Studies on the Blackleg Disease of Potato in the Libyan Jamahiriya

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ABSTRACT

Symptoms of blackleg disease have been found recently on potato (*Solanum tuberosum* L.) in the Libyan Jamahiriya. These symptoms occur at different parts of the country's north-west region where most of potato plantations are located.

Isolation trials of the causal organism revealed the presence of a Gram-negative, non-spore former, motile, rod-shaped bacterium, $0.5 - 0.7 \times 1 - 2\mu$. Its colonies on nutrient agar were round, white, smooth, glistening and butyrous. It grew at 25°C but not at 37°C, liquified gelatin, utilized citrate as a sole source of carbon, reduced nitrate to nitrite and was able to produce hydrogen sulfide but not indole or phenylpyruvic acid. Also it was able to ferment glucose, sucrose, fructose, maltose, mannito, with the production of acid only. Voges Proskauer test was positive while the methyl red test was negative.

The isolated bacterium was able to rot potato tubers and to cause the development of blackleg symptoms when stems of vigorously growing plants were inoculated under green house conditions. The symptoms developed after 4–5 days of inoculation.

The results obtained indicate that the isolated organism was the blackleg bacterium, *Erwinia carotovora* var. *atroseptica* (Hellmers and Dowson) Dye, 1969.

The sensitivity of two isolates of the causal bacterium to different antibiotics was also studied. Both isolates were found sensitive to tetracyclin, gentamycin, ampicillin, kanamycin, neomycin. However, one isolate was resistant to penicillin and chloramphenicol, and the other one was resistant to penicillin and streptomycin.

INTRODUCTION

Blackleg caused by *Erwinia carotovora* var. *atroseptica* (Hellmers and Dowson) Dye is one of the important potato diseases in nearly every country where this crop is grown (9). This disease occurs in the wet seasons and in places where the soil apt to be water logged. Dowson (9) and Walker (17) reviewed the early investigations on blackleg and its etiological organism. Burkholder and Smith (6), Smith (16) and Buchnan *et al.*, (15) reported the pathological and biochemical characteristics manifested by *E. caratovora* var. *atroseptica*.

Potato plantations in the Socialist Peoples Libyan Arab Jamahiriya expand every

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year. Recent statistics (3) reveal that 19000 hectares were under potato cultivation in 1974–1975 and 95% of this area is located in Tripoli and Zawia area.

In the Socialist Peoples Libyan Arab Jamahiriya, Pucci (15) reported the occurrence of blackleg symptoms for the first time in 1968. This disease seemed to be obscure in the Jamahiriya in the past. No previous investigation was undertaken in this country to study this disease and its causal organism. Symptoms similar to those of blackleg disease were detected during the wet season of 1975–1976 in many parts of Tripoli and Zawia area. Diseased plants were stunted with blackened stems and their tops were curled inwards. Such plants were easily lifted out of the soil and their roots were rotted. The severely affected plants collapsed and rotted away leaving blank spaces. Also at harvest, some of the slightly infected plants gave infected tubers that had no external sign of disease but a dark brown to black discoloration at the heel end.

This study was undertaken to isolate and identify the causal organism of blackleg of potato and investigate its physiological characteristics and sensitivity to antibiotics.

MATERIALS AND METHODS

I. Isolation of the causal organism

Samples of infected potato plants were collected from Vegetable Farms of the Secretariat of Agriculture at Sidi El-Masri, Tripoli. Presence or absence of a causal organism was accomplished by stripping off the outermost layers of the epidermis of infected stems with a sharp sterile scalple. Fragments from cortex tissue were removed with a sterile needle and deposited in small amount of sterile water. Few drops from the obtained suspension were streaked onto nutrient agar (9,12) and incubated at 25°C for 48 hr. Several bacterial colonies that appeared on the medium were picked up and streaked onto nutrient agar plates. Pure cultures from several isolates were obtained.

II. Pathogenicity test

The ability of the isolated bacteria to cause infection and soft rot was investigated as follows:

(a) Potato tubers were planted in sterilized sandy soil in pots of 20 cm diameter. Pots were placed in the green house at 25–27°C. Inoculation was done according to the method described by Afanaseiv and Stevens (2) when plants were 30 to 35 cm long. Inoculated and check plants were held into moistened plastic bags for 48 hr. Later, they were removed and plants were observed for symptom's development.

(b) Potato tubers were surface sterilized with 0.1% mercuric chloride solution for one minute then washed with sterile distilled water. Inoculation was done by dipping a sterile needle into 48 hr bacterial growth on nutrient agar and puncturing the tubers up to 1 cm deep. Inoculated and check tubers were placed into moistened plastic bags, kept under the laboratory conditions and checked daily for disease development.

III. Cultural and Morphological Characteristics

The isolated bacterium was grown for 48 hr at 25°C on nutrient agar plates, nutrient agar slants, endo-agar and sodium deoxycholate agar. Gram stain was performed and used for shape and size determination. Staining of spores was done by the method of Bartholomew and Mittwer (12). Motility was tested on the motility test medium (8).

IV. Physiological characteristics

Temperature Relation: Cultures were grown for 48 hr at 25°C on nutrient agar medium. The growth of the bacterium was recorded under a range of temperatures.

Hydrogen sulfide production: Tubes of peptone water were inoculated with the bacterium. Dry strips of filter-paper previously moistened in saturated solution of lead acetate were hanged inside the tubes (9). These were then incubated at 25°C for seven days. Black colouration on the edges of the paper strips indicates the formation of H_2S.

Indole test: Peptone water medium was inoculated and incubated at 25°C for one week (12). 0.5 ml of Kovac's indole reagent was then added. Development of a deep red colour indicates the presence of indole.

Phenylalanine deaminase production: Slants of phenylalanine agar were heavily inoculated and incubated for 24 hr at 30°C. Few drops of 10% ferric chloride solution were added over the surface of the agar slopes (12). The development of a green colour in the slope and in the liquid was used as an indication of a positive reaction.

Urease test: Tubes of Christensen's medium (12) were inoculated and incubated at 30°C for four days. Development of reddish-purple colour in the medium was used as an indication of a positive urease test.

Gelatin Liquification: A medium of nutrient agar combined with 0.4% gelatin of a final pH 7.2 was poured in plates, streaked with the bacterium and kept at 25°C for 10 days (12). Plates were then flooded with mercuric chloride solution. Development of clear zones was used as an indication of gelatin hydrolysis.

Utilization of citrate: Slopes of Simmon's citrate agar were inoculated and incubated at 25°C for one week (12). Turbidity of the medium indicates the utilization of citrate as the sole source of carbon.

Nitrate reduction: Tubes of nitrate peptone water medium were inoculated and incubated at 25°C for one week. The production of nitrite was tested by Griess–Ilosvay's reagent (12). Development of red colour within few minutes indicates the presence of nitrite.

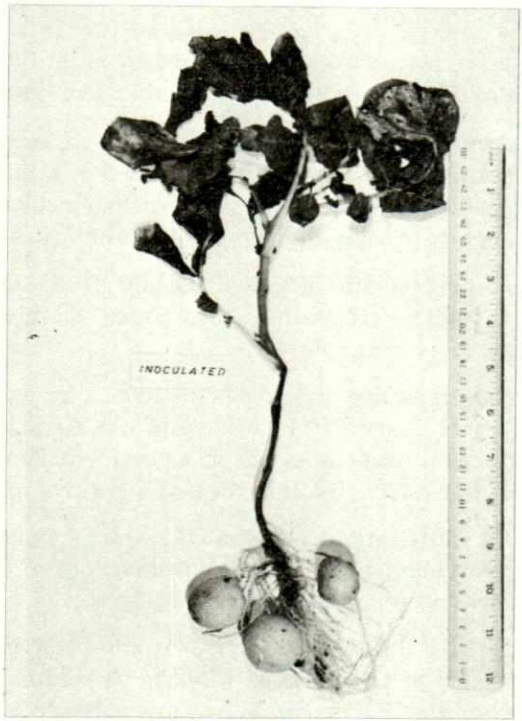
Methyl Red test: Glucose phosphate broth was inoculated and incubated at 25°C for one week (12). Drops of methyl red solution were added to 5 ml of culture as indicator.

Voges–Proskauer test: Glucose phosphate broth was inoculated and incubated at 25°C for one week. A knife point of creatine was added to the culture followed by 5 ml of 40% sodium hydroxide (12). The development of a pink colour in the medium within 30 minutes indicates a positive reaction.

Fermentation of carbohydrate compounds: 1% of fermentable substrates were added to peptone water medium; 0.0025% bromocresol purple was incorporated in the medium to detect acid production. These were inoculated and incubated at 25°C for one week (12). The change in the colour of the indicator to yellow was used as a positive test for acid production.

V. Sensitivity to antibiotics

The effect of different antibiotics on the growth of two isolates of the bacteria was investigated (11). The Oxoid multidisc code no. 1915E was placed on a nutrient agar



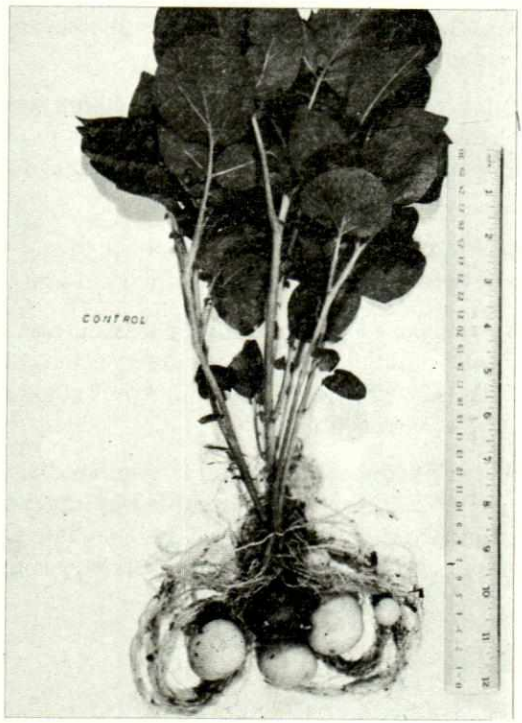


Fig. 1. Healthy (control) and infected potato plants after artificial inoculation.

Table 1 Cultural and morphological characteristics of the isolated *Erwinia caratovora* var. *atroseptica*.

A. Cultural characteristics	Nutrient agar plates	Colonies round, white, smooth, glistening and butyrous.
	Nutrient agar slants	Growth moderate, filiform, grayish-white, iridescent and butyrous; medium unchanged.
	Endo agar	Colonies round, at first pink, later deep red with a metallic laster, medium turns red.
	Sodium deoxycholate agar	Good growth, colonies pink.
B. Morphological characteristics	Shape	Rods, 0.5–0.8 × 1–2 μ.
	Gram stain	Gram negative rods, non-spore former.
	Motility	Motile.

Table 2 Physiological characteristics of the isolated *Erwinia caratovora* var. *atroseptica*.

Tests	Results
Temperature relation	Good growth at 25°C, no growth at 37°C
Hydrogen sulfide production	$+^a$
Indole production	$-^b$
Phenylalanine deaminase production	−
Urease production	−
Liquification of gelatin	+
Utilization of citrate	+
Nitrate reduction	+
Methyl red test	−
Voges-Proskauer test	+
Fermentation of carbohydrates: (Acid production)	
glucose	+
fructose	+
sucrose	+
mannitol	+
maltose	+
lactose	−
inositol	−

$+^a$ = positive test
$-^b$ = negative test

place seeded with the test bacteria. Sensitivity was shown by a zone of clearing around each disc. Diameter of zones of inhibition was determined after 48 hr of incubation at 25°C. The sensitivity of the bacterial isolate to the action of antibiotic compounds was determined according to those reported by Bauer *et al.*, (4).

RESULTS AND DISCUSSION

The isolated organism obtained from the infected stems of potato plants grown at Vegetable Farm of Secretariat of Agriculture, Sidi El-Masri, Tripoli, revealed to be a bacterium. Several bacterial isolates were obtained in pure cultures on nutrient agar plates. Colonies were round, white, smooth, glistening and butyrous. The isolated

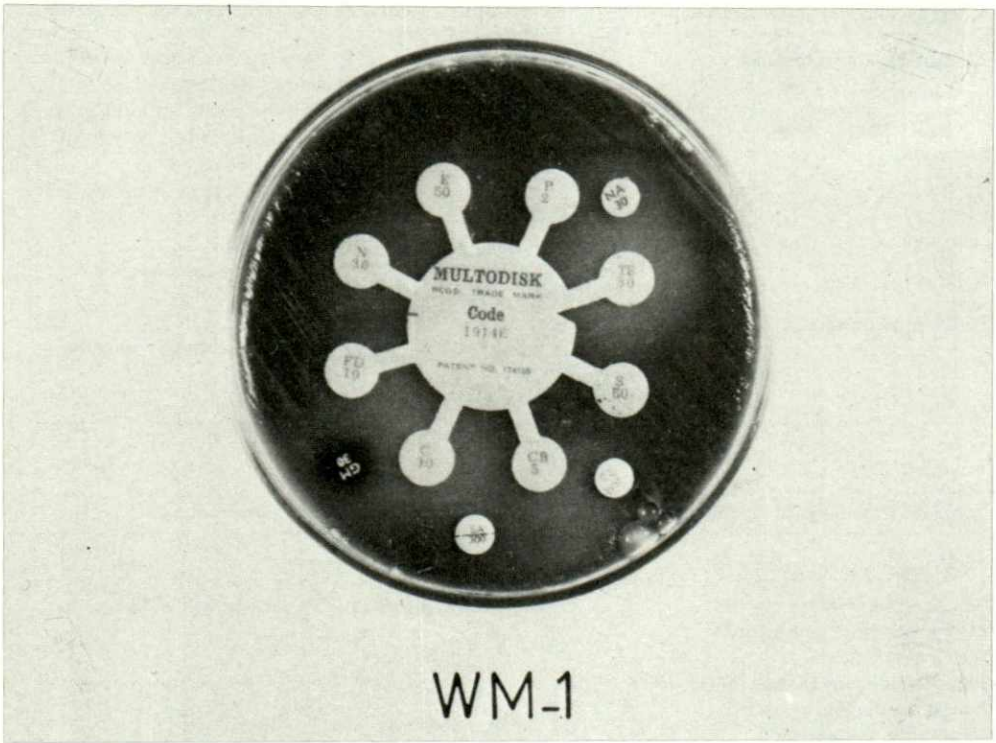


Fig. 2. The multidisc technique showing inhibition effect of some antibiotics on an isolate of *E. carotovora* var. *atroseptica*.

bacteria were found to be Gram-negative, rod shaped ($0.5 - 0.8 \times 1 - 2\mu$), non-spore former, and motile.

Pathogenecity tests showed that inoculated plants developed blackleg symptoms after 4–5 days (Fig. 1). The bacteria was reisolated from the artificially inoculated plants. The organism was also able to cause soft rot when inoculated into potato tubers.

The growth and physiological characteristics of the isolates under study (Tables 1 and 2) were similar to those reported by other investigators (5,6,9,10) and indicated that the isolates under study belong to *Erwinia carotovora* var. *atroseptica* (Hellmers and Dowson) Dye, 1969.

The sensitivity of two isolates of the bacteria (Fig. 2) indicated that both isolates were sensitive to ampicillin, gentamycin, kanamycin, neomycin and tetracyclin. On the other hand, one isolate was resistant to penicillin and chloramphenicol while the other one was resistant to penicillin and stereptomycin (Table 3).

It was concluded that symptoms observed on diseased potato plants in the Socialist Peoples Libyan Arab Jamahiriya were that of blackleg disease. This disease is caused by the bacterium *E. carotovora* var. *atroseptica*. Favourable weather conditions, presence of insects, and nematodes may play a role in the spread of this disease (9,13,14,17). Under prevelence of these factors, the disease could be a threat to potato production in the country.

Sanitation measures and the use of healthy potato seeds as well as crop rotation and rouging of diseased plants constitute major factors recommended for controlling this disease.

Table 3 The sensitivity of two isolates of *E. carotovora* var. *atroseptica* to different antibiotic compounds.

Compound	Disc potency	Isolate I.Z.D.[a]	SI–7 S[b]	Isolate I.Z.D.	WM–I S
Ampicillin	10 mg	14	S	14	S
Chloramphenicol	30 mg	0.0	R	18	S
Gentamycin	10 mg	13	S	13	S
Kanamycin	30 mg	18	S	18	S
Neomycin	30 mg	16	S	16	S
Penicillin	10 mg	0.0	R	0.0	R
Streptomycin	10 mg	16	S	0.0	R
Tetracyclin	30 mg	26	S	26	S

[a]The inhibition zone diameter in mm.
[b](S) implies that the bacteria is inhibited by antibiotic compound, while (R) implies that the bacteria is resistant to antibiotics.

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دراسات على مرض الساق الاسود فى البطاطس

فى ليبيـــــــــا

م٠م٠ الزيات ، ج٠ل٠ عمر ، م٠م٠ طاهـــــــر

المستخلـــــص

يعتبر مرض الساق الاسود من الامراض الهامة التى تصيب محصول البطاطس والتى ينتشر زراعتـــه فى مناطق مختلفة من طرابلس والزاوية ٠ وبدراسة صفات البكتريا المعزولة من النباتات المصابة تبين انهـــا عضوية الشكل سالبة لصبغة جرام وغير متجرثمة ، ومتحركة وتبين انها تستطيع أن تنمو على درجة حـــرارة ٣٥ م ولكنها لا تنمو عند ٣٧ م٠ كما انها تسيل الجلاتين وقادرة على اختزال النترات وتكـــوين كبريتور الايدروجين والاندول ٠ كما تستطيع ان تستخدم السترات كمصدر وحيد للكربون بالاضافة الى انها سالبة لاختبار أحمر المثيل وموجبة لاختبار فاجس بروسكر وقادرة على انتاج حامض من الجلوكوز ، الفركتوز ، السكروز ، المالتوز والمانيتول ٠

ولقد وجد ان هذه البكتريا تعفن درنات البطاطس ، كما سببت ظهور أعراض مرض الساق الاسود عند حقنها فى سيقان نباتات البطاطس بالصوبة الزجاجية ٠

وعليه يتضح من هذه الدراسة بان البكتريا مشابهة فى صفاتها مع بكتريا *Erwinia carotovora* *var. astroseptica* (Hellmers and Dowson) Dye كما درست حساسية البكتريا المعزولة لعدة مواد حيوية مضادة باستعمال طريقة الاقراص المتعددة ٠