Erwinia carotovora pv. *carotovora*, causal agent of soft rot disease in some crops in Puerto Rico[1]

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ABSTRACT

The bacterium *Erwinia carotovora* pv. *carotovora* (Jones) was identified from bacterial isolates from decaying material of tobacco, tomato, tanier, head lettuce, cabbage, *Dracaena* spp, *Pothos* spp, and calla lily as the causal agent of soft rot disease. Physiological characteristics, biochemical reactions and antibiotic response were similar among strains. The ability of the isolates to produce soft rot symptoms on healthy plants when artificially inoculated was demonstrated by pathogenicity tests.

RESUMEN

***Erwinia carotovora* pv. *carotovora*, agente causante de la podredumbre blanda en algunas cosechas en Puerto Rico**

Erwinia carotovora pv. *carotovora* es el causante de la podredumbre de cultivares en Puerto Rico. Se estudió el organismo causante de la podredumbre blanda aislado en tabaco, tomate, yautía, lechuga repollada, repollo y las ornamentales *Pothos* sp., *Dracaena* sp. y lirio cala. Las características fisiológicas, reacciones bioquímicas y sensitividad a antibióticos fueron similares entre los distintos aislamientos obtenidos. Pruebas de patogenicidad demostraron síntomas de podredumbre blanda en las distintas hospederas inoculadas. El organismo se identificó como *E. carotovora* pv. *carotovora* (Jones).

INTRODUCTION

Erwinia carotovora, described by Jones (11) as *Bacillus carotovora*, was known to produce soft rot disease in carrots as early as 1901 when he studied and described it. In 1904 a bacterial pathogen producing soft rot in tomato was named *Bacillus aroideae* by Townsend (13). Numerous synonyms (1,12) have since been accepted for this organism which causes soft rot in various plants and vegetables (9).

In Puerto Rico, in 1935, Cook (2) reported a soft rot of tanier caused by *E. carotovora* (Jones). No records of the identification studies exist. Throughout the years, soft rot of local crops and ornamentals has been extensively observed. Plantings of tobacco, tomato, tanier, head lettuce, and cabbage are among the cultivars infected throughout different areas in Puerto Rico (table 1). Commercial nurseries of ornamentals such as *Dracaena* spp, *Pothos* spp, and calla lily have evidence of the same dis-

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TABLE 1.—*Plant diseases caused by* E. carotovora *pv.* carotovora *in some localities in Puerto Rico*

Plant disease	Source of isolation	Occurrence
1. Hollow stem	*Nicotiana tabacum* L. (tobacco)	San Lorenzo
	Lycopersicon lycopersicum L. (tomato) var. Ace 500	Guayama
	Floradel	Guayama
	Walter	Río Grande
2. Soft rot of rhizome	*Zantedeschia aethiopica* (Spreng) (Calla lily)	Aibonito
	Xanthosoma sagittifolium (L.) Schott (Tanier)	Río Piedras
		Corozal
		Orocovis
		Villalba
3. Soft rot of stem	*Lactuca sativa* L. (lettuce)	Juana Díaz
	Brassica oleracea var. *capitata* L. (cabbage)	Juana Díaz
	Epiprenum aureum (Lindé & André) Bunt. (*Pothos*)	Dorado
	Dracaena sp.	Río Piedras

ease. Rot is a symptom response of a plant to invasion by bacteria or fungi. Bacterial soft rot organisms are destructive in the field whether they invade a whole plant or part of it. Having invaded before harvest, the organism multiplies while in storage or transit (under favorable temperature conditions) thus affecting marketability.

The disease caused by this bacteria is characterized by moist or watery tissue of a brownish color. Gradually, the tissue becomes soft and slimy giving off a putrid offensive odor which is due to the destroyed cells and the disintegration of the pectin by the enzyme pectinase produced by the organism.

In this paper I describe the results of the studies of physiological characters and biochemical activites performed to identify and classify the organism.

MATERIAL AND METHODS

Source and symptoms

Tobacco—Mature plants in the field presented a withered appearance. Longitudinal sections of the stem revealed a dark brown discoloration with a decomposing mass in the vascular pith that gradually became hollow. Younger plants exhibited only a slimy soft rot.

Head lettuce and cabbage—Plants presented a severe rot infection characterized by a slimy decay, brown discoloration, eventually becoming a wet mass of rotten tissue. Split stem showed longitudinal brownish streaks, early rot and foul smell.

Tomato—Cultivars of Floradel, Ace 500, and Walter presented stem cracks, moderate wilt and hollow stem. Few plants appeared bent down.

Tanier—Rhizomes presented soft areas with decomposing tissue and strong putrid odor.

Calla lily—Young plants were slightly chlorotic, stunted and presented a soft area in the bulb or rhizome.

Dracaena spp.—The stem of young plants presented a soft area with broken tissue a few inches above the top soil.

Pothos spp.—The whole plant presented a rotting appearance, wilted leaves and collapsing stem and roots.

Cultural studies

Isolations were performed using diseased material collected from each of the crops mentioned in the preceding section. Whenever possible, samples were washed under water faucet to rinse away dirt and surface contamination. Pieces from the area between infected and healthy tissue were cut, immersed in a 5.25% solution of sodium hypochlorite for 1 to 2 min, and washed in sterile distilled water. The tissue was teased apart in 1-2 ml tryptone glucose broth (TGB) and allowed to stand for 1 h at 28° C. Plates of tryptone glucose agar (TGA) were smeared with a loopful of the suspension and incubated at 28° C. Individual colonies visible after 24 h were restreaked into McConkey (Mc) agar (6,7) plates and Logan's (4,10) differential medium for *Erwinias*. Standard media and methods used in the identification of the causal agent were followed as previously described by Cortés-Monllor and Ruiz (3).

Pathogenicity test

Distinct colony cultures were tested for ability to produce soft rot in potato and carrot slices. The test was performed by pricking through a drop of bacterial suspension (10^7 cells/ml) with a sterile needle. Inoculated tissue was placed in humid plastic chambers at room temperature and observed for the production of soft rot. Those soft rotting bacteria were inoculated onto healthy plants of tobacco, tomato and potato by the stem pricking method. Plants were kept in a chamber with controlled humidity (86%) and temperature (85° C).

Sensitivity test

The susceptibility disk system (5) was used to measure *in vitro* susceptibility of the organism to different antibiotics. Performance of the test was followed as stated in Difco (6). After 24 h incubation at 37° C, the plates were observed for zones of inhibition of bacterial growth which

would indicate the susceptibility of the organism to the antibiotic tested. Resistant organisms produce no zone of inhibition.

RESULTS

The organism is a gram negative bacillus devoid of capsule. In a motility test medium a diffuse zone of growth spreads from the line of inoculation.

Colonies visible on TGA 24 h after incubation were small, approximately 0.5-1 mm in diameter, grayish-white, glistening, slightly raised, wet and round with crenated edge. On Mc agar, in 24 h, minute grayish colonies were observed. Gradually in 36 to 48 hours, the colonies developed a red dot center surrounded by a grayish border. Eventually, the colony became totally red with an opaque zone of precipitation. Mc agar is suitable for detecting members of the enterobacteria group because it inhibits gram positive bacteria. Furthermore, the lactose-fermenting organisms, due to the action of the acids, precipitates the bile salts, and thus changes the color of the colonies to red (6,7). Logan's medium (10) is based on the reduction of triphenyl tetrazolium chloride, an indicator useful for detecting fermentative organisms. The nonfermentant strains are stained deep red and the fermenters remain colorless. *E. carotovora* pv. *carotovora* colonies approximately 1-1.5 mm in diameter developed a pink to dark red center surrounded by a grayish border after 24 h.

Among the biochemical characteristics which distinguished this group was the ability to produce acid without gas from lactose, glucose, salicin and trehalose but not from maltose, and the rapid liquefaction of gelatin and pectate degradation by action of proteolytic enzymes.

In litmus milk, as the organism acted upon lactose and glucose, the color changed to red (acid) and the lactic acid formed coagulated the caseinogen. It utilized citrate and esculin as a source of carbon but not malonate or starch, and tolerated 5% to 8% salt. It was able to reduce nitrates to nitrites but not to split fats and oils. From the break-out of peptone H_2S evolved turning the lead acetate paper strip black, but no ammonia nor indole was produced. The oxidase and catalase tests were negative. Tables 2, 3A and B show the results obtained from the different tests performed.

Pathogenicity test

Slices of potato and carrots inoculated *in vitro* presented a watery light cream exudate 24 h after infected. Gradually, in 48 h, the macerated area on the potato slices developed a dark brown edge, extending beyond the point of inoculation. Healthy tomato, tobacco and potato plants inoculated with the various isolates (table 4) presented similar symptoms. Three days after inoculation, affected plants were slightly wilted; leaves became flaccid with brown watery veins; the stem was brownish and

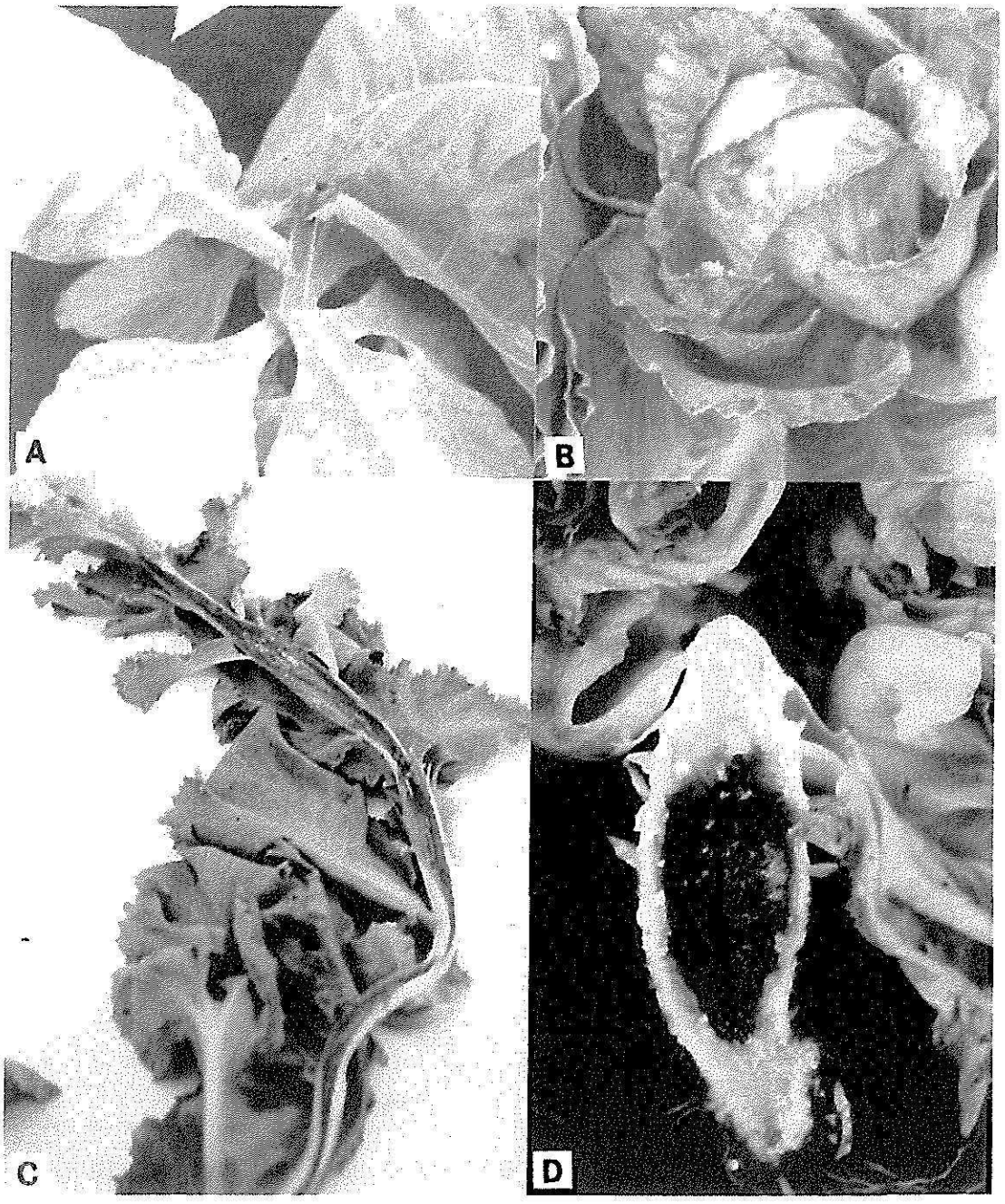

PLATE 1. Symptoms expressed by different crops after inoculating with a suspension of *E.c.* pv. *carotovora* isolated from diseased head lettuce.
A. Initial symptoms of hollow stem rot of tobacco.
B. Slimy decay, brown discoloration and soft rot of cabbage.
C. Brown soft rot, hollow stem of leaf lettuce.
D. Split open stem of head lettuce showing severe soft rot, black discoloration.

slimy at touch. Eventually, the top of a few plants dropped. Longitudinal sections of the stem, showing dark-brown discoloration of the vascular tissue, became hollow. Affected tomato plants also presented stem cracks

TABLE 2.—*Behaviour* E. carotovora *pv.* carotovora

Test	Response
Gram stain and morphology	Negative, rod
Colony appearance	
TGA—24h	Small, grayish, glistening, crenated edge and grow at 37° C
MC Agar	Small, red center surrounded by grayish border.
Logan's medium	Small, dark red center surrounded by grayish border.
Motility medium	Diffuse zone of growth
Capsule stain	Negative
Oxidase	Negative
Catalase	Negative
Maceration of potato slice	Soft rot extending throughout the area

and a few necrotic spots on the leaves. Black soft rot was observed on infected potato plants, which finally collapsed. Plate 1 presents symptoms on various hosts inoculated.

Sensitivity test

The various strains were constantly sensitive to tetracycline, aureomycin, chloroamphenicol, streptomycin, neomycin and novobiocin. Variable responses were obtained to bacitracin and polymyxin B, and insensitiveness or resistance to erythromycin, penicillin and nystatin. Results of the susceptibility test are stated in table 5.

DISCUSSION

Diagnoses of *Erwinia* spp., based primarily on disease symptoms, colony and pigment characteristics and some specific biochemical reactions are criteria used to classify an organism into a genus. The soft rot bacteria isolates from local decaying cultivars proved to be an *Erwinia* spp. The different strains produced similar symptoms in the various cultivars inoculated, some severer than others. Some differences in their biochemical activities and in their responses to antibiotics were observed. Although no differentiation between strains can be sustained, variability within the pathovars has been reported (1,4,8).

The genus *Erwinia* has been a controversial group because of its common and differential characters. Related strains of *Erwinia* were grouped as the "soft rot or carotovora group" within the Enterobacter family. This has led many scientists to support Waldee's suggestion (14) to restrict those pathogens which attack the middle lamella causing soft rots due to the presence of pectolytic enzymes in a new genus *Pectobacterium*. To date, there exists general confusion, and no final agreement has been reached. Lelliott, in Bergey's edition (12), states a compromise

TABLE 3.—*reactions of* E. carotovora *pv.* carotovora *isolated from various sources*

A. Biochemical Test

TEST	SOURCE Cabbage	Calla lily	*Dracaena* sp.	Lettuce	*Pothos* sp.	Tanier	Tobacco	Tomato
Indole	–	–	–	–	–	–	–	–
NH_3	–	–	–	–	–	–	–	–
MR-VP	–, +[1]	–, –	–, +	+, +	+, –	+, –	+, –	–, +
NO_3	+	+	+	+	+	+	+	+
Milk (Litmus)	AC[2]	AC	AC	AC	AC	AC	AC	AC
Citrate	+	+	+	+	+	+	+	+
Malonate	–	+	–	–	–	–	–	–
Urea	–	–	–	–	–	–	–	–
H_2S (Peptone)	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+
Pectate	+	+	+	+	+	+	+	+
Esculin	.[3]	+	.	+	.	+	+	+
Lypolisis	–	–	–	–	.	–	–	–
Starch	–	–	–	–	.	–	–	–
Salt Tolerance	5%	.	7%	.	5%	5%	8%	7%

B. Acid production on carbohydrates

Sucrose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Maltose	–	–	–	–	–	–	–	–
Salicin	+	+	+	+	+	+	+	+
Glycerol	+	–	+	.	+	+	+	.
Dextrine	+	.	–	–	–	–	–	–
Dulcitol	+	.	–	–	–	–	–	—
Sorbitol	–	–	–	–	–	–	–	–
Trehalose	.	+	.	+	.	+	+	+

[1] + = positive; – = negative
[2] AC = acid, coagulation
[3] . = reaction not recorded

retaining the genus *Erwinia* divided in 3 groups (amylovora, herbicola and carotovora). The nonfermenter soft rot bacterium *E. carotovora* var. *aroideae* (Townsend) has been placed under the carotovora group, *E. carotovora* var. *carotovora*. Since none of the local strains were able to produce gas from carbon, we will identify this pathogen *Erwinia carotovora* pv. *carotovora* as the causal agent of bacterial soft rot disease in tomato, tobacco, head lettuce and cabbage, and ornamentals calla lily,

TABLE 4.—*Symptoms expressed by various hosts after inoculation with soft-rot isolates of* Erwinia *spp*

Hosts tested	Source of *Erwinia* spp. isolates						
	Dracaena sp.	*Pothos* sp.	Cabbage	Lettuce	Tanier	Tobacco	Tomato
Tomato	Wilt Rot Hollow stem	Rot Hollow stem	*	Brown Soft-rot Hollow stem	Wilt Rot Hollow stem Death	*	Stem crack Stem rot Necrotic spots on leaves
Tobacco	Soft-rot stem	Rot Hollow stem	*	Soft-rot Hollow stem Strong putrid odor	*	Rot Hollow stem	Stem crack Hollow stem
Potato	*	*	Stem rot (Black) Hollow stem Death	*	Soft-rot (Black) Death	*	*

*No record

TABLE 5.—*Susceptibility test of* E. carotovora *pv.* carotovora, *isolates from various sources, to different antibiotics*

Antibiotic		Concentration per disk	Source							
			Tobacco	Tomato	Cabbage	Lettuce	Tanier	Calla lily	Dracaena	Pothos
Tetracycline	(TE_{30})	30 ug	S[1]	S	S	S	S	S	S	S
Aureomycin	(A_{30})	30 ug	S	S	S	S	S	S	S	S
Chloroamphenicol	(C_{30})	30 ug	S	.	S	S	S	S	.	.
Streptomycin	(S_{10})	10 ug	S	S	S	S	S	S	S	S
Polymyxin B	(PB_{300})	300 units	R	.	S	S	S	S	S	S
Neomycin	(N_{30})	30 ug	S	.	S	S	S	S	S	S
Erythromycin	(E_{15})	15 ug	R	R	R	R	R	R	R	R
Bacitracin	(B_{10})	10 units	S	.	R	R	S	S	R	R
Novobiocin	(NB_{30})	30 ug	S	.	S	S	S	S	.	.
Nystatin	(NY_{100})	100 units	R	.	R	R	R	R	.	.
Penicillin	(P_{10})	10 units	R	R	R	R	R	R	R	R

[1]S = Susceptible or zone of inhibition around disk. R = Resistant or no zone of inhibition around disk. . = Test not performed.

Dracaena and *Pothos* spp. in Puerto Rico. At the same time, identification studies for the bacterial soft rot disease in taniers, previously reported by Cook (2) in Puerto Rico, are presented.

LITERATURE CITED

1. Bradbury, J. F., 1977. *Erwinia carotovora* pv. *carotovora*, Commonwealth Mycological Institute (CMI) Descriptions of Pathogenic Fungi and Bacteria No. 552, Kew, Surrey, England.
2. Cook, M. T., 1937. Ann. Rep. Univ. P. R., Agric. Exp. Stn., 1935-36, p. 135.
3. Cortés-Monllor, A. and H. Ruiz, 1987. Bacterial leaf spot of pigeon pea in Puerto Rico. *J. Agric. Univ. P. R.* 71 (2): 177-84.
4. Cother, E. J. and K. Sivasithamparam, 1983. *Erwinia*: The "Carotovora" group. *In*: Plant Bacterial Diseases: a diagnostic guide. P. C. Fahy and G. J. Persley (Ed). Academic Press, Australia, 393 pp.
5. Difco Laboratories, 1988. Quality Products for Microbiology, P. O. Box 1058, Detroit, MI. 48232.
6. Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures, 1977, Ninth Ed., Detroit 1, MI.
7. Dowson, W. J., 1957. Plant Diseases Due to Bacteria, 2nd ed., Cambridge University Press, Cambridge, England.
8. Dye, D. W., 1983. *Erwinia*: The "Amylovora" and "Herbicola" Groups. *In*: Plant Bacterial Diseases: a diagnostic guide. P. C. Fahy and G. J. Persley (Ed). Academic Press, Australia, 393 pp.
9. Elliott, C., 1951. Manual of Bacterial Plant Pathogens, 2nd ed., Chronica Botanica Company, Waltham, MA.
10. Fahy, P. C. and A. C. Hayward, 1983. Media and Methods for Isolation and Diagnostic Test. *In*: Plant Bacterial Diseases: a diagnostic guide. P. C. Fahy and G. J. Persley (Ed). Academic Press, Australia, 393 pp.
11. Jones, L. R., 1893. A bacterial stem rot of lettuce, Vermont Agric. Exp. Stn. 6th Ann. Rep., 1892, pp. 82-7.
12. Lelliott, R. A., 1974. Genus *Erwinia*. *In*: Bergey's Manual of Determinative Bacteriology, 8th ed., The Williams and Wilkins Co., Baltimore, MD.
13. Massey, A. B., 1924. A study of *Bacillus aroideae* Townsend, the cause of a soft rot of tomato, and *B. carotovorus* Jones, *Phytopath.* 14: 460-77.
14. Waldee, E. L., 1945, *in* Dowson, W. J., 1957. Plant diseases due to Bacteria, 2nd ed., Cambridge University Press, Cambridge, England.