Acidovorax valerianellae sp. nov., a novel pathogen of lamb's lettuce [*Valerianella locusta* (L.) Laterr.]

Louis Gardan,[1] David E. Stead,[2] Catherine Dauga[3] and Moniek Gillis[4]

Correspondence
Louis Gardan
gardan@angers.inra.fr

[1]INRA, UMR de Pathologie Végétale, INRA-INH-Université 42, rue G. Morel, 49071 Beaucouzé cedex, France

[2]Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK

[3]Unité des Entérobactéries, Institut Pasteur, Paris, France

[4]Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium

Bacterial spot disease of lamb's lettuce [*Valerianella locusta* (L.) Laterr.] was first observed in fields in 1991. This new bacterial disease is localized in western France in high-technology field production of lamb's lettuce for the preparation of ready-to-use salad. Nineteen strains isolated in 1992 and 1993 from typical black leaf spots of naturally infected lamb's lettuce were characterized and compared with reference strains of *Acidovorax* and *Delftia*. The pathogenicity of the 19 strains was confirmed by artificial inoculation. Biochemical and physiological tests, fatty acid profiles, DNA–DNA hybridization and other nucleic acid-based tests were performed. A numerical taxonomic analysis of the 19 lamb's lettuce strains showed a single homogeneous phenon closely related to previously described phytopathogenic taxa of the genus *Acidovorax*. DNA–DNA hybridization studies showed that the lamb's lettuce strains were 91–100 % related to a representative strain, strain CFBP 4730^T, and constituted a discrete DNA hybridization group, indicating that they belong to the same novel species. Results from DNA–rRNA hybridization, 16S rRNA sequence analysis and fatty acid analysis studies confirmed that this novel species belongs to the β-subclass of the *Proteobacteria* and, more specifically, to the family *Comamonadaceae* and the genus *Acidovorax*. The name *Acidovorax valerianellae* sp. nov. is proposed for this novel taxon of phytopathogenic bacteria. The type strain is strain CFBP 4730^T (=NCPPB 4283^T).

Lamb's lettuce, also known as corn salad [*Valerianella locusta* (L.) Laterr.], was traditionally cultivated in France for use in salads in autumn and winter. Since 1985, advanced technology has been used for continuous, year-round cultivation; 90 % of French production (representing 75 % of world production) is concentrated in western France, near the Atlantic coast. The lettuce produced is used for sale traditionally, in trays, and for the preparation of ready-to-use salad alone or mixed with other salad leaves in sealed plastic bags (Péron & Rees, 1998).

The advanced technology of cultivation of lamb's lettuce has changed the profile of plant disease, since it has created microclimatic conditions more favourable for the development of disease. Diseases due to fungi, e.g. *Peronospora valerianellae* and *Phoma valerianellae*, remain important (Péron & Rees, 1998).

Published online ahead of print on 18 October 2002 as DOI 10.1099/ijs.0.02404-0.

The EMBL accession number for the 16S rDNA sequence of strain CFBP 4730^T is AJ431731.

An expanded version of Fig. 3 including more reference taxa is available as supplementary material in IJSEM Online.

In 1991, water-soaked spots that became black after 3–4 days were observed on cotyledons and leaves of crops in western France. Bacteria were consistently isolated from water-soaked spots. The pathogenicity of the strains was confirmed by artificial spray leaf inoculations. The bacterium was initially identified as a non-fluorescent *Pseudomonas* sp. (Rat & Gardan, 1993; Rat *et al.*, 1994).

The present study was initiated to make a formal description of this novel phytopathogenic group of bacteria, based on phenotypic and physiological tests, fatty acid profiles, DNA–DNA and DNA–rRNA hybridization and 16S rRNA sequence analysis. Our conclusion is that this group of strains represents a novel species within the genus *Acidovorax*, for which the name *Acidovorax valerianellae* sp. nov. is proposed.

A collection of 19 strains were isolated from naturally infected plants in 1992 and 1993 from samples taken in different plots of cultivation of lamb's lettuce over a large area in western France, where around 2000 ha are grown. Reference strains included in this study are listed in Table 1. All the strains were grown at 25 °C on YBGA (0·7 % yeast extract, 0·7 % bactopeptone, 0·7 % glucose and 1·5 % agar; pH 7·3) (Gardan *et al.*, 2000).

Table 1. Strains used in this study

Strain(s) as received	Other designation	Host plant/origin, place and year of isolation
Acidovorax valerianellae sp. nov. CFBP 4720–CFBP 4739		*Valerianella locusta* L., western France, 1992 and 1993
Acidovorax anthurii NCPPB 4104^T	CFBP 3232^T	*Anthurium* sp. (hybrid), Martinique, 1991
Acidovorax avenae subsp. *avenae* NCPPB 1011^T	CFBP 2425^T	*Zea mays*, USA, 1958
Acidovorax avenae subsp. *cattleyae* NCPPB 961^T	CFBP 2423^T	Unknown orchid, USA, 1961
Acidovorax avenae subsp. *citrulli* NCPPB 3679^T	CFBP 4459^T	*Citrullus lanatus*, USA, NK
Acidovorax delafieldii DSM 64^T		NK
Acidovorax facilis DSM 649^T		NK
Acidovorax konjaci NCPPB 3698^T	CFBP 4460^T	*Amorphophallus konjaci*, Japan?
Delftia acidovorans NCPPB 1967		Pharyngeal swab, 1966
Delftia acidovorans NCPPB 1968		NK, 1966

NK, Not known.

For pathogenicity tests, bacterial suspensions adjusted to approx. 5×10^8 c.f.u. ml^{-1} were applied on the adaxial and abaxial parts of the leaves by rubbing the leaf surfaces with a piece of sterile cotton wetted with bacterial suspension. Plants were used at the fifth to sixth leaf stage and were maintained under high relative humidity ($\geqslant$90 %) in plastic tunnels.

The 20 classical biochemical and physiological tests and 147 carbon-assimilation sources used were those enumerated by Gardan *et al.* (2000). In total, 167 characteristics were included in the numerical taxonomy analysis. A distance matrix was calculated using the Jaccard coefficient (Sneath & Sokal, 1973). Cluster analysis was done using UPGMA.

A total of 100 fatty acid profiles were prepared for 10 strains isolated from *V. locusta* and from representative strains of *Acidovorax* species with validly published names and known to cause plant disease, together with *Delftia acidovorans*, *Acidovorax facilis* and *Acidovorax delafieldii* (Table 2). These were all from the National Collection of Plant-Pathogenic Bacteria (NCPPB) and comprised almost all the *Acidovorax* and *Delftia* strains in the collection. They are not listed in Table 1. Cells were harvested from trypticase soy broth agar plates grown for 24 h at 28 °C. Replicate runs were made for some strains to ensure that differences in the profiles were not due to problems with reproducibility. Fatty acids were extracted as methyl esters by using methods described elsewhere (Stead, *et al.*, 1992;

Table 2. Fatty acids detected in selected *Acidovorax* and *Delftia* species

Species: 1, *A. valerianellae* sp. nov. (10 strains analysed/10 profiles obtained); 2, *A. anthurii* (2/9); 3, *A. avenae* subsp. *avenae* (28/35); 4, *A. avenae* subsp. *cattleyae* (1/4); 5, *A. avenae* subsp. *citrulli* (5/11); 6, *A. delafieldii* (1/10); 7, *A. facilis* (1/5); 8, *A. konjaci* (4/7); 9, *D. acidovorans* (2/2). Values are mean percentages ± SD of the total named peak area.

Fatty acid	1	2	3	4	5	6	7	8	9
9:0 3-OH		0·2±0·1							
10:0			0·2±0·1	0·2±0·2				0·2±0·2	
10:0 3-OH	2·1±0·2	1·9±0·3	3·1±0·5	3·7±0·2	3·2±0·4	2·6±0·6	2·3±0·1	3·0±0·1	2·7±0·1
11:0		0·3±0·1			0·1±0·1				
12:0	2·9±0·1	2·3±0·2	2·3±0·1	2·3±0·1	2·4±0·2	3·1±0·5	3·1±0·2	2·5±0	2·6±0·2
12:1 3-OH				0·6±0·1	0·2±0·3				
13:0		0·4±0·1			0·1±0·1				
14:0	3·6±0·1	1·6±0·2	2·2±0·2	1·7±0·1	1·6±0·2	3·5±0·4	3·0±0·1	3·0±0	0·7±0·1
15:1ω6*c*	0·4±0·3	2·8±1·0			1·6±0·9				0·2±0·3
15:0	3·8±0·9	12·5±2·6	0·2±0·1	0·5±0·1	5·5±2·2	0·1±0·2	0·1±0·1	0·4±0·1	1·8±1·6
16:1ω7*c*	46·3±0·5	37·9±1·6	42·9±1·0	40·6±0·3	41·9±0·3	40·9±0·3	43·3±0·6	43·1±0·4	36·3±3·9
16:0	29·4±1·0	20·4±1·7	32·2±1·2	34·9±0·4	32·9±2·7	25·9±0·7	26·0±0·1	29·3±0·5	31·9±1·2
17:1ω8*c*	0·1±0·2	1·0±0·2							0·1±0·1
17:1ω6		1·4±0·3			0·1±0·1				
17:0 cyclo	0·2±0·3		0·3±0·3	0·5±0·2		0·2±0·3	0·1±0·2		6·2±3·2
17:0	0·9±0·2	5·0±0·8	0·1±0·1	0·3±0·2	1·4±0·5			0·3±0	0·7±0·8
18:1ω7*c*	10·3±0·5	11·8±1·9	15·9±0·8	14·4±0·6	9·1±1·6	23·8±0·8	21·9±0·5	17·8±0·6	15·9±1·8
18:0		0·1±0·1	0·2±0·1	0·2±0·2			0·2±0·2	0·2±0	0·2±0·1

Stead, 1992a, b). Fatty acid methyl ester profiles were obtained by GC (model 6890 gas chromatograph; Hewlett Packard). Peaks were named and quantified using the Microbial Identification System (MIDI). Only profiles with total peak areas of $>50\,000$ U were accepted, to avoid problems of dilution. Profiles of selected strains were compared by principal component analysis and by dendrogram analysis based on UPGMA and expressed as Euclidean distances.

Extraction of DNA and DNA–DNA hybridization were done as indicated by Gardan *et al.* (2000). Native DNA of two lamb's lettuce strains, CFBP 4730^T and CFBP 4723, was labelled with tritiated nucleotides (Amersham) by nick-translation. The S1 nuclease/trichloroacetic acid method was used as indicated by Gardan *et al.* (2000). The reassociation temperature was 70 °C.

The DNA–rRNA hybridization method of De Ley & De Smedt (1975) was used. ^{3}H-labelled rRNA from *Acidovorax avenae* subsp. *avenae* NCPPB 1011^T was used. Since the lamb's lettuce strains were related by phenotypic characteristics to the other phytopathogenic *Acidovorax* species and subspecies, we verified the assignment of the lamb's lettuce strains to the branch of *A. avenae* within the family *Comamonadaceae*.

16S rRNA gene (rDNA) fragments were generated by a PCR and the amplified products were then purified, cloned and sequenced (Gardan *et al.*, 2000). Multiple sequence alignment and phylogenetic analysis were performed as previously described (Gardan *et al.*, 2000).

Pathogenicity tests

The pathogenicity of the 19 strains was confirmed on young plants of lamb's lettuce, based on the presence of symptoms recorded for 2 weeks after inoculation. The typical water-soaked spots appeared after 3 days incubation, becoming grey to black after 6 days. The leaf spots are circular with a regular margin and can reach 3 mm in diameter. They can also be surrounded by a bright-yellow halo. Pathogenicity was confirmed for all 19 strains. We observed some slight variations in aggressiveness (number of leaf spots per plant), which were probably due to the method of inoculation. We demonstrated that the lamb's lettuce strains were not pathogenic to anthurium. Pure cultures were easily reisolated, especially from the water-soaked lesions. On YBGA, colonies are white/cream, circular with a clearer margin and reach diameters of 1–2 mm after 4 days incubation at 25 °C. A clear brown pigment can diffuse around the colonies after 10 days.

Biochemical and physiological tests

A dendrogram displaying the distance relationships amongst the 23 strains is shown in Fig. 1. At a distance of 0·12, the 19 lamb's lettuce strains were clustered in one phenon. The small distance (0·12) of clustering for the lamb's lettuce strains indicated a highly similar phenotypic profile, demonstrating that they constitute a phenotypically homogeneous group of strains.

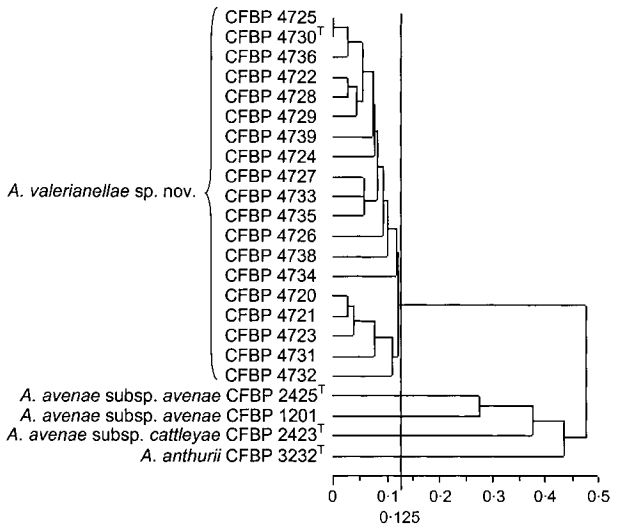

Fig. 1. Dendrogram obtained using UPGMA of distances of the Jaccard coefficient, among 32 strains tested from *Acidovorax valerianellae* sp. nov., *Acidovorax*, *Burkholderia*, *Herbaspirillum* and *Pseudomonas*.

Gardan *et al.* (2000) indicated the phenotypic characteristics that differentiate the lamb's lettuce strains from phytopathogenic *Acidovorax* species and other species of *Acidovorax*. Four additional biochemical tests can be used to distinguish the lamb's lettuce strains from *Acidovorax anthurii*. The lamb's lettuce strains assimilate sebacate and tryptophan but do not assimilate D-arabitol or DL-5-aminobutyrate. The reverse responses are obtained for *A. anthurii*.

Fatty acid extraction and analysis

All strains from *V. locusta* gave very similar profiles. All strains contained 3-hydroxydecanoic acid (10:0 3-OH), which was the only hydroxy fatty acid present in the profile. All strains also contained dodecanoic acid (12:0), tetradecanoic acid (14:0), pentadecanoic acid (15:0), hexadecanoic acid (16:0), *cis*-9-hexadecenoic acid (16:1ω7*c*), heptadecanoic acid (17:0) and *cis*-11-octadecenoic acid (18:1ω7*c*). In addition, some strains contained small amounts of *cis*-9-pentadecenoic acid (15:1ω6*c*), *cis*-9,10-methylene hexadecanoic acid (17:0 cyclo) and *cis*-9-heptadecenoic acid (17:1ω8*c*). Fatty acid data for all plant-pathogenic *Acidovorax* species and some other *Acidovorax* species and *D. acidovorans* are shown in Table 2. The *V. locusta* profiles were very similar to each other, and standard deviations divided by mean values were $<0{\cdot}56$ for all fatty acid methyl esters present in all strains. The proportions of 10:0 3-OH and 18:1ω7*c* in the profiles of the *V. locusta* strains were generally smaller than for almost all other taxa, while the proportions of

14:0, 15:0 and 16:1ω7*c* were generally greater than for other taxa (Table 2). These results show that the profiles were typical of the genus *Acidovorax* and of *D. acidovorans* in having 3-hydroxydecanoic acid as the major hydroxy acid (Oyaizu & Komagata, 1983; Willems *et al.*,1990; Stead, 1992a; Gardan *et al.*, 2000.) In fact, whereas some other *Acidovorax* taxa have traces of other hydroxy acids, 3-hydroxydecanoic acid was the sole hydroxy acid in the lamb's lettuce strains tested. Although there were no fatty acids unique to the *V. locusta* strains, quantitative differences supported genomic data favouring a novel taxon. Dendrogram analysis of strains of most taxa included in the study (Fig. 2) also showed a discrete cluster within 4 Euclidean distance units of each other, but which was 7 Euclidean distance units away from other taxa. Principal component analysis (not presented) showed that the 10 *V. locusta* strains formed a discrete homogeneous cluster. Fatty acid analysis provided phenotypic support for differentiation of all taxa included in the study. The two strains of *A. anthurii* clustered separately in both analyses. These analyses also showed that the proposed type strain (CFBP 4730^T) is a typical member of the panel of strains.

DNA–DNA hybridization

Results of DNA hybridization studies are shown in Table 3. Eleven of 19 lamb's lettuce strains from the phenon (derived from phenotypic analysis) in which all the lamb's lettuce strains clustered are 84–100 % (mean 99·7 %, SD 8·8) related to CFBP 4730^T. The same strains were 91–100 % related to CFBP 4723 (mean 96·5 %, SD 5·8). Thus, the lamb's lettuce strains are members of a single DNA hybridization group.

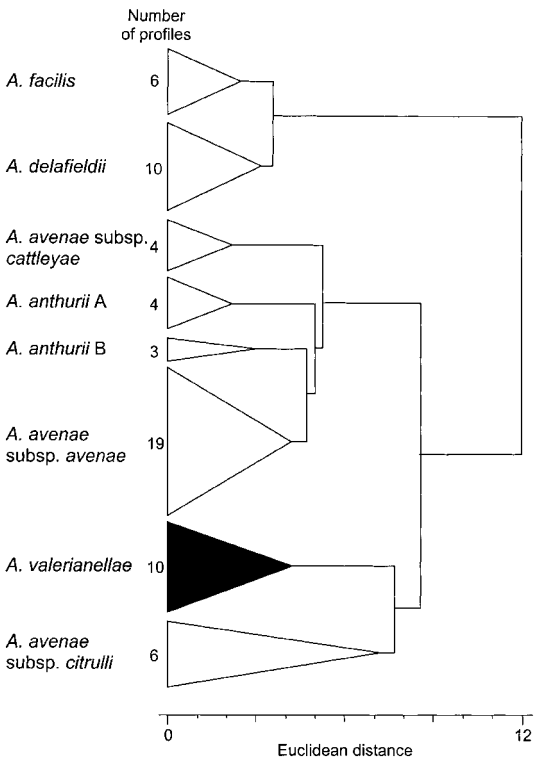

Fig. 2. Clustering of *Acidovorax valerianellae* sp. nov. and related strains by fatty acid profiling.

Table 3. Levels of DNA relatedness among *Acidovorax valerianellae* sp. nov. and related strains

Hybridization was determined at 70 °C. ND, Not determined.

Source of unlabelled DNA	Relative binding with labelled DNA from:	
	CFBP 4730^T	CFBP 4723
A. valerianellae sp. nov.		
CFBP 4730^T	100	91
CFBP 4720	100	100
CFBP 4721	84	100
CFBP 4723	100	100
CFBP 4725	100	98
CFBP 4726	95	99
CFBP 4728	89	88
CFBP 4731	100	100
CFBP 4732	100	93
CFBP 4733	92	89
CFBP 4734	100	100
A. anthurii CFBP 3232^T	24	ND
A. avenae subsp. *avenae*		
CFBP 2425^T	19	ND
CFBP 1201	23	ND
A. avenae subsp. *cattleyae* CFBP 2423^T	35	ND
A. avenae subsp. *citrulli* CFBP 4459^T	29	ND
A. konjaci CFBP 4460^T	15	ND

Representative strains of the phytopathogenic species *A. anthurii*, *Acidovorax konjaci* and three subspecies of *A. avenae* were 15–35 % related to strain CFBP 4730^T. Seven strains of *Burkholderia* sp., one strain of *Herbaspirillum* and four strains of non-fluorescent *Pseudomonas* sp. were very distantly related (0–5 %) to strain CFBP 4730^T (data not shown). Other non-phytopathogenic *Acidovorax* species were not included in this study because they belong to another rRNA branch. Thus, the lamb's lettuce strains constitute a discrete genomospecies.

DNA base composition

The G+C content of the DNA was determined from the thermal denaturation temperature as described by Gardan *et al.* (2000). The G+C content of strain CFBP 4730^T was found to be 64·5 mol%.

DNA–rRNA hybridization

The ΔT_m(e) values of two strains tested, CFBP 4723 and CFBP 4730^T, were respectively 1·3 and 1·6 °C, and showed that these strains belong to the *Acidovorax* rRNA branch.

They were more closely related to *A. avenae* subsp. *avenae* than to *A. konjaci*, which had a ΔT_m(e) of 3·1 °C. We could assign the lamb's lettuce strains to the genus *Acidovorax* within the family *Comamonadaceae*, in the β-subclass of the *Proteobacteria sensu* Stackebrandt *et al.* (1988), and to superfamily III *sensu* De Ley (1978).

16S rRNA sequencing and sequence analysis

A total of 1492 nucleotides of 16S rDNA sequence of strain CFBP 4730^T were determined. Phylogenetic sequence analysis confirmed the affiliation of the newly isolated strains to the genus *Acidovorax* (Fig. 3). Our sequence branched in the vicinity of *A. anthurii* regardless of the phylogenetic method used. These sequences were included in a cluster of phytopathogens, *A. avenae* subsp. *avenae*, subsp. *citrulli* and subsp. *cattleyae* and *A. konjaci*, described previously (Wen *et al.*, 1999) (Fig. 3; an expanded tree is available as supplementary material in IJSEM Online). This tree topology was confirmed by using a maximum-parsimony method (data not shown). The maximum-likelihood analysis led to a different topology, in which *A. avenae* subsp. *avenae*, subsp. *citrulli* and subsp. *cattleyae* rooted the newly described *A. anthurii* phylogenetic group (data not shown).

The similarity values calculated for the 16S rDNA sequences of the novel *Acidovorax* isolate and its closest relatives (*A. avenae* subsp. *avenae*, subsp. *citrulli* and subsp. *cattleyae* and *A. anthurii*) ranged from 97·6 to 98·4 %. Because of the microheterogeneity of 16S rRNA genes within species, these similarity values cannot be used for delimiting species (Rossello-Mora & Amann, 2001). However, DNA relatedness levels showed unambiguously that the lamb's lettuce strains constitute a novel species.

On the basis of pathogenicity tests, phenotypic properties, fatty acid profiles, DNA–DNA reassociation values, 16S rDNA sequencing, DNA–rDNA hybridization and G+C content, the lamb's lettuce strains should be considered as a novel species of the genus *Acidovorax*. The name *Acidovorax valerianellae* sp. nov. is proposed.

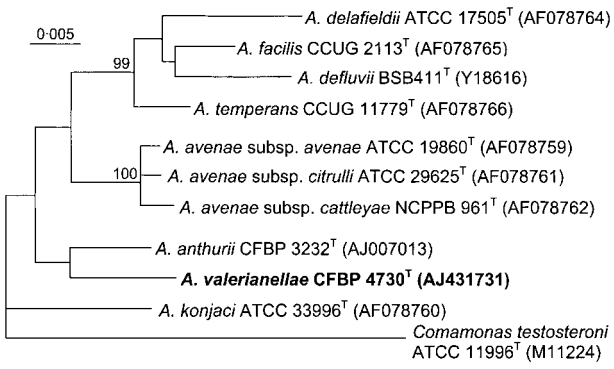

Fig. 3. Neighbour-joining tree obtained from 16S rRNA gene sequences. The scale bar represents 1 estimated base substitution per 200 nucleotide positions. Percentages refer to bootstrap values of 100 calculated trees. EMBL/GenBank accession numbers are shown in parentheses. An expanded version of this tree, showing more taxa, is available as supplementary material in IJSEM Online.

Description of *Acidovorax valerianellae* sp. nov.

Acidovorax valerianellae (va.le.ri.a.nel′lae. N.L. fem. gen. n. *valerianellae* of *Valerianella*, the genus name of lamb's lettuce, the host plant).

On YBGA, colonies are slow-growing, 2 mm in diameter after 4 days incubation, circular, slightly raised and white/cream with a clear margin. Cells are Gram-negative, straight rods, motile by means of a long polar flagellum. Oxidase, catalase and urease tests are positive. Strictly aerobic: there is no anaerobic acid production from glucose in a Hugh and Leifson test. Poly-β-hydroxybutyrate is accumulated. Nitrates are not reduced and arginine, gelatin and aesculin are not utilized. No growth at 4 or 41 °C. Glycerol, D-fructose, gluconate, succinate, fumarate, glutarate, adipate, pimelate, suberate, azelate, sebacate, DL-lactate, DL-3-hydroxybutyrate, D-malate, L-malate, pyruvate, 2-ketoglutarate, L-alanine, L-leucine, L-isoleucine, L-phenylalanine, L-tyrosine, L-histidine, L-tryptophan, L-aspartate, L-glutamate, L-proline and DL-4-aminobutyrate are assimilated. All strains possess 3-hydroxydecanoic acid, which is the only hydroxy fatty acid present. Many other carbon sources are not utilized. All strains elicit a hypersensitive reaction on tobacco leaves and are pathogenic on *V. locusta*, producing leaf-spot symptoms.

The type strain is strain CFBP 4730^T (=NCPPB 4283^T). The G+C content of the DNA of the type strain is 64·5 mol% (thermal denaturation method).

Acknowledgements

We thank Andy Aspin, John Heeney, Solange Belouin and Alain Huard for technical assistance.

References

De Ley, J. (1978). Modern molecular methods in bacterial taxonomy: evaluation, application, prospects. In *Proceedings of the International Conference on Plant Pathogenic Bacteria*, vol. 1, pp. 347–357. Tours: Gilbert.

De Ley, J. & De Smedt, J. (1975). Improvements of the membrane filter method for DNA:rRNA hybridization. *Antonie van Leeuwenhoek* **41**, 287–307.

Gardan, L., Dauga, C., Prior, P., Gillis, M. & Saddler, G. S. (2000). *Acidovorax anthurii* sp. nov., a new phytopathogenic bacterium which causes bacterial leaf-spot of anthurium. *Int J Syst Evol Microbiol* **50**, 235–246.

Oyaizu, H. & Komagata, K. (1983). Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. *J Gen Appl Microbiol* **29**, 17–40.

Péron, J. Y. & Rees, D. C. (1998). High-tech production of corn salad (*Valerianella locusta* (L.) Laterr.), a local, French vegetable crop. *Acta Hortic* **467**, 259–268.

Rat, B. & Gardan, L. (1993). La tache bactérienne due à un *Pseudomonas* sp. *Fruits Légumes* **11**, 42–43.

Rat, B., Gardan, L. & Bollet, C. (1994). Black spot of lamb lettuce: a new bacterial disease. In *Plant Pathogenic Bacteria*, p. 183. Edited by M. Lemattre, S. Freigoun, K. Rudolph & J. G. Swing. Paris: INRA.

Rossello-Mora, R. & Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol Rev* **25**, 39–67.

Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical Taxonomy: Principles and Practice of Numerical Classification.* San Francisco: W. H. Freeman.

Stackebrandt, E., Murray, R. G. E. & Trüper, H. G. (1988). *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the ''purple bacteria and their relatives''. *Int J Syst Bacteriol* **38**, 321–325.

Stead, D. E. (1992a). Grouping of plant-pathogenic and some other *Pseudomonas* spp. by using cellular fatty acid profiles. *Int J Syst Bacteriol* **42**, 281–295.

Stead, D. E. (1992b). Techniques for detecting and identifying plant pathogenic bacteria. In *Techniques for Rapid Diagnosis of Plant Pathogens*, pp. 76–111. Edited by J. Duncan & L. Torrance. Oxford: Blackwell Science.

Stead, D. E., Sellwood, J. E., Wilson, J. & Viney, I. (1992). Evaluation of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. *J Appl Bacteriol* **72**, 315–321.

Wen, A., Fegan, M., Hayward, C., Chakraborty, S. & Sly, L. I. (1999). Phylogenetic relationships among members of the *Comamonadaceae*, and description of *Delftia acidovorans* (den Dooren de Jong 1926 and Tamaoka *et al.* 1987) gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 567–576.

Willems, A., Falsen, E., Pot, B., Jantzen, E., Hoste, B., Vandamme, P., Gillis, M., Kersters, K. & De Ley, J. (1990). *Acidovorax*, a new genus for *Pseudomonas facilis*, *Pseudomonas delafieldii*, E. Falsen (EF) group 13, EF group 16, and several clinical isolates, with the species *Acidovorax facilis* comb. nov., *Acidovorax delafieldii* comb. nov., and *Acidovorax temperans* sp. nov. *Int J Syst Bacteriol* **40**, 384–398.