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BACTERIAL LEAF SPOT OF ANTHURIUM (**Anthurium andreanum**) CAUSED BY A **Pseudomonas** sp. IN THE FRENCH WEST INDIES

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ABSTRACT

A **Pseudomonas** species is reported as a new pathogen of the leaves and spathe of anthurium. This vascular disease may be characterized by black necrotic spots surrounded by greasy margins and a bright chlorotic halo on leaves and a violet halo on the spathe. Symptoms observed on naturally infected material, as well as in pathogenicity tests, showed both local and systemic infections. Morphological, cytological, physiological, biochemical and serological relationships of the pathogen are presented. There is evidence that this bacterium may be a new species. The species **Pseudomonas aracearum** is proposed.

INTRODUCTION

Anthurium andreanum Lind and hybrid anthuriums are an economically important ornamental crop in many humid, tropical countries. Flower production has shown great potential in the crop diversification programme of the French West Indies. Since its successful development, anthuriums have remained relatively disease-free. In 1982, however, disease symptoms appeared on hybrid anthuriums in Guadeloupe and Martinique. The conclusions of a phytosanitary inquiry (Hostachy et al., 1985), indicate that severe damage was caused by **Xanthomonas campestri** pv. **dieffenbachiae** (Hayward, 1972; Nishijima and Fujiyama, 1985). The present paper reports another bacterial disease of anthurium incited by a **Pseudomonas** sp. A search of the literature has revealed no other references to this bacterial leaf spot.

The studies reported were undertaken to isolate and identify the causal organism of bacterial leaf spot of anthurium. Distribution, symptomatology, inoculation host range and serological studies are reported.

MATERIALS AND METHODS

Isolation of the Causal Organism

Tissues were excised from the margins of lesions on affected petioles, leaves, stems and flowers of anthuriums from ten locations in Guadeloupe and Martinique. Samples were surface-sterilized with alcohol, crushed in sterile distilled water (SDW) in sterile petri dishes and streaked onto King's medium (King et al., 1954), and Kelman's medium (Kelman, 1954)

without tetrazolium chloride and amended with 1 g l^{-1} yeast extract (DIFCO) (KY). Plates were incubated for 48 hr at 38º C. Single colonies were restreaked on KY for purification.

Characterization of the Pathogen

Eighteen isolates of the bacterium were collected from different locations in Guadeloupe and Martinique and compared for biochemical, physiological and cultural characteristics. All isolates were Gram stained, observed for motility and stained for flagella (Rhode's method) (Fahy and Hayward, 1983) and for poly-beta-hydroxybutyrate (PHB) inclusions (Burdon, 1946). Pigmentation, growth and colony characteristics were observed on KY medium and King's medium A (KA) and B (KB). Other physiological and biochemical tests included in this study are described in Tables 1 and 2.

Hypersensitivity in tobacco (**Nicotiana tabacum** cv. Xanti and Samsun N.N.) was treated by injection of 10^3 - 10^9 colony-forming units (CFU) per milliliter of an aqueous suspension of each isolate into leaves (Fahy and Hayward, 1983). Growth at 4ºC and 41ºC was observed on liquid KY medium and salt tolerance (2% and 5% NaCl) determined after incubation for 14 days at 28ºC on solid YDA (yest extract 5 g, dextrose 10 g, bactopeptone 5 g, agar 20 g p^{-1}, pH 7).

Antisera Production

Two representative isolates from Guadeloupe, (P1G6) and Martinique (P4M9) were chosen for antisera production. The isolates were grown in Roux bottles on KY medium (48 hr, 28ºC) then collected and rinsed twice by centrifugation in SDW. Suspensions were prepared from each pellet and optically adjusted to 10^9 bacteria ml^{-1} and heat-killed (2 hr, 100ºC) in order to inactivate common flagellar antigens (Schaad, 1979). Antisera were obtained from six month old rabbits and prepared against whole cell antigens following the procedure described in Table 3 and stored frozen at -20ºC.

Indirect immunofluorescence (Faure et al., 1977) was used for determining titer and specificity of antisera. Specificity of anti-P1G6 and anti-P4M9 was tested against 18 isolates of the pathogen causing leaf spot of anthurium, six non-fluorescent, and six fluorescent species of pathogenic **Pseudomonas** spp. (Table 4).

Pathogenicity Tests

Healthy hybrid anthuriums from **in vitro** culture were cultivated in a moist chamber (75-100% RH, 25-28ºC) with a 12 hr photoperiod. Inoculum was prepared by flooding 48 hr-old KY agar cultures of the bacterium with SDW. Suspensions of 10^3 CFU ml^{-1} were sprayed over both leaf surfaces. All isolates were tested for pathogenicity by injection of 10^7 CFU ml^{-1} into juvenile and adult leaves. Soil inoculations were made by incorporating the pathogen into the substrate (fresh distillery bagasse) at a rate of 10^6 CFU g^{-1} dry weight before transplanting healthy anthuriums. SDW was used to inoculate control plants. Host range was tested by infiltrating P1G6 and P4M9 into the leaves of **Xanthosoma saggitifolium, Dieffenbachia sequine,** and **Caladium bicolor.**

Table 1. Characteristics differentiating **Pseudomonas** sp. pathogen on anthurium from other non-fluorescent **Pseudomonas** species isolated. a/

Character	Species (b,c,d)																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
No. polar flagella	1-6	1	1-2	>1	1-2	>1	1	>1	>1	>1	>1	1	1	1-2	>1	>1	1
PHB (e)	-	+	+	+	ND	+	+	+	+	-	-	+	d	+	+	+	+
Kovac´Oxidase	-	-	+	+	ND	+	+	+	+	d	-	+	+	+	+	+	+(g)
Arginine dihydrolase	-	-	-	+	ND	-	-	-	-	-	ND	-	-	-	-	-	-(n)
Growth at 4ºC	+	ND	ND	-	ND	-	ND	ND	-	ND	-	-	-	ND	ND	ND	-
Growth at 41ºC	-	-	+	+	ND	d	-	-	+	ND	-	-	+	+	+	-	-
Gelatin hydrolysis	-	-	d	d	-	d	+	+	+	+	-	-	+	+	-	+	-(h)
Starch hydrolysis	-	-	+	-	ND	-	+	-	-	-	ND	d	-	-	-	-	-(n)
Potato soft rot	-	-	-	-	ND	ND	-	-	ND	ND	ND	ND	ND	-	-	ND	-
Denitrification	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	d	-(g)
Nitrite from nitrate	-	-	+	-	+	d	-	+	d	d	-	-	-	+	+	+	+(g)
Tween 88 hydrolysis	+	-	+	d	ND	+	ND	ND	+	+	-	+	+	+	-	+	+(g)
Oxydative on glucose	+	+	+	+	ND	+	+	+	+	+	+	d	-	+	+	+	+(i)
Inert on glucose	-	-	-	-	ND	-	-	-	-	-	-	d	+	-	-	-	-(i)
Acid from sucrose	+	-	-	+	+	d	+	+	+	-	-	+	-	-	-	+	-
Diffusible pigment (f)	-	-	-	gr,yl	-	d	-	gr,yl	gr,yl	-	-	-	-	-	-	d(br)	d(br)

(a) Data from Hayward (1983)
(b) Species code: 1, **P. amygdali**; 2, **P. andropogonis**, 3. **P. avenae**; 4. **P. caryophylli**; 5. **P. cattleyae**; 6. **P. cepacia**; 7. **P. cissicola**; 8. **P. corrugata**; 9. **P. gladioli**; 10. **P. maltophila**; 11. **P.mesophilica**; 12. **P. paucimobilis**; 13. **P. pseudoalcaligenes** subsp. **citrulii**; 14. **P. rubrilineans**; 15. **P. rubrisubalbicans**; 16. **P. solanacearum**; 17. **Pseudomonas** sp. from anthurium.
(c) All are Gram negative rods, not producing endospores, or a pigment which fluoresces under long wavelength ultra violet light (368 nm) when grown on medium B of King et al. (1954).
(d) +, 98% or more strains positive; -, 98% or more strains negative; ND, no data available; d, 11-89% strains positives or variable results obtained by different authors.
(e) PHB, Poly-b-hydroxybutyrate inclusions.
(f) gr, green, yl, yellow; pl, purple; pk, pink; br, brown.
(g) Method described in Fahy & Hayward (1983).
(h) Method of Frazier (1926).
(i) Method of Hugh & Leifson (1953).
(j) Method described in Schaad (1980).
(k) Method of Dye (1962).
(l) Method of Oshiro et al. (1964)
(m) Method of Kado & Herkett (1970).
(n) Method of Ayers et al. (1919)
(o) Method described in Gardan & Luisetti (1982).

Table 2. General characteristics of **Pseudomonas** sp. pathogen of Anthurium in the French Este Indies[1/]

Positive Tests	Negative Tests	Variable Tests
+ strict aerobe	- indole production (o)	- production of acids (n) from:
- catalase (j)	- levan production (g)	. trehalose
- arginine (o) and asparagine (k) used as a sole source of C & N	- acetoine production (g)	. glucose
- H_2S from cysteine (g)	- aesculin hydrolysis (o)	
- tryptophane deaminase (f)	- casein (k)	
- urease activity (o)	- DNase activity (o)	- utilization of (o):
- cellulose hydrolysis (l)	- pectinolyse (o) potato soft rot (m)	. D tartrate
- growth on 2% NaCl YDA	- growth on 5% NaCl YDA	. L tartrate
- production of acids (n) from:	- production of acids (n) from:	
. galactose	. inositol	
. arabinose	. sorbitol	
. glycerol	. mannose	
- utilization of (o):	. sucrose	
. acetate	. cellobiose	
. formiate	. rhamnose	
. citrate	- utilization of (o):	
	. propionate	
	. benzoate	

1/ Se footnotes Table 1.

RESULTS

Symptoms

Early foliar symptoms of bacterial leaf spot of anthurium may be characterized as small, angular, greasy spots on the abaxial surface near veins, leaf margins, and on spathes. These lesions develop rapidly resulting in large, black necrotic spots which become grey-black when older and distort the leaves. Necrotic spots are surrounded by greasy margins and narrow, bright chlorotic halos. High humidity is required to observe bacterial slime oozing from lesion margins. Typical black or brown necrotic spots surrounded by violet halos usually appear on spathes. Infections may progress into veins causing a soft rot of infected tissue resulting in the abscission of leaves and spathe petioles.

Table 3. Immunization schedule to obtain antisera[1/]

Day No.	Dose (ml) (a)	Method (b)
0	0.5	IV
1	1	IV
2	1.5	IV
6	1.5	IV
7	2	IV
8	2 + 2	IV + SC
18	-	HPB

1/ Race of rabbit:

Isolate from Guadeloupe (PlG6): New Zealand
Isolate from Martinique (P4M9): "Fauve de Bourgogne"

(a) 10^9 heat killed whole cells ml^{-1}
(b) IV = intraveneous, SC = subcutaneous,
HPB = heart puncture bleeding

Symptoms of plants with systemic infections display a general yellowing of the entire lamina and typical black, necrotic lesions progressing from the leaf petioles into major veins. In this case petioles may rot at the base or top depending on the rapidity of bacterial colonization. Plants with systemic or local infections in the stem, petiole, leaves and spathes usually die.

Characterization of the Pathogen

All isolates displayed similar characteristics which most closely associate them with the genus **Pseudomonas.** The isolates were motile, Gram-negative rods with a single polar flagellum. Colonies were circular, raised with an entire margin, and creamy white on KY, KA, KB and YDA medium. No fluorescent pigment was produced on KA or KB. Some isolates produced a brown diffusible pigment when grown at 41ºC on solid KY medium. No significant visible turbidity was determined after 10 days incubation at 4ºC or 41ºC in liquid KY medium. All isolates elicited hypersensitivity in Xanthi and Samsun N.N. tobacco. Other tests are presented in Tables 1 and 2.

Serological Identification of the Pathogen

All isolates of the anthurium pathogen reacted positively when stained by the indirect fluorescent antibody technique (Table 4). Anti-PlG6 and anti P4M9 cross-reacted with **Pseudomonas rubrilineans**, and **Pseudomonas tolaasii** stained slightly when using anti-P4M9 sera. **Ps. rubrilineans** and **Ps. tolaasii** were not pathogenic on anthurium when infiltrated into leaves.

Table 4. Immunofluorescence indirect staining of **Pseudomonas** sp. pathogen of anthurium compared with other pathogenic **Pseudomonas** species 1/

Antigen	Strain	Antisera (a)	
		Anti-P4M9	Anti-P1G6
Pseudomonas sp. from Martinique (16 isolates)		+++ (b)	+++
Pseudomonas sp. from Guadeloupe (2 isolates)		+++	+++
Ps. solanacearum	2000 (c)	-	-
Ps. corrugata	20701 (c)	-	-
Ps. rubrilineans	1201 (d)	++	++
Ps. gladioli pv alliicola	2422 (d)	-	-
Ps. cattleya	2423 (d)	-	-
Ps. pseudoalcaligenes	2435 (d)	-	-
Ps. syringae pv **syringae**	1393 (d)	-	-
Ps. syringae pv **tabaci**	3700 (e)	-	-
Ps. syringae pv phaseolicola	20501 (d)	-	-
Ps. cichorii	1005 (e)	-	-
Ps. agarici	2063 (d)	-	-
Ps. tolaasii	2068 (d)	+	-

1/ (a) Anti-P4M9: titer 1/18248, working dilution 1/1288.
Anti-P1G6: titer 1/28488, working dilution 1/2568.

(b) Symbols: +++ bright green fluorescence; ++ green fluorescence + light fluorescence; cells not visible.

(c) Cultures obtained from M. Lemattre, INRA, Versailles, France.
(d) Cultures obtainedfrom R. Samson, INRA, CNBP, Angers, France.
(e) Cultures formthe laboratory.

Pathogenicity Tests

Plants inoculated with the isolated bacteria displayed symptoms identical to those observed under natural conditions. Symptoms on spray-inoculated anthurium usually appeared within 5-6 days under conditions of high humidity. Necrotic lesions grew rapidly to form large dead areas and leaf burning symptoms. Narrow, bright, chlorotics halos developed around black lesions and bacteria were easily reisolated from advancing margins.

Soil-borne inoculations were positive resulting in vascular colonization and the development of characteristic symptoms: yellowing of leaves and black necrotic areas located on both sides of the major veins were observed within three weeks. At this time, systematically infected inflorescences showed large black spots surrounded by violet halos. All symptomatic and soil incubated plants died 5 weeks after inoculation.

All isolates were pathogenic on **Anthurium** sp. When infiltrated into leaves isolates P1G6 and P4M9 where also pathogenic on **Dieffenbachia sequine.**

Water-soaked lesions appeared within 24 hr and progressed out of infiltrated areas 48 hr after inoculation. On **Anthurium**, typical black, necrotic lesions developed with yellow margins after 4 days. After 21 days, bacteria moved down the petiole and became systemic resulting in new leaf and spathe infections. Lesions on **Dieffenbachia** were brownish and surrounded by a tiny white halo on the abaxial leaf surface, and a diffuse yellow area on the upper leaf surface at 4 days.

Inocula of P1G6 and P4M9 elicited a hypersensitive-like response when infiltrated into leaves of **Caladium bicolor** and **Xanthosoma saggitifolium.** Black, necrotic lesions appeared within two days surrounded by a bright yellow margin limited to the infiltrated areas.

DISCUSSION

Bacterial leaf spot of anthurium is caused by a **Pseudomonas** sp. A comparison of this bacterium with other **Pseudomonas** species was conducted in order to determine relationships based upon physiological, cytological, morphological and biochemical characteristics. It appears that the bacterium belongs to non-fluorescent Pseudomonads according to Hayward (1983), i.e., isolate from plants, fluorescent pigment not produced and PHB is accumulated (Table 1). The anthurium pathogen also possess characteristics which distinguish it from other **Pseudomonas** species. The most notable of these are the presence of only one polar flagellum; the production of nitrite from nitrate; the absence of growth at 41ºC; and the inability to hydrolyse gelatin or to produce acids from sucrose. The anthurium pathogen does not correspond with any other non-fluorescent **Pseudomonas** species previously described.

Using immunofluorescence techniques, the Guadeloupe and Martinique isolates of the pathogens did not reveal any serological differences and were easily distinguished from other **Pseudomonas** species. However, it is worth noting that cross-reactions could be detected between **Ps. rubrilineans** and the pathogen using low dilutions of antisera (1/1280 and 1/2568 with anti-P4M9 and anti-P1G6). **Ps. tolaasii** cross-reacted weakly with P4M9 antisera. None of the cross-reacting strains were pathogenic on anthurium. This specific serological test is useful for the quick and accurate identification of the **Pseudomonas** pathogen of anthurium.

This disease can cause important losses in commercial anthurium houses in Guadeloupe and Martinique, particularly during the rainy season. Splashing rain spreads the pathogen from infected to healthy plants causing the common foliar symptoms. Systemic infections are sporadic in appearance under natural conditions which may be due to the variable level of inoculum in the substrate.

Under natural conditions, only hybrid **Anthurium** spp. seems susceptible to the **Pseudomonas** pathogen, though the bacterium has been isolated from **Anthurium martinicense** Engler (**A. lanceolatum** auct. non Kth.) (Fournet, 1979) in Martinique (isolate P1M15).

Differences in varietal susceptibility of hybrid **Anthurium** spp. were observed, but no variety appeared resistant. This bacterium occurs naturally on **Anthurium** and is pathogenic to **Dieffenbachia sequine** when artificially inoculated. **Caladium bicolor** and **Xanthosoma saggitifolium** appeared resistant

because they presented a hypersensitivity-like response when artificially inoculated.

Attempts to control this disease with bactericides have been ineffective, and copper compounds have been reported to be phytotoxic to anthurium (Hostachy et al., 1985; Nishijima and Fujiyama, 1985). Presently, strict sanitation is the best control strategy for management of this disease.

This is the first report of bacterial leaf spot of **Anthurium** caused by **Pseudomonas** species. Considering it's different morphological, physiological, cytological, biochemical, serological and pathological characteristics we propose naming this new bacterial species **Pseudomonas aracearum.**

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