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Pathological and bacteriological characteristics of bacterial isolates causing soft rot of potato in the Sudan

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ABSTRACT

Isolation of soft rot bacteria was made from potato tubers and soil samples collected from different districts of potato production. The bacterial isolates induced hypersensitive reactions on tobacco leaves and produced typical blackleg and soft-rot symptoms on potato plants and tubers, respectively. A wide range of fruits and vegetables were also found susceptible to the isolates.

Morphological, biochemical and physiological characterization of the isolates proved that they all belong to the *Erwinia carotovora* group. All are gram negative, motile, non-spore forming and capable of producing various chemical compounds and enzymes. Although all were able to produce acid and gas from the tested carbohydrates, only two of them utilized maltose and glycerine.

INTRODUCTION

Potato, *Solanum tuberosum* L., is grown mainly as a winter crop in the Sudan. The main areas of production are along the Nile banks in both Khartoum and Northern Provinces. The production depends greatly on seed tubers imported annually from the Netherlands.

The soft rot disease, caused by *Erwinia carotovora* pv. *carotovora* (Jones 1901) and *E. carotovora* pv. *atroseptica* (Van Hall 1902), is the most serious disease of potato. It occurs wherever potatoes are grown in the country and causes high economical losses of the crop. On a world wide basis, losses are extremely high (Perombelon & Kelman 1980).

The causal organisms have been thoroughly investigated by Dye (1969), and Graham (1964, 1972) and were found to be gram-negative, motile, non-spore forming and facultative anaerobes. Very high populations of the bacteria were detected frequently from fields in which potato had been grown (Mew *et al.* 1976; De Boer *et al.* 1979). Studies of the disease in the Sudan have not been made since an early report by Dowson (1957).

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Bacterial isolates obtained from tubers and soil samples of different areas of potato production in the present studies, were compared for their morphological, biochemical and physiological characteristics. Pathogenicity and host range of each isolate were also investigated.

MATERIALS AND METHODS

ISOLATION FROM INFECTED TUBERS

Potato tubers exhibiting soft-rot symptoms were collected from different areas of production in the Sudan and also from seed tubers imported from the Netherlands during 1982 and 1983. Rotted tissue from the heart of an infected tuber was removed with a sterile spatula or a needle and transferred immediately to a test tube containing sterile distilled water. The suspension was shaken to disperse clumps before a dilution series was prepared. A loopful from each bacterial suspension was streaked on nutrient agar medium. After 24 hours incubation at 25°C, single colonies were selected on the basis of their similarity to reference cultures of *E. carotovora* pv. *carotovora* (IRB 264) or *E. carotovora* pv. *atroseptica* (IRB 263) kindly supplied from Northern Ireland by Dr Logan. Representative isolates of each area of production were kept at 5°C on nutrient agar slants in capped tubes before use.

ISOLATION FROM SOIL

Soil samples were collected at random, immediately after harvest from fields of three different areas in Khartoum Province. The samples were removed from the depth where potato tubers were found and kept in sterile polythene bags. A subsample of 25 g on an oven-dry basis was suspended in 250 ml of sterile distilled water. A range of dilutions was made from which aliquots (0·1 ml) were removed and spread on the surface of crystal violet pectate agar (Cuppels & Kelman 1974) or nutrient agar. All plates were incubated at 25°C and the number of colonies per gram oven-dry soil was determined for each area of potato production.

HYPERSENSITIVE REACTION ON TOBACCO

Bacterial colonies developing on isolation plates were suspended in sterile water to produce suspensions of 10^9 cells/ml. Suspensions of each isolate were injected into intercellular spaces of intact leaves of one-month old tobacco (*Nicotina tabaccum* L.) plants, using a hypodermic syringe fitted with a fine needle (No. 30). Ten areas each of 3–5 cm^2 were made for each isolate. Inoculated plants were maintained at high humidity and after 24 hours were examined for the development of necrotic tissues.

PATHOGENICITY TESTS ON POTATO TUBERS

Bacterial isolates, from rotted tubers or soil, capable of producing necrosis on tobacco leaves, were tested for their pathogenicity to healthy tubers.

Sound healthy tubers of 'alpha' potato of almost equal sizes were surface-sterilized with mercuric chloride solution (0·1% w/v), washed and weighed individually. A plug was removed with a cork borer (4 mm diameter) from a hole (1 cm deep) made mid-

way between the apex and basal end of each tuber. An inoculum of 0·2 ml of 10^9/ml suspension of cells from 1–2-day-old cultures of each isolate on nutrient agar, was introduced into each hole. After treatment, each hole was covered by its plug and sealed with melted paraffin wax (Bourne *et al.* 1981). Drops of the same volume of sterile water were introduced as controls. Three treatments of 10 tubers each were used for each bacterial isolate. Inoculated tubers in each treatment were incubated at 30°C in a plastic bag moistened with sterile water to maintain high humidity. Eight days later, tubers were removed and examined for rot development. Rotted tissues were separated from healthy parts and weighed. Percent rot was calculated from the total weight of the tuber.

PATHOGENICITY TESTS ON POTATO PLANTS

Healthy alpha potato tubers were washed and cut by a sterile knife into small pieces, each containing at least two eyes. The pieces were then sown in sterile soil (mixture of 1:2 sand and river silt) in plastic pots (25 cm diameter) which were placed in a glasshouse and left to grow. Leaves and stems of four-week-old plants were inoculated by spraying with bacterial suspensions (10^9 cells/ml). Inoculated plants were kept under humid conditions for 46 h and left in the glasshouse to allow disease development. Disease symptoms were rated on a 0 to 4 scale in which 0 = no symptoms and 4 = maximum symptoms.

HOST RANGE STUDIES

Some fruits and vegetables grown in the Sudan were tested for their susceptibility to infection by potato soft-rot bacterial isolates. The edible part of each fruit or vegetable was surface-sterilized and inoculated using the cork borer method as was described for potato tubers. Inoculated parts were kept at 30°C under humid conditions and examined for rot development after seven days.

CHARACTERIZATION OF ISOLATES

Isolates obtained from each production district were compared for their bacteriological characteristics with reference cultures of *E. carotovora* pv. *carotovora* and *E. carotovora* pv. *atroseptica* on the following bases.

MORPHOLOGY

Morphological studies were made on cells and colonies of each isolate after growth on nutrient agar. Oxygen requirements for growth were investigated under aerobic and anaerobic conditions. Adding sterile oil on the top of the medium was employed in order to secure anaerobic conditions. Smears from 1–2-day-old cultures on glass slides were stained for gram reaction, capsulation, flagellation and sporulation using the procedures described by Norris & Ribbons (1970).

BIOCHEMICAL AND PHYSIOLOGICAL TESTS

Isolates were subjected to the following biochemical tests: hydrolysis of starch and

gelatin, ammonia production and the Voge–Proskauer test as described by Kiraly *et al.* (1974); catalase and hydrogen sulphide production (Cowan & Steel 1979); sodium chloride tolerance; nitrate reduction and indole production (Dye 1969); the litmus milk reaction and methyl red test (Dowson 1957).

The ability of each isolate to grow and utilize different carbon sources was also tested. Acid and gas production was detected using the method of Dye (1969). Solutions of all the tested carbon sources except salicin were sterilized by filtration using a membrane filter (0·45 μ) and were added to autoclaved peptone water, to give a final concentration of 1% of each sugar. Salicin, at the same concentration, was autoclaved directly in the basic broth medium. The standard inoculum for all the tests was 0·1 ml of bacterial suspension (10^9 cells/ml).

RESULTS AND DISCUSSION

In the present studies, almost pure cultures of *Erwinia carotovora* were obtained from the heart of rotted tissues. This did not require the usual purification techniques. The reason may be that the pathogen in rotted tissues had already been selected by the host tissues and occurred in large numbers (Webb & Wood 1974). Isolation from rotted tissues yielded, with few exceptions, pathogens belonging to the genus *Erwinia* capable of producing the soft-rot symptoms described by Burkholder & Smith (1949).

Soil samples collected from El Geili, Wad Ramli and Wawisi, were tested for the presence of soft-rot bacteria. Colonies were recovered from all the samples. The percent of soft-rot bacteria from the total was higher in Wad Ramli compared to the other two localities (Fig. 1). The crystal violet pectate medium greatly inhibited other soil bacteria but permitted greater recovery of the soft-rot pathogen than the nutrient agar (Fig. 1). The presence of *E. carotovora* has also been demonstrated by other workers in soils where potatoes were grown (Kerr 1953; De Boer *et al.* 1979). The difficulty in explaining why seed tubers obtained from apparently healthy plants, often produced plants that developed blackleg, led many workers to conclude that the pathogen is soil-borne (Perombelon & Kelman 1980).

The selected isolates were able to induce a hypersensitive reaction in tobacco leaves. Since only pathogenic bacteria are able to cause necrosis of tobacco leaves, the method was considered by many authors as the most rapid and simple method for obtaining pathogenic isolates (Klement *et al.* 1964; Kiraly *et al.* 1974).

Reactions of bacterial isolates representing different districts of potato production on both potato tubers and plants are shown in Table 1. Considerable differences in pathogenicity were found among the isolates. Isolate NLB15 proved the least pathogenic, whereas, isolate SB12 was the most virulent. Disease symptoms produced by the tested isolates were similar to those reported for *E. carotovora* by Dowson (1957). A strong fishy smell was also produced. According to Ratuszniak (1984), the smell is usually variable and depends on the potato variety used.

Table 2 shows the reactions of different fruits and vegetables towards infection with the selected isolates that infect potato. All tested edible parts were found susceptible to the seven isolates, except sweet potato which was not infected by isolates NLB15 and IRB263/22. Carrots and some other fruits and vegetables have been reported susceptible to the soft-rot bacteria (Burkholder & Smith 1949).

Cell and colony characteristics of the tested isolates showed that all isolates produced white, round colonies with entire margins on nutrient agar. All were

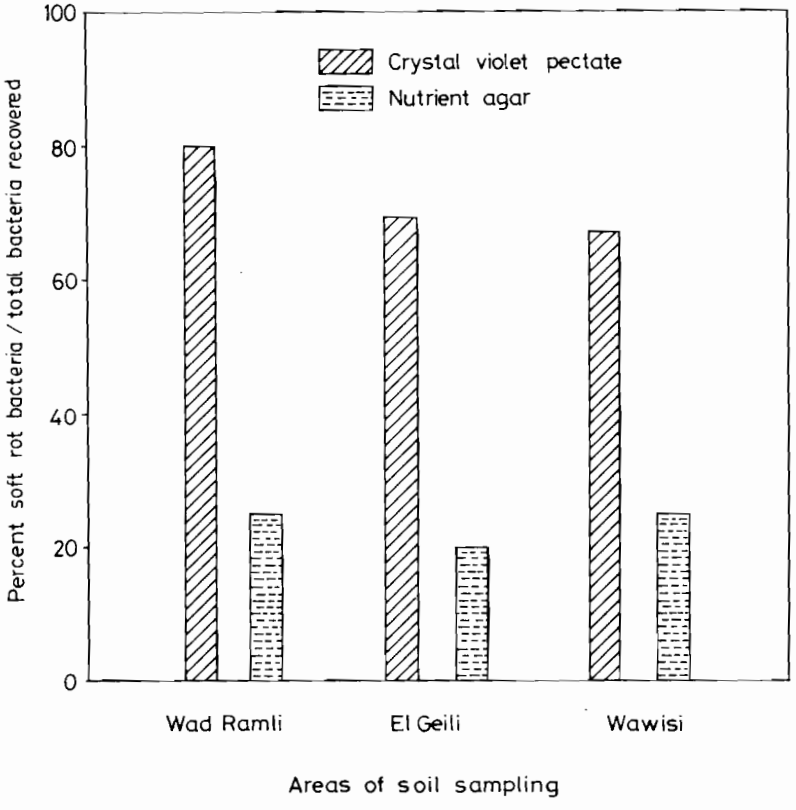

Fig. 1. Percent recovery of soft-rot bacteria in soils of different areas of potato production and on two culture media.

Table 1. Pathogenicity of soft-rot bacterial isolates obtained from different areas of potato production. Data refer to averages of 10 replicates

Isolate	Location	% Rot/tuber (w/w)	Disease index†
IRB263	Northern Ireland	27·0 ± 3·35*	2·5
IRB264	Northern Ireland	38·5 ± 1·99	3·3
SB11	El Geili (Sudan)	32·3 ± 7·24	3·6
SB12	Wad Ramli (Sudan)	43·5 ± 10·29	4·0
SB13	Wawisi (Sudan)	28·5 ± 1·75	2·0
SB12	Jebel Mara (Sudan)	35·2 ± 3·04	3·5
NLB15	The Netherlands	18·3 ± 0·67	1·5
Control	Sterile water	0·0	0·0

* Standard deviation.
† 0 = no symptoms; 4 = maximum symptoms.

facultative anaerobes and did not produce pigments. The isolates produced deep pits in crystal violet pectate agar and liquified Star medium. The cells are gram-negative, motile, capsulated and non-spore forming. These results are in agreement with those reported for *E. carotovora* (Dye 1969; Graham 1972; Cuppels & Kelman 1974).

Table 2. Susceptibility of the edible parts of some fruits and vegetables to the soft-rot bacterial isolates (+ = positive, − = negative)

Fruits and vegetables	Bacterial isolates						
	IRB263	IRB264	SB11	SB12	SB13	SB14	NLB15
Daucus carota L.	+	+	+	+	+	+	+
Cucumis sativus L.	+	+	+	+	+	+	+
Allium cepa L.	+	+	+	+	+	+	+
Lycopersicon esculentum Mill.	+	+	+	+	+	+	+
Solanum melongena L.	+	+	+	+	+	+	+
Cucurbita pepo L.	+	+	+	+	+	+	+
Beta vulgaris L.	+	+	+	+	+	+	+
Solanum tuberosum L.	−	+	+	+	+	+	−
Musa sapientum L.	+	+	+	+	+	+	+
Citrus limon Burm.f.	+	+	+	+	+	+	+
Citrus sinensis Osbeck	+	+	+	+	+	+	+
Psidium guajava L.	+	+	+	+	+	+	+
Mangifera indica L.	+	+	+	+	+	+	+

Table 3. Chemical and physiological characterization of the soft-rot bacterial isolates (+ = positive, ± = weakly positive, − = negative)

Test	Bacterial isolates						
	IRB263	IRB264	SB11	SB12	SB13	SB14	NLB15
Starch hydrolysis	−	−	−	−	−	−	−
H_2S production	±	±	+	+	+	±	±
Indole production	−	−	−	−	−	−	−
Ammonia production	±	+	+	±	+	+	±
Voge–Proskauer test	+	±	+	+	±	+	±
Methyl red test	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+
Litmus milk	+	+	+	+	+	+	+
Growth at 5% NaCl	+	+	+	+	+	+	+
Acid production from:							
Salicin	+	±	+	±	±	+	+
Maltose	+	−	−	−	−	−	+
Galactose	±	+	+	+	+	+	+
Manitol	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Cellobiose	±	+	+	+	+	+	+
Lactose	+	+	+	+	+	±	+
Glycerine	+	−	−	−	−	−	+
Fructose	±	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+

Results of the biochemical and physiological studies are shown in Table 3. None of the isolates hydrolysed starch or produced indole. All tolerated 5% sodium chloride solution, produced ammonia from peptone, produced catalase, reduced nitrate and produced variable amounts of hydrogen sulphide. Similar results were reported in the

literature for isolates of *E. carotovora* (Burkholder & Smith 1949; Dye 1969; Graham 1972).

In the present work, positive results were indicated for the Voge–Proskauer test and methyl red test. However, Waldee (1945) and Burkholder & Smith (1949) reported variable results with these two tests. The majority of their results have been negative with only a few positive ones. Coagulation of litmus milk and acid production by the different isolates were observed.

Carbohydrate utilization by the isolates is also shown on Table 3. All the isolates produced acid from salicin, galactose, manitol, glucose, cellobiose, fructose, arabinose, lactose and mannose with variable amounts of gases evolved. This was also reported by Burkholder & Smith (1949), and Graham & Dowson (1960). On the other hand, glycerine and maltose were only used by isolates NLB15 and IRB263/22 but not by the other five isolates. According to Perombelon & Kelman (1980) utilization of maltose was one of the key characteristics distinguishing *E. carotovora* pv. *carotovora* from pv. *atroseptica*. While the former does not attack maltose the latter utilizes it to produce acid.

From these results it can be concluded that bacterial isolates obtained from potato producing areas in the Sudan belong to *E. carotovora* pv. *carotovora* group and those obtained from potato tubers imported from the Netherlands belong to *E. carotovora* pv. *atroseptica* group.

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دراسة البكتيريا المسببة لمرض العفن الطري للبطاطس في السودان

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خلاصة

تم في هذا البحث اجراء دراسة ميدانية للبكتيريا التي تسبب مرض العفن الطري للبطاطس ، حيث جمعت درنات مصابة وكذلك عينات من التربة من مناطق الانتاج المختلفة . ثم عزلت البكتيريا من أجزاء الدرنات المصابة ومن التربة ، على بيئات غذائية .

أثبتت العدوى الاصطناعية بالبكتيريا التي تم عزلها أن بعضها قادر على تحليل نسيج ورقة التبغ وعلى احداث الاصابة في درنات ونباتات البطاطس السليمة .

وقد أكدت الدراسة التصنيفية لسلالات البكتيريا المعدية للبطاطس أنها تنتمي جميعها الى النوع أروينيا كاروتوفورا . فهي سالبة لصبغة جرام ، متحركة ، وليست لها القدرة على التجرثم ، ويمكنها افراز بعض المواد الكيماوية والانزيمات المختلفة .

كذلك أثبتت هذه الدراسة أن معظم ثمار الفاكهة والخضروات التي تم احداث العدوى بها اصطناعيا بالبكتيريا المعدية للبطاطس قد أصيبت بمرض العفن الطري .

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