected from Tripoli, El-Zawia, Azizia, Ameria and Bengashir. The samples consisted of two onion cultivars: Texas Yellow Grano 502 and Red D-amposta. One hundred recently harvested and marketed bulbs were taken randomly of each cultivar from the indicated localities.

Isolations and maintenance of bacteriual cultures were carried out using Difco nutrient agar, and modified peptone yeast extract agar medium (PGY) (5).

Isolation:

Isolations were made from rotted onion bulbs by the folowing methods:

a. Pieces from individual leaf bases (fleshy leaf) near the advanced margins of the lesions were cut to small cubes (2mm) and placed in sterile distilled water for 2 hours. A loopful of liquid was streaked on Difco nutrient agar contained in plastic petri dishes. Plates were incubated at 27°C for 48 hours. Results were taken as described by Cother (4).
b. Small pieces of decayed tissue from infected onion bulbs were placed on healthy onion slices. The slices were incubated at 27°C for 1-4 days in plastic petri-dishes containing moist filter paper. After the rot had developed, tissue from the margin of the infected area was plated on nutrient agar and incubated at 27°C for 24 hours as described by Kawamoto and Lorbeer (8).

Different colonies of bacteria were isolated and pure cultures were obtained by repeated subculturing on slants of nutrient agar as described (12).

Morphological characteristics:

Cultures were grown at 27°C for 18, 24, and 48 hours on PGY plates. Gram stain was performed for shape and size measurment (3). Staining of flagella was done by Leifson method (3). Detection of endo-spores was carried out as described by Shaeffer and Fulton (12). For capsule staining, the isolates were grown on PGY medium in which 1% sucrose was substituted for 0.5% glucose, then stained according to Anthony's tecnique (12).

Difco-semisolid motility sulfied medium was used for motility tests. Inoculated plates were incubated at 27°C for 3 days. The motility was evidenced by presence of diffuse growth away from the inoculum.

Physiological characteristics:

1. Sugar metabolism:
   The basal medium used in this test was composed of 0.2% tryptone, 0.5% sodium chloride, 0.03% dipotasium hydrogen phosphate, 0.008% bromothymol blue in distilled water. One percent of the tested sugar was used for differentiating fermentation from oxidative metabolism of sugar by the organisms. Two tubes each of glucose, sucrose, lactose and mannitol were stab inoculated with each bacterial species. One of the inoculated tubes of each sugar medium was then covered with 2 ml of sterile mineral oil to exclude oxygen and the other was left uncovered. The tubes were incubated at 27°C for two days for acid and gas formation.
2. Production of hydrogen sulfide:
   Difco lead acetate agar medium was used to grow cultures for 24 hours at 27°C. Browning of the medium along the line of growth indicated hydrogen sulfide production.
3. Starch hydrolysis:
   Cultures were grown for 5 days on Difco-starch agar of the following composition: 1% soluble starch, 1% casein hydrolyzate, 0.1% glucose, 0.3 monosodium hydrogen phosphate and 1.5% sugar in distilled water. The presence of a clear zone around the growth line of flooding with iodine solution indicate a positive reaction.
4. Gelatin liquefaction:
   Tubes of nutrient gelatin medium were inoculated and incubated at 27°C for 5 days, then tested for liquefaction by refrigeration for 15 minutes (12).

5. Reduction of nitrate:
   Cultures were grown at 27°C for 3 days on a medium composed of 1% glucose, 0.1% yeast extact, 0.2% dipotasium hydrogen phosphate, 0.5% potasium nitrate, 0.01% magnesium sulfate and 1.5% agar in distilled water. Production of nitrate was detected by flooding the plates with a nitrite test solution (12). The presence of nitrite is indicated by a pink color formation.
6. Indole test:
   Cultures were grown for 4 days on tryptone medium and were tested for indole production by Kovac's reagent (12).
7. Catalase test:
   Cultures were grown on PGY agar plates at 27°C for 24 hours and tested for enzyme activity using 2% H_2O_2 solution (12).
8. Urease test:
   Difco urea agar slants were inoculated and incubated at 27°C for 24 hours. The change in color of the mediun from yellow to red is an indication of positive urease test.
9. Growth in 5% sodium chloride:
   Tubes of nutrient broth containing 5% NaCl were inoculated and incubated at 27°C for 48 hours, until growth was demonstrated (12).

Phytopathological characteristics:

a. Pathogenicity test:
   Bulbs and slices of both onion cultivars were stab inoculated with each bacterial species. Also slices of squash, carrot, cucumber and potatoes were surface inoculated with the bacteria. Control bulbs and slices were inoculated with sterile distilled water. Inoculated bulbs were placed in sterile plastic bags and incubated at 28°C for two weeks and examined daily for rot development. Other inoculated material were placed on moist filter paper in sterile plates and incubated at the same temperature and for the same duration.

Infection and disease development studies:

These studies were conducted by using the following methods:

1. Intact bulbs:
   a. Ten healthy bulbs from each onion cultivar were injected through the neck with 1 ml of each bacterial suspension containing 10^7 cell/ml. Inoculated bulbs were then put in plastic bags and incubated at room temperature for 4 weeks. At the end of the incubation period each bulb was cut and examined for disease development.
   b. One ml of bacterial suspension of each isolate was surface sprayed on healthy bulbs of each cultivar. Inoculated and control bulbs were treated as mentioned previously.
   c. Ten bulbs of each cultivar were injected through the neck with 1 ml (10^7 cell/ml) of mixed suspension of both isolates, and were then treated as described in (a).
2. Sliced bulbs:
   Slices of 3 mm in thickness were obtained by transverse sectioning of healthy bulbs of each cultivar, each slice was composed of 5 to 8 leaf bases. These slices were then placed on sterile moist filter paper contained in petri-dishes. The outermost leaf bases and the inner ones were inoculted with each isolate separately. Uninoculated slices were left as control. All slices were incubated at 27°C for six days, and examined for rot development.

Effect of temperature on growth of bacteria:

The plate count technique (12) was used in this experiment. Cultures were grown on PGY medium and incubated at the following degrees: 4, 15, 20, 30, 40 and 42°C for 24 hours. The optimum growth temperature was, therefore, determined for each culture.

RESULTS AND DISCUSSION

Isolation and characterization of the bacteria:

Different types of bacteria were isolated from onion samples, but only two isolates of bacteria with different colony morphology were predominant. These two bacteria produced characteristic symptoms in pathogenicity tests and were designated as A and B isolates.

Morphological, cultural, physiological and pathogenic properties (Table 1 and 2) of these two isolates suggest that isolate A and B could be designated as *Pseudomonas cepacia* (Burkholder) and *Erwinia cartovora* (Jones) respectively (2). However, *P. cepacia* showed negative nitrate reduction and growth in 5% NaCl contrary to those properties listed in Bergey's manual. *Erwinia cartovora* differed in gelatin liquefaction only.

Incidence of bacterial soft-rot on onion in western parts of Libya:

Table 3 showed that the disease incidence was highest in Tripoli followed by El-Zawia, Ameria, Bengashir and Azizia. Data also indicated that onion cultivar Red D-amposta was more resistant to natural infection by the bacteria than Texas Yellow Grano 502. However, losses were high in both cultivars. This could be attributed to intensive planting in these areas without proper sanitation. Table 1 - 2.

Pathogenicity studies indicated that each bacterium caused rot of both onion cultivars. However, inoculation with a mixture of both bacteria produced severe rotting of both cultivars. This suggests the presence of a synergestic action. Surface-sprayed bulbs with each bacterial species separately, produced no lesion, but when injected throug the neck, lesions developed within four weeks. This could be explained on the basis that the bacteria are unable to causes infection in the absencc of wounds. This confirms the results obtained by Kawamoto and Lorbeer (8). They found that *Pseudomonas cepacia* can enter the bulb wounds of the upper parts of the onion. They also concluded that the system of irrigation could be considered as an important factor in aiding infection. Cother and co-workers (4) and Irwin and Vaughan (6) found that irrigation of onion by

Table 1 — Selected characters of isolate A

I.	**Morphological characteristics:**	
	Gram stain	Gram negative
	Shape and arrangement	Rods single or in pairs
	Motility	Motile by (1-2) polar flagella
	Size	0.8-1.2 × 1.5-2.0 um
	Capsule	(−)
	Spore	(−)**
II.	**Physiological characteristics:**	
	H_2S productgion	(−)
	Nitrate reduction	(−)
	Indole test	(−)
	Catalase	(+)*
	Urease	(−)
	Gelatin liquefaction	(−)
	Starch hydrolysis	(−)
	Growth in 5% NaCl	(−)
	Acid production from:	
	Glucose	(+)
	Sucrose	(+)
	Lactose	(−)
	Mannitol	(+)
III.	**Phytopathological characteristics:**	
	Pathogenecity test	Induced discloration, soft rot on onion bulbs, and slices of squash, carrots and cucumber.
	Hypersensitivity test	(−)

* (+) Positive test ** (−) Negative test

Table 2 — Selected characters of isolate B

I. Morphological characteristics:	
Gram stain	Gram negative
Shape and arrangement	Rods single or in pairs
Motility	Motile by (1-2) polar flagella
Size	0.6-0.8 × 1.5-2.0 um
Capsule	(+)*
Spore	(−)**
II. Physiological characteristics:	
H_2S productgion	(+)
Nitrate reduction	(+)
Indole test	(−)
Catalase	(+)
Urease	(−)
Gelatin liquefaction	(−)
Starch hydrolysis	(−)
Growth in 5% NaCl	(+)
Acid production from:	
Glucose	(+)
Sucrose	(+)
Lactose	(+)
Mannitol	(+)
III. Phytopathological characteristics:	
Pathogenicity test	Induced soft rot on onion bulbs, slices of squash, carrots, onion and potato.
Hypersensitivity test	(−)

* (+) Positive test ** (−) Negative test

Table 3 — Incidence of bacterial soft-rot of onion in the western parts of Libya

Location	% disease incidence* onion cultivar	
	Red D-amposta	Texas Yellow Grano 502
Ameria	21	51
Azizia	30	40
Bengashir	20	43
Tripoli	32	64
El-zawia	33	61

* Based on 100 bulbs of each cultivar

sprinkler systems caused splashing of soil, particularly organic matter, onto the bulbs and leaves resulting in water congested areas in the neck region. They concluded that bacteria in the soil may be conveyed onto the wet plant, and infection occurs through water-soaked and wounded tissue.

Data obtained from inoculting onion bulbs and slices of both onion cultivars with *Pseudomonas cepacia* indicate that lesion devolopment was restricted to the outer leaf-bases. On the other hand, when onion bulbs and slices of both onion cultivars were inoculated with *Erwinia cartovora*, lesion devolopment was not as so restricted, but advanced to the inner ones as well. The data obtained in this study do not explain this phenomenon. Therefore, more work is required to determine this particular effect.

Table 4 — The effect of temperature on the growth of bacteria isolated from infected onion

Incubation temperature °C	Bacteria, million/ml medium	
	Pseudomonas cepacia	*Erwinia cartovora*
4	3	4
15	6	8
20	8	13
25	19	21
30	21	18
40	16	7
41	14	3
42	0	0

Studies of temperature effect on bacterial growth (Table 4) showed that *E. cartovora* and *P. cepacia* were able to grow at 4°C with optimal temperatures of 25 and 30°C, respectively, and a maximal of 41°C. This might suggest that under Libyan conditions, onion should be stored under 4°C to minimize losses caused by these bacteria.

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دراسات عن مرض التعفن البكتيرى للبصل فى المناطق الغربية بليبيا

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المستخلص

اثبتت الدراسات ان شدة الاصابة بمرض التعفن البكتيرى فى البصل لصنف تكساس يلوجرانو 502 أعلى منها فى الصنف رد دى أمبوستا كما اتضح من النتائج ان نسبة انتشار المرض كانت عالية فى كل من طرابلس، الزاوية، العامرية وبن غشير والعزيزية على الترتيب . تم عزل نوعان من البكتيريا من الابصال المصابة هما *Erwinia cartovora* و *Pseudomonas cepacia* وكلاهما له القدرة على احداث تعفن لكل من صنفى البصل المستعملين فى هذه الدراسة، غير أن خلط نوعى البكتيريا فى معاملة واحدة أدى الى احداث عدوى بصورة مضاعفة عند مقارنة العدوى التى تحدث من استخدام كل بكتيريا على حدة.