Comparison Between *Xanthomonas campestris* pv. *manihotis* (ISPP List 1980) and *X. campestris* pv. *cassavae* (ISPP List 1980) by Means of Phenotypic, Protein Electrophoretic, DNA Hybridization and Phytopathological Techniques

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(Received 1 April 1986; revised 16 June 1986)

Xanthomonas campestris pv. *manihotis* (ISPP List 1980) and *X. campestris* pv. *cassavae* (ISPP List 1980) strains, isolated from cassava (*Manihot esculenta*) plants of different geographical origin, were studied by numerical analysis of 267 phenotypic features, computer-assisted comparison of gel electrophoregrams of soluble proteins, mol% G+C determinations, DNA:DNA hybridizations and virulence tests. *X. campestris* pv. *manihotis* and pv. *cassavae* constituted separate biological entities which could be differentiated from each other by four biochemical features, their symptoms on cassava, their soluble protein electrophoregrams and their DNA characteristics. Within each pathovar no correlation was found between phytopathogenicity, geographic origin and year of isolation of the strains, on the one hand, and the biochemical, physiological and protein electrophoretic properties on the other. Two yellowish *Xanthomonas* strains, CIAT 1164 and CIAT 1165, isolated from cassava in Colombia were genetically and electrophoretically similar to *X. campestris* pv. *poinsettiicola*, but were unable to infect *Euphorbia pulcherrima*. *X. campestris* pv. *poinsettiicola* was genetically more related to the *X. campestris* pv. *manihotis* cluster than to the *X. campestris* pv. *cassavae* cluster.

INTRODUCTION

Two pathovars of *Xanthomonas campestris* (Pammel 1958) Dowson 1939 have been recorded on cassava (*Manihot esculenta* Crantz). The white *X. campestris* pv. *manihotis* (ISPP List 1980: see Dye *et al.*, 1980) originated from South America but now has a worldwide distribution (Maraite *et al.*, 1981). It causes the severe cassava bacterial blight disease characterized by angular spots extending into blight areas on the leaves and a systemic infection of the stem, leading to wilt and die-back. The yellow *X. campestris* pv. *cassavae* (ISPP List 1980: see Dye *et al.*, 1980) is at present restricted to the East African highlands. It causes cassava bacterial necrosis disease, which is generally much less severe than the cassava bacterial blight disease. Cassava bacterial necrosis is characterized by angular leaf spots and a cortical infection of the stem; but, leaf blight or systemic invasion of the plant do not occur.

Robbs *et al.* (1972) isolated in Brazil a pv. *manihotis* strain, with reduced virulence, that caused symptoms similar to those induced by pv. *cassavae*; they suggested that pv. *cassavae* might be considered as a synonym of pv. *manihotis*, and simply referred to it as a yellow variant of the latter taxon. In other studies, comprising physiological, biochemical and inoculation tests (Maraite & Weyns, 1979; Maraite & Perreux, 1979), the strain used by Robbs *et al.* (1972), which is the neopathotype strain of pv. *manihotis* (Bradbury, 1984), behaved in a similar fashion to other strains of pv. *manihotis* and was clearly different from pv. *cassavae* strains.

Sabet *et al.* (1969) reported an overlap in the host-range among isolates from *Euphorbiaceae*: an isolate from *Euphorbia pulcherrima* also infected *M. esculenta*, and an isolate from the common weed *Euphorbia acalyphoides* also infected *Ricinus communis* and *E. pulcherrima*, but not *M. esculenta*.

The present study was undertaken in order to clarify the relationships between the various xanthomonads occurring on cassava, using a numerical analysis of 267 phenotypic features, gel electrophoresis of soluble cellular proteins, mol% G+C determinations, DNA:DNA hybridizations and phytopathological tests.

METHODS

Organisms used. The strains studied are listed in Table 1. They were grown at 28 °C on GYCA medium (modified from Dye, 1962), containing 1% (w/v) D-glucose, 0.5% (w/v) yeast extract (Oxoid), 3% (w/v) CaCO₃ and 2% (w/v) agar in tap water, or on GYEA medium, containing 0.5% (w/v) D-glucose, 0.1% (w/v) yeast extract (Oxoid), 0.0001% (w/v) FePO₄ and 2% (w/v) agar. The isolates were maintained at 4 °C on GYCA medium and transferred monthly.

Phenotypic features tested. A total of 267 phenotypic features were examined. Liquid media were inoculated with two drops of a suspension containing approximately 7×10^8 cells per ml distilled water and incubated with shaking, unless stated otherwise. Solid media in Petri dishes were inoculated with a multipoint inoculator, and incubated at 28 °C for 7 d, unless stated otherwise. The colony morphology (diameter, profile, slime production, pigmentation, growth rate and formation of a brown water-soluble pigment) was examined for 9 d on GYCA medium (Dye, 1962). Cell morphology was examined on 24- to 48-h-old GYCA grown-cells. Gram-staining was done according to Hucker's method (Doetsch, 1981). Formation of a fluorescent pigment and growth on *Pseudomonas*-F agar (Oxoid) were tested. Acetoin production was examined after 5 d in YS medium (Dye, 1962) supplemented with 0.5% (w/v) D-glucose, by using Barritt's reagent (Barritt, 1936). Indole production was tested after 5 d in a medium containing 0.5% (w/v) yeast extract and 2% (w/v) tryptone (Oxoid) using Kovacs' reagent (Kovac, 1928). The production of H₂S from L-cysteine was examined in a medium containing 0.05% (w/v) NH₄H₂PO₄, 0.05% (w/v) K₂HPO₄, 0.02% (w/v) MgSO₄·7H₂O, 0.5% (w/v) yeast extract to which L-cysteine (autoclaved separately for 10 min) was added to give a final concentration of 0.02% (w/v). H₂S production was detected by the blackening of lead acetate paper strips (tubes were not shaken).

Nitrate reduction was tested after 5 d in a medium containing 0.05% (w/v) KH₂PO₄, 0.05% (w/v) K₂HPO₄, 0.02% (w/v) MgSO₄·7H₂O, 0.2% (w/v) sodium succinate, 0.3% (w/v) KNO₃ and 0.5% (w/v) yeast extract. Catalase was tested by suspending a loopful of cells in 10% (v/v) H₂O₂. Oxidase was tested by Kovacs' procedure (Kovac, 1956). Gelatinase was detected with charcoal gelatin discs (Oxoid) in a liquid medium containing 0.5% (w/v) yeast extract, 0.5% (w/v) neutralized peptone (Oxoid) and 0.5% (w/v) D-glucose. Urease activity was tested according to Dye (1962). Starch hydrolysis was tested on a medium containing 0.5% (w/v) yeast extract, 0.5% (w/v) neutralized peptone, 0.3% (w/v) soluble starch and 2% (w/v) agar in distilled water. The Petri dishes were heavily inoculated with a loopful of cells, and after 2 d incubation, Lugol's iodine solution was poured on the plates. Aesculin hydrolysis was tested by heavily inoculating GYEA medium to which 0.3% (w/v) aesculin and 0.05% (w/v) ferric ammonium citrate had been added. Hydrolysis of Tween 80 and Tween 60 was observed on a medium consisting of 1% (w/v) peptone (Oxoid), 0.01% (w/v) CaCl₂·2H₂O, 0.1% (w/v) yeast extract, 2% (w/v) agar and 1% (v/v) Tween. The Tweens were sterilized and added separately. Lecithinase activity was checked on GYEA medium supplemented with 11% (v/v) egg yolk emulsion (Oxoid). All media were heavily inoculated with a loopful of cells. β-Glucosidase was tested in a medium containing 0.05% (w/v) K₂HPO₄, 0.05% (w/v) NH₄H₂PO₄, 0.02% (w/v) MgSO₄·7H₂O, 0.1% (w/v) yeast extract and 0.7 ml of a 1.5% (w/v) bromocresol purple solution in ethanol to which filter-sterilized arbutin was added to a final concentration of 1% (w/v). To detect polygalacturonase a modified Paton's medium (Paton, 1959) was used, consisting of two layers: layer A containing 0.5% (w/v) yeast extract, 0.5% (w/v) neutralized peptone, 0.5% (w/v) calcium lactate, 0.5% (w/v) D-glucose and 2% (w/v) agar; and layer B containing 2% (w/v) sodium polygalacturonate and 0.1% (w/v) disodium ethylenediaminetetra-acetate. Enzymic activity was observed after 5 d. DNAase activity was tested on DNase agar (Oxoid) by pouring 1 M-HCl on the plates. Clear zones around the cultures indicated DNAase activity. The action in litmus milk was observed for 18 d. The medium contained 10% (w/v) skim milk powder (Oxoid) and 0.07% (w/v) soluble litmus. It was autoclaved for 10 min and was not shaken. The O/F (oxidation/fermentation) test was done by stab-inoculation in a medium containing 0.01% (w/v) yeast extract, 0.1% (w/v) NH₄H₂PO₄, 0.02% (w/v) MgSO₄·7H₂O, 0.02% (w/v) KCl, 0.3% (w/v) agar, 0.003% (w/v) bromthymol blue and 1% (w/v) D-glucose (sterilized separately). Reactions were observed daily for 2 weeks. Acid formation from carbohydrates was detected after 9 d in a basal medium (Dye, 1962) containing the following filter-sterilized carbohydrates at a final concentration of 1% (w/v): L-arabinose, cellobiose, D-fructose, D-galactose, maltose, D-mannose, D-ribose, sucrose, D-xylose, salicin, *meso*-erythritol, raffinose, melibiose, trehalose, L-rhamnose, lactose, D-glucose, sorbitol,

adonitol, L-sorbose, dulcitol, amygdalin and *meso*-inositol. Utilization of carbon sources was tested using the basal medium of Starr (1945). The following carbon sources were sterilized separately by filtration and were tested at a final concentration of 0.2% (w/v), unless stated otherwise: L-rhamnose (0.18%), sodium 2-ketogluconate, salicin, inulin, glycolic acid, L-leucine (0.1%), L-threonine (0.1%), glycine (0.1%), L-tryptophan (0.1%), L-lysine (0.08%), L-cysteine (0.1%), L-arginine (0.1%), L-methionine (0.1%), L-isoleucine (0.1%), ethanolamine (0.1%), sodium hippurate (0.09%), acetamide (0.1%), n-butyrate (0.1%), DL- β -hydroxybutyrate (0.1%), pelargonate (0.1%), ethanol (0.1%), n-butanol (0.1%), iso-butanol (0.1%), diethyleneglycol (0.1%), adonitol, D-glucose, D-mannose, trehalose, cellobiose, D-fructose, D-galactose, maltose (0.19%), melibiose (0.19%), sucrose, glycerol, D-alanine (0.1%), L-alanine (0.1%), L-glutamic acid (0.1%), saccharic acid, mucic acid and DL-glyceric acid. All stock solutions of carbon sources, except sugars, were adjusted to pH 7.0 by addition of 1 M-NaOH or 1 M-HCl. Growth on some organic acids was tested using a basal medium containing 0.005% (w/v) yeast extract, 0.01% (w/v) vitamin-free Casamino acids, 0.05% (w/v) $\text{NH}_4\text{H}_2\text{PO}_4$, 0.05% (w/v) K_2HPO_4 , 0.02% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0016% (w/v) bromthymol blue and 2% (w/v) agar (final pH 6.8). The organic acids (pH adjusted with 1 M-NaOH to pH 6.8) were sterilized by filtration and added to a final concentration of 0.2% (w/v). The following organic salts were tested: sodium acetate, sodium malate, trisodium citrate, calcium D-gluconate, potassium-sodium tartrate, sodium fumarate, calcium lactate, sodium benzoate, sodium oxalate, sodium succinate and sodium propionate. The requirement for organic nitrogen and/or growth factors was determined by testing growth on the following media: (i) 0.1% (w/v) NH_4Cl and 1% (w/v) D-glucose; (ii) 0.1% (w/v) NH_4Cl , 1% (w/v) D-glucose, 1.6% (v/v) mineral solution A [6% (w/v) KH_2PO_4 and 1.2% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, pH 6.8] and 0.5% (v/v) mineral solution B [0.0001% (w/v) H_3BO_3 , 0.002% (w/v) CaCO_3 , 0.0002% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.00002% (w/v) KI, 0.0002% (w/v) $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.0002% (w/v) MoO_3 and 0.0032% (w/v) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$]; (iii) medium (ii) plus 5% (v/v) vitamin solution [0.0015% (w/v) thiamin, 0.0015% (w/v) riboflavin, 0.001% (w/v) calcium D-pantothenate, 0.00001% (w/v) biotin, 0.001% (w/v) pyridoxal hydrochloride, 0.001% (w/v) *p*-aminobenzoic acid, 0.001% (w/v) vitamin B12, 0.001% (w/v) folic acid and 0.005% (w/v) nicotinic acid in distilled water]; (iv) medium (ii) plus 5% (v/v) vitamin solution and 0.3% (w/v) vitamin-free Casamino acids; (v) medium (iv) from which NH_4Cl was omitted. Growth on 0.2% (w/v) vitamin-free Casamino acids, 0.5% (w/v) peptone or 0.5% (w/v) yeast extract was also verified. The utilization of 22 single L-amino acids as sole nitrogen source was tested in a basal medium containing 1% (w/v) D-glucose, 1.6% (v/v) mineral solution A, 0.5% (v/v) mineral solution B and 5% (v/v) of the filter-sterilized vitamin solution. The amino acids were sterilized separately and added to the basal medium to a final concentration of 0.1% (w/v). The utilization of L-alanine, glycine, L-glutamine, L-glutamic acid, L-asparagine, L-hydroxyproline and L-proline as sole source of carbon and nitrogen was tested in the same basal medium from which D-glucose was omitted. The stimulatory effect of low concentrations (0.02%, w/v) of single L-amino acids on the growth was verified on a basal medium containing 0.1% (w/v) NH_4Cl , 1% (w/v) D-glucose, 1.6% (v/v) mineral solution A, 0.5% (v/v) mineral solution B and 5% (v/v) vitamin solution to which the L-amino acid was added.

Growth was tested after 8 d on a methionine/glutamic acid (MG) medium containing 0.5% (w/v) D-glucose, 1.6% (v/v) mineral solution A, 0.5% (v/v) mineral solution B, 5% (v/v) vitamin solution, 0.2% (w/v) L-methionine, 0.2% (w/v) L-glutamic acid and 2% (w/v) agar. Growth on SX agar (Schaad, 1980) was checked after 10 d. Anaerobic growth was tested on GYEA medium in an anaerobic jar. Growth in the presence of 10 and 20% (w/v) D-glucose was observed in GYEA medium. Concentrated D-glucose solution was autoclaved separately for 10 min. The tolerance towards filter-sterilized dyes and metals was tested in GYEA medium. The tolerance towards triphenyltetrazolium chloride (0.005, 0.01, 0.05 and 0.1%, w/v) or 0.1% (w/v) Actidione was tested in GYEA medium. Growth at different temperatures (4, 28, 37 °C), the ability to grow at initial pH values of 4.5, 5.5, 6.5, 7.5 and 8.5, and the tolerance towards 1, 2, 3 and 4% (w/v) NaCl was tested on GYEA medium. The *in vitro* susceptibility towards 20 antibiotics was determined by the Kirby-Bauer technique (Bauer *et al.*, 1966) using Oxoid sensitivity discs. The basal medium was YPGA [0.5% (w/v) yeast extract, 0.5% (w/v) neutralized peptone, 0.5% (w/v) D-glucose and 2.5% (w/v) Oxoid no. 1 agar]. Inhibition zones were recorded after 36 to 48 h; diameters of 12 mm or more were interpreted as susceptible. The following antibiotics were tested: 10 μg ampicillin, 10 U bacitracin, 25 μg cephaloridin, 30 μg chloramphenicol, 10 μg colistin sulphate, 10 μg erythromycin, 10 μg fucidic acid, 10 μg gentamycin, 2 μg lincomycin, 30 μg kanamycin, 10 μg methicillin, 200 μg nitrofurantoin, 30 μg neomycin, 30 μg nalidixic acid, 30 μg novobiocin, 10 U penicillin G, 300 U polymyxin, 10 μg streptomycin, 100 μg sulfafurazole and 30 μg tetracycline.

Numerical analysis of phenotypic features. All 267 features were coded as 2 (positive) or 1 (negative). Missing features were coded as 0 (no comparison). Similarities between the strains were calculated with the similarity coefficient S_{SM} of Sokal & Michener (1958) using a modified program of Bonham-Carter (1967). The clustering of the strains was done according to the unweighted average pair-group method (Sneath & Sokal, 1973) with the Clustan 1C program (Wishart, 1978) using the Siemens 7551 (BS2000) computer of the Centraal Digitaal Rekencentrum, Rijksuniversiteit, Gent, Belgium.

Numerical analysis of protein gel electrophoregrams. The preparation of the soluble protein fractions, PAGE and

Table 1. *Strains used and information on their origin and isolation*

Name (host plant)	Strain no.*	Strain no. in other collections*	Place and year of isolation	Isolated by	Pathogenicity† on cassava
<i>X. campestris</i> pv. <i>manihotis</i> (from <i>Manihot esculenta</i>)	HMB 3	LMG 766	Gandajika, Zaire, 1973	H. Maraite	5
	HMB 6	LMG 767	Kijaka, Zaire, 1973	H. Maraite	5
	HMB 9	ICPB XM144, NCPPB 3058, LMG 768	Yangambi, Zaire, 1973	H. Maraite	4
	HMB 10	ICPB XM145, NCPPB 3059, LMG 769	Mvuazi, Zaire, 1973	H. Maraite	6
	HMB 23	ICPB XM147, Pereira BM12, LMG 770	Socorro, São Paulo, Brazil, 1973	A. L. G. Pereira	6
	HMB 25	ICPB XM146, NCPPB 3060, LMG 771	Ibadan, Nigeria, 1976	H. Maraite	5
	HMB 27	LMG 629	Nyombe, Cameroon, 1977	H. Maraite	5
	HMB 36	LMG 772	Ibadan, Nigeria, 1977	H. Maraite	5
	HMB 55‡	NCPPB 1834, PDDCC 5741, ICPB XM125, Robbs ENA-300, LMG 773	Brazil, 1965	C. F. Robbs	4 (5)
	HMB 60	LMG 774	Taiwan, 1978	H. Maraite	6
	HMB 68§	NCPPB 1159, PDDCC 6934, ATCC 23380, ICPB XM4, Burkholder SM1, LMG 5274	USA, 1941	W. H. Burkholder	4
	HMB 70§	NCPPB 1160, PDDCC 222, ICPB XM12, LMG 5273	Brazil, 1941	O. A. Drummond & O. Hipolito	5
	HMB 71	NCPPB 1161, ICPB XM105, LMG 775, Orian M3	Mauritius, 1946	G. Orian	5
	HMB 72t1 and t2	NCPPB 2443, LMG 776t1 and t2 , Lozano 23L	Colombia, 1970	J. C. Lozano	5
	HMB 78	Takatsu B35, LMG 777	Goiania, Brazil, 1978	A. Takatsu	6
	HMB 79	Takatsu Xm6b, LMG 778	Bahia, Brazil, 1978	A. Takatsu	4
	HMB 80t1 and t2	Takatsu B7, LMG 779t1 and t2	Brasilia, Brazil, 1978	A. Takatsu	5
	HMB 81	LMG 780	Uganda, 1979	H. Maraite	5
	HMB 83	LMG 781	Uganda, 1979	H. Maraite	5
	HMB 93	LMG 782	Ngetta, Uganda, 1979	H. Maraite	5
	HMB 148	LMG 783	Nairobi, Kenya, 1979	H. Maraite	5
	HMB 195§	LMG 5247	Uganda, 1981	H. Maraite	5
	HMB 202§	LMG 5248	Mvuazi, Zaire, 1981	H. Maraite	5
	HMB 203§	LMG 5249	Toura, Ivory Coast, 1981	H. Maraite	5
	HMB 205§	LMG 5250	Nairobi, Kenya, 1981	H. Maraite	5
	GSL	LMG 765	Serdang, Selangor, Malaysia, 1980	Geh Swee Lan	5

	NCPBPB 1834†	ICPB XM125, PDDCC 5741, HMB 55, Robbs ENA-300, LMG 784	Brazil, 1965	C. F. Robbs	4 (5)
<i>X. campestris</i> pv. <i>cassavae</i> (from <i>Manihot esculenta</i>)	NCPBPB 3161§	Persley C6, LMG 5287	Cameroun, 1976	G. Persley	5
	NCPBPB 3194§	Bradbury B7797, LMG 5288	Nigeria, 1978	J. F. Bradbury	5
	HMB 29	NCPBPB 3061, LMG 671	Rwanda, 1977	H. Maraite	3
	HMB 37§	LMG 5264	Rwanda, 1977	H. Maraite	3
	HMB 38	NCPBPB 3062, LMG 672	Rwanda, 1977	H. Maraite	2
	HMB 41§	LMG 5265	Rwanda, 1977	H. Maraite	3
	HMB 42§	LMG 5266	Rwanda, 1977	H. Maraite	3
	HMB 44§	LMG 5267	Rwanda, 1977	H. Maraite	3
	HMB 45§	LMG 5268	Rwanda, 1978	H. Maraite	3
	HMB 51§	LMG 5269	Rwanda, 1978	H. Maraite	3
	HMB 53§	LMG 5270	Rwanda, 1978	H. Maraite	3
	HMB 54†	NCPBPB 101, PDDCC 204, LMG 673	Malawi, 1951	P. O. Wiehe & W. J. Dowson	3
	HMB 59	LMG 674	Tanzania, 1978	H. Maraite	3
	HMB 146	LMG 5271	Nairobi, Kenya, 1979	H. Maraite	3
	HMB 179§	LMG 5246	Nairobi, Kenya, 1979	H. Maraite	3
	ICPB XC110	LMG 670			
Yellowish isolate from <i>Manihot esculenta</i>	CIAT 1164	HMB 137, LMG 585	Carimagua, Colombia, 1976	J. C. Lozano	0 (1)
Yellowish isolate from <i>Manihot esculenta</i>	CIAT 1165	HMB 136, LMG 586	Carimagua, Colombia, 1976	J. C. Lozano	0 (1)
<i>X. campestris</i> pv. <i>euphorbiae</i> (from <i>Euphorbia acalypthoides</i>) <i>X. campestris</i> pv. <i>poinsetticola</i> (from <i>Euphorbia pulcherrima</i>) <i>X. campestris</i> pv. <i>poinsetticola</i> <i>X. campestris</i> pv. <i>ricini</i> (from <i>Ricinus communis</i>) <i>X. campestris</i> pv. <i>campestris</i> (from <i>Brassica oleracea</i>)	NCPBPB 1828†	PDDCC 5730, LMG 863, Sabet strain Euph 1	Sudan, 1965	K. A. Sabet	0
	ICPB XP137†	ATCC 11643, NCPBPB 581, PDDCC 5779, LMG 849	India, 1950		0 (1)
	PDDCC 332	LMG 5401	India		0 (1)
	NCPBPB 1063†	ATCC 19317, PDDCC 5747, LMG 861, Hayward B651	Ethiopia, 1961	A. C. Hayward	0
	NCPBPB 528¶	ATCC 33913, PDDCC 13, LMG 568	UK, 1957	E. Billing	0

* Abbreviations: ATCC, American Type Culture Collection, Rockville, Md., USA; CIAT, Centro Internacional de Agricultura Tropical, Cali, Colombia; HMB, H. Maraite's Bacterial Collection, Louvain-la-Neuve, Belgium; ICPB, International Collection of Phytopathogenic Bacteria, Davis, Calif., USA; LMG, Culture Collection Laboratorium Microbiologie Gent, Belgium; NCPBPB, National Collection of Plant Pathogenic Bacteria, Harpenden, UK; PDDCC, Plant Diseases Division Culture Collection, Auckland, New Zealand.

† Scale of symptom severity is from 0 to 6: 0-1 is non-pathogenic and 6 is highly virulent. Occasional reactions are given in parentheses.

‡ Neopathotype strain.

§ Strains not included in the phenotypic analysis.

|| t1 and t2 denote different stable colony variants.

¶ Type strain.

computer-assisted comparisons of normalized protein electrophoregrams were done as previously described (Kerstens & De Ley, 1975; Vera Cruz *et al.*, 1984).

DNA base composition. DNA was prepared according to the method of Marmur (1961). The mean mol % G + C content of the overall genome DNA of representative *Xanthomonas* strains was determined by the thermal denaturation method (De Ley & Van Muylem, 1963) and calculated by the equation of Marmur & Doty (1962) as modified by De Ley (1970).

DNA: DNA hybridizations. The degree of DNA-binding (%D) was determined spectrophotometrically from the initial renaturation rates using the method of De Ley *et al.* (1970). Renaturations were done in $2 \times \text{SSC}$ ($1 \times \text{SSC}$ is 0.15 M-NaCl, 0.015 M-trisodium citrate, pH 7.0) at optimal renaturation temperature (81 °C) with a DNA concentration of approximately $60 \mu\text{g ml}^{-1}$. We used a Gifford 2600 spectrophotometer equipped with a thermostatted cuvette chamber and a Hewlett-Packard 7225A plotter.

Phytopathogenicity tests. Pathogenicity of all strains was tested on cassava plants, cultivar Eala Amer 07, multiplied from cuttings provided by the Institut des Sciences Agronomiques du Rwanda, Rubona, Rwanda. Selected strains were also inoculated in *Euphorbia pulcherrima* plants purchased in Belgium as ornamental plants (without cultivar specification) and in *Ricinus communis* plants (without cultivar specification) grown from seeds provided by the Unité de Phytotechnie tropicale, Université Catholique de Louvain. The plants were grown and the leaf and stem inoculations done according to previously described methods (Maraite *et al.*, 1981).

The evolution of the symptoms was assessed during 6 weeks and the observed reactions rated according to the following scale: 0, no reaction on leaf or stem inoculation; 1, small water-soaked area extending less than 1 mm around the inoculation point and becoming necrotic within 1 week; 2, water-soaked angular spots (on the leaves) larger than 1 mm and extending during incubation; 3, formation on the leaves of secondary spots along the veins and of dark areas on the stem extending more than 5 mm from the inoculation point; 4, formation of blighted areas on the lamina after 2 weeks and slow systemic infection of the stem leading to the formation of exudates and wilt that often stopped after 3 weeks; 5, appearance of the blight symptoms between 9 and 12 d after inoculation, and regular systemic infection of the stem leading generally to wilt of the apical part within 1 month; 6, appearance of blight symptoms 7 d after leaf inoculation and fast stem infection leading to tip die-back within 3 weeks.

RESULTS

Numerical analysis of phenotypic features

Twenty-three *X. campestris* pv. *manihotis* strains, six *X. campestris* pv. *cassavae* strains, the type strain of the type species *X. campestris* (NCPPB 528), and three *X. campestris* strains isolated from other *Euphorbiaceae* (*X. campestris* pv. *poinsettiiicola* ICPB XP137, *X. campestris* pv. *euphorbiae* NCPPB 1828 and *X. campestris* pv. *ricini* NCPPB 1063) were examined for 267 phenotypic features. The S_{SM} values between the replicates of three duplicated strains had a mean ($\pm$ SD) of $96.4 \pm 1.6\%$. The dendrogram of the numerical analysis using the S_{SM} coefficient and the unweighted average pair group method is shown in Fig. 1. The 23 *X. campestris* pv. *manihotis* strains clustered together in phenon 1 at 80% S_{SM} . Phenon 2 contained five *X. campestris* pv. *cassavae* strains at the 83% S_{SM} level. The type strain of *X. campestris* and the neopathotype strains of *X. campestris* pv. *euphorbiae*, pv. *poinsettiiicola* and pv. *ricini* grouped together at 83% S_{SM} . *X. campestris* pv. *cassavae* strain HMB 38 occupied a separate position in the dendrogram.

Morphological, physiological and biochemical features

The following features occurred in all 33 strains examined: the cells were Gram-negative motile rods, occurring singly, measuring 0.3 to 0.5 μm by 1.3 to 2.1 μm ; on GYCA medium they formed shiny, slimy, convex and circular colonies with a diameter of 2 to 8 mm, visible after 6 d of incubation; catalase; DNAase; peptonization in litmus milk; growth at initial pH 5.5 to 8.5; growth in the presence of 1% (w/v) NaCl, 10% (w/v) D-glucose, 0.1% (w/v) Actidione, 0.005% (w/v) triphenyltetrazolium chloride, 0.001% (w/v) $(\text{NH}_4)_2\text{CuCl}_4 \cdot 2\text{H}_2\text{O}$, 0.01% (w/v) sodium salicylate, 0.01% (w/v) KIO_3 , 0.01% (w/v) KSCN , 0.01% (w/v) $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.01% (w/v) $(\text{NH}_4)_2\text{S}_2\text{O}_8$, 0.001% (w/v) $\text{C}_4\text{H}_4\text{KO}_7\text{Sb}$, 0.001% (w/v) CuI , 0.0015% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.012% (w/v) $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01% (w/v) $\text{Ba}(\text{NO}_3)_2$, 0.015% (w/v) $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.011% (w/v) $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.01% (w/v) H_3BO_3 , 0.01% (w/v) K_2SO_4 , 0.01% (w/v) methylene blue, 0.005% (w/v) methyl green, 0.0001% (w/v) crystal violet, 0.01% (w/v) Nile blue, 0.01% (w/v) Bismarck brown and 0.01% (w/v) Congo red; growth on *Pseudomonas*-F agar; acid formation from D-fructose, D-galactose, sucrose; growth on D-glucose, D-mannose, trehalose,

Fig. 1. Dendrogram obtained after numerical analysis of 267 phenotypic features of 32 *X. campestris* (*X. c.*) strains isolated from cassava and some other *Euphorbiaceae*, and the type strain of *X. campestris*. The S_{SM} coefficient and the unweighted average pair-group method were used. The pathogenicity on cassava is given on a 0 to 6 scale: 0–1 is non-pathogenic and 6 is highly virulent.

cellobiose, D-fructose, trisodium citrate, sodium fumarate, sodium acetate, calcium gluconate, calcium lactate; the use of L-alanine as sole source of carbon and nitrogen; resistance towards 2 µg lincomycin.

The following features were uniformly absent in the 33 strains: nitrate reduction; formation of indole and acetoin; fermentative utilization of D-glucose; production of a fluorescent pigment on *Pseudomonas*-F agar; anaerobic growth; acid formation in litmus milk; growth at pH 4.5; growth at 4 or 37 °C; growth in the presence of 20% (w/v) D-glucose, 4% (w/v) NaCl, 0.01% (w/v) CuCl₂, 0.01% (w/v) cupric acetate, 0.02% (w/v) cadmium acetate, 0.013% (w/v) Cu(NO₃)₂·3H₂O, 0.015% (w/v) CuSO₄·5H₂O, 0.018% (w/v) CoSO₄·7H₂O, 0.01% (w/v) CoCl₂, 0.01% (w/v) ZnCl₂, 0.07% (w/v) methyl green; acid formation from *meso*-erythritol, L-rhamnose, L-sorbose, sorbitol, salicin, adonitol, dulcitol and *meso*-inositol; growth on L-rhamnose, sodium 2-ketogluconate, salicin, inulin, glycolic acid, L-leucine, L-arginine, L-methionine, L-isoleucine, L-threonine, glycine, L-tryptophan, L-lysine, L-cysteine, ethanolamine, sodium hippurate, acetamide, n-butyrate, DL-β-hydroxybutyrate, pelargonate, ethanol, n-butanol, iso-butanol, diethyleneglycol, adonitol, sodium-potassium tartrate, sodium oxalate and sodium benzoate; utilization of L-asparagine, glycine or L-glutamine as sole source of carbon and nitrogen; resistance towards 10 µg erythromycin, 10 µg streptomycin or 30 µg novobiocin.

Features giving different reactions are compiled in Table 2.

Only minor biochemical and physiological differences were noted between the two colony types (t1 and t2; large and small colony diameter, respectively) of *X. campestris* pv. *manihotis* strains HMB 72 and HMB 80 (Fig. 1); their protein patterns were almost identical (Fig. 2).

Table 2. *Different phenotypic reactions for the strains of X. campestris pv. manihotis and pv. cassavae*

+, 90% or more of strains are positive; —, 90% or more of strains are negative; D, 11–89% of strains are positive.

The following features occurred in more than 90% of the *X. campestris* pv. *manihotis* strains and in all pv. *cassavae* strains: acid formation from D-glucose, D-mannose and cellobiose; growth on sucrose, D-alanine, L-alanine, sodium succinate, sodium L-malate and sodium propionate; hydrolysis of starch; H₂S formation from L-cysteine; oxidative utilization of D-glucose; the use of sodium L-glutamate as sole C- and N-source; tolerance of 10 µg colistin sulphate.

The following features were absent in more than 90% of the *X. campestris* pv. *manihotis* strains and in all pv. *cassavae* strains: acid formation from D-ribose; utilization of L-serine as sole N-source; tolerance of 0.001% (w/v) malachite green, 0.01% (w/v) thionin, 30 µg nalidixic acid, 30 µg kanamycin, 10 µg gentamycin, 10 µg fusidic acid, 30 µg chloramphenicol and 30 µg tetracyclin.

The following features occurred in 11–89% of the *X. campestris* pv. *manihotis* strains and in one to five of the six pv. *cassavae* strains: cells in chains; occurrence of filaments, oxidase and polygalacturonase; tolerance of 2% (w/v) NaCl and 0.01% (w/v) triphenyltetrazolium chloride; acid from amygdalin; growth on glycerol; utilization of L-glycine, L-histidine and L-tyrosine as sole N-source; tolerance of 0.001% (w/v) CuO, 0.005% (w/v) crystal violet, 0.01% (w/v) basic fuchsin, 100 µg sulfafurazole, 10 U penicillin G, 25 µg cephaloridin, 10 µg methicillin, 30 µg neomycin, 200 µg nitrofurantoin and 300 U polymyxin B.

Phenotypic feature	No. of strains . . .	<i>X. campestris</i> pv.	
		<i>manihotis</i>	<i>cassavae</i>
Colonies yellow; growth on D-saccharic acid		—	+
Colonies white; hydrolysis of Tween 60; growth on DL-glyceric acid		+	—
Hydrolysis of Tween 80 and aesculin; gelatinase, lecithinase and β -glucosidase; acid from melibiose and trehalose; growth on MG medium, D-galactose, melibiose, L-glutamic acid, NH ₄ Cl + glucose + minerals, NH ₄ Cl + glucose + minerals, NH ₄ Cl + glucose + minerals + growth factors, NH ₄ Cl + glucose + minerals + vitamin-free Casamino acids + vitamins, glucose + minerals + vitamins + vitamin-free Casamino acids; growth on NH ₄ Cl + glucose + minerals + vitamins + 0.02% L-alanine, L-arginine, L-asparagine, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-threonine, L-tyrosine, L-tryptophan, L-lysine or L-valine; utilization of L-alanine, L-arginine, L-asparagine, L-cysteine, L-glutamic acid, L-glutamine, L-hydroxyproline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-threonine, L-tryptophan, L-lysine, L-valine or L-ornithine as sole N-source; utilization of L-hydroxyproline or proline as sole C- and N-source; tolerance of 0.001% HgCl ₂ , 0.001% Hg ₂ (NO ₃) ₂ , 0.001% Cu ₂ Cl ₂ , 0.005% safranin T, 0.005% methyl green, 0.0001% brilliant green		+	D
Coagulation in litmus milk; acid from lactose; growth on mucic acid		—	D
Alkalinization of litmus milk; growth on SX medium; acid from L-arabinose, maltose, D-xylose; growth on maltose; tolerance of 0.005% diamant fuchsin		D	+
Reduction in litmus milk; tolerance of 3% NaCl; acid from raffinose; tolerance of 0.001% cadmium acetate, 0.001% pyronin Y, 10 U bacitracin or 10 µg ampicillin		D	—

Numerical comparison of protein gel electrophoregrams

The protein electrophoregrams of 55 strains listed in Table 1 were prepared at least in duplicate. The reproducibility was excellent for the majority of the strains ($r > 0.95$). However, several *X. campestris* pv. *cassavae* strains showed, even after recultivation, reproducibility levels of $r = 0.90$. The most typical electrophoregram of each strain (Swings *et al.*, 1976) was used for the final clustering shown in Fig. 2. Meaningful taxonomic conclusions from numerical analyses of protein electrophoregrams can only be drawn above $r = 0.85$ (Kerstens, 1985); consequently, clustering levels below this value are represented as dashed lines in Fig. 2. Four electrophoretic clusters could be delineated at $r = 0.85$. All the *X. campestris* pv. *manihotis* strains grouped in the tight cluster 1, whereas the *X. campestris* pv. *cassavae* strains grouped in two electrophoretic clusters, 2 and 3. The protein patterns of the yellow strains CIAT 1164 and CIAT 1165, isolated

Fig. 2. Clustering of the protein electrophoregrams of *X. campestris* (*X. c.*) strains isolated from cassava and some other *Euphorbiaceae*, and the type strain of *X. campestris*, based on the correlation coefficient r and the unweighted average pair-group method.

from cassava in Colombia (Elango *et al.*, 1981), grouped in cluster 4 together with two strains of *X. campestris* pv. *poinsetticola*. The type strain of *X. campestris*, NCPPB 528, and the neopathotype strains of *X. campestris* pv. *euphorbiae* and pv. *ricini* occupied a separate position in the dendrogram (Fig. 2). Protein electrophoregrams of 16 representative strains are shown in Fig. 3.

DNA base composition and DNA:DNA hybridizations

DNA was prepared from eight representative strains selected from the phenotypic and protein electrophoretic clusters. The mol % G+C values of the DNAs and the results of the DNA:DNA hybridizations are compiled in Table 3. Three DNA-hybridization groups were recognized. As expected from their phenotypic and protein electrophoretic similarities, *X. campestris* pv. *manihotis* strains NCPPB 1834 and HMB 25 were genetically highly related to each other (99% DNA-binding). A high degree of DNA-binding (95%) was observed between

Fig. 3. Normalized protein electrophoregrams from 15 *X. campestris* strains isolated from cassava and some other *Euphorbiaceae*. Lanes (a)–(e): *X. campestris* pv. *manihotis* HMB 25, HMB 71, NCPPB 1834, HMB 27, HMB 60. Lanes (f)–(j): *X. campestris* pv. *cassavae* HMB 54, HMB 146, HMB 38, HMB 44, HMB 51. Lanes (k) and (l): yellowish isolates of Colombia CIAT 1164, CIAT 1165. Lane (m): *X. campestris* pv. *poinsettiiicola* ICPB XP137. Lane (n): *X. campestris* pv. *ricini* NCPPB 1063. Lane (o): *X. campestris* pv. *euphorbiae* NCPPB 1828. Lane (p): *X. campestris* NCPPB 528.

Table 3. DNA:DNA hybridizations between representative strains from the different *X. campestris* clusters studied

The results are shown in a half matrix. The degree of DNA binding (%D) is expressed versus the homologous reaction.

Strain	Mol % G+C	Degree of DNA binding:							
		1	2	3	4	5	6	7	8
1. <i>X. campestris</i> pv. <i>manihotis</i> NCPPB 1834*	65.9	100							
2. <i>X. campestris</i> pv. <i>manihotis</i> HMB 25	—	99	100						
3. Yellowish isolate CIAT 1164	66.9	60	—	100					
4. Yellowish isolate CIAT 1165	—	—	—	98	100				
5. <i>X. campestris</i> pv. <i>poinsettiiicola</i> ICPB XP137*	66.4	62	61	99	—	100			
6. <i>X. campestris</i> pv. <i>cassavae</i> HMB 54*	66.2	35	—	36	—	—	100		
7. <i>X. campestris</i> pv. <i>cassavae</i> HMB 38	—	—	—	—	—	—	95	100	
8. <i>X. campestris</i> pv. <i>campestris</i> NCPPB 528†	65.2‡	18	—	37	—	—	34	—	100

—, Not determined.

* Neopathotype strain.

† Type strain.

‡ From De Vos & De Ley (1983).

X. campestris pv. *cassavae* HMB 54 (neopathotype strain; member of phenon 2 and electrophoretic cluster 2) and *X. campestris* pv. *cassavae* HMB 38, which belonged to electrophoretic cluster 3 (Fig. 2) and occupied a separate position in the phenogram (Fig. 1). The DNA-relatedness between *X. campestris* pv. *manihotis* and pv. *cassavae* was low (35% DNA-binding; Table 3). The yellow cassava isolates from Colombia (strains CIAT 1164 and CIAT 1165) were genetically highly related to each other and to the neopathotype strain of *X. campestris* pv. *poinsettiiicola*, ICPB XP137, reflecting the protein electrophoretic similarities of

these strains. They have a higher DNA:DNA homology (60%*D*) with the *X. campestris* pv. *manihotis* cluster than with the *X. campestris* pv. *cassavae* cluster (36%*D*) and the type strain of *X. campestris* pv. *campestris* (37%*D*).

Pathogenicity

On cassava leaves, all the pv. *manihotis* strains induced angular leaf spots evolving into blight areas, and caused a systemic infection in the stem leading to formation of bacterial pockets, followed by exudation, wilting and abscission of the leaves (Table 1). Differences in the speed of symptom formation were evident: strains HMB 10, HMB 23, HMB 60 and HMB 78 were the most virulent ones, whereas strains HMB 9, HMB 68 and HMB 79 were weakly virulent. The neopathotype strain NCPPB 1834, a subculture of the original isolate (ENA-300) reported as inducing only necrotic angular leaf spots (Robbs *et al.*, 1972), showed a low to intermediate virulence that was different from the pv. *cassavae* strains. *X. campestris* pv. *cassavae* strain HMB 38 showed a lower virulence than the other pv. *cassavae* strains.

The Colombian yellowish isolates occasionally induced small spots, which rapidly became necrotic, on the leaves and a restricted browning of the vessels. Similar restricted lesions were induced on cassava by pv. *poinsettiicola*, but not by pv. *euphorbiae*, pv. *ricini*, nor pv. *campestris*.

On *E. pulcherrima*, restricted lesions were observed after inoculation with all strains tested except pv. *poinsettiicola* and pv. *ricini*, which induced water-soaked greasy spots extending more than 4 mm around the inoculation point (Table 4). On *R. communis*, only pv. *ricini* induced this type of symptom, the other strains causing no reaction.

DISCUSSION

All strains of phenons 1 and 2 possessed both the generic characteristics of *Xanthomonas* and all the features described for the species *X. campestris* (Bradbury, 1984; Van den Mooter, 1984). The mol % G + C of representative strains of *X. campestris* pv. *manihotis* and pv. *cassavae* (Table 3) fit in the mol % G + C range of *X. campestris* (63.5–69.2%; Bradbury, 1984). The generic assignment of *X. campestris* pv. *manihotis* HMB 25 and pv. *cassavae* HMB 38 to the genus *Xanthomonas* was also confirmed by DNA:rRNA hybridizations using ¹⁴C-labelled rRNA of the type strain *X. campestris* NCPPB 528 (P. Segers & J. De Ley, unpublished results).

A good overall correlation was found between the grouping obtained by numerical analysis of phenotypic features, protein electrophoregrams and DNA:DNA hybridizations. All *X. campestris* pv. *manihotis* strains clustered in the homogeneous phenon 1 (Fig. 1) and the tight electrophoretic cluster 1 (Fig. 2). All the *X. campestris* pv. *cassavae* strains of phenon 2 (Fig. 1) grouped in electrophoretic cluster 2, whereas the phenotypically aberrant strain HMB 38 grouped with seven other *X. campestris* pv. *cassavae* strains in the protein electrophoretic cluster 3 (Fig. 2). Yet, strain HMB 38 has 95% DNA homology (Table 3) with strain HMB 54 (the neopathotype strain of *X. campestris* pv. *cassavae*), and is thus considered to be an authentic member of this pathovar (Table 2).

X. campestris pv. *manihotis* (phenon 1, electrophoretic cluster 1)

In addition to the features of *X. campestris* already described by Bradbury (1984), the following characteristics were detected in more than 90% of the *X. campestris* pv. *manihotis* strains: hydrolysis of Tween 60, Tween 80 and starch; growth in the presence of 0.001% (w/v) Hg(NO₃)₂ but not in the presence of 0.05% (w/v) triphenyltetrazolium chloride or 0.001% (w/v) malachite green; β -glucosidase activity; acid formation from melibiose but not from D-ribose and lactose; growth on DL-glyceric acid but not on mucic acid, saccharic acid or ethanol; use of L-threonine as sole source of nitrogen and susceptibility against 10 μ g gentamycin and 10 μ g fusidic acid. None of the *X. campestris* pv. *manihotis* strains produces a yellow pigment.

Biochemical and physiological variations within this pathovar have already been mentioned by Maraite *et al.* (1981). No correlation was found between biochemical and physiological features or a group of features and protein electrophoretic patterns on the one hand and the geographical origin, year of isolation or virulence on the other. Highly virulent and weakly

Table 4. Host range of several *X. campestris* strains on three Euphorbiaceae

Name	Strain no.	Original host	Reaction* on:		
			<i>Manihot esculenta</i>	<i>Euphorbia pulcherrima</i>	<i>Ricinus communis</i>
<i>X. campestris</i> pv. <i>manihotis</i>	HMB 9, HMB 10, HMB 23, HMB 36, HMB 55,† HMB 72, HMB 78	<i>Manihot esculenta</i>	4-6	1 (0)	0
<i>X. campestris</i> pv. <i>cassavae</i>	HMB 29, HMB 38, HMB 54,† HMB 146	<i>Manihot esculenta</i>	2-3	1 (0)	0
Colombian yellowish isolates	CIAT 1164, CIAT 1165	<i>Manihot esculenta</i>	0 (1)	1 (0)	0
<i>X. campestris</i> pv. <i>poinsetticola</i>	ICPB XP137,† PDDCC 332	<i>Euphorbia pulcherrima</i>	0 (1)	2	0
<i>X. campestris</i> pv. <i>ricini</i>	NCPFB 1063†	<i>Ricinus communis</i>	0	2	2
<i>X. campestris</i> pv. <i>euphorbiae</i>	NCPFB 1828†	<i>Euphorbia acalyphoides</i>	0	1	0
<i>X. campestris</i> pv. <i>campestris</i>	NCPFB 528†	<i>Brassica oleracea</i>	0	1 (0)	0

* Reaction on lamina pricked with a contaminated needle. Scale of symptom severity is from 0 to 6: 0-1 is non pathogenic and 6 is highly virulent. In parentheses: occasional reaction.

† Neopathotype strain.

‡ Type strain.

virulent strains were situated at random in the dendrograms (Figs 1 and 2). A similar situation has also been observed within *X. oryzae* and *X. campestris* pv. *oryzicola* (Vera Cruz *et al.*, 1984; Van den Mooter, 1984) and *X. graminis*, *X. campestris* pv. *phlei*, pv. *arrhenatheri* and pv. *poae* (Van den Mooter, 1984; Van den Mooter *et al.*, 1986).

The strains HMB 10, HMB 23 and HMB 27 grouped in the phenogram (Fig. 1) somewhat separate from the other *X. campestris* pv. *manihotis* strains, but were scattered over the electrophoretic cluster 1 (Fig. 2). Upon visual inspection, the protein patterns of these three strains were almost identical to those of the other pv. *manihotis* strains. The high degree of genetic relatedness between strains NCPPB 1834 and HMB 25 (Table 3) consolidates the phenotypic and protein electrophoretic similarities of the investigated *X. campestris* pv. *manihotis* strains. All our data indicate that the pv. *manihotis* strains constitute a phenotypically and genetically homogeneous group of bacteria, clearly distinct from *X. campestris* pv. *cassavae*.

X. campestris pv. *cassavae* (phenon 2, electrophoretic clusters 2 and 3)

In addition to the features of the species *X. campestris* already described by Bradbury (1984), the following characteristics were found in all the strains of *X. campestris* pv. *cassavae*: yellow pigmentation; hydrolysis of starch; lack of growth in the presence of 0.05% (w/v) triphenyltetrazolium chloride, 0.001% (w/v) pyronin Y or 0.001% (w/v) malachite green; growth on SX agar; acid formation from maltose or xylose but not from raffinose or ribose; growth on maltose, saccharic acid but not on DL-glyceric acid and ethanol; susceptibility to 10 U bacitracin.

Phenotypically (Fig. 1) and when compared by protein electrophoresis (Fig. 2), *X. campestris* pv. *cassavae* was more heterogeneous than *X. campestris* pv. *manihotis*. Although the protein patterns of the pv. *cassavae* strains were similar (Fig. 3), two electrophoretic clusters were delineated by numerical analysis (Fig. 2). Only strain HMB 38 of electrophoretic cluster 3 was investigated in the phenotypic analysis; it occupied a separate position in the phenogram (Fig. 1). This strain differed from the other pv. *cassavae* strains by its inability to utilize amino acids as sole source of nitrogen and by its lower virulence, yet it showed a high degree of DNA-relatedness with the neopathotype strain HMB 54. Within the pathovar *cassavae* no correlation was detected between the phenotypic features and the protein electrophoretic patterns on the one hand and the geographical origin, year of isolation or pathogenicity on the other.

Differentiation of *X. campestris* pv. *manihotis* from pv. *cassavae*

A clearcut differentiation between the pathovars *manihotis* (phenon 1) and *cassavae* (phenon 2 and strain HMB 38) is possible by growth on saccharic acid, growth on DL-glyceric acid, hydrolysis of Tween 60 and the formation of yellow pigment (pv. *manihotis* gives negative, positive, positive and negative results, and pv. *cassavae* the opposite) (Table 2). Until now, the only differential character between the pathovars *manihotis* (white) and *cassavae* (yellow) was pigmentation (Maraite & Weyns, 1979). It should be noted, however, that the absence of the typical yellow pigment is not limited to the pathovar *manihotis*; e.g. some strains belonging to the pathovars *phyllanthi*, *ricini*, *vitiswoodrowii*, *vesicatoria*, *mangiferaeindicae*, *azadiractae* and *pedalii* and to *Xanthomonas maltophilia* are also white (Van den Mooter, 1984). Maraite & Weyns (1979) differentiated the pathovars *manihotis* and *cassavae* by the acidification of maltose: the former showing a delayed (5–16 d) and the latter a rapid (2 d) acidification. In the present work, *X. campestris* pv. *manihotis* strains showed differences in acid production from maltose (see Table 2). Growth on different carbon sources has not received much attention in previous publications. From our results it is obvious that growth on saccharic acid and glyceric acid are useful features to differentiate the two pathovars. No differences were found between the two phenons in the susceptibility towards antibiotics *in vitro*. The susceptibility patterns agree with those found for the strains of the species *X. campestris* (Van den Mooter *et al.*, 1981).

X. campestris pv. *poinsettiiicola* and the yellowish strains isolated from cassava in Colombia (electrophoretic cluster 4)

Elango *et al.* (1981) found that the yellow strains, isolated from cassava in Colombia were

serologically similar to *X. campestris* pv. *cassavae*. However, our results show that the two Colombian strains CIAT 1164 and CIAT 1165 were electrophoretically different from the pv. *cassavae* strains, but very similar to the investigated *X. campestris* pv. *poinsetticola* strains (Figs 2 and 3). DNA:DNA hybridizations (Table 3) confirmed that the Colombian cassava isolate CIAT 1164 is genetically highly related to the neopathotype strain of *X. campestris* pv. *poinsetticola* ICPB XP137 (99% DNA-binding) and clearly different from *X. campestris* pv. *cassavae* HMB 54. The ability of *X. campestris* pv. *poinsetticola* ICPB XP137 to induce active lesions on *E. pulcherrima* differentiated it, however, from the non-pathogenic or only weakly pathogenic Colombian yellowish isolates (Table 4). The reported overlap in host-range of pv. *poinsetticola* (infecting *E. pulcherrima* and *M. esculenta*) and pv. *euphorbiae* (infecting *E. acalyphoides* and *R. communis*) (Sabet *et al.*, 1969) was not confirmed by our results. However, *X. campestris* pv. *ricini* NCPB 1063 and other pv. *ricini* strains (H. Maraite & N. de Bonhome, unpublished data) induced active lesions on *E. pulcherrima*, similar to those caused by pv. *poinsetticola*. DNA-binding values (Table 3) indicate that the yellowish Colombian isolates and pv. *poinsetticola* strain ICPB XP137 are genetically more closely related to pv. *manihotis* (60% *D*) than to strains of *X. campestris* pv. *cassavae* (36% *D*). On the basis of the protein profiles of the outer cell membranes, Dos Santos & Dianese (1985) also concluded that the Colombian yellowish isolates are more related to pv. *manihotis* than to pv. *cassavae*. Based on the analysis of the soluble enzymes, Kimura & Dianese (1983) even suggested that the Colombian isolates could be yellow strains of pv. *manihotis*; but our data demonstrate clear differences between these strains and pv. *manihotis*.

The rather low DNA-relatedness values, the phenotypic features, the protein electrophoregrams and phytopathogenic symptoms allow a clearcut differentiation between *X. campestris* pv. *cassavae*, pv. *manihotis* and pv. *poinsetticola*. More *X. campestris* strains isolated from other *Euphorbiaceae* need to be compared before definite conclusions can be drawn concerning the relationships of pv. *manihotis*, pv. *cassavae* and pv. *poinsetticola* versus pv. *euphorbiae*, pv. *phyllanthi* and pv. *ricini*.

J.D.L. is indebted to the Fonds voor Geneeskundig Wetenschappelijk Onderzoek and to the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw (IWONL) (Belgium) for research and personnel grants. H.M. is grateful to the International Institute of Tropical Agriculture, Ibadan, Nigeria, and the Administration de la Coopération au Développement, Brussels, for collaboration and funding, and to Nadine de Bonhome for technical assistance. K.K. and J.S. are grateful to the Nationaal Fonds voor Wetenschappelijk Onderzoek for research grants. M.V.d.M. is indebted to the IWONL for scholarships.

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