Diseases caused by *Pseudomonas* spp in some cultivars in Puerto Rico—an updating[1]

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ABSTRACT

A revision of the species of *Pseudomonas* affecting cultivars of economic importance in Puerto Rico is presented. The pathogen has been isolated from various vegetables, fruits and ornamentals. Studies of the isolates include affected plants, cultural characters, hypersensitivity reaction and other pathogenicity tests. Some saprophytic *Pseudomonas* spp. producing leaf spot disease in some cultivars are reported herein for the first time.

RESUMEN

Enfermedades causadas por *Pseudomonas* spp. en algunos cultivares en Puerto Rico - una actualización

Se presenta una revisión de la especie *Pseudomonas* spp., la cual afecta cultivares de importancia económica en Puerto Rico. El patógeno se ha aislado de varios vegetales, frutas y ornamentales. Los estudios realizados incluyen hospederas afectadas, caracteres culturales, reacción de hipersensibilidad y pruebas de patogenicidad. Algunos *Pseudomonas* saprofíticos que causan manchas necróticas en algunos cultivares se informan por vez primera.

INTRODUCTION

The genus *Pseudomonas* comprises an important group of bacteria which are associated with plants. They are widespread in many natural environments. They attack a number of plant species and cause some of the most serious diseases of plants.

In 1903 (27) bacterial wilt caused by *Bacillus solanacearum* Sm. was reported affecting tomatoes and eggplants in Puerto Rico. In 1925, Tucker (29) mentioned bacterial wilt as one of the most destructive diseases of tomatoes in Puerto Rico. In 1929 (1) pepper, potatoes and zinnias were also affected by the disease but it was less severe than in tomatoes and eggplants. In 1930 (5) *P. maculicola* (McCullogh) causing leaf spot of cabbage and *P. syringae* causing bacterial blight of *Phaseolus* sp. were reported.

A year later Roque (24) reported bacterial wilt of tobacco and developed tomato and eggplant varieties resistant to the disease. In 1935

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(1), *Bacterium lachrymans*, Erw. F. Sm. and Bryan, was found causing bacterial fruit rot of cucumber (*Cucumis sativus* L.). *Phytomonas ananas* (Serrano) causing brown fruit rot (fruitlet black rot) of pineapple and *P. angulata* (Fromme and Murray) Holland, causing angular leaf spot (black fire) of tobacco have been also reported (17, 28).

Throughout the years, other *Pseudomonas* have been recorded in the island causing disease to various cultivars and ornamentals. Strains of plant pathogenic and saprophytic *Pseudomonas* have been isolated and studied and some are herein reported for the first time. In an effort to summarize information of plant diseases caused by *Pseudomonas* species on the island, this paper presents a compendium of most of the *Pseudomonas* species recorded in Puerto Rico.

General chracteristics

Members of the genus *Pseudomonas* are Gram negative bacilli, usually straight but at times slightly curved, non-capsulated and motile (one to several polar flagella). The organism grows well in most of the culture media, at 5° to 45°C, on 24-h incubation. Colonies are grayish-white, smooth, low convex and slightly butyrous. They are strictly aerobic organisms, catalase positive and usually oxidase positive (some pathovars give a negative oxidase response). Many of them are weak or non-fermenters of carbon compounds. The majority are lactose negative. Litmus milk reaction is usually alkaline and some strains may liquify gelatin but do not degrade pectates. The genus includes many non-pigmented species but some produce fluorescein (yellow-green) and pyocyanin (blue-green) pigments, soluble in water, that with ageing may fade to brownish color diffusing into the medium. Two groups of *Pseudomonas* have been differentiated: fluorescent and non-fluorescent. The fluorescent pathogenic *Pseudomonas* recorded in Puerto Rico include *P. ananas*, *P. angulata*, *P. cichorii*, *P. marginalis*, and *P. syringae* pathovars. Among the non-fluorescent pathogenic *Pseudomonas* the following species have been recorded locally: *P. cattleyae*, affecting orchid plants, *P. cepacia* (sour skin of onions), *P. rubrilineans*, and *P. rubrisubalbicans* (red stripe and mottle stripe of sugarcane, respectively) and *P. solanacearum* which causes diseases in a variety of crops of economic importance (table 1).

Although the *Pseudomonas* are common in soils, a few species are considered human and animal pathogens: *P. aeruginosa*, *P. mallei*, and *P. pseudomallei*. *P. cepacia* is an opportunistic phytopathogen which has been isolated from clinical material and infected tissue in humans (21). The phytopathogenic *Pseudomonas* species can be separated into various groups and from non-pathogenic strains according to their response to the LOPAT test. These responses include the levan production, oxidase reaction, potato rot, arginine dihydrolase and tobacco hypersen-

TABLE 1.—*Plant diseases caused by* Pseudomonas *species isolated in Puerto Rico from cultivars of economic importance*

	Cultivar	Pathogen	Disease
1.	Bean - *Phaseolus vulgaris*	*P. s. phaseolicola*	Halo blight (Halo spot)
		P. s. syringae	Bacterial blight (Brown spot)
2.	Cabbage - *Brassica oleracea* var. *capitata* L.	*P. marginalis*	Margin leaf blight
		P. s. maculicola	Leaf spot
3.	Chayote - *Sechium edule* (Jacq.) Swartz	*P. s. lachrymans*	Angular leaf spot
4.	Coffee - *Coffea arabiga* L.	*P. s. syringae*	Bacterial spot
		P. fluorescens	Leaf spot
5.	Cucumber - *Cucumis sativus* L.	*P. s. lachrymans*	Angular leaf spot Fruit rot
6.	Eggplant - *Solanum melongena* L.	*P. solanacearum*	Bacterial wilt
7.	Lettuce - *Lactuca sativa* L. cvs. Market Price and Romaine	*P. s. marginalis*	Margin leaf blight (Bacterial soft rot)
		P. cichorii[1]	Bacterial blight (Leaf spot)
	Leaf Lettuce	" "	" "
		P. aeruginosa	Leaf spot
		P. fluorescens	" "
8.	Melon - *Cucumis melo* L. cv. Honeydew	*P. s. lachrymans*	Angular leaf spot
		P. aeruginosa	Leaf spot
9.	Mushrooms - *Volvariella volvacea*	*P. chlororaphis*	Bacterial basal rot
		P. fluorescens	Bacterial spot
10.	Onions - *Allium* spp.	*P. cepacia*	Sour skin (Soft rot)
11.	Pepper - *Capsicum annuum* L.	*P. s. syringae*[1]	Bacterial blight (Brown spot)
		P. solanacearum	Bacterial wilt
		P. aeruginosa	Leaf-spot
12.	Pineapple - *Ananas comosus* (L.) Merrill	*P. ananas*	Brown fruit rot
13.	Plantain - (*Musa* spp.)	*P. fluorescens*	Leaf-spot

TABLE 1.—*(Continued)*

	Cultivar	Pathogen	Disease
14.	Potato - *Solanum tuberosum* L.	*P. solanacerum*	Bacterial wilt
15.	Soybean - *Glycine max* (L.) Merr.	*P. s. glycines*	Bacterial blight
16.	Squash - *Cucurbita* spp.	*P. s. lachrymans*	Angular leaf spot
17.	Sugarcane - *Saccharum officinarum* L.	*P. rubrilineans*	Red stripe
		P. rubrisubalbicans	Mottle stripe
		P. aeruginoss	Leaf-spot
18.	Sweetpotato - *Ipomea batatas* (L.) Lam.	P. aeruginosa	Leaf-spot
19.	Tobacco - *Nicotiana tabacum* L.	*P. solanacearum*	Bacterial wilt
		P. s. tabaci	Wildfire
		P. angulata	Angular leaf spot
		P. aeruginosa	Leaf-spot
		P. fluorescens	Leaf-spot
20.	Tomato - *Lycopersicon lycopersicum* L.	*P.s. syringae*[1]	Bacterial blight
		P. solanacearum	Bacterial wilt
		P. fluorescens	Leaf-spot

[1]Reported hereinfor the first time.

sitivity (HR) reaction (13). Most of the *Pseudomonas* species are resistant to penicillin and sensitive to tetracylines. There are various saprophytic species that may cause leaf spot disease in some cultivars. They are distinguished from the phytopathogenic species by some characteristics such as faster growth in media and positive arginine dihydrolase reaction. Most of them do not induce a hypersensitive reaction when injected on tobacco leaves.

DISEASES

Pathogenic and non-pathogenic *Pseudomonas* coexist together within the plant surface. The pathogenic organisms invade their host producing toxic substances and killing the cells. The plant thus produces visible symptoms. Usually it enters through the stomatas but being common inhabitants of soil, they can also enter through leaf and root injuries. Symptoms of desintegration of parenchyma, leaf spots or halo blights, necrosis, cankers, and galls are common in *Pseudomonas* infection.

Black rot or brown fruit rot of pineapple: *P. ananas* (Serrano)

The disease was reported in Puerto Rico (17, 28) caused by *Phytomonas ananas* (Serrano). Fruitlets of pineapple develop internal dark brown rot extending to the vascular bundles of the core. The disease is similar to brown rot caused by *Erwinia ananas* (Serrano) (11), but, when isolated, *P. ananas* produces greenish pigmentation on King's medium B (KB).

Blackfire or angular leaf spot of tobacco: *P. angulata* (Fromme and Murray) Holland.

The disease was reported by Stevenson (28) in Puerto Rico in tobacco, but is rarely observed. Symptoms on leaves of tobacco are irregular dark brown areas, sometimes surrounded by a narrow yellow border. The center of the spot becomes pale brown, brittle and may fall out, leaving holes. Some scientists (13) consider this organism a non-toxin strain of *P. syringae* pv. *tabaci* which causes "wildfire" disease. Both diseases may occur at the same time; both pathogens are seed-borne and fluorescent organisms. Application of copper solution has been found effective to control the organism (19). The use of resistant varieties of tobacco is recommended.

Brown spot of orchid: *P. cattleyae* (Pavarino) Savulescu.

The organism has been isolated in Puerto Rico by the author from flowers and leaves of *Cattleya* and *Phalaenopsis* spp. The spots are small, brownish and dry on the petals and sepals of the flower. Leaves develop water-soaked lesions with a dark brown center. They may be surrounded by a light brown or chlorotic rim. The organism belongs to the non-fluorescent *Pseudomonas* group. Overhead irrigation should be avoided; disinfection of tools is mandatory.

Sour skin or soft rot of onions: *P. cepacia* (Burkholder).

The organism belongs to the non-fluorescent *Pseudomonas* group. It affects the outer skin of the bulb producing a mild shrinkage. The organism is considered an opportunistic pathogen in man and plants. It has been isolated from clinical material in hospitals (15). In Puerto Rico, Campo et al. (3) evaluated the pathogenicity of the organism in four commercial onion genotypes.

Bacterial blight or leaf spot of lettuce: *P. cichorii* (Swingle) Stapp.

First recorded in Puerto Rico in 1986 by the author (unpublished data) on lettuce leaves presenting dark brown necrosis at the edge or margins of the blade. The stem develops longitudinal necrotic streaks of

a light brown color and dry appearance. This pathogen is considered a common soil organism. It has been associated with *P. marginalis*, which causes similar symptoms in lettuce and related vegetables. The organism belongs to the fluorescent *Pseudomonas* group and is usually found as an epiphyte.

Marginal leaf spot of lettuce: *P. marginalis* (Brown) Stevens.

This organism is considered to be *P. fluorescens* biotype B (biovar II) (21). It has been isolated in Puerto Rico from cabbage and head lettuce (9). Dark brown necrosis develops at the margin of the leaf extending toward the center throughout the veins. It is often mistaken as leaf burn. The tissue is at first soft and wet but then dries out. Minute reddish spots can be observed near the leaf margin. It is a soil organism and may be found in association with *P. cichorii*. It belongs to the fluorescent *Pseudomonas* group. As main control, removal of infected debris is recommended.

Red stripe of sugarcane (*S. officinarum* L.) is caused by *P. rubrilineans*, (Lee et al.) Stapp (2).

Reported in Puerto Rico in 1929 (1) caused by *Phytomonas rubrilineans* (Lee et al.). Mentioned by Elliott (12) as *Xanthomonas rubrilineans* and by Cortés-Monllor (10) as *X. campestris* pv. *rubrilineans*. Primary symptoms consist of watery dark green short strips which spread up and down the leaf. Gradually the strips unite and turn into bright red stripes. Some of them may coalesce to form broad bands. When young central shoots become infected top rot may occur. The organism also infects maize and sorghum. It belongs to the non-fluorescent *Pseudononas*.

Mottle stripe of sugarcane (*S. officinarum* L.): *P. rubrisubalbicans* (Christopher and Edgerton) Krasilnikov (2).

It was reported in Puerto Rico in 1932 (1) as caused by *Phytomonas rubrisubalbicans*. Elliott (12) and Cortés-Monllor (10) described it under *X. rubrisubalbicans* and *X. campestris* pv. *rubrisubalbicans*. The leaf blade develops stripes which are predominately red with white areas or white margins. The stripes are parallel to the central leaf vein. Stripes may coalesce to form mottle red and white bands. Symptoms are similar to the red stripe caused by *P. rubrilineans*. The organism belongs to the non-fluorescent *Pseudomonas*.

Bacterial wilt: *P. solanacearum* Erw. F. Sm.

According to Stevenson (27, 28) the disease was first recorded in Puerto Rico in 1906 affecting eggplants and tomatoes. The casual organism

was identified originally as *Phytomonas solanaceara*, E.F.S. This organism has also been referred to as *Bacillus solanacearum, Bacterium solanacearum* and *Xanthomonas solanacearum* (28). The disease has been intensively studied in Puerto Rico by several scientists (20,22,28). It has been recorded affecting solanaceous plants (pepper, tobacco, tomato, and potato) and zinnias. On tomato it was found to be one of the most destructive diseases affecting 60% of the varieties tested (29). In 1933, Roque (24) developed tomatoes and eggplants resistant to the organism. The first symptom observed on infected plants is a general wilting, followed by drying and chlorosis of the lower leaves. A whitish exudate may become evident on transversely cut stems. Occasionally the stem may become darker brownish green. Symptoms can vary according to the host. In tomato plants, adventitious roots can be produced; in potato, tubers may develop brown ring rot. In Puerto Rico, the organism has not been found affecting bananas and plantains with Moko disease, which is considered a very serious disease in other tropical countries. Typical symptoms on bananas and plantains include wilting, blackening of the pseudostem, stunting, necrotic and twisted leaves. The fruits present yellow immature fingers, black and dry rot. The Moko disease can be effectively controlled by strict quarantine measures. The organism belongs to the non-fluorescent *Pseudomonas*, but some strains produce a brown pigment which diffuses into the medium. Hayward (15) and Palleroni (21) have widely studied *P. solanacearum* behavior and the species have been divided into races and bio-types according to the pathogenic characteristics and biochemical properties, respectively.

Bacterial blight of soybean: *P. syringae* pv. *glycinea* Coerper.

Leaves of soybean, *Glycine max* L. (Merr.) initially develop symptoms consisting of small pale yellow spots with water-soaked center. Eventually the spots turn brown to dark brown, become irregular and surrounded by a yellow rim. Small lesions tend to coalesce to form larger spots. Stems and pods may be affected and the seeds may become infected. The organism may remain in the soil and on leaf debris for many months after harvest. It has been found as an epiphyte and belongs to the fluorescent *Pseudomonas* group. According to Leypon (19) chemical control with Kocide 101 was effective.

Angular leaf spot: *P. syringae* pv. *lachrymans* (Smith and Bryan) Carsner.

This disease was first recorded in Puerto Rico in 1935 (1), affecting cucumbers. Fruit rot of cucumbers was a severe disease and the cause of heavy losses when in transit. Other cucurbits (squash, honeydew melon and chayote) have been recently reported affected by the organism (8). General symptoms include small water-soaked spots on the leaves,

which become dry and necrotic. As the infection progresses, the leaves become broken and ragged in appearance. Petioles and stems present dry, brown epidermal tissue. The spots on the fruits are small, circular and water-soaked. Gradually they enlarge and may crack open exuding a whitish liquid. In severe infections the seeds may become affected. The organism belongs to the fluorescent *Pseudomonas* and produces a blue-green pigmentation on King's medium B. Control includes the use of certified seeds of resistant or tolerant varieties, insect spray, irrigation and crop rotation with non-cucurbits for 2 to 3 years as this organism remains in the soil for a few years.

Leaf spot of lettuce: *P. syringae* pv. *maculicola* (McCullogh) Stevens.

The disease was first reported affecting cabbage in Puerto Rico by Cook (5). The leaves present minute dark green spots located between the veins. The spots become irregular, turning purple-brown. Some may coalesce to form large necrotic areas. The disease is seed-borne and it affects a variety of crucifers. The organism belongs to the fluorescent *Pseudomonas.*

Halo blight or bacterial blight of *Phaseolus* spp: *P. syringae* pv. *phaseolicola* (Burkholder) Dowson.

In Puerto Rico the disease was found in 1931 (5) affecting *Phaseolus* spp. and *P. lunatus* (5,28). The organism was then known as *P. vignae* Gard. and Kendr. and as *Phytomonas viridifaciens* Tiesd. and Williamson. Symptoms on leaves start as irregular reddish brown spots with pale center. In some cases the lesion breaks, leaving broken areas. Stems present reddish brown streaks which may crack and exude a viscid liquid. Infected pods present irregular brownish areas, like greasy spots, and the infected seeds become wrinkled and dry. The organism belongs to the fluorescent *Pseudomonas* group. Use of certified seeds or those obtained from disease-free crops is highly recommended.

Bacterial leaf spot (brown spot): *P. syringae* pv. *syringae* van Hall.

The disease has been recorded in Puerto Rico in beans (5), coffee (7) and recently in pepper and tomato (6). Symptoms on bean, pepper and tomato leaves are reddish-brown small spots which may be surrounded by a narrow yellow rim. The spots are dry and irregular, mostly scattered on the surface of the leaf. On coffee leaves the lesion starts as a dark brown area at the tip of the leaf which continues spreading down toward the central area of the leaf. A narrow yellow rim can be observed surrounding the necrotic area. This organism usually is recorded as an epiphyte and its pathogenic activity has to be confirmed by inoculation tests and ice nucleation activity (INA) (7,13). It belongs to the fluores-

cent *Pseudomonas* group and induces a hypersensitive reaction in tobacco. Cultural and sanitation measures are recommended to avoid spreading the disease agent.

Wildfire of tobacco and soybean: *P. syringae* pv. *tabaci* (Wolf and Foster) Stevens.

The organism has been mentioned by some scientists as identical to *P. angulata,* which causes "blackfire" or angular leaf spot of tobacco (2,13,28). The symptoms consist of reddish-brown spots scattered on the leaves. The spots enlarge and become surrounded by a broad yellow halo produced by a toxin (13). Sometimes only chlorotic spots appear and eventually the center becomes brown and necrotic. Few of the spots may coalesce to form larger areas. The necrotic spot in soybean is surrounded by an intense yellow border (19). The disease has been associated with soybean leaf spot (bacterial pustule) caused by *Xanthomonas campestris* pv. *glycines* (Nakano). *P. tabaci* is a fluorescent organism which produces greenish pigmentation on King's medium B. The toxin in it produces a severe hypersensitive reaction in tobacco leaves (13). Application of copper solution has been found effective to control the organism (19).

Tables 1 and 2 present cultivars and ornamentals which have been affected by *Pseudomonas* species in Puerto Rico.

TABLE 2.—*Plant diseases of ornamentals caused by* Pseudomonas *species isolated in Puerto Rico.*

Source	Pathogen	Disease
Aglaonema spp.	*P. aeruginosa*[1]	Leaf-spot
	P. fluorescens[1]	" "
cv. Maria	*P. putida*[1]	" "
Aloe spp.	*P. aeruginosa*[1]	" "
Cattleya spp.	*P. cattleyae*	Brown spot
	P. aeruginosa[1]	Leaf-spot
Dracaena marginata	*Pseudomonas* spp.	" "
D. massengena	*P. aeruginosa*[1]	" "
D. warneckii	*P. aeruginosa*[1]	" "
Dieffenbachia roehrsi	*Pseudomonas* spp.	" "
Euphorbia spp.	*P. fluorescens*[1]	" "
Ficus pandurata	*Pseudomonas* spp.	" "
Monstera spp.	" "	" "
Phalaenopsis spp.	*P. aeruginosa*[1]	" "
	P. cattleyae	Brown spot
Spatyphyllum spp.	*P. fluorescens*[1]	Leaf-spot
Syngonium spp.	*P. aeruginosa*[1]	" "
Zinnias spp.	*P. aeruginosa*	" "
	P. solanacearum	Bacterial wilt

[1]Saprophytic species reported herein for the first time in Puerto Rico as causal agent of leaf-spot disease.

Saprophytic species

It is believed that some leaf-spot pathogens may originate from saprophytic populations of soil *pseudomonads* (*P. fluorescens* and *P. putida*) (11). In Puerto Rico, few known saprophytic species have been recorded as causal agents of leaf spot disease of some cultivars. Most of the isolates screened were classified as *Pseudomonas* according to their response to the bacteriological tests. The organisms were Gram-negative bacilli and produced fluorescent pigment on KB medium. Other characteristics in common include positive arginine dihydrolase reaction, catalase positive and lactose negative. Some were unable to induce a hypersensitive reaction in tobacco leaves. The non-fluorescent isolates could not be identified to the species.

P. aeruginosa (Schroeter), Migula, *P. pyocyanea* (Migula), *P. polycolor* (Clara).

It is a common soil and plant saprophyte, although it is considered the only species, together with *P. mallei* and *P. pseudomallei*, pathogenic to man and animals. Some scientists (11,12,13) consider this organism the same as *P. polycolor* (Clara) which has been isolated from leaf spot of tobacco and has similar characters (11). It was reported in Puerto Rico in 1956 by Pérez et al., as quoted by Stevenson, (28) isolated from foot rot of cattle. The author has isolated it from leaf spot of various cultivars (table 2). The diseased leaves present light brown spots surrounded by a narrow yellow halo. In some instances a wet rot may develop. Among the outstanding cultural characteristics of this organism is the production of the yellow-green pigment fluorescin, which diffuses into the medium and is soluble in water but not in chloroform. It also produces a second pigment, pyocyanin, which is blue-green, soluble in water and in chloroform. Hence, this organism is also known clinically as *P. pyocyaneus* or "blue-pus" organism (21).

P. chlororaphis (Guignard and Sauvageau).

This organism is a soil and water saprophyte. It has been reported in Puerto Rico as the cause of bacterial basal rot of straw mushroom *Volvariella volvacea* (16) and leaf spot of *Cattleya* species. In media culture, it produces an emerald-green pigment (chlororaphin), which crystalizes to form fine needles either single or in bundles (16).

P. fluorescens, Migula.

This organism is commonly found in soil and water occasionally associated with food spoilage. Strains have been differentiated into biotypes I to V. *P. marginalis*, which causes marginal leaf spot of let-

tuce, is considered to be *P. fluorescens* biotype II (13). Both organisms possess very similar characters. In Puerto Rico, it has been isolated from leaf spot of many cultivars.

P. putida (Trevisan) Migula.

This organism is found in soil and water. Strains have been differentiated into biovars A and β (21). In Puerto Rico, it was isolated from leaf spot of *Aglaonema conmutatum* cv. Maria, an ornamental plant.

Cultural characteristics

Characterization of some of the organisms was made by following standard procedures and media (10). Selective media King's medium B (KB) or Pseudomonas Agar F (PAF) (14,25), MacConkey agar (Mc) (11) and Thornley's arginine dihydrolase (21) were included for pigment production and character differentiation.

Pseudomonas isolates grow well on tryptone glucose agar (TGA) producing mucoid (formation of levan), entire, grayish-white small colonies in 24 hr incubation at 37°C. *P. solanacearum* colonies are rather slow appearing in 36 h to 48 hr incubation. Initially the colonies are of a dirty white color but with aging they become creamy, opaque and buttery. A brown pigmentation which diffuses into the medium is produced. In TGA slants the medium becomes dark brown or black.

Most of the fluorescent *Pseudomonas* strains produce a greenish tinge which fades with aging into a greenish brown and later turns to a brownish hue. The pigment pyocyanin is better observed in KB or PAF, producing blue fluorescense under UV light. Saprophytic *P. aeruginosa* and *P. fluorescens* produce a blue-green and yellow-green pigmentation on TGA, respectively.

In Mc agar (selective medium for Gram negative bacteria) *Pseudomonas* strains growth is slower. Pin point grayish colonies appear after 24 hr incubation. Medium underneath the growth decolorizes (reduction of glucose). *P. solanacearum*, tomato strain, in many cases does not grow, or only a few colonies appear after 3-day incubation.

The isolates are Gram negative bacilli, aerobic, motile and catalase positive. Some are able to form ammonia from peptone and use citrate and malonate as carbon sources. Most of the strains cause an alkaline (blue) reaction on litmus milk although *P.s. glycines* and *P.s. tabaci* digest casein. Gelatin is hydrolyzed by most of the isolates except *P. cichorii* and *P.s. syringae*. None hydrolyze pectates nor liberate H_2S from cysteine and peptone. The arginine dihydrolase reaction is found in some of the *Pseudomonas* species. *P. aeruginosa*, *P. chlororaphis*, *P. cichorii*, *P. fluorescens*, *P. marginalis*, and *P.s. lachrymans* are able to use arginine as a carbon source for growth thus liberating ammonia.

Negative reaction is obtained from *P. ananas*, *P. cattleyae*, *P. solanacearum*, and *P.s. syringae*. Acid production is observed on glucose, xylose and arabinose, but all strains except *P. ananas* were lactose, salicine and sorbitol negative.

Table 3 presents response to biochemical tests performed on some fluorescent *Pseudomonas* species recorded in Puerto Rico.

Pathogenicity tests

Successful inoculation can be attained with various procedures. Inoculation *in-vitro* of vegetable slices is a useful quick method when dealing with soft rot organisms. Bacterial suspension of a pure culture of *Pseudomonas* isolate inoculated in potato, carrot, onions or cucumber slices is commonly employed to demonstrate the desintegration of tissue (*P. solanacearum*). Organisms producing necrotic symptoms, *P.s. syringae* (7), *lachrymans* (8) and *glycines* (19), are usually inoculated by the needle prick method (14) on limes, cucurbits and bean pods, respectively.

Vascular pathogens are inoculated by spray or wound prick methods (14) on leaves, leaf-axil, stems and roots (7,8,19) in plants grown under greenhouse conditions. Reference cultures as well as non-inoculated plants should be included. Table 4 presents results of the pathogenicity tests performed with some of the local isolates of *Pseudomonas*.

Hypersensitive reaction

The hypersensitive reaction (HR) is used as a determinant screening procedure to separate pathogen from non-pathogenic fluorescent strains of *Pseudomonas*. It is also included in the LOPAT test as a rapid diagnosis of the organism involved. Usually tobacco is used as test plant but pepper plants can also induce the reaction (14). When bacterial suspension (14,25) are infiltrated into the leaf veins, phytopathogenic *Pseudomonas* will produce, within 24 hours, dry necrosis in the infected area. Many strains induce a severe reaction because of production of toxins. *P. syringae* pv. *tabaci*, causal agent of wildfire disease in tobacco, induces severe hypersensitivity (HR) symptoms of brown rot, wilting of the leaf and eventually of the plant. Saprophytic bacteria induce mild yellowing of the injected area 72 hours after inoculation.

Table 5 presents the hypersensitivity reaction induced by some of the local isolates of *Pseudomonas*.

Antiobiotic sensitivity

Susceptibility of *Pseudomonas* species to antimicrobial agents may be determined by using sensitivity discs and *in-vitro* procedures (7, 26). Local isolates of *Pseudomonas* species are sensitive to tetracyclines (tet-

racycline and aureomycin). *P.s. syringae* exhibited 50 mm inhibition zone diameter to both antibiotics at a concentration of 30 ug each disc. The isolates are resistant to penicillin (10 units) and nystatin (100 units). Other organisms show various degrees of susceptibility to some antimicrobial agents (table 6). The effect of antibiotics in the *in-vitro* test has been observed in the field. A combination of terramycin and streptomycin (Agri-Strep, Agrymycin) has been used to control wildfire of tobacco (11), bacterial wilt of tomato (22) and leaf spot of ornamentals (4).

Control

Dissemination of phytopathogenic bacteria can occur in various ways. *Pseudomonas* species are soil organisms and can remain in manure for many years. Infected seed-pieces, seedlings in plant beds in infected soil, drainage water and movement of soil from high ground to lower areas will spread the disease agent into new fields. Cultural practices where the laborer carries soil on his implements and shoes or uses contaminated pruning knives aid in the spread of disease.

Insects are considered primary agents of dissemination for many organisms but of little importance for the transmission of bacteria. In Puerto Rico, Nolla (20) stated that the green beetle *Diabrotica graminea* Baly transmitted *P. solanacearum* to eggplants.

Changes in cultural practices and a combination of control measures are of great importance and often required to develop a healthy agricultural program or one of low disease incidence. Crop rotation, fertilization, moisture control and other means of plant cultivation can lead to healthy plantations and better yields. The use of certified seeds or of pathogen-free vegetative seed pieces is highly recommended.

Crop rotation has been recommended for at least 2 years with non-cucurbits to avoid angular leaf spot disease caused by *P.s. lachrymans* (8). Rotations and use of disease free pods to erradicate *P.s. glycines* and *P.s. phaseolicola* (12) are recommended for beans and soybeans. Chemical control using Kocide 101 was found to be effective for *P.s. glycines* (19). Removal of debris, ground irrigation and application of various concentration of fertilizer (4) can reduce severity of leaf spot disease caused by *P. cichorri* and *P. marginalis* in crops grown near the ground (lettuce, cabbage). Soil heat treatment, chemical treatment or use of resistant potato seeds have been effective against *P. solanacearum*-caused wilt in potato. Wilt disease was considerably reduced with sea-shell grit (CaO) mixed in soil in a tomato field in Suriman (23). Preventive application by dipping cuttings in antibiotic solution can prevent many bacterial diseases . Although many *Pseudomonas* species are resistant to a number of antimicrobial agents, others are known to be very sensitive to the tetracyclines (11) (table 6). In Puerto Rico, Pérez (22) applied streptomy-

TABLE 3.—*Diagnostic test response of some fluorescent pathogenic* Pseudomonas *spp. isolated in Puerto Rico*

TEST	ISOLATES and SOURCE						
	P. ananas (Pineapple)	*P. cichorii* (Lettuce)	*P. marginalis* (Cabbage) (11)	*P. s. glycines* (Soybean) (19)	*P. s. lachrymans* (Melon) (10)	*P. s. syringae* (Coffee) (9)	*P. s. tabaci* (Soybean) (19)
Oxidase	−	+	+	−	−	+	−
Catalase	+	+	+	+	+	+	+
Ammonia (from Peptone)	+	+	+	−	+	+	+
Arginine Dihydrolase	−	−	+	•	−	−	•
Levan production in Sucrose Media	+	•	+	•	+	+	•
Citrate utilization	+	+	+	+	+	+	+
Malonate utilization	+	+	+	-	+	+	+
Nitrite from Nitrate	+	−	−	+	v	+	v
H_2S from Cysteine and Peptone	−	−	−	−	−	−	−
Action on Litmus Milk	Alkaline	Alkaline	Alkaline	Alkaline C.D.	Alkaline Clear	Alkaline	Alkaline C.D.
Hydrolysis of gelatin	+	−	+	v	+	−	+
" "starch	−	−	−	v	−	+	v
" "esculin	+	−	−	+	−	+	+
" "cotton seed oil	+	−	v	−	+	−	−
" "pectates	•	−	−	−	−	−	−
Salt tolerance (1-5%)	2%	4%	4%	4%	4%	4%	5%
Acid production from: (21 days observation)							
Arabinose	+	+	+	•	•	+	+
Glucose	+	+	+	+	+	+	+
Lactose	+	−	v	v	−	−	−
Maltose	−	−	−	−	−	−	−
Salicine	+	−	−	−	−	−	−

Sorbitol	+	–	–	–	–	–	–
Sucrose	–	+	v	v	+	+	+
Trehalose	+	–	–	+	•	–	•
Xylose	+	+	+	+	+	+	+

Data:
+ = Positive response
– = Negative response
v = Variable response
CD = Casein digested
• = No record

TABLE 4.—*Symptoms observed in various hosts after inoculation with some* Pseudomonas *isolated in Puerto Rico*

Isolate and Source	Inoculated Host	Symptom
P. cichorii (Lettuce)	Head lettuce	Dark brown spots on veins and leaves.
	Lettuce (del país)	Brown necrotic lesions on stem.
P. marginalis (Cabbage)	Head lettuce (9)	Yellowish spots scattered on leaves, dry brown margin which may develop in soft wet area
P. solanacearum (Tomato)	Potato slices	Brown soft area.
	Carrot slices	Brown, cracked soft area.
	Tomato plants	Wilt, branches cupping down, rot and death.
P. s. glycines (Soybean)	Soybean (19)	Scatter, minute spots with yellow halo.
	Bean (*Phaseolus* spp.)	Watery small, irregular spots with a yellow halo.
P. s. lachrymans (Melon)	Honeydew melon (8)	Brown spots, irregular, and tear-drop exudation emerging from lesion
	Limes	Brownish irregular necrotic spots.
P. s. syringae (Coffee)	Limes (7)	Crater-like sunken spots brown-black and dry.
	Coffee plants (7)	Brown and dry tips of leaves

cin sulfate to the main tank of the nutrient solution of a tomato hydroponic system affected by *P. solanacearum* and obtained adequate control. On the United States mainland, *P.s. tabaci*, which causes wildfire of tobacco, was controlled with streptomycin and the use of resistant

TABLE 5.—*Tobacco hypersensitivity reaction induced by some* Pseudomonas *strains isolated in Puerto Rico*

Isolate and Source	HR Symptom
P. cattleyae (*Phalaenopsis* spp.)	Brown necrotic, slight dry area surrounded by yellow halo.
P. solanacearum (Tomato)	Light brown necrotic large area surrounded by yellow border.
P. s. glycines (Soybean)	Necrotic area and slightly dry. (19)
P. s . syringae (Coffee)	Initially the leaf develops light brown wet area. Later necrosis develops along the central vein, down to the peduncle and lateral veins. Chlorosis. Rot and death.
P. s. tabaci (Soybean)	Large dry necrotic area surrounded by yellow border. (19)

TABLE 6.—*Susceptibility of some pathogenic* Pseudomonas *strains isolated in Puerto Rico to different antibiotics*

Antibiotic		Concentration per disk	ISOLATES *P. ananas*	*P. cattleyae*	*P. cichorii*	*P. s. lachrymans*	*P. s. syringae*	*P. solanacearum*	*P.s. tabaci*
Tetracycline	(TE_{30})	30 ug	S	S	S	S	S	S	S
Aureomycin	(A_{30})	30 ug	S	S	S	S	S	S	S
Chloroamphenicol	(C_{30})	30 ug	S	S	S	S	S	S	S
Streptomycin	(S_{10})	10 ug	R	R	S	S	S	S	S
Polymyxin B	(PB_{300})	300 units	R	S	S	S	S	R	•
Neomycin	(N_{30})	30 ug	S	S	S	S	S	S	•
Erythromycin	(E_{15})	15 ug	S	S	R	R	S	•	S
Bacitracin	(B_{10})	10 units	R	S	R	R	R	•	•
Novobiocin	(NB_{30})	30 ug	S	S	S	S	R	•	S
Nysatatin	(NY_{100})	100 units	R	R	R	R	R	•	•
Penicillin	(P_{10})	10 units	R	R	R	R	R	•	R

S = Susceptible or zone of inhibitition around disk.
R = Resistant or no zone of inhibition observed.
• = No record.

tobacco varieties (11). Leypon (19) found that a copper solution was effective for controlling *P.s. tabaci* in a soybean plantation in Puerto Rico.

There is need for new control methods for bacterial diseases. Better knowledge of the relation between pathogen and cultivars for the application of biological control techniques, breeding for disease resistance, new bactericides with reduced phytotoxicity, better farming systems and more enforcement of sanitation practices will eventually reduce bacterial diseases or incidence of the phytopathogens.

Bacterial diseases are more severe in tropical and subtropical areas than in temperate areas. Environmental factors, temperature and moisture, can influence the growth of phytopathogenic organisms. Some *Pseudomonas* species overwinter in soil and can survive for many years, thus increasing disease levels. Severe losses due to bacterial diseases can occur after high levels of soil moisture result from continuous or heavy rainfall. In Puerto Rico, bacterial wilt of tomato (20) and tobacco (24) caused by *P. solanacearum* appeared after periods of heavy rainfall followed by dry spells.

Losses can be minimized and disease incidence reduced by adapting crops to selected farming sites with climatic conditions unfavorable to the pathogen and with adequate soil drainage.

Quarantine precautions prevent introduction of pathogens into fields, regions or countries where they do not exist. There are many *Pseudomonas* species that have not yet been recorded in Puerto Rico (table 7), but could be introduced because they exist in nearby tropical countries (30).

Preventive methods and precautions are the most logical solutions to reduce or avoid any disease.

TABLE 7.—*Some* Pseudomonas *spp. not recorded in Puerto Rico*

Organism	Host	Disease	Symptoms
P. agarici Young	Mushrooms (*Agaricus bisporus*)	Drippy gill	Dark brown, water-soak spots on the gills (13).
P. alboprecipitans Rosen	Grasses	Bacterial leaf blight (Stalk rot)	Brown spots and streaks on blades. When entering causes stalk rot (12).
P. andropogonis (E. F. Sm) Stapp	Grasses Leguminous Ornamentals	Bacterial leaf stripe	Reddish brown leaf spots, streaks, irregular, water-soaked and chlorotic halo (15).
P. caricapapayae Robbs	Papaya (*Carica papava*)	Leaf spot	Angular leaf spots on leaves, water-soaked (13).

TABLE 7.—*(Continued)*

Organism	Host	Disease	Symptoms
P. caryophylli Starr & Burkholder	Carnation	Bacterial wilt	Wilt and stem crack (15).
P. corrugata Roberts and Scarlett	Tomato (*Lycopersicon esculentum* Mill)	Pith necrosis	Brown discoloration of pith, vascular browning, brown stem lesions, exudate, adventicious roots and collapsation of plant (18).
P. setariae Okabe	Rice	Bacterial stripe (Brown stripe)	Leaf spots on seedlings and young plants (15).
P. solanacearum Race 2 E. F. Smith	Musa spp.	Moko disease	Variable symptoms, chlorosis of young leaves, wilting, stunting, dry brown rot of fruits (5, 15, 30).
P. syringae pv. *passiflora* Reid	Passion fruit (*Passiflora edulis*)	Grease spot	Round greasy patches on fruits, brownish lesions with pale yellow halo on leaves, water-soaked brown areas on stem (13).
P. syringae pv. *pisi* (Sackett)	Peas (*Pisum sativum*)	Bacterial blight	Small angular spots, water-soaked. Stems, pods, and seeds may become infected (13).
P. syringae pv. *syringae* (van Hall)	Citrics	Black pit (fruit) Blast (leaves)	Dark brown to black sunken spots on fruits and leaves (5, 13).
	Ornamentals	Bacterial blight	Spotting of leaves, flowers and stems (6, 30).
P. syringae pv. *tomato* (Okabe)	Tomato (*L. esculentum*, Mill	Bacterial speck	Very small black spots with narrow yellow halo in leaves and fruits. Seedlings, stems and seeds may become infected (13, 30).
P. tolaasii Paine	Mushrooms (*Agaricus bisporus*)	Bacterial blotch	Dark brown sunken lesions on caps (13).

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