

**BIOLOGICAL STUDIES ON BACTERIA
PATHOGENIC TO MELONS**



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PREFACE

Melons (Cucumis melo L.) are cultivated in various parts of the world for their valuable delicious fruits. Different sorts of melons are usually cultivated in lower and upper Egypt; occupying about 11% of the cultivated area with cucurbitaceae; the latter represents 27% of all cultivated lands of Egypt (Statistics data, 1979).

In Egypt, melons are considered one of the most indispensable summer fruits. Unfortunately, the best qualities are always attacked by several diseases. Pseudomonas lachrymans (Smith and Bryan), Carsner, is one of the common bacterial pathogens known to attack melons in North America and Africa. Erwinia tracheiphila (Smith) Holland, and Erwinia carotovora var. carotovora (Jones) Holland, f. citrullis (Sizova) are known to cause leaf spots, wilting and rotting of melon and water melon fruits (Saleh and Korobko, 1981). The bacterial diseases, attacking melons, in the Egyptian country, have seldom been studied, though, in 1980, the wilting percentage in melon plantations, in Ismailia Governorate (lower Egypt), reached 40-50%. Accordingly, etiological studies of these pathogens may be considered an important step in identifying the causal organisms, as well as finding out possible protective measures against them. Such measures would save both humans and animals from the toxic effects of such

bacteria (Shavarnevaii, 1950), since melon fruits are directly consumed without being cooked.

The aim of this investigation is:

- (a) to detect and describe the symptoms of bacterial diseases of melons in several localities of the Egyptian fields and
- (b) to isolate and study, in detail, the pathogenicity of the causal organisms as well as morphology, biochemical activity and serological characters, in order to determine the systematic position of these pathogens, as the first step in combat against such diseases.

The work was carried out in the bacteriology labs of the Botany Department, Faculty of Science, Cairo University, during 1979-1982.

PART I

INTRODUCTION

- A. Historical Review.**
- B. Material and Methods.**

I N T R O D U C T I O N

A - Historical Review:

Identification of the organism causing the soft rot disease of carrot, in the United States, was first established by Jones (1901). He described the pathogen as a motile peritrichously flagellated bacillus to which he gave the name "Bacillus carotovorus". As the generic name "Bacillus" can no longer be used for non-sporing rod shaped bacteria (Report of Second International Congress for Microbiology, London, 1936 - published 1937 -, and J. Bact. XXXIII, 1937, p. 445) since Dowson used the name Bacterium in 1939.

However, in 1917, the genus Erwinia replaced the genus Bacterium, as recommended by the Committee of the Society of American Bacteriologists. Later Bergey et al. (1923) published their first Manual of Determinative Bacteriology and it included the genus Erwinia which was restricted to the peritrichously flagellated organisms.

Rahn (1937) recommended the establishment of a family "Enterobacteriaceae" with a genus "Enterobacter" to include all members of the genus "Erwinia", Enterobacteriaceae, as a family, was accepted in the fifth edition of Bergey's Manual (1939) but the family was

divided, according to biochemical activities, into five tribes. The tribe *Erwinieae* remained segregated from the other tribes on the basis of pathogenicity of its members to the plants; and remained as a separate tribe in the sixth (1948), seventh (1957), and eighth (1974) edition of Bergey's Manual.

Waldee (1945) suggested that the genus Erwinia consisted, at least, of two distinct types of organisms. Though both were morphologically similar, yet they differ in pathogenicity, nutritional requirements and biochemical properties. He retained the name Erwinia to the organisms responsible for dry necrosis and wilt diseases. These do not produce pectolytic enzymes. He proposed a separate genus "Pectobacterium" for the other organisms responsible for the true soft rots. These produce pectolytic enzymes. Such differentiations have been accepted by Dowson (1949, 1957) and by other phytobacteriologists (Patel and Kulkarani, 1951; Gorlenko and He, 1961; Martinec and Koem, 1963; Graham, 1964; Ercolani, 1968; Komagata; 1970).

Dye (1969) performed a systematic study on the different members of the genus Erwinia and came to the conclusion that these organisms were very closely related to Erwinia carotovora (Jones) Bergey et al. (1957). He suggested that the several species of this genus should

be classified as five varieties of E. carotovora namely var. carotovora; var. atroseptica; var. chrysanthemi; var. rhapontici and var. cyprapedii.

Graham (1972) supported this classification and carried out several studies on the phenotypic characters of all supposed members of this genus in order to distinguish between the different varieties of E. carotovora.

The biochemical breakdown of the host tissues, induced by the soft rot bacteria, renders such tissues very wet and slimy. Waldee (1945) confirmed that these bacteria attack the middle lamella of plant cells and produce soft rots.

Walter et al. (1949) noticed that E. carotovora was also able to attack other plants causing soft rot, including cucumber. Sabet (1954) succeeded to isolate a soft rot organism from the Egyptian soil. The bacterium was similar, in characters, to Erwinia carotovora. Since the strain was able to infect maize, he proposed to give it the name E. carotovora f. sp. zeae. Kryvets' (1975) found that E. carotovora, causing lupine bacteriosis, could survive in the rhizosphere of the host plant during the whole vegetative period.

Burr and Schorth (1977), in their survey studies on the soft rot bacterium, E. carotovora var. carotovora were

able to detect the organism, in abundance without the need of enrichment, in the rhizosphere soils of many weeds and several crop plants, as well as from field soils containing plant residues but not from fallow soils, devoid of vegetation or recognizable plant debris.

Moliva and Harrison (1980) noticed that the rise of soil temperature from 26°C to 35°C, during the first 30 days of planting, caused a severe attack by E. carotovora var. carotovora causing the post emergency black leg disease of potato.

Cother (1980) found that the pectolytic activity of the soil borne bacteria was more important in initiating and causing rotting of the plants than the seed-potato tuber borne bacteria.

According to Elliott (1951), E. carotovora is Gram-negative rod-shaped bacterium, 0.6-0.9 x 1.5-5.0 μ . It forms chains, devoid of capsules and can move by 2-5 peritrichiate flagella. On nutrient agar, it forms gray white rounded smooth colonies and can infect melons (Cucumis melo). It can liquefy gelatin, reduce nitrate, coagulate milk and produce hydrogen sulphide gas (Waldee, 1945) but cannot form indole (Stapp, 1928).

The toxigenic bacterium, Erwinia carotovora f. sp. citrullis is a well known pathogen confined and restricted to USSR. It was first recorded in 1941 and never traced in

any other country. Shavarnevaii (1950) isolated and described this pathogen. It infects water melon fruits forming raised brown spots with a decaying center that gradually deepens in the mesocarp though the epicarp seems unchanged. At the early stages of infection, the outer colour of the fruits is almost unchanged but turns yellow at later stages. When eaten, such infected fruits stimulate vomiting, diarrhoea and headaches that may lead to death. Saleh (1977) succeeded in isolating this pathogen from both melons and water melons. The isolated bacterium could cause rotting of both fruits as well as leaf spots and wilting of both plants that become brown to reddish brown in colour. The leaf blades acquire necrotic spots that may be irregularly distributed or occupy the leaf margin.

Ceratanena and Papova (1948) isolated a toxigenic form of the bacterium and identified it as a species of Proteus. A similar toxic bacterium was isolated from three sorts of water melons by Petrovich and Shustova (1951). These isolates were weakly agglutinated by the antiserum prepared by Ceratanena for her toxic bacterium. Accordingly the authors classified this bacterium as a variety of Escherichia coli referring to their similarity in serological and biochemical characters. However, this bacterium bears very close resemblances to Erwinia carotovora var. carotovora, but differed in its inability to decompose carrot,

or potato slices (Petrovich and Shustova, 1951; Saleh and Korobko, 1982). Also this pathogen was very toxic to man and animals (Petrovich and Shustova, 1951).

Fedrova (1964) reported that the nucleic acid content of this bacterial was identical to that of the other members of enterobacteriaceae viz. Proteus vulgaris, P. morganii, Escherichia coli, Erwinia carotovora. Sizova (1965) compared the morphological, biochemical, serological and toxic effects of the toxigenic bacterium isolated from rotted water melons with those of Erwinia carotovora, Escherichia coli, and Proteus sp. She reached the conclusion that this toxic bacterium was a form species of Erwinia carotovora and gave it the name E. carotovora f. citrullis (Sizova).

Gnytenko (1966, 1968) showed that the different strains of E. carotovora f. citrullis were not identical in their antigenic structure, but could be scrutinized into different groups. Bogorodniskaya (1972, 1975) observed the presence of common components in the antigenic complex of the different strains of this pathogen thus proving the presence of close relationships between them.

The origin of the toxic bacterium, Erwinia carotovora f. sp. citrullis, is still under consideration. Gorlenko (1964) was of the opinion that this bacterium is an inter-

mediate link in the evolution of Escherichia coli to Erwinia carotovora or it may be regarded as a modified form of E. carotovora.

According to Sizova (1966) E. carotovora f. sp. citrullis cannot survive, in the soil, beyond the limits of the rhizosphere of water melon roots. If inoculated into the soil, it would totally disappear within 3-14 days. The destruction of the organism, in the soil, depended, among other factors, on microbial antagonism, biological and nutritional criteria, the adsorptive power of the soil, soil temperature ... etc. The author was also able to isolate the organism from seeded soil, during the winter season. This points to the possibility of arresting de novo infection of water melons by using prophylactic methods of agricultural techniques. In the meantime, Saleh and Korobko (1982) noticed that the virulence of this pathogen was weakened or even lost by maintaining the bacterium, in the laboratory, on synthetic medium.

B - Material and Methods

The material of this study was collected, during 1979-1982, from infected melon in the open fields of Ismailia(North east Cairo, 100 km); El-Tahrir Governorate (North west Cairo, 80 km) and Imbaba, 20 km and Ayyat fields (South Cairo, 40 km).

The infectious bacteria were isolated from 200 specimens of infected leaves, stems and roots where 1146 pathogenic cultures were obtained. The detailed biological and biochemical studies were carried out on 20 of the strongly virulent isolates. The characteristics of the isolated pathogen were compared with standard cultures of Erwinia carotovora var. carotovora, provided by the Pathological Research Institute, Ministry of Agriculture, Giza, Egypt and Erwinia carotovora f. citrullis (strain 777a), isolated and identified by Saleh (1977) from infected melons and water-melons in Ukrainian SSR.

Isolation of bacteria from the infected plants was performed according to one of the following procedures:

- a - Infected parts were squeezed, with few drops of sterile distilled water, in a porcelain mortar and the sap was plated on nutrient agar medium.
- b - Small fragments of the infected parts were directly placed on the surface of nutrient medium.

- c - The pulp of the rotted parts was directly smeared on the surface of nutrient medium.

The produced colonies were separately isolated on slants. The pathogenicity of the isolated strains was studied on cultivated melon plants, grown in the experimental fields of the Botany Department, Faculty of Science, Cairo University. Healthy leaves and stems of melon plants were punched, 3 times, by a fine needle, then one drop of the bacterial suspension (10^8 cells/ml) was placed on the formed holes. Healthy fruits were infected by inoculating the bacterial suspension in their inner tissues.

Identification and classification of the isolated cultures were based on the biological, biochemical and serological characteristics of each isolate. The morphological characters and structure of the colonies were studied on beef peptone agar medium. The bacterial cells were stained with Gram-staining. The optimum and critical temperatures were determined on beef peptone bouillon.

The following media and methods were adopted for biochemical and physiological characterization of the isolates:

1 - Nutrient Broth:

Beef extract	3.0 g
Peptone	5.0 g
Sodium chloride	3.0 g
Distilled water	to 1000 ml

Nutrient agar is nutrient broth, gelled by addition of 2% agar.

2 - Carbohydrate Utilization:

The medium, described by Collins and Lyne (1974) was used. It consists of:

Ammonium dihydrogen phosphate	1.0 g
Potassium chloride	0.2 g
Magnesium sulphate	0.2 g
Bromothymol blue (0.2% solution)	4.0 ml
Carbohydrate source	10.0 g
Distilled water	to 1000 ml

3 - Organic Acid Utilization:

This was performed on Gordon and Mihm's (1957) medium which has the following composition:

Sodium chloride	1.0 g
Magnesium sulphate	0.2 g
Diammonium hydrogen phosphate	1.0 g
Potassium dihydrogen phosphate	0.5 g
Organic acid (sodium salt)	2.0 g
Agar	20.0 g
Phenol red (0.2% solution)	4.0 ml
Distilled water	to 1000 ml

The organic acids used were malic, oxalic, benzoic, acetic, pyruvic, lactic, tartaric, succinic and propionic. Citric acid was supplied in Simmon's medium (1926) having the following constitution:

Sodium chloride	5.0 g
Magnesium sulphate	0.2 g
Ammonium dihydrogen phosphate	1.0 g
Citric acid	2.0 g
Bromothymol blue (0.2% solution)	4.0 ml
Agar	20.0 g
Distilled water	to 1000 ml

4 - Litmus solution:

The bacterial reaction to litmus solution was studied by growing the organisms on the following medium:

Lactose	20.0 g
Glucose	0.4 g
Disodium hydrogen phosphate	0.5 g
Diammonium sulphate	1.0 g
Sodium citrate	2.0 g
Sodium chloride	5.0 g
Peptone	0.05 g
Distilled water	up to 1000 ml

5 - Lipolysis of Tween 80:

The method of Sierra (1957) was used. The medium consisted of:

Bacto-peptone	10.0 g
Sodium chloride	5.0 g
Calcium chloride	0.1 g
Agar	17.0 g
Distilled water	up to 1000 ml
PH 7.4	

The above medium was sterilized then cooled to 40-50°C. Tween 80 was autoclaved separately and added to a final concentration of 1% before plating. Plates were inoculated and incubated at 28°C. The formation of a white precipitate around the bacterial growth, within 10 days, was scored as positive result.

6 - Oxidation-Fermentation (O/F) test:

This test was carried out using the medium of Hugh and Leifson (1953). It consisted of:

Peptone	2.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	0.3 g
Bromothymol blue (0.2% solution)	15.0 ml
Agar	3.0 g
Distilled water	up to 1000 ml

Duplicate tubes containing 3 ml of the above medium were inoculated by stabbing with a straight wire. The surface of one tube was covered with a layer of molten soft paraffin or sterile glycerol. The tubes were incubated, for a maximum of two weeks, at 28°C, observing daily the colour change.

7 - Nitrate Reduction:

The following medium was used:

Potassium nitrate	1.0 g
Peptone	5.0 g
Beef extract	3.0 g
Distilled water	up to 1000 ml
PH 7.0-7.2	

The above medium was inoculated and incubated for 48 hours. The presence of nitrite, in the media was confirmed by applying the modified Griess Ilosvay's reagent which consists of:

- a) 0.8% sulphanilic acid in 5N acetic acid;
- b) 0.6% dimethyl- α -naphthylamine in 5N acetic acid

1 ml from both solutions were added to the medium. If a distinct colour did not appear within one hour, zinc dust was added to determine whether or not complete denitrification had occurred.

8 - Phenyl alanine Deaminase:

The medium of Ewing et al. (1957) was used.

It consisted of:

DL-phenyl alanine	2.0 g
Yeast extract	3.0 g
Disodium hydrogen phosphate	1.0 g
Sodium chloride	5.0 g
Agar	20.0 g
Distilled water	up to 1000 ml

Phenyl alanine agar slopes were prepared, inoculated and incubated overnight. 0.2 ml aqueous 10% ferric chloride solution was seen over the bacterial growth. A green colour on the slope and in the free liquid, at the base of the slant tube, indicated positive deamination.

9 - Tyrosine Hydrolysis:

It was detected in the medium of Gordon and Smith (1955) which consisted of:

Peptone	5.0 g
Beef extract	5.0 g
L-Tyrosine	5.0 g
Agar	20.0 g
Distilled water	up to 1000 ml

The formation of red to reddish brown pigments, in the medium, denoted a positive result.

10 - β -Galactosidase Activity:

This test was performed using Lowe (1962) method. The reaction solution consisted of 0.6% of o-nitrophenyl- β -D-galactopyranoside dissolved in 0.01 M disodium hydrogen phosphate (ONPG). 250 ml of this solution were mixed with 750 ml peptone water (peptone 10 g ; NaCl, 5 g ; water, 1000 ml.) before being distributed, in 3 ml aliquots, in test tubes, inoculated and incubated at 28°C for 24 hours. The reaction was positive with the appearance of a yellow colour due to the formation of O-nitrophenol.

11 - Methyl Red (MR) and Voges-Proskauer (V.P.) Reaction:

The method of Cowan and Steel (1965) was used.

The medium consisted of:

Peptone	5.0 g
Dipotassium hydrogen phosphate	5.0 g
Glucose	5.0 g
Distilled water	up to 1000 ml
PH 7.5	

The medium was distributed, in 1.5 ml aliquots, into test tubes, sterilized and inoculated. After 5 days incubation, at 30°C, the presence of acetyl methyl carbinol (acetoin) was tested by adding 0.6 ml of 5% α -naphthol solution (in absolute alcohol) and 0.2 ml of 40% aqueous potassium hydroxide solution (Barritt, 1936). The tubes were vigorously shaken and the final reading was taken after 2 hours. The final pH, in this test, was determined by adding two drops of methyl red solutions (0.04 g % in 40% alcohol) to one ml of the grown medium.

12 - Decarboxylase Activity:

The decarboxylase enzyme was detected, in the bacteria, with the help of Möller's (1955) medium which consisted of:

Peptone	5.0 g
Beef extract	5.0 g
Pyridoxal	5.0mg
Glucose	0.5 g
Bromocresol purple (0.2% solution)	5 ml
Cresol red (0.2% solution)	2.5 ml
Amino acid	10.0 g
Distilled water	up to 1000 ml

1.5 ml portions were distributed in small vials, covered with sterile paraffin oil, incubated at 28°C and daily examined for 4 days. The medium turned, at first, to yellow colour due to acid production from the glucose. Later, if decarboxylation occurred, the medium became violet. The control tubes should remain yellow.

13 - Lecithinase Activity:

The medium of Macfarlane et al. (1941) was used. It consisted of:

Hen egg yolk	4
Sodium chloride 0.85%	1000 ml

The yolk was beaten vigorously and mixed with saline to form a homogenous solution, then distributed among sterile vials and inoculated by 24 hours bacterial growth. The flocculation and separation of a thick curd of fats on the surface of the medium indicated a positive reaction.

14 - Hydrogen Sulphide Production

Dye's medium (1968) was used. It consisted of:

Ammonium dihydrogen phosphate	0.5 g
Dipotassium hydrogen phosphate	0.5 g
Magnesium sulphate	0.2 g
Sodium chloride	5.0 g
Yeast extract	5.0 g
Sodium thiosulphate	0.5 g
Distilled water	up to 1000 ml

The production of hydrogen sulphide was detected by lead acetate paper strips, suspended between the plug and the tube. Blackening of the strip was examined within 7 days incubation.

15 - Indole Production:

This could be easily detected by inserting an oxalic acid paper between the plug and the inoculated test tube containing nutrient broth medium. After 7 days incubation, at 28°C, a pink colour may develop on the paper to confirm a positive reaction.

16 - Protopectinase Activity:

1 mm thick slices of peeled potato tuber were prepared, under aseptic conditions, and placed in Petri dishes, on sterile moist filter paper. Slices were inoculated, at the centre, with a loopful of organisms from a nutrient agar slope and examined for rotting after 48 hours of incubation.

17 - Oxidase Activity:

It was detected by Steel's (1961) modification of Kovac's method (1956) in which one platinum loop of 24-48 hours bacterial culture was placed onto a filter paper, impregnated with 1% aqueous solution of tetra methyl-p-phenylenediamine dihydrochloride.

The production of a distinct purple colour, in 10 seconds, was recorded as a positive result.

18 - Gelatin Liquefaction:

Stab inoculation with a loop suspended 24 hours bacterial growth was performed in test tubes containing

nutrient broth to which 12% gelatin was added. The tubes were incubated at 28°C and observed after 7, 14 and 21 days. Tubes were kept at 4°C for 30 minutes before detecting the liquefaction of gelatin.

19 - Levan Production:

Streaks of 24 hours old bacterial growth were produced on nutrient agar containing 5% sucrose. The formation of large white domed mucoid colonies was assumed to indicate the production of levan (Dye, 1968).

20 - Coagulation and Peptonization of Milk:

This was carried out by inoculating the bacteria in 3 ml sterile skimmed milk (defattened milk). Tubes were incubated for 14 days at 28°C.

21 - Catalase Activity:

A loopful from a 24 hours old biomass, grown on nutrient agar slopes was smeared on a slide covered with few drops of 3% hydrogen peroxide. Oxygen bubbles were evolved if catalase was active.

22 - Starch Hydrolysis:

Nutrient agar containing 0.2% soluble starch was poured in Petri-dishes. The plates were inoculated by streaking the bacterial cells on the surface of the medium. Hydrolysis was detected after 4 days by flooding the plates with Lugol's iodine solution.

Serological Reactions

Serological reactions were carried out to provide information on the relationship between the isolated pathogens and the standard museum strains.

Preparation of Antisera:

To obtain immunized antisera, rabbits, weighing approximately 4 lb., were used. They were infected via the marginal ear view, after disinfection with alcohol. On the first day, 1 ml containing 5×10^8 cells was intravenously injected. After 6 days, 1 ml containing 10×10^8 cells was again intravenously injected and the animals rested for another six days before they were intravenously and subcutaneously injected by 1 ml of 2×10^9 cells, three times at six days intervals. The animals were bled, by cardiac puncture, thirteen days after the last injection and the sera were preserved in 0.5% phenol.

Agglutination Reactions:

The agglutination reactions were set up in 12 test tubes each containing 1 ml of a serial dilution beginning with 1:50, and carried past titre of the serum to 1:51200. The control tube contained physiological saline and normal serum. 0.1 ml of a heavy antigen of bacterial suspension (20×10^8 cells/ml) was added to each tube then incubated

overnight, at 28°C. The titre was determined as the least dilution where agglutination was still present. The intensity of agglutination was given the arbitrary signs "4 + ", "3 + ", "2 + ", " + ", " - " to indicate strong, good, moderate, mild and negative reaction respectively.

The identification and systematic classification of the isolated strains were determined with the help of Bergey's Manual of Determinative Bacteriology (1934, 1957, 1974), Elliott (1951), as well as consultation of publications by Dye (1968, 1969); Graham (1964, 1972) Lelliott et al. (1966).

PART I I
RESULTS

A. ERWINIA CAROTOVORA
(Jones) Holland var.
CAROTOVORA Dye

R E S U L T S

A. ERWINIA CAROTOVORA (Jones) Holland var. CAROTOVORA Dye

1 - Symptoms:

Under natural field conditions, E. carotovora var. carotovora infected melons causing wilting and rotting of the vegetative parts of the plants. Infection began at the junction of the root to the stem (cotylar region) forming a rotted oily area. The bacterium penetrated through the parenchymatous tissues and invaded the inner vessels which appeared brown to reddish brown as a result of the necrotic effect of the pathogen. Dark brown exudates appeared on the surface of the rotted stems. Infection of the fruits caused the decay of the cortical tissues changing them to soft rotted, non-edible mass (Fig. 1).

Artificial inoculation of E. carotovora var. carotovora, either under field conditions or the green house caused similar symptoms to those induced naturally. Under both conditions, the wilt appeared within 24 hours. Furthermore, the potency or virulence of the pathogen did not fade with storage. After three years (the research period), the organism was still capable of inducing complete wilting and rotting of the plants within 24-48 hours.



Fig.(1) Rotted Melon's Fruit in the Natural Field Conditions Compared with a Non-infected Fruit . Ismailia Governorate , 1981.

Inoculated leaves only, developed necrotic pale brown to yellow spots that occupied the intraveinal areas leading to complete wilting of the whole plant within three days.

Nine virulent pathogenic strains were selected for detailed morphological, biochemical and serological studies.

2. Biology of the Pathogen:

a - Morphology:

Erwinia carotovora var. carotovora flourished on beef-peptone agar (BPA) medium within 48 hours incubation, at 28°C, it formed small rounded non-mucoid colonies, reaching 0.6-1 mm diameter. In day light, the colonies are transparent white or pale gray in colour with a slightly elevated upper surface forming a low convex and an entire or slightly undulated margin (Fig. 2) 7 days old colonies reached 1.2-2 mm diameter.

The non-spore forming, rod-shaped bacterial cells of E. carotovora var. carotovora appeared single or in pairs, rarely in short chains. In all cases they acquire peritrichous flagella and stain Gram-negative (Fig. 3).

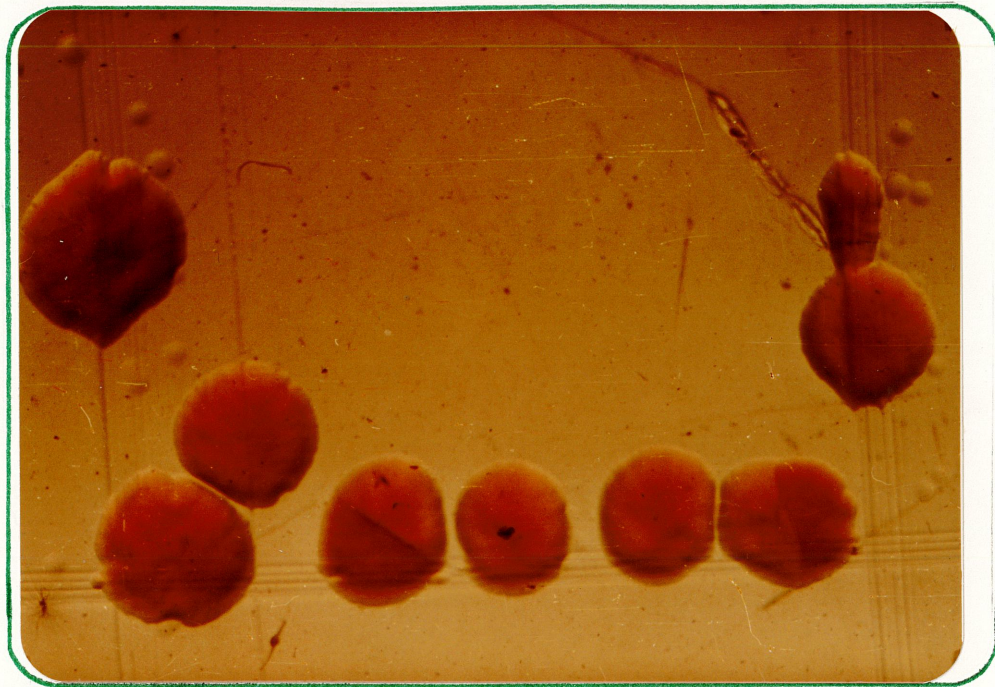


Fig.(2) Colonies of E. carotovora var. carotovora (Strain 124)after 4 days Incubated at 28 °C on Beef-Peptide Agar Medium (x 20).

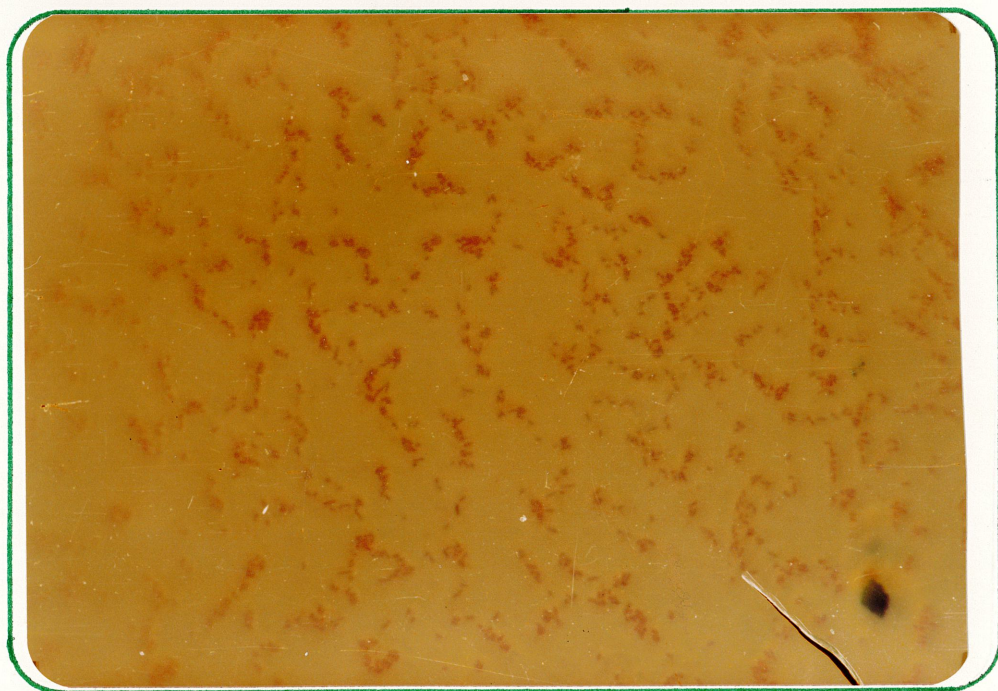


Fig.(3) Cells of E. carotovora var. carotovora (Strain 124) after 24 Hours on Beef-Peptide Agar Medium Stained by Gram (x 2000).

Table 1: Utilization of different carbon sources by
E. carotovora var. carotovora

Isolate No	Arabinose	Xylose	Rhamnose	Fructose	Galactose	Glucose	Mannose	Sorbose	Cellobiose	Lactose	Maltose	Dextrine	Sucrose	Trehalose	Raffinose	Salicin	Dulcitol	Glycerol	Mannitol	Sorbitol	Inositol
124	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
119 a	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
499 B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
503	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
524	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
525	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
525 a	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
527 B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
527 C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

A: acid was produced

- : negative reaction

Table 2: Utilization of different organic acid by
E. carotovora var. carotovora

Isolate No	Malate	Oxalate	Benzoate	Acetate	Pyruvate	Lactate	Tartrate	Succinate	Propionate	Citrate
124	+	-	-	+	+	+	-	+	-	+
119 a	+	-	-	+	+	+	-	+	-	+
499 B	+	-	-	+	+	+	-	+	-	+
503	+	-	-	+	+	+	-	+	-	+
524	+	-	-	+	+	+	-	+	-	+
525	+	-	-	+	+	+	-	+	-	+
525 a	+	-	-	+	+	+	-	+	-	+
527 B	+	-	-	+	+	+	-	+	-	+
527 C	+	-	-	+	+	+	-	+	-	+

+ : positive reaction

- : negative reaction

Table 3: Biochemical characteristics of the isolated strains of E. Carotovora var carotovora

Isolate No.	Litmus reaction	Nitrate reduction	H ₂ S evolution	Indole formation	Gelatin liquefaction	Milk Coagulation	Protopectinase	Levan Production	Oxidase	Phenylalanine deaminase	Tyrosinase	Leucithinase	Tween 80	Methyl red	Acetoin	Glucose fermentation	B-Galactosidase	Starch hydrolysis	Arginine	Lysine	Glutamic acid	Ornithine
124	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
119a	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
499B	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
503	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
524	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
525	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
525a	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
527B	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
527C	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

A: Acid reaction

C: Coagulation of milk.

+ : positive reactions

- : negative reactions

b - Biochemical Activities:

The freshly isolated strains of Erwinia carotovora var. carotovora were able to produce acid without gas, on the following carbohydrate sources: arabinose, xylose, rhamnose, fructose, galactose, glucose, mannose, sorbose, cellobiose, lactose, trehalose, raffinose, salicin, glycerol, mannitol, inositol but not maltose, dextrin, sucrose or dulcitol (Table 1).

In the meantime, Table 2 shows that all strains could utilize malate, acetate, pyruvate, lactate, succinate and citrate but not oxalate, benzoate, proprionate or tartrate.

The freshly isolated strains of E. carotovora var. carotovora had an acidic reaction to litmus medium, reduced nitrate, produced hydrogen sulphide, liquefied gelatin, possessed protopectinase and β -galactosidase activities, coagulated milk, produced acetoin and red colour with methyl red but were unable to produce indole (Table 3). All strains could not produce levan, had no oxidase, deaminase, tyrosinase, lecithinase, amylase activities. Also arginine, lysine, glutamic acid and ornithine decarboxylase were not detected.

Variation of incubation temperature showed that, on beef peptone bouillon (BPB), the organism could tolerate a maximum temperature for growth of 41°C for the incubation period of 24 hours.

c - Serological Reactions

The agglutination reaction was carried out between the antigens of the freshly isolated strains and the antiserum prepared for the strain 119 a. The results showed that all strains were agglutinated at titre 1: 25600 which indicated the one and same identity of these cultures. Furthermore, the antiserum 119 a could agglutinate the authentic (standard) culture of E . carotovora provided by the Phytopathology Research Institute, Ministry of Agriculture, Giza, Egypt- at a dilution of 1:12800 (Table 4). Comparing the data recorded for freshly isolated strains of E . carotovora var. carotovora with these recorded for the same strains in Bergey's Manual (1974) and the data reported by Dye (1969) showed that they were identical in most characteristics (Table 5). Minor differences in the utilization of the carbohydrate sources (glycerol and sorbitol) coincided more with Dye (1969) observations rather than those of Bergey (1974). These differences might be attributed to differences in environment, locality and/or purity of the chemicals used to carry out the various tests.

In conclusion one cannot escape to confirm that the

isolated pathogen might be identified as Erwinia carotovora (Jones) Holland var. carotovora Dye which could infect melon plants causing rotting and wilting besides the usual known symptoms of rotting other different plants.

Table 4: Agglutination of the different isolates of E. carotovora var. carotovora with the antiserum of strain 119 a

Isolate N°	Dilution of antiserum										
	50	100	200	400	800	1600	3200	6400	12800	25600	51200
119 a	4+	4+	4+	4+	4+	4+	3+	2+	2+	+	±
124	4+	4+	4+	4+	3+	3+	2+	2+	2+	±	-
499 B	4+	4+	4+	4+	4+	3+	3+	2+	+	±	-
503	4+	4+	3+	3+	3+	3+	2+	2+	+	+	±
524	3+	3+	2+	2+	+	+	+	+	±	±	-
525	3+	3+	2+	2+	+	+	+	+	±	-	-
525 a	2+	2+	2+	+	+	+	+	±	±	-	-
527 B	3+	2+	2+	2+	2+	+	+	+	+	-	-
527 C	2+	2+	2+	2+	+	+	+	+	±	-	-
Standard	3+	3+	2+	+	+	+	+	±	±	-	-

(+, +2, +3, +4) : the relative intensity of agglutination
 (-) : negative reaction
 (±) : doubted results
 c : control

Table 5: Characteristics of the freshly isolated strains of E. carotovora var. carotovora compared with those recorded in other literature data

Test	Isolates of <u>E. carotovora</u> var <u>carotovora</u>	Bergey's Manual (1974)	Dye (1969)
Utilization of			
Arabinose	+	+	+
Mannitol	+	+	0
Salicin	+	+	+
Xylose	+	+	+
Raffinose	+	+	+
Rhamnose	+	0	+
Dulcitol	-	-	-
Inositol	+	V	+
Lactose	+	+	+
Maltose	-	V	V
Cellobiose	+	+	0
Dextrin	-	-	0
Glycerol	+	-	+
Mannose	+	+	0
Sorbitol	+	+	-
Sucrose	-	-	-
H ₂ S production	+	+	0
Protopectinase	+	+	+
Acetoin	+	+	+
Nitrate reduction	+	+	+
Gelatin liquefaction	+	+	+
Phenyl alanine deaminase	-	-	-

1	2	3	4
Indol formation	-	-	-
Gas from glucose	-	V	V
Lecithinase	-	-	0
Tartrate	-	-	-
Lactate	+	+	0
Citrate	+	+	0
Maximum temperature (°C) for growth	37-41	37-40	37-40

+ : Positive reaction

V: variable results

- : Negative reaction

0: not tested

B. ERWINIA CAROTOVORA
(Jones) Holland f.
CITRULLIS Sizova

B - ERWINIA CAROTOVORA (Jones) Holland f. CITRULLIS Sizova

1 - Symptoms:

The pathogenic bacterial disease of melons, caused by Erwinia carotovora f. citrullis, causes complete wilting of the whole plant, beginning by the root, extending upwards to the stem and leaves; thus changing the plant into a dry wrinkled dead mass (Fig. 4). Prior to death the stem acquires an oily appearance and yellow brown exudates ooze out. Irregular or triangular dark-green or brown spots appear at the apex, margin or intraveinal areas of the upper surface of the leaves. Sometimes these spots are chaotically distributed on the leaf blade (Fig. 5). On the lower surface, opposite to the spots, oily patches were observed. The xylem vessels, in wilted roots and stems, change to a dark brown colour and sometimes were surrounded by decayed rotted tissues. In the meantime, the pathogen could induce a soft rot decay to the fruit tissues changing them to a non-edible mass.

Eleven pathogenic strains, causing these symptoms were isolated. These isolates, when artificially inoculated into cultivated melons, in the experimental field or the green house, they induced the same symptoms. However some of these isolates, though may have lost their wilting



Fig.(4) Wilted Plants of Melon under Field Conditions. Ismailia District (June ,1981).



A- Necrotic Spots Under
Field Conditions.

Fig.(5) Development of Necrotic
Spots on Melon's leaf.



B- Necrotic Spots Artificially Infected
in the Green House.

potency yet they still have the capacity to cause necrotic spots on leaves, reaching, in some cases, 15 x 12 mm diameter. Under natural field conditions, the severity of the disease reached, (in 1979) 5-15% leaf spots and 2-4% wilt. In 1980-1981, leaf spots increased to 10% while the wilt reached 50-75%.

2 - Biology of the Pathogen:

a - Morphology:

When cultured for 48 hours, at 28°C, on beef peptone agar (BPA), the colonies of E. carotovora f. citrullis appeared pale yellow to buff bright, rounded, non-mucoid with entire margin and slightly raised centre forming a low convex. In diffused day light, the colonies seemed homogenous, transparent, reaching about 1.5 mm in diameter (Fig. 6). After 7 days incubation, the colonies slightly increased in size reaching 2 mm in diameter.

The cells of E. carotovora f. citrullis are short rod-shaped, Gram negative, with peritrichous flagella. These non-spore forming rod were either single or arranged in pairs, sometimes in short chains (Fig. 7).

b - Biochemical Activities:

The freshly isolated strains of E. carotovora f. citrullis could produce acids and gas on arabinose, xylose, rhamnose, fructose, galactose, glucose, mannose, cellobiose, lactose, maltose, sucrose, raffinose, salicin, mannitol,

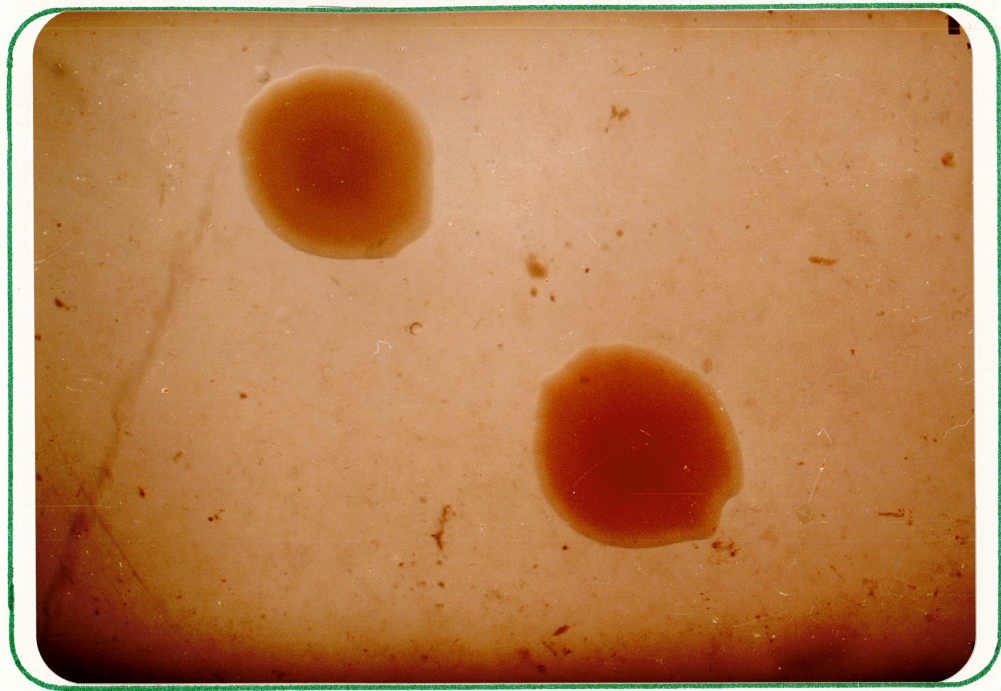


Fig.(6)Colonies of E. carotovora f. citrullis (Strain 84)
after 48 Hours Incubated at 28 °C on Beef -
Peptone Agar Medium (x 20).

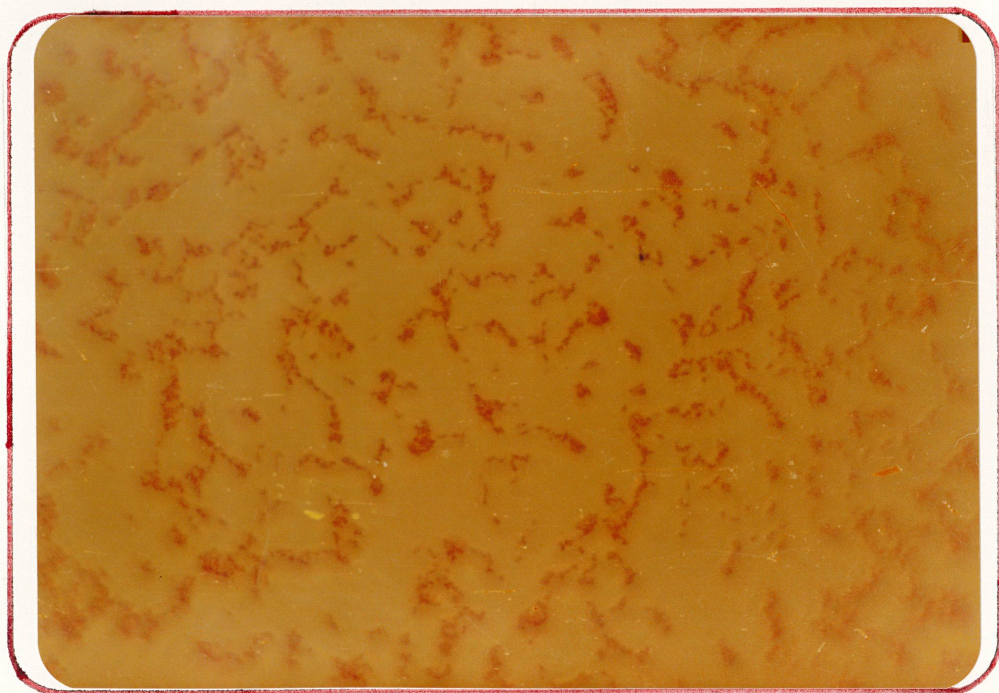


Fig.(7)Cells of E. carotovora f. citrullis (Strain 84)
after 24 Hours on Beef -Peptone Agar Medium
Stained by Gram (x 2000).

trehalose. Acids were produced without evolution of gas when the organisms were cultured on glycerol or inositol. Some strains produced acid and gas whereas others were unable to grow when incubated with either dulcitol or sorbitol. Similarly some strains could produce very weak acids, on dextrin but all organisms could not utilize sorbose (Table 6). All isolated strains of E. carotovora f. citrullis could utilize malate, acetate, pyruvate, lactate, succinate or citrate; variably utilized tartrate but were unable to utilize oxalate, benzoate or propionate (Table 7).

Furthermore, freshly isolated strains of E. carotovora f. citrullis had acidic reactions to litmus (Table 8), reduced nitrate, liquefied gelatin, produced β -galactosidase enzyme, hydrogen sulphide gas; could ferment glucose, coagulate milk, decarboxylate arginine but not lysine, ornithine or glutamic acid; variably produced levan, tyrosinase and acetoin but not indole, oxidase, deaminase, protopectinase, lecithinase or lipase (Tween 80). They formed red colour with methyl red and could not hydrolyse starch. Verification of temperature conditions showed that E. carotovora f. citrullis could tolerate the maximum temperature of 47°C when cultured on beef peptone bouillon (BPB).

Table 6: Utilization of different carbon sources
by E. carotovora f. citrullis

Isolate No.	Arabinose	Xylose	Rhamnose	Fructose	Galactose	Glucose	Mannose	Sorbose	Cellobiose	Lactose	Maltose	Dextrine	Sucrose	Trehalose	Raffinose	Salicin	Dulcitol	Glycerol	Mannitol	Sorbitol	Inositol
428 C	AG	AG	A	AG	AG	AG	AG	-	AG	AG	AG	AW	AG	A	AG	AG	AG	A	AG	AG	A
428 d	AG	AG	AG	AG	AG	AG	AG	-	AG	AG	AG	AW	AG	A	AG	AG	AG	A	AG	AG	A
428 E	AG	AG	A	AG	AG	AG	A	-	AG	AG	AG	AW	AG	A	AG	AG	AG	A	AG	AG	A
428 F	AG	AG	A	AG	AG	AG	AG	-	AG	AG	AG	AW	AG	A	AG	AG	AG	A	AG	AG	A
70 B	AG	AG	A	AG	AG	AG	AG	-	AG	AG	AG	AW	AG	AG	AG	AG	AG	A	AG	AG	A
478 d	AG	AG	AG	AG	AG	AG	AG	-	AG	AG	AG	AW	AG	A	AG	AG	-	A	AG	AG	A
522 E	AG	AG	A	AG	AG	AG	AG	-	AG	A	AG	AW	AG	A	AG	AG	-	A	AG	AG	A
84	AG	AG	AG	AG	AG	AG	AG	-	AG	AG	AG	AW	AG	A	AG	AG	-	A	AG	AG	A
67 a	A	AG	A	AG	AG	AG	AG	-	AG	AG	A	AW	AG	AG	AG	A	-	A	AG	AG	-
416 a	A	AG	AG	AG	AG	AG	AG	-	A	AG	AG	-	AG	A	AG	AG	-	A	AG	AG	-
448	A	AG	A	A	A	A	A	-	A	AG	A	-	AG	A	AG	AG	-	A	AG	-	-

A: acid formation

Aw: weak acid formation

AG: acid and gas formation

--: negative result

Table 7: Utilization of organic acid by the different strains of E. carotovora f. citrullis

Isolate No.	Malate	Oxalate	Benzoate	Acetate	Pyruvate	Lactate	Tartrate	Succinate	Propionate	Citrate
428 c	+	-	-	+	+	+	+	+	-	+
428 d	+	-	-	+	+	+	+	+	-	+
428 E	+	-	-	+	+	+	+	+	-	+
428 F	+	-	-	+	+	+	+	+	-	+
70 B	+	-	-	+	+	+	+	+	-	+
478 d	+	-	-	+	+	+	-	+	-	+
522 E	+	-	-	+	+	+	-	+	-	+
84	+	-	-	+	+	+	-	+	-	+
67 a	+	-	-	+	+	+	-	+	-	+
416 a	+	-	-	+	+	+	-	+	-	+
448	+	-	-	+	+	+	-	+	-	+

- : negative result

+ : positive result

c - Serological Reactions:

The serological tests were carried out on the antisera for E. carotovora f. citrullis, prepared from the freshly isolated strain 84 and compared with that of strain 777a which represents the stock culture isolated by Saleh (1977) from the Ukrainian SSR. Agglutination of the freshly isolated strains with the antiserum 84 occurred at dilution 1600 and 6400 (Table 9). While the antiserum 777a was agglutinated with the same strains in the average titre 1:1600-1:12800 (Table 10). This indicated that both antisera belonged to one serotype. The wide range of agglutination may be attributed to the heterogenic genetic structure of the different strains of E. carotovora f. citrullis.

Table 11 compares the biochemical characteristics of the freshly isolated strain of E. carotovora f. citrullis with those recorded for the same organism, isolated from the Ukrainian SSR by Saleh (1977). A great similarity between these pathogens concerning the utilization of the different carbon sources, organic acids and enzymatic activities of the different strains could be easily observed. Some negligible differences were reported concerning the utilization of sucrose (variably utilized by reference strain; 777a) and sorbitol (variably utilized by the freshly isolated strains) as well as liquefaction of gelatin, being liquified only by some of the literature strains. These minor differences may be attributed to the difference in

localities as well as other environmental factors and/or differences in the varieties of the cultivated melons in both Ukrainian SSR and Egypt, though both were similarly attacked by this pathogen. All these factors may affect some strains to lose or gain some enzymatic activities.

The above results lead to the conclusion that the pathogenic organism, infecting melons in Egypt, causing leaf spots, wilting of the vegetative plants and soft rot of the fruits bears great similarities or even identical to that pathogen isolated previously from USSR fields (Sizova, 1965; Saleh, 1977) and identified as Erwinia carotovora f. citrullis infecting both melons and water-melons causing the same symptoms. Accordingly the newly isolated pathogen may be given the same name.

Table 9: The agglutination of different isolates of E. carotovora
f. citrullis with the antiserum 777a

Isolate No	Dilution of the antiserum										
	50	100	200	400	800	1600	3200	6400	12800	25600	51200 C
777a	4+	4+	4+	4+	4+	3+	2+	2+	+	+	-
84a	4+	4+	4+	4+	3+	2+	+	±	±	-	-
428a	4+	3+	3+	3+	3+	2+	2+	2+	+	+	-
428d	4+	3+	3+	3+	2+	2+	+	±	±	-	-
428E	3+	3+	3+	3+	2+	2+	+	±	-	-	-
428F	4+	4+	3+	3+	3+	2+	2+	+	±	-	-
67a	3+	2+	2+	+	+	+	±	-	-	-	-
70B	2+	2+	2+	2+	+	+	±	±	-	-	-
416a	4+	3+	3+	3+	2+	+	±	±	-	-	-
478d	3+	3+	2+	2+	2+	+	+	±	-	-	-
522E	+	+	+	+	+	±	-	-	-	-	-
448	+	+	+	-	-	-	-	-	-	-	-

(+, +2, +3, +4): The relative intensity of agglutination

(-): Negative reaction

(±): Doubtful results

C : Control.

Table 10: The agglutination of the different isolates
of E. carotovora f. citrullis with the antiserum
84.

Isolate No.	Dilution of the antiserum										
	50	100	200	400	800	1600	3200	6400	12800	25600	51200 C
84	4+	4+	4+	3+	3+	3+	3+	2+	+	±	-
777a	4+	4+	4+	3+	3+	2+	+	±	±	-	-
428c	4+	4+	4+	3+	3+	3+	3+	2+	+	±	-
428d	3+	3+	3+	3+	2+	2+	2+	2+	+	±	-
428E	4+	3+	3+	3+	3+	3+	2+	2+	+	±	-
428F	4+	4+	3+	3+	3+	3+	3+	+	+	±	-
67a	4+	3+	3+	2+	+	+	-	-	-	-	-
70B	4+	4+	3+	2+	+	+	-	-	-	-	-
416a	3+	3+	2+	2+	+	+	-	-	-	-	-
478d	2+	2+	2+	2+	+	+	-	-	-	-	-
522E	+	+	+	+	±	±	-	-	-	-	-
448	+	+	+	±	-	-	-	-	-	-	-

(+, +2, +3, +4) : The relative intensity of agglutination

(-) : Negative reaction

(±) : Doubtful results

C : Control

Table 11: Comparison of the biochemical characteristics of the freshly prepared isolates with those of reference strain of E. carotovora f. citrullis.

Test	Saleh, Korobko, 1982	Freshly isolated strains
1	2	3
Utilization of		
Arabinose	AG	AG
Galactose	AG	AG
Glucose	AG	AG
Fructose	AG	AG
Rhamnose	AG	AG or A
Mannose	AG	AG
Sorbose	-	-
Lactose	AG	AG
Maltose	AG	AG
Sucrose	AG (V)	AG
Cellobiose	AG	AG
Raffinose	AG	AG
Mannitol	AG	AG
Sorbitol	AG	AG (V)
Salicin	AG	AG
Inositol	A (V)	A (V)
Lactate	+	+
Pyruvate	+	+

Test	Saleh, Korobko, 1982	Freshly isolated strains
1	2	3
Succinate	+	+
Malate	+	+
Acetate	+	+
Citrate	+	+
Tartrate	V	V
Propionate	-	-
Oxalate	-	-
Reaction on litmus medium	A	A
Nitrate reduction	+	+
Gelatin liquefaction	V	+
Protopectinase	-	-
Indol	V	-
Deaminase	-	-
Oxidase	-	-
Lecithinase	-	-
Lipase (Tween 80)	-	-
MR	-	-
Acetoin	V	V
Glucose fermentation	+	+
β -galactosidase	+	+
Arginine decarboxylase	+	+
Maximum temperature for growth on (BPB)	47° C	47°C

A: Acid production

+: Positive result

AG: Acid and gas production -: Negative result

V: Variable result

D I S C U S S I O N

DISCUSSION

Search for the causal organisms inducing bacterial disease to the Egyptian melons, during 1979-1981 indicated that melon was infected by two microbes namely: E. carotovora var. carotovora, causing wilting of the plants and rotting of the fruits and E. carotovora f. citrullis causing spotting of leaves, wilting of the plants and rotting of the fruits.

The coliform soft rot bacterium E. carotovora var. carotovora is one of the very early known pathogens - discovered by Jones (1901) - infecting a wide variety of plants (Elliott, 1951).

This bacterium can easily destroy the pectic substances in the wall matrix or middle lamella of plant cells (Kiraly et al. 1974), leading to soft rot and thus changing the plant part to a mascerated useless mass. Accordingly the pathogenecity and virulence of this species depends entirely on the activity of the enzymes decomposing pectin.

In this investigation we were able to isolate this bacterium from wilted plants as well as from their rotten fruits. Artificial inoculation of this pathogen into the

vegetative plants caused their wilting and rotting within 24-48 hours.

The widespreading of this pathogen and its capability to infect several plant species belonging to cucurbitaceae and its capacity to attack the vegetative plant parts as well as fruits, attracted the attention to the large damage, it may cause to the crop particularly if wilting was severe before flowering and/or fruit setting.

Kryvets' (1975) reported that E. carotovora var. carotovora may exist in the rhizosphere of lupins, during the whole vegetative period. Accordingly the presence of this pathogen in the rhizosphere, under different environmental conditions, accompanying other microbes, would urge the bacterium to struggle for its existence through increasing its enzymatic potency or ability to infect a wide variety of host plants or by changing to another more virulent form viz. E. carotovora f. citrullis.

E. carotovora f. citrullis is a dangerous pathogenic variety of E. carotovora. Since its discovery, isolation and identifications by Ceratanena and Papova (1948) in the Ukrainian SSR as the causal agent of the rotting of water melon fruits, it aroused serious problems, being a toxic-bacterium, to man and animals (Petrovich and Shustova, 1951). Later this pathogen was recorded, in wide distribution, in other states and governorates of USSR (Saleh, 1977) causing

well developed symptoms of wilting and spotting of leaves of melons and water-melons plants.

The isolation of this pathogen from the Egyptian soil, for the first time outside USSR, attracts great attention and requires urgent careful study of the factors causing its widespread and rapid development in other countries. In the meantime, the increased virulence of the pathogen from rotting water-melon fruits only (Ceratanena and Papova, 1948) to further wilting and spotting of leaves of both melons and water-melons (Saleh, 1977) as well as probably other members of cucurbitaceae would urge for immediate search for control measures against this pathogen especially if we take into consideration the progressive increase in cultivation of cucurbitaceae plants in different countries.

The isolation of these two pathogens E. carotovora var. carotovora and E. carotovora f. citrullis at the same time from melon plants, cultivated in the same field, and the possibility of their existence for long periods in the soil (Kyrrets', 1975), lead to the conclusions that some changes had occurred in E. carotovora var. carotovora leading to its transformation to the other form species of E. carotovora f. citrullis.

Such changes may have resulted as a response to environmental conditions or to the biological effects of the surrounding bacterial community in the soil.

This adaptation lead to two main changes in E. carotovora var. carotovora, namely: inability to produce protopectinase enzyme and its tolerance to survive and grow at high temperatures, reaching 47°C (Saleh and Korobko, 1982). These two main differences are the principal criteria differentiating E. carotovora var. carotovora from E. carotovora f. citrullis.

E. carotovora f. citrullis is the adapted well developed new pathogen. Its danger lies in its ability to infect different cucurbitaceae plants in other countries than USSR. Still infection of plants belonging to other families is quite possible. Accordingly it is of almost importance to study the environmental and biological factors that may lead to the conversion of E. carotovora var. carotovora to E. carotovora f. citrullis in order to find out some measures for the control of such disease.

It is worth mentioning that Ceratanena and Papova (1948), Gnytenko (1966, 1968) reported that E. carotovora f. citrullis had a toxic effect on warm blooded animals.

This adds a new serious obligation to study the pathogen as a strong invader to the plants causing poisoning to humans and food animals.

S U M M A R Y

SUMMARY

The study of the bacterial diseases of melon plants in different localities of Egypt revealed that there are two main pathogens responsible for spotting, wilting and rotting of the fruits. There are E. carotovora (Jones) Holland var. carotovora Dye, causing wilting and soft rotting of the fruits and E. carotovora (Jones) Holland f. citrullis Sizova, causing spotting, wilting and rotting of the fruits.

These two pathogens caused a great damage to melons crop where, in some instances, wilting reached 75%.

Identification of these two pathogens was confirmed by comparing their biological and biochemical characteristics with certified isolates offered by Ministry of Agriculture, Egypt and isolate 777a(Saleh, 1977) as well as by the records reported in Bergey's Manual of Determinative Bacteriology.

Storage of E. carotovora var. carotovora for 3 years did not affect its virulence. It could cause wilting and rotting within 24-48 hrs after inoculation. On the other hand, the virulence of E. carotovora f. citrullis was lost when kept in synthetic media for a long time.

The main differences between the two pathogens lie in the absence of protopectinase and tolerance of E. carotovora f. citrullis to high temperatures (47°C) compared with E. carotovora var. carotovora.

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دراسات بيولوجية للأمراض البكتيرية المنتشرة على الشمام

ملخص عربى

يتضمن البحث دراسة الأمراض البكتيرية التى تصيب نبات الشمام فى مناطق مختلفة من مصر . وقد أوضحت الدراسة وجود نوعين من البكتيريا الممرضة يسببان حدوث بقع الأوراق وذبول النبات وتعطن الثمار .

وقد أثبتت الدراسة أن هذين النوعين هما :

١ - اروينيا كاروتوفورا صنف كاروتوفورا .

ويسبب ذبول النبات وتعطن الثمار .

٢ - اروينيا كاروتوفورا صنف سيتروليس .

ويسبب مرض تبقع الأوراق وذبول النبات وتعطن الثمار .

وينتج عن هذين الكائنين خسارة كبيرة فى محصول الشمام حيث تصل

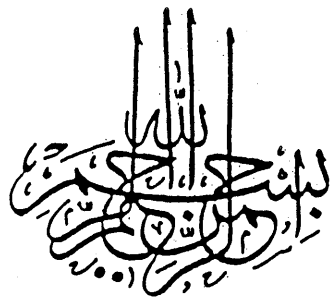
نسبة الذبول فى بعض الاحيان الى ٧٥ ٪ .

وقد أمكن التعرف على هذين النوعين بمقارنة صفاتهما البيولوجية والبيوكيميائية والسيرولوجية مع صفات عينات معرفة سابقا مأخوذة من وزارة الزراعة المصرية ونوع آخر (٧٧٧ أ) عزل بواسطة د . يسرى صالح ١٩٧٧ كما قورنت النتائج مع ماهو مذكور فى مرجع برجى لتعريف البكتريا (Bergey's Manual)

كما أنه قد وجد أن بكتيرة الاروينيا كاروتوفورا صنف كاروتوفورا تحتفظ بقدرتها على أحداث ذبول نبات الشمام وتعطن ثماره بعد ٢٤ - ٤٨ ساعة من حقنها بعد مضي ٣ سنوات على حفظ العينة .

ومن جهة أخرى وجد أن بكتيرة الاروينيا كاروتوفورا صنف سيتروليس تفقد كثيرا من صفاتها الممرضة عند حفظها على وسط غذائى صناعى لمدة طويلة .

ويكمن الفرق الرئيسى بين النوعين فى أن بكتيرة الاروينيا كاروتوفورا صنف سيتروليس لا يحتوى على انزيم البروتوبكتينيز كما أنها تتحمل درجات الحرارة العالية التى تصل الى ٤٧°م بينما لا تستطيع أن تتحملها بكتيرة الاروينيا كاروتوفورا صنف كاروتوفورا .



دراسات بيولوجية للأمراض البكتيرية المنتشرة على الشمام

رسالة مقدمة لكلية العلوم - جامعة القاهرة
للحصول على درجة الماجستير في العلوم
(نباتات - كائنات حية دقيقة)

مقدمة من

هدى حامد الهنداوى

بكالوريوس علوم ١٩٧٧

جامعة القاهرة

كلية العلوم

قسم النبات

١٩٨٣