***Acidovorax anthurii* sp. nov., a new phytopathogenic bacterium which causes bacterial leaf-spot of anthurium**

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The bacterial leaf-spot of anthurium emerged during the 1980s, in the French West Indies and Trinidad. This new bacterial disease is presently wide spread and constitutes a serious limiting factor for commercial anthurium production. Twenty-nine strains isolated from leaf-spots of naturally infected anthurium were characterized and compared with reference strains belonging to the Comamonadaceae family, the genera *Ralstonia* and *Burkholderia*, and representative fluorescent pseudomonads. From artificial inoculations 25 out of 29 strains were pathogenic on anthurium. Biochemical and physiological tests, fatty acid analysis, DNA–DNA hybridization, 16S rRNA gene sequence analysis, DNA–16S RNA hybridization were performed. The 25 pathogenic strains on anthurium were clustered in one phenon closely related to phytopathogenic strains of the genus *Acidovorax*. Anthurium strains were 79–99% (ΔT_m range 0.2–1.6) related to the strain CFBP 3232 and constituted a discrete DNA homology group indicating that they belong to the same species. DNA–rRNA hybridization, 16S rRNA sequence and fatty acid analysis confirmed that this new species belongs to the β -subclass of *Proteobacteria* and to rRNA superfamily III, to the family of *Comamonadaceae* and to the genus *Acidovorax*. The name *Acidovorax anthurii* is proposed for this new phytopathogenic bacterium. The type strain has been deposited in the Collection Française des Bactéries Phytopathogènes as CFBP 3232^T.

Keywords: *Acidovorax anthurii*, plant pathogen, rDNA sequencing, bacterial leaf-spot, DNA–DNA hybridization

INTRODUCTION

Anthurium andreanum Lind. is an important ornamental cash-crop found in most tropical-humid countries in the world and in particular the Caribbean, Latin America and Hawaii. Anthuriums have remained relatively disease free, however several bacterial, fungal and viral diseases may damage production without becoming a serious limiting factor for anthurium production (Chase, 1987). During the 1980s, hybrid and standard anthuriums, in Hawaii, Venezuela, Trinidad, the French West Indies, Philippines and Jamaica (Nishijima *et al.*, 1980; Rott & Prior, 1987; Natural, 1990; Young, 1990), were

heavily infested by a vascular bacterial blight disease caused by *Xanthomonas axonopodis* pv. *dieffenbachiae*. However, a distinct bacterial disease, the bacterial leaf-spot, causing only limited damage compared to those due to bacterial blight, has emerged recently in some anthurium farms in the French West Indies and Trinidad (Prior & Rott, 1987; Saddler *et al.*, 1995). Following the first observations in restricted areas in Guadeloupe, bacterial leaf-spot was confirmed in Trinidad (Dilbar *et al.*, 1992) and is becoming more widespread. It has been identified as a serious limiting factor for commercial anthurium production.

The new disease of anthurium is described as bacterial leaf-spot, because the first symptoms consist of necrotic lesions close to veins and leaf margins, which blacken and turn grey. From these lesions, bacteria enter the leaf and spathe parenchymas and become systemic, resulting in tissue discoloration and plant

Abbreviation: UPGMA, unweighted pair group method with averages.

The EMBL accession number for the 16S rRNA gene sequence of *Acidovorax anthurii* strain CFBP 3232^T is AJ007013.

Table 1. Origin of the strains of *Acidovorax*, *Burkholderia*, *Herbaspirillum* and fluorescent and non-fluorescent *Pseudomonas* examined in this study

CFBP, Collection Française des Bactéries Phytopathogènes, Angers, France; ATCC, American Type Culture Collection, Manassas, VA, USA; ICMP, International Collection of Microorganisms from Plants, Auckland, New Zealand; IMI, International Mycological Institute, Egham, Surrey, UK; LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie Ghent, Belgium; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, UK; NCPPB, National Collection of Plant-Pathogenic Bacteria, Harpenden, UK.

Name	Strain no. as received	Other designation	Host plant or origin and place and year of isolation
<i>Acidovorax anthurii</i>	CFBP 3231		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3232 ^T		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3233 A*		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3233 B*		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3234 A*		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3234 B*		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3236		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3237		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3240		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3241		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
<i>Acidovorax anthurii</i>	CFBP 3242		<i>Anthurium</i> sp. (hybrid.), Martinique, 1984
<i>Acidovorax anthurii</i>	CFBP 3243		<i>Anthurium</i> sp. (hybrid.), Martinique, 1984
<i>Acidovorax anthurii</i>	CFBP 3244		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 3245		<i>Anthurium</i> sp. (hybrid.), Martinique, 1984
<i>Acidovorax anthurii</i>	CFBP 3246		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 3247		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 3248		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 3249		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 3250		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 3251		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 3252		<i>A. lanceolatum</i> (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 3282		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 10907		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 10908		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 10909		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax anthurii</i>	CFBP 10910		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
Unknown	CFBP 3235		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
Unknown	CFBP 3238		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
Unknown	CFBP 3239		<i>Anthurium</i> sp. (hybrid.), Martinique, 1991
Unknown	CFBP 10908		<i>Anthurium</i> sp. (hybrid.), Martinique, 1985
<i>Acidovorax avenae</i> subsp. <i>avenae</i> (= <i>Pseudomonas rubrilineans</i>)	NCPBP 920	CFBP 1201	<i>Saccharum officinarum</i> , Réunion, 1960
<i>Acidovorax avenae</i> subsp. <i>avenae</i>	NCPBP 1011 ^T	CFBP 2425 ^T	<i>Zea mays</i> , USA, 1961
<i>Acidovorax avenae</i> subsp. <i>avenae</i>	NCPBP 1392	CFBP 2426	<i>Oryza sativa</i> , Japan, 1963
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i>	NCPBP 961 ^T	CFBP 2423 ^T	Host and place unknown, 1961
<i>Acidovorax avenae</i> subsp. <i>citrulli</i>	NCPBP 3679 ^T	CFBP 10880 ^T	<i>Citrullus lanatus</i> , USA, year unknown
<i>Acidovorax delafeldii</i>	NCIMB 11476 ^T		
<i>Acidovorax facilis</i>	NCIMB 9938 ^T		
<i>Acidovorax konjaci</i>	NCPBP 3698 ^T	CFBP 10881 ^T	<i>Amorphophalus konjac</i> , Japan, year unknown
<i>Acidovorax</i> sp.	CFBP 4692		<i>Valerianella locusta</i> , France, 1992
<i>Acidovorax</i> sp.	CFBP 4693		<i>Valerianella locusta</i> , France, 1992
<i>Acidovorax</i> sp.	CFBP 4694		<i>Valerianella locusta</i> , France, 1992
<i>Acidovorax</i> sp.	CFBP 4695		<i>Valerianella locusta</i> , France, 1992
Unknown	CFBP 4696		<i>Valerianella locusta</i> , France, 1992
<i>Burkholderia caryophylli</i>	NCPBP 2151 ^T	CFBP 2429 ^T	<i>Dianthus caryophyllus</i> , USA, 1951
<i>Burkholderia plantarii</i>	ICMP 9424 ^T	CFBP 3199 ^T	<i>Oryza sativa</i> , Japan, 1982
<i>Burkholderia glumae</i>	NCPBP 2391 ^T	CFBP 2430 ^T	<i>Oryza sativa</i> , Japan, 1967

Table 1 (cont.)

Name	Strain no. as received	Other designation	Host plant or origin and place and year of isolation
<i>Burkholderia cepacia</i>	NCPPB 2993 ^T	CFBP 2227 ^T	<i>Allium cepa</i> , USA, 1948
<i>Burkholderia gladioli</i>	IMI 347513		<i>Gladiolus</i> sp., Israël, 1958
<i>Burkholderia gladioli</i> pv. <i>alliiicola</i>	NCPPB 947	CFBP 2422 ^T	<i>Allium cepa</i> , USA, 1939
<i>Burkholderia gladioli</i> pv. <i>gladioli</i>	NCPPB 1891 ^T	CFBP 2427 ^T	<i>Gladiolus</i> sp., place unknown 1966
<i>Burkholderia andropogonis</i>	NCPPB 934 ^T	CFBP 2421 ^T	<i>Sorghum officinale</i> , USA, 1961
<i>Delftia acidovorans</i>	NCIMB 9681 ^T		
<i>Commamonas terrigena</i>	NCIMB 8193 ^T		
<i>Commamonas testosteroni</i>	NCIMB 8955 ^T		
<i>Herbaspirillum rubrisubalbicans</i>	NCPPB 1026 ^T	CFBP 1202 ^T	<i>Saccharum officinarum</i> , Jamaica, 1961
<i>Herbaspirillum rubrisubalbicans</i>	IMI 347532		<i>Saccharum officinarum</i> , USA, 1961
<i>Pseudomonas cissicola</i>	NCPPB 2982 ^T	CFBP 2432 ^T	<i>Cissus japonicus</i> , Japan, 1974
<i>Pseudomonas meliae</i>	ICMP 6289 ^T	CFBP 3225 ^T	<i>Melia azedarach</i> , Japan, 1974
<i>Pseudomonas pseudoalcalignes</i> subsp. <i>pseudoalcalignes</i>	NCPPB 1971 ^T	CFBP 2435 ^T	Sinus drainage, France, 1956
<i>Pseudomonas alcaligenes</i>	ATCC 14909 ^T	CFBP 2437 ^T	Water, place unknown, 1961
<i>Pseudomonas syzygii</i>	NCPPB 3446 ^T	CFBP 11053 ^T	<i>Syzygium aromaticum</i> , Sumatra, 1980
<i>Pseudomonas aeruginosa</i>	ATCC 10145 ^T	CFBP 2466 ^T	Host, origin, place and year unknown
<i>Pseudomonas agarici</i>	ICMP 2656 ^T	CFBP 2063 ^T	<i>Agaricus bisporus</i> , New Zealand, 1969
<i>Pseudomonas asplenii</i>	ICMP 3944 ^T	CFBP 3279 ^T	<i>Asplenium nidus</i> , place unknown, 1966
<i>Pseudomonas aureofaciens</i>	ATCC 13985 ^T	CFBP 2133 ^T	Host, origin, place and year unknown
' <i>Pseudomonas blatchfordae</i> '	ICMP 8933	CFBP 3280	<i>Phaseolus vulgaris</i> , USA, 1980
<i>Pseudomonas caricapapayae</i>	NCPPB 1873 ^T	CFBP 3204 ^T	<i>Carica papaya</i> , Brazil, 1966
<i>Pseudomonas chlororaphis</i>	ATCC 9446 ^T	CFBP 2132 ^T	Plate contaminant, place and year unknown
<i>Pseudomonas cichorii</i>	NCPPB 943 ^T	CFBP 2101 ^T	<i>Cichorium endivia</i> , Germany, 1929
<i>Pseudomonas cichorii</i>	NCPPB 906		<i>Chrysanthemum morifolium</i> , USA, 1957
<i>Pseudomonas flectens</i>	ICMP 745 ^T	CFBP 3281 ^T	<i>Phaseolus vulgaris</i> , Australia, 1956
<i>Pseudomonas fluorescens</i> biotype A	ATCC 13525 ^T	CFBP 2102 ^T	Water, UK, 1951
<i>Pseudomonas fuscovaginae</i>	CFBP 2065 ^T	NCPPB 3085	<i>Oryza sativa</i> , Japan, 1976
<i>Pseudomonas marginalis</i> pv. <i>alfalfae</i>	NCPPB 2644	CFBP 2039	<i>Medicago sativa</i> , USA, 1971
<i>Pseudomonas marginalis</i> pv. <i>marginalis</i>	CFBP 3300 ^T	LMG 2210 ^T	<i>Cichorium intybus</i> , USA, 1949
<i>Pseudomonas marginalis</i> pv. <i>pastinacae</i>	CFBP 2038 ^T	NCPPB 806	<i>Pastinaca sativa</i> , USA, 1959
<i>Pseudomonas putida</i> biotype A	CFBP 2066 ^T	ATCC 12633 ^T	Soil, USA, year unknown
<i>Pseudomonas</i> sp.	CFBP 2022		<i>Allium sativum</i> , France, 1976
<i>Pseudomonas stutzeri</i>	ATCC 17588	CFBP 2443	Spinal fluid, USA, 1970
<i>Pseudomonas syringae</i> pv. <i>atrofaciens</i>	NCPPB 2612		<i>Triticum aestivum</i> , Zimbabwe, 1973
<i>Pseudomonas syringae</i> pv. <i>delphinii</i>	NCPPB 2666	CFBP 1641	<i>Delphinium</i> sp., UK, 1974
<i>Pseudomonas syringae</i> pv. <i>helianthi</i>	NCPPB 1229		<i>Helianthus annuus</i> , Zambia, 1962
<i>Pseudomonas syringae</i> pv. <i>morsprunorum</i>	IMI 349210		<i>Prunus domestica</i> , UK, 1967
<i>Pseudomonas syringae</i> pv. <i>pisi</i>	NCPPB 123		Host unknown, Australia, 1938
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	CFBP 1542	NCPPB 2784	<i>Euonymus japonicus</i> , France, 1974
<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	NCPPB 214		<i>Nicotiana tabaccum</i> , Zimbabwe, 1948
<i>Pseudomonas tolaasii</i>	CFBP 2068 ^T	NCPPB 2192 ^T	<i>Agaricus bisporus</i> , UK, 1965
<i>Pseudomonas tolaasii</i>	CFBP 2810	NCPPB 3146	<i>Agaricus bisporus</i> , UK, 1981
<i>Ralstonia solanacearum</i>	CFBP 2047 ^T	NCPPB 325 ^T	<i>Lycopersicum esculentum</i> , USA, 1953
<i>Ralstonia solanacearum</i>		IMI 347554	<i>Solanum tuberosum</i> , Egypt, 1959

* A and B represent two different clones of the strain.

death. Prior & Rott (1989) have shown by biochemical characteristics that bacterial leaf-spot symptoms of anthurium were associated with an undescribed *Pseudomonas* sp. related to the previous large group of non-fluorescent pseudomonads now assigned to the

Comamonadaceae, and the genera *Ralstonia*, *Burkholderia* and *Herbaspirillum* (Kerstens *et al.*, 1996). Moreover they showed that this bacterium differs from other previously described phytopathogenic bacteria. Saddler *et al.* (1995) confirmed

the characterization of this bacterium, and suggested a similarity with the genus *Acidovorax*, using fatty acid profile and PCR-RAPD analyses.

Because of the incomplete description of this bacterium, and some uncertainty about its phylogeny, the present study was initiated to produce a full description based on phenotypic and physiological tests, fatty acids, DNA–DNA and DNA–rRNA hybridization analyses and 16S rRNA sequence analysis. Our results indicate clearly that the bacterium is a new phytopathogenic bacterium within the genus *Acidovorax*, and for which the name *Acidovorax anthurii* has been proposed.

METHODS

Bacteria. The data detailed below are based on a collection of 29 isolates from naturally infected *Anthurium lanceolatum* L. and hybrid anthuriums from Martinique (Table 1). The strains were isolated from leaf-spots and vascular necrosis of petioles and spathes on YBGA (0.7% yeast extract; 0.7% bacto-peptone; 0.7% glucose and 1.5% agar; pH 7.3). White-grey colonies evident on agar plates after 48 h reach a diameter of 1.5–2.0 mm. The cultures were preserved either by freeze-drying or on agar slopes under oil at 4 °C. In addition, reference strains from a variety of culture collections were also included in this study (Table 1).

Pathogenicity tests. Inocula were prepared by suspending 24-h-old bacterial growth from slants in sterile distilled water and adjusting the cell density to 5×10^8 c.f.u. ml⁻¹. Inoculation tests were made with all 29 isolates listed in Table 1 on both the leaf and spathe lamina by infiltration of a bacterial suspension in the mesophyll near the vein of leaves and spathes lamina of hybrid anthurium. Sterile distilled water was used as control. The inoculated plants were incubated for 6 weeks in a closed humid chamber in a greenhouse under a relative humidity of 90% and a mean temperature of 26 °C.

Biochemical and physiological tests. The presence of flagella was confirmed by the method of Rhodes (1958). Oxidation-fermentation of glucose was performed by the method of Hugh & Leifson (1953). Growth at 37 and 41 °C was observed using liquid nitrate medium and recorded after 5 d incubation. The presence of oxidase, gelatinase and arginine dihydrolase activities, fluorescent pigment on agar medium King's B, levan formation, acid production from sucrose, sorbitol, erythritol and mannitol, reduction of nitrates, hydrolysis of Tween 80 and aesculin and the hypersensitivity reaction on tobacco leaves were tested as described by Leliott *et al.* (1966). Pectinolytic activity was tested using the method of Prunier & Kaiser (1964) and Hildebrand (1971). Utilization of lactate L(+)tartrate and D(–)tartrate was tested on the basal medium of Ayers *et al.* (1919) supplemented with 0.1% (w/v) organic acid sodium salts. The presence of DNase was tested on DNA agar (Diagnostic Pasteur). Assimilation of 49 carbohydrates, 49 organic acids and 49 amino acids were studied using API 50CH, API 50AO and API 50AA strips (bioMérieux). The accumulation of granules of PHB was observed by the method of Ostle & Holt (1975) using Nile blue A. *Ralstonia solanacearum* CFBP 2047^T was used as a positive control.

Numerical taxonomy. A total of 167 characters was included in the numerical taxonomic analysis. The distance matrix

was calculated using the Jaccard coefficient (Sneath & Sokal, 1973). Cluster analysis was achieved using unweighted pair group method with averages (UPGMA) (Sneath & Sokal, 1973).

At a given distance level and for the different phenotypes, the amount of information for each test was measured by calculating the diagnostic capacity coefficient (DCC) (Descamps & Veron, 1981) to determine discriminating biochemical characteristics.

DNA extraction. Extraction and purification of DNA were performed using previously described methods (Brenner *et al.*, 1982). The strains studied are listed in Table 3.

DNA–DNA hybridization. Native DNAs were labelled *in vitro* by nick translation with tritium-labelled nucleotides (Amersham International). The S1 nuclease-trichloroacetic acid hybridization method was used as previously described (Crosa *et al.*, 1973). The reassociation temperature was 70 °C. DNA–DNA hybridization tests were carried out using labelled DNA from one strain CFBP 3232^T isolated from anthurium. DNA–DNA hybridization tests were repeated at least twice.

Thermal stability of DNA reassociation. The temperature at which 50% of reassociated DNA became hydrolysable by nuclease S1 (T_m) was determined using the method of Crosa *et al.* (1973). The T_m values were determined only if the percentage of DNA–DNA hybridization was between 65 and 70%.

16S rRNA gene sequencing analysis. 16S rRNA gene (rDNA) fragments were amplified by PCR as previously described (Dauga, 1993). Primers including deoxy-UMP residues at the 5' end were designed to amplify a 1500 base fragment of 16S rDNA. The amplified product was purified using a Jet Pure system (Genomed), then cloned in *Escherichia coli* MC1061 with pAMP1 plasmid from the Cloneamp system (Gibco-BRL). Three clones were sequenced using [γ -³²P]-labelled dATP and Hot-Tub polymerase (Amersham International) by cycle sequencing (Blakesley, 1993).

16S rRNA gene sequence analysis. The sequences of the organisms used for alignment and the similarity matrix were obtained from GenBank/EMBL through DNASAR. The reference sequences were also taken from the Ribosomal Database Project (Maidak *et al.*, 1997) and the rRNA WWW server of the University of Antwerp (Belgium) (Van de Peer *et al.*, 1994). The sequences being compared were aligned manually.

Distance analyses were performed using the PHYLIP package in the DNADIST program (Felsenstein, 1989). Divergence between two sequences was estimated using the following formula: $K_{nuc} = -1/2 \ln [(1 - 2P - Q)(1 - 2Q)^{1/2}]$ (Kimura, 1980). A phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). Parsimony analysis was performed with the PAUP program using the heuristic option (Swofford, 1990). The robustness of each topology was evaluated under the two methods described through 100 bootstrap replications. A maximum-likelihood analysis was also done with fastDNAML (Felsenstein, 1981; Olsen *et al.*, 1994).

Nucleotide sequence accession numbers. The EMBL/GenBank accession numbers of the 16S rRNA sequences used in the phylogenetic analyses were as follows: *Burkholderia cepacia* ATCC 25416^T, M22518 and M22467; *Burkholderia* sp. GSY, U16140; *Burkholderia* sp. 2 K172, X77118; *Burkholderia* sp. 3 KB740, X77679; *Burkholderia*

Fig. 1. Dendrogram of the distances among the 71 strains studied.

andropogonis ATCC 23061, X67037; *Ralstonia solanacearum* biovar 2 ACM 158, X67035; *Ralstonia solanacearum* biovar 3 ATCC 11696^T, X67036; *Ralstonia solanacearum* biovar 4 ACH 092, X67040; *Ralstonia solanacearum* biovar 5 ACH 0171, X67041; *Ralstonia pickettii* ATCC 27511^T, S55004; *Telluria chitinolytica* sb. 2 K172, X65590; *Telluria mixta* str. ACM17, X65589; *Acidovorax facilis* DSM 649^T, X59169; *Variovorax paradoxus* IAM 12373, D30793; *Comamonas testosteroni* ATCC 11996, M11224; *Delftia acidovorans* DSM 39^T, X59168; *Pseudomonas putida* mt2, L28676; *Pseudomonas mendocina* ATCC 25411, M59154; *Pseudomonas aeruginosa* ATCC 10145^T, X06684; *Brevundimonas diminuta* ATCC 11568, M59064; *Halomonas halophilum* DSM 4770, M93353; *Halomonas marina* ATCC 25374, M93354; *Halomonas aquamarina* ATCC 14400, M93352; *Stenotrophomonas maltophilia* ATCC 13637, M59158.

DNA base composition. To determine the G+C content, purified DNA was diluted in 0.1 × SCC buffer at a final concentration of 50 µg ml⁻¹. The G+C content was determined by the thermal denaturation temperature (Marmur

& Doty, 1962) and was calculated using the equation of Owen & Lapage (1976). The DNA from *P. corrugata* CFBP 2431^T (G+C content 60 mol %) was used as a standard.

DNA-rRNA hybridization. The hybridization method of De Ley & De Smedt (1975) was used. Each rRNA-DNA hybrid was characterized by its *T_m*(e) (the temperature at which 50% of rRNA-DNA hybrid was denatured). Labelled (³H) rRNA from *Acidovorax avenae* subsp. *avenae* NCPPB 1011^T was used.

Fatty acid extraction and analysis. Biomass was prepared by harvesting cultures grown on Trypticase Soy Broth Agar (TSBA) for 48 h at 28 °C as described previously (Sasser, 1990). Fatty acids were extracted and derivatised using the method of Miller & Berger (1985). The resultant fatty acid methyl esters were analysed by GC (Hewlett Packard 5890) in conjunction with the Microbial Identification System (MIDI). Each fatty acid profile was repeated six times, with a fresh cultivation/extraction conducted on each occasion. The mean profile of strains were clustered using the UPGMA

protocol from Euclidean distance measurements using software from MVSP (Anglesey, UK).

RESULTS

Pathogenicity

The pathogenicity of 25 out of 29 strains studied was confirmed using anthurium plants. Three to five days after inoculation, tissues appeared brown to black. Subsequently the margin of the necrotic lesion became water-soaked. After more than 10 d, the development of the disease was halted and the necrotic lesions were surrounded by a typical bright yellow halo. At the

same time the vascular development of the disease was observed resulting in abscission of leaf and spathe petioles. After 4 weeks incubation we observed typical symptoms on leaves and spathes which had not been artificially inoculated. The bacteria were easily reisolated in pure culture especially from water-soaked tissues, throughout the incubation period. Strains CFBP 3235, 3239, 10908 and 3238 did not induce any symptoms.

The pathogenic strains isolated from anthurium displayed characteristics common to the genus *Acidovorax*: straight rods, 0.2–0.7 × 1.0–5.0 µm, occurring

Table 2. Characteristics that differentiate *Acidovorax anthurii* from other *Acidovorax* species and other plant-pathogenic species: *Pseudomonas*, *Herbaspirillum*, *Burkholderia* and *Ralstonia* tested

Strain	Phenon	Utilization of:*																		HR† on tobacco
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
DCC‡ ...		0.97	0.91	0.85	0.83	0.81	0.81	0.80	0.79	0.77	0.76	0.71	0.71	0.69	0.68	0.68	0.65	0.64	0.53	0.75
<i>Acidovorax anthurii</i>	1	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	–	–	+
<i>Acidovorax</i> sp. (lambs' lettuce)	2	–	–	–	–	–	d	–	–	–	–	–	–	–	+	+	+	–	–	d
<i>Acidovorax</i> sp.	3	–	d	+	+	–	d	d	d	+	d	–	d	d	+	+	+	–	+	+
<i>A. avenae</i>		–	–	d	+	–	+	–	–	d	–	–	d	NT	+	+	+	–	d	NT
<i>A. konjaci</i>		–	–	+	–	–	+	–	–	–	–	–	–	NT	+	+	+	–	+	NT
<i>A. delafieldii</i>		–	–	+	+	–	+	–	–	+	–	–	–	NT	+	–	+	–	+	NT
<i>A. temperans</i>		–	–	–	+	–	+	–	–	d	–	–	–	NT	+	+	+	–	+	NT
<i>A. facilis</i>		–	–	+	+	–	–	–	–	–	+	–	–	NT	+	+	+	–	–	NT
Fluorescent pseudomonads	4	+	+	+	+	+	+	+	+	d	+	+	d	+	d	–	–	d	+	–
<i>Burkholderia</i> sp.	5	+	+	+	+	+	+	d	+	+	d	–	+	d	d	+	+	+	+	d
Strains from anthurium	6	+	–	+	+	+	+	+	+	+	+	–	+	d	–	–	–	+	+	–
<i>P. agarici</i>		–	+	+	+	–	+	–	–	–	–	+	–	–	–	–	–	+	+	–
<i>P. caricapapayae</i> CFBP 3204 ^T		–	+	+	+	–	+	+	+	+	+	–	+	+	–	–	–	–	+	+
<i>P. caricapapayae</i> CFBP 2429		+	–	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	+	–
<i>P. cichorii</i>		–	+	+	+	–	+	–	+	–	+	+	+	+	+	–	–	–	+	+
<i>P. putida</i>		+	+	+	+	–	+	+	–	–	+	+	–	+	–	–	–	+	–	–
<i>P. meliae</i>		–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	+	–	+
<i>B. andropogonis</i>		–	–	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–	+	+
<i>P. cissicola</i>		+	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	+	–	–
<i>P. pseudoalcaligenes</i>		–	–	–	–	–	+	+	–	–	–	+	–	+	–	+	–	–	–	–
<i>H. rubrisubalbicans</i>		–	+	+	+	–	–	+	–	+	–	+	+	–	–	+	–	–	+	–
<i>P. stutzeri</i>		–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	+	–
<i>B. plantarii</i>		+	+	+	+	+	–	+	+	+	–	–	+	+	+	–	+	+	+	–
<i>R. solanacearum</i>		–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–
Strain CFBP 3228 (from anthurium)		+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Strain 4696 (lambs' lettuce)		+	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–

NT, Not tested.

* Numbers represent: 1, trehalose; 2, caprylate; 3, D-ribose; 4, D-glucose; 5, N-acetylglucosamine; 6, L-arginine; 7, saccharose; 8, inositol; 9, sorbitol; 10, sarcosine; 11, itaconate; 12, D-xylose; 13, DL-5-aminobutyrate; 14, D(–)tartrate; 15, L-tryptophan; 16, azelate; 17, aesculin; 18, mannitol.

† HR, Hypersensitive reaction.

‡ DCC, Diagnostic Capacity Coefficient.

Table 3. Levels of DNA relatedness among *A. anthurii*, *Acidovorax avenae* and *A. konjaci*, *Burkholderia* and plant-pathogenic *Pseudomonas* strains

Relative binding (%) at 70 °C with labelled DNA from *A. anthurii* CFBP 3232^T.

Taxon	CFBP no.	Rel. binding to CFBP 3232 ^T DNA (%)	ΔT_m (°C)
<i>A. anthurii</i>	3232 ^T	100	0.1
<i>A. anthurii</i>	3237	96 (5.7)	0.9
<i>A. anthurii</i>	3244	99 (4)	0.2
<i>A. anthurii</i>	3248	85 (9)	0.9
<i>A. anthurii</i>	3249	86 (7.8)	1.6
<i>A. anthurii</i>	3250	86 (10.5)	0.3
<i>A. anthurii</i>	3282	84 (6.4)	1.0
<i>A. anthurii</i>	10910	79 (3.2)	0.9
<i>A. anthurii</i>	3239	3	
<i>A. avenae</i> subsp. <i>avenae</i>	1201	34	
<i>A. avenae</i> subsp. <i>avenae</i>	2425	34	
<i>A. avenae</i> subsp. <i>cattleyae</i>	2423	37	
<i>A. avenae</i> subsp. <i>citrulli</i>	10880	34	
<i>A. konjaci</i>	10881	44	
<i>Acidovorax</i> sp. (lambs' lettuce)	4694	27	
<i>R. solanacearum</i>	2047	7	
<i>B. cepacia</i>	2027	7	
<i>B. gladioli</i> pv. <i>alliiicola</i>	2422	7	
<i>B. gladioli</i> pv. <i>gladioli</i>	2427	8	
<i>B. caryophylli</i>	2429	3	
<i>B. glumae</i>	2430	5	
<i>B. plantarii</i>	3199	8	
<i>B. andropogonis</i>	2421	3	
<i>H. rubrisubalbicans</i>	1202	6	
<i>P. cissicola</i>	2432	3	
<i>P. pseudoalcaligenes</i> subsp. <i>pseudoalcaligenes</i>	2435	3	
<i>P. alcaligenes</i>	2437	0	
<i>P. syzygii</i>	11053	4	
<i>P. meliae</i>	3225	1	
<i>P. aeruginosa</i>	2466	0	
<i>P. agarici</i>	2063	0	
<i>P. asplenii</i>	3279	0	
<i>P. aureofaciens</i>	2133	3	
' <i>P. blatchfordae</i> '	3280	1	
<i>P. caricapapayae</i>	3204	1	
<i>P. chlororaphis</i>	2132	1	
<i>P. cichorii</i>	2101	0	
<i>P. flectens</i>	3281	0	
<i>P. fuscovaginae</i>	2065	0	
<i>P. fluorescens</i>	2102	0	
<i>Pseudomonas</i> sp.	2022	0	
<i>P. marginalis</i> pv. <i>marginalis</i>	1387	1	
<i>P. marginalis</i> pv. <i>pastinacae</i>	2038	4	
<i>P. tolaasii</i>	2068	1	
<i>P. tolaasii</i>	2810	0	
<i>P. putida</i>	2066	0	

* Values in parentheses are standard deviation.

Table 4. $T_m(e)$ values of hybrids between DNAs from *Acidovorax anthurii* CFBP 3240 and 3232^T and labelled rRNA from *A. avenae* subsp. *avenae* NCPPB 1011^T

DNA from strain:	$T_m(e)$ (°C) vs [³ H]rRNA from <i>A. avenae</i> subsp. <i>avenae</i> NCPPB 1011 ^T	$\Delta T_m(e)$
<i>A. avenae</i> subsp. <i>avenae</i> NCPPB 1011 ^T	78.8	0
<i>A. konjaci</i> ICMP 7851	75.7	3.1
<i>A. anthurii</i> CFBP 3240	78.4	0.4
<i>A. anthurii</i> CFBP 3232 ^T	77.2	1.6

Fig. 2. rRNA cistron similarity dendrogram of *Acidovorax anthurii* within the Comamonadaceae, based on $T_m(e)$ values (in °C) of DNA-rRNA hybrids.

mostly singly, motile by long polar flagella, Gram-negative, oxidase-positive, urease-positive, strictly aerobic, poly- β -hydroxybutyrate is accumulated intracellularly. Colonies were circular, raised with an entire margin and white-creamy on medium King's B and YPGA. No fluorescent pigment was produced on

medium King's B but a brown diffusible pigment is generally produced when grown on YBGA. All isolates elicited a hypersensitive reaction on tobacco leaves cv. samsun.

Numerical analysis

The dendrogram displaying the distance relationships amongst the 71 strains, studied is shown in Fig. 1. At the distance 0.39, six phena and 15 isolated strains can be distinguished. The distribution of species and pathovars in the six phena and isolated strains is shown in Table 2. Phenon 1 contained 25 out of the 29 of the strains isolated from anthurium. Phenon 2 included four out of the five strains of *Acidovorax* sp. isolated from a necrosis on lambs' lettuce, the fifth strain, CFBP 4696 demonstrated a separate phenotype. Phenon 3 encompasses four strains of *Acidovorax avenae* and the type strain of *A. avenae* subsp. *cattleyae*, CFBP 2423. Phenon 4 includes 15 type strains of fluorescent and non-fluorescent *Pseudomonas* spp. belonging to the genus *Pseudomonas sensu stricto*. Phenon 5 includes strains of three *Burkholderia* spp. Phenon 6 contains three non-pathogenic strains isolated from necrotic lesions of anthurium whose identity is not known. Fifteen strains did not cluster at the distance of 0.39. Among these strains are 11 type strains of fluorescent and non-fluorescent *Pseudomonas* spp., two type strains of *Burkholderia*, *B. andropogonis*, *B. plantarii* and two unidentified strains, one isolated from anthurium and the other isolated from lambs' lettuce (Fig. 1).

Using the Diagnostic Capacity Coefficient (DCC) we selected 19 biochemical and physiological tests that differentiated the strains clustered in the different phena or isolated type strains (Table 2). *A. anthurii* could be distinguished from *Acidovorax* sp. (lambs' lettuce) and reference strains of *Acidovorax* of phenon 3 by two and five tests, respectively (Table 2). The differentiation between *A. anthurii* and strains of fluorescent *Pseudomonas* (phenon 4), *Burkholderia* (phenon 5) and other isolates from anthurium is possible by a range of biochemical tests (Table 2). This was also true for the rest of the strains tested here (Table 2).

DNA-DNA hybridization

Results of DNA hybridization studies are shown in Table 3. Nine representative *A. anthurii* strains from phenon 1 are 79–100% similar to the strain CFBP 3232^T. The ΔT_m values ranged from 0.1 to 1.6 °C. Strain CFBP 3239 from phenon 6, isolated from anthurium hybridized only 3.0% with the pathogenic strain CFBP 3232^T.

Representative strains of the three subspecies of *Acidovorax avenae*, *Acidovorax konjaci*, *Acidovorax* sp. CFBP 4694 were 27–44% related to *A. anthurii* CFBP 3232^T. Seven species of *Burkholderia* and seven species of non-fluorescent *Pseudomonas* were related only 0–8% to strain CFBP 3232^T. All strains of

Fig. 3. Phylogenetic tree obtained with the maximum likelihood analysis (fastDNAmI) showing the clustering of *Acidovorax anthurii* and *Acidovorax facilis*.

fluorescent *Pseudomonas* spp. included were distantly related (0–4%) to strain CFBP 3232^T.

DNA base composition

The DNA G+C base composition of strain CFBP 3232^T was found to be 63.5 mol %.

DNA–rRNA hybridization

To verify the assignment of *A. anthurii* to the branch of *A. avenae* within the family *Comamonadaceae*, DNA from *A. anthurii* CFBP 3232^T and 3240 and one strain of *A. konjaci* ICMP 7851 were hybridized with labelled rRNA of *A. avenae* subsp. *avenae* NCPPB 1011^T. The results are shown in Table 4.

Two strains CFBP 3232^T and 3240 belonged to the *Acidovorax* rRNA branch and are thus members of the genus *Acidovorax*. Because of $\Delta T_m(e)$ values of 0.4–1.6 °C, this new species is more closely related to *A. avenae* subsp. *avenae* than to *A. konjaci* (Fig. 2). We could position *A. anthurii* within the family *Comamonadaceae* and the genus *Acidovorax* (Fig. 2).

16S rRNA gene sequence analysis

The sequence of 1487 nucleotides of 16S rDNA was determined, corresponding to positions 8–1509 in the *E. coli* numbering system (Brosius *et al.*, 1978). Alignment of the sequence with related members of the

Fig. 4. Dendrogram of mean fatty acid profiles from strains of *Acidovorax anthurii* and reference strains from the families *Comamonadaceae*, *Burkholderiaceae* and *Pseudomonadaceae*, as calculated from Euclidean distance with clustering achieved by UPGMA.

β subclass of the *Proteobacteria* reveals common signatures at positions 50, 812, 955, 976, 1233 and 1234 (Woese, 1987).

The phylogenetic tree obtained with the maximum-likelihood method is shown in Fig. 4. The overall phylogenetic structure obtained fits with known information on the *Pseudomonadaceae* (Kerstens *et al.*, 1996). Members of the rRNA group I, considered to contain the authentic genus *Pseudomonas* are in the γ subclass of the *Proteobacteria*. Members of the rRNA group II and III, the pathogens or opportunistic pathogens of animals and plants are in the β subclass of the *Proteobacteria* (De Vos *et al.*, 1985). Our strain branched in the vicinity of *Acidovorax facilis*, *Variovorax paradoxus*, *Delftia acidovorans* and *Comamonas testosteroni*, which represent the *Comamonadaceae*. Clustering of these four species with *A. anthurii* was also observed in the phylogenetic tree obtained with the parsimony method and the neighbour-joining method (data not shown) done only on partial sequences because long stretches of rRNA sequences were not available for *C. acidovorans* and

Table 5. Fatty acid profiles for the type strains of the genus *Acidovorax*

Data are mean percentage compositions, with standard deviations in parentheses. Species: 1, *A. anthurii* CFBP 3232^T; 2, *A. anthurii*; 3, *A. a. subsp. avenae* (= *P. rubrilineans*) CFBP 1201; 4, *A. avenae* subsp. *avenae* CFBP 2425; 5, *A. avenae* subsp. *cattleyae* CFBP 2423; 6, *A. avenae* subsp. *citrulli* CFBP 10880; 7, *A. delafieldii* NCIMB 11476; 8, *A. facilis* NCIMB 9938; 9, *A. konjaci* CFBP 10881. Abbreviations: CFBP, Collection Française des Bactéries Phytopathogènes, Angers, France; NCIMB: National Collection of Industrial and Marine Bacteria, Aberdeen, UK.

Fatty acids*	Species:								
	1	2†	3	4	5	6	7	8	9
10:0 3-OH	1.9(0.1)	2.0(0.2)	3.0(0.1)	3.4(0.2)	3.4(0.3)	3.0(0.2)	2.8(0.1)	2.7(0.1)	3.3 (0.3)
12:0	2.5(0.2)	2.8(0.2)	2.2(0.0)	2.6 (0.4)	2.3(0.1)	2.3(0.1)	2.8(0.1)	2.8(0.1)	2.8 (0.1)
14:0	1.8(0.2)	3.0(0.1)	2.0(0.1)	2.4(0.3)	1.8(0.1)	1.6(0.2)	3.4(0.2)	2.9(0.1)	3.5 (0.1)
15:1	2.0(0.8)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	2.6(1.1)	0.0(0.0)	0.9(0.2)	0.0(0.0)
15:0	11.1(2.2)	1.9(0.3)	0.4(0.1)	0.1(0.1)	0.6(0.1)	6.7(2.2)	0.3(0.2)	2.0(0.1)	0.4(0.2)
16:1	37.8(1.6)	42.7(0.8)	41.0(1.0)	42.9(3.2)	40.8(0.6)	40.7(1.6)	39.5(0.4)	43.3(0.2)	42.8(0.7)
16:0	22.6(2.8)	28.2(1.6)	33.1(0.2)	32.2(2.1)	35.2(0.6)	33.7(2.3)	27.4(1.6)	28.1(0.1)	31.1(0.9)
17:0cyclo	0.0(0.0)	0.0(0.0)	0.7(0.1)	0.2(0.3)	0.5(0.7)	0.0(0.0)	0.0(0.0)	1.1(0.1)	0.0(0.0)
17:0	4.8(1.0)	0.8(0.2)	0.1(0.2)	0.0(0.1)	0.3(0.2)	1.6(0.3)	0.1(0.1)	1.2(0.2)	0.2(0.2)
18:1cis11	12.7(1.3)	18.4(1.8)	15.5(0.7)	15.7(1.7)	14.5(1.0)	7.6(0.3)	23.4(1.0)	15.1(0.2)	15.6(0.5)
18:0	0.2(0.3)	0.1(0.1)	0.4(0.0)	0.3(0.1)	0.1(0.1)	0.0(0.1)	0.1(0.2)	0.0(0.0)	0.0(0.1)

* Fatty acids are listed using standard abbreviations (no. carbon atoms:no. double bonds). Each strain was extracted and analysed six times and data represent the mean value (standard deviation).

† Raw fatty acid data collected from CFBP 3231, 3240, 3245, 3248, 3251, 3252 and 3282.

A. facilis. The bootstrap value of this clustering was significantly high: 95.4% with the parsimony method, 76% with the neighbour-joining method (partial sequences) and the branch was significantly positive at $P < 0.01$ % in the maximum-likelihood analysis.

Similarity values were calculated for the closest relatives to the new *Acidovorax* species. The level of similarity value (approx. 92% with *A. facilis*) would indicate that this organism is a new species (Stackebrandt & Goebel, 1994).

Fatty acid analysis

Analysis of quantitative fatty acid data are presented in the form of a dendrogram (Fig. 3). All of the anthurium isolates cluster with reference strains from the family *Comamonadaceae*, and are clearly separated from species of the families *Burkholderiaceae* and *Pseudomonadaceae*. Closer inspection reveals that CFBP 3232^T is distinct from other *A. anthurii* strains.

The fatty acid data for all species of the genus *Acidovorax* studied here are shown in Table 5. Profiles were highly reproducible and the standard deviation was rarely more than 10% of the mean value. Qualitatively the profiles were very similar, with large amounts of hexadecanoic (16:0) and hexadecenoic (16:1) acids found in all strains. In addition, 3-hydroxydecanoic acid (10:0 3-OH) was the only hydroxylated fatty acid present in all the members of the genus *Acidovorax* studied here. CFBP 3232^T was found to have a slightly different fatty acid profile from

other anthurium isolates with significant amounts of pentadecanoic (15:0) and pentadecenoic (15:1) acids.

DISCUSSION

Twenty-nine strains isolated from bacterial leaf-spot of anthurium were compared with 42 strains including phytopathogenic type strains belonging to the genera *Acidovorax*, *Burkholderia*, *Ralstonia* and *Pseudomonas*. The analysis by numerical taxonomic methods of the 167 physiological and biochemical characteristics, summarized in a dendrogram demonstrated that 25 out of 29 strains pathogenic for anthurium, fell into phenon 1 defined at the distance 0.39. The closest relative belonging to phenons 2 and 3 corresponded to strains of a new *Acidovorax* sp. isolated from lambs' lettuce (distance level 0.45) and strains of *A. avenae* subsp. *avenae* and *A. avenae* subsp. *cattleyae* (distance level 0.48), respectively. On the basis of these phenotypic characteristics strains isolated from anthurium can be easily identified.

DNA–DNA hybridization results confirmed the findings of phenotypic analysis, since nine strains from phenon 1 showed a high level of mean relative binding with strain CFBP 3232^T (89.7 SD 7.2). One strain CFBP 3239 representative of phenon 6, isolated from anthurium but not pathogenic on this host, showed very low hybridization with CFBP 3232^T. Five reference strains of *A. avenae* (subsp. *avenae*, subsp. *cattleyae*, subsp. *citrulli*) and *A. konjaci*, showed low levels of relatedness ranging from 27 to 44% to members of phenon 1. *A. facilis*, *A. delafieldii* and *A.*

temperans were not included because they belong to another rRNA branch (Fig. 2). All the other strains including those belonging to the genera *Burkholderia*, *Ralstonia* and *Pseudomonas* exhibited levels of hybridization below 8%.

The G + C value of strain CFBP 3240 was 66.2 mol% compared to 57.4–70.8 mol% for strains of *A. avenae* subsp. *avenae* and *A. avenae* subsp. *cattleyae* (Willems *et al.*, 1992). Therefore the G + C ratio of this new bacterium is within the range of the genus *Acidovorax*. The results from our DNA studies clearly indicate that the strains isolated from anthurium constitute a homogeneous new species under the species definition of Wayne *et al.* (1987).

By rRNA–DNA hybridization this new species can be assigned to the β subclass *sensu* Stackebrandt *et al.* (1988), and to rRNA superfamily III *sensu* De Ley (1978), more specifically the *Comamonadaceae* family and the genus *Acidovorax*. It belongs in the *A. avenae*–*A. konjaci* sub-branch. Despite the lack of complete 16S rRNA sequences in databases the phylogenetic trees obtained agree with this result.

Comparison of the fatty acid data reveal the presence of 3-hydroxydecanoic acid as the sole hydroxylated fatty acid present in all anthurium isolates (Table 5), which is typical of members of the genus *Acidovorax* in particular (Oyaizu & Komagata, 1983; Willems *et al.*, 1990; Stead, 1992). Examination of the raw data from this study has shown that the presence or absence of pentadecenoic acid (15:1) is the major qualitative difference between CFBP 3232^T and other *A. anthurii*. Differences in the fatty acid profiles of strains of the same species have been recorded previously, Stead (1992) found that *Burkholderia* (*Pseudomonas*) *glumae* comprised two distinct populations, similarly in a previous study of *A. anthurii* strains isolated from diseased anthuriums from Trinidad, isolates were recovered in two distinct clusters (Saddler *et al.*, 1995).

Thus all our results are in favour of the description of a new species and we propose the name *Acidovorax anthurii*. The description below is based on the results presented above, as well as on those obtained by Prior & Rott (1989).

Description of *Acidovorax anthurii* sp. nov.

Acidovorax anthurii (an.thu.rii. L. fem. n. *Anthurium* anthurium; L. gen. n. *anthurii* of anthurium, referring to the plant from which this phytopathogenic bacterium was first isolated).

On YBGA, colonies are circular, raised with an entire margin and white-creamy and brown diffusible pigment is produced. Cells are Gram-negative, straight rods, 0.2–0.7 × 1.0–5.0 μ m, motile by a long polar flagellum. Oxidase, catalase and urease are positive. Strictly aerobic, poly- β -hydroxybutyrate is accumulated in the cell, arginine is used as sole source of carbon. H₂S is produced from cysteine and cellulose is hydrolysed. Indole, levane and acetoin are not

produced. Casein and aesculin are not hydrolysed. Acid is produced from galactose, arabinose and glycerol. Acetate, formate, glycerol, DL-5-amino-butyrates, D(–) tartrate and azelate are utilized. Trehalose, caprylate, D-ribose, D-glucose, N-acetylglucosamine, L-arginine, saccharose, inositol, sarcosine, itaconate, D-xylose, L-tryptophan, aesculin and mannitol are not utilized. The sole hydroxylated fatty acid present is 3-hydroxy-decanoic acid. The G + C content of the strain CFBP 3232^T determined by thermal denaturation method is 63.5 mol%. All strains elicit a hypersensitive reaction on tobacco leaves (HR) and are pathogenic on anthurium, producing typical leaf-spot symptoms. The type strain has been deposited in the CFBP, Angers, France, as CFBP 3232^T.

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