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Metabolic diversity of *Xanthomonas axonopodis* pv. *vignicola*, causal agent of cowpea bacterial blight and pustule

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Abstract

Fifty-five strains of *Xanthomonas axonopodis* pv. *vignicola*, isolated from blight and pustule symptoms of cowpea leaves, originating from 11 countries, were characterized for their carbon-source metabolization pattern using the Biolog GN microplate system. Great variation was found between strains according to origin. Dextrin, glycogen and succinamic acid were not used by strains from Benin, Uganda or Thailand, but by all the other strains (excluding two strains from Mozambique), whereas N-acetyl-D-glucosamine and malonic acid were used by the strains from Benin, Uganda and Thailand, but generally not by the other strains. The strains from Benin, Uganda and Thailand, as well as strains from Venezuela, Brazil and Mozambique, clustered separately from the others in multivariate analysis. Nineteen substrates were used by all the strains, 47 not by any strain and 29 only by some strains. No considerable differences were found between strains isolated from blight symptoms and from pustules. Virulence of strains was not related to the metabolic pattern. The Biolog database was not representative of the diversity of *X. axonopodis* pv. *vignicola*, since all strains were identified as *Xanthomonas campestris*, although belonging to eight pathovars, while only eight of nine strains from Benin and both strains from Thailand were identified as *X. campestris* pv. *vignicola*. The Biolog system appeared to be useful for characterizing the diversity of *X. axonopodis* pv. *vignicola* strains. A set of representative strains based on metabolic and molecular diversity, virulence and geographic origin is suggested for screening for resistant cowpea cultivars.

Introduction

Cowpea bacterial blight and pustule caused by *Xanthomonas axonopodis* pv. *vignicola* (Vauterin et al., 1995), former *Xanthomonas campestris* pv. *vignicola* (Burkholder). In the following paper the earlier name is used for convenience with the description in the Biolog database. *X. campestris* pv. *vignicola* is one of the most important diseases of cowpea in all areas where cowpea is grown. High seedling mortality due to seedborne inoculum and secondary infection in growing plants causes heavy losses (Kishun, 1989; Sikirou, 1999). Reductions in seed yield of more than 64% were observed under favourable conditions (Sikirou, 1999; Wydra and Rudolph, 1999). Control is difficult and integrated control measures are recommended (Sikirou et al., 2001; Wydra et al., 2001; Wydra, 2002). Cowpea bacterial blight has been described in Latin America (Vakili et al., 1975), North America (Gitaitis et al., 1986), Africa (Kaiser and Ramos, 1979; Adam, 1990), Asia (Jainkittivong et al., 1989) and Europe (Severin and Stancescu, 1990), while cowpea bacterial pustule has only been reported from Nigeria (Williams, 1975) and East Africa (Patel, 1975; Kaiser and Ramos, 1979; Singh and Allen, 1979; Ouko and Buruchara, 1989). Mixed symptoms of blight and pustules are often

observed on cowpea leaves (Khatri-Chhetri, 1997). Most of the strains isolated from leaves with blight and/or pustule symptoms collected from West-Africa, Uganda and Venezuela, and their re-isolates, produced both blight and minute pustules or specks, irrespective if they were isolated from blight or pustule symptoms (Khatri-Chhetri, 1999).

Patel and Jindal (1982) proposed a separate pv. '*vignaeunguiculatae*' of *X. campestris* for the pustule-causing strain, but a pathotype strain has never been designated. The differentiation between pathovars causing either bacterial blight or bacterial pustule was not accepted internationally, and some authors still describe different strains within *X. campestris* pv. *vignicola* to be responsible for the different symptoms (Gitaitis, 1983; Khatri-Chhetri et al., 1998a; Wydra et al., 2003). Hence, confusion exists between the symptoms called pustule and blight, the systemicity of the disease and their causal agents. Additionally, considerable variations of *X. campestris* pv. *vignicola* in virulence (Khatri-Chhetri et al., 1998a; Wydra et al., 2003) and genetic composition (Verdier et al., 1998) are reported. Molecular characterization of the strains by RFLP analysis using the plasmid probe pthB revealed nine haplotypes, which were independent of pathogenic variation (virulence and origin of isolation from blight or pustule symptom) and geographic origin.

The Biolog GN microplate system developed by Biolog, Inc. (Hayward, Calif., USA) is a quick and easy method for the identification and characterization of bacteria. Metabolism patterns of 95 carbon compounds in a microplate serve for identification using a computerized database. Vauterin et al. (1995) used the Biolog System as one of the criteria to classify pathovars of *Xanthomonas* spp. and Hildebrand et al. (1993) used nutritional screening to identify DNA homology groups of *X. campestris* pathovars. Thus, a comparison of the metabolic diversity of a wide range of *X. campestris* pv. *vignicola* strains using the Biolog GN microplate system could (i) characterize the diversity of *X. campestris* pv. *vignicola* strains varying in virulence, geographic origin and genetic grouping (Verdier et al., 1998), (ii) contribute to further confirmation that the xanthomonads causing blight and pustule on cowpea are only strains of the same pathovar (Verdier et al., 1998; Khatri-Chhetri, 1999), and, thus (iii) further define and complete a set of representative strains to be used for inoculation tests in breeding for resistance to cowpea bacterial blight and pustule.

Materials and methods

Bacteria

Fifty *X. campestris* pv. *vignicola* strains freshly isolated from blight symptoms (38 strains from blight symptoms, of which strain no. 42 was isolated from a leaf where pustules were present) and from pustule symptoms (12 strains isolated from pustule symptoms with blight symptoms on the same leaf, while strains numbers 54 and 55 were isolated from pustule symptoms without blight symptoms on the same leaf) originating from seven countries and five reference strains of *X. campestris* pv. *vignicola* from four countries (Table 1) were selected. The strains were evaluated for virulence (highly, medium and low) on the susceptible cowpea genotype IT84D-449 (Verdier et al., 1998; Khatri-Chhetri et al., 1998a; Khatri-Chhetri, 1999).

Metabolization of organic substrates

Fifty-five strains of *X. campestris* pv. *vignicola*, *X. campestris* pv. *phaseoli* (Göttinger Sammlung Phytopathogener Bakterien, Göttingen, Germany, GSPB 351) and *X. campestris* pv. *phaseoli* var. *fuscans* (GSPB 271) were tested for their metabolism of 95 organic substrates using Biolog GN microplates. Bacteria grown for 48 h at 29 ± 1 °C on tryptic soy agar (tryptic soy broth 30.0 g, Bacto agar 15.0 g, in 1 l of distilled water) were suspended in 25 ml sterile NaCl (0.85%) solution (Von Kietzell et al., 1994). Bacterial cells were washed (6000 × *g*, 18 min) and the pellet resuspended in 20 ml sterile saline to remove extracellular polysaccharides, cell debris and other exogeneous substances, which may be metabolized by the bacteria resulting in false positive readings (Adams and Martin, 1964; Black and Sweetmore, 1994). Bacterial suspensions were adjusted to an OD of 0.3 at 590 nm (corresponding to 5×10^8 cfu/ml, verified by counting colonies of eight strains) (Fessehaie, 1997). The Biolog microplates were prewarmed at 29 ± 1 °C for about 5 min, inoculated with 150 µl bacterial suspension per well and incubated at 29 ± 1 °C. Metabolism of substrates by *X. campestris* pv. *vignicola* was visually evaluated 24 h after incubation. For introduction into the database for identification, three categories, positive, negative and borderline, were formed, and classes 1–3 (see below) were evaluated as positive.

Table 1. Origin of 55 strains of *X. campestris* pv. *vignicola*, symptom on cowpea leaf, from which strains were isolated, and virulence class of strains

Origin	Isolated from	Designation of strains	Virulence[1]
Cameroon	Blight[2]	1[3]	L
		3 (2403[4]), 7	M
	Pustule + blight[5]	2, 5, 6	L
		4	M
		8 (2404)	H
Nigeria	Blight	13, 22, 25 (2795), 27	L
		10, 12, 14, 16	M
		9, 19, 23, 24 (2408), 26 (2409), 28 (2411), 29, 30 (2412)	H
	Pustule + blight	15, 20	L
		18, 21 (2407)	M
		11 (2405), 17 (2406)	H
Niger	Blight	32	M
		31 (2414), 33 (2413), 35 (2416)	H
	Pustule + blight	34 (2415)	H
Benin	Blight	37, 39, 43, 38	L
		36, 40, 41 (2517), 44	M
	Blight + pustule[6]	42	M
Uganda	Blight	45	M
Venezuela	Blight	46 (534), 48 (536b)	M
		47 (536a)	H
Mozambique	Pustule[7]	54 (2417), 55	L
Reference strains[8]			
Sudan	Unknown	49 (NCPPB 2061[9], 2331)	L
Thailand	Blight	52 (2459)	L
		51 (2455)	H
Brazil	Unknown	50 (NCPPB 3187, 2332)	L
USA	Unknown	53 (ICMP 333, 2327)	AV

[1]L = lowly virulent, M = medium virulent, H = highly virulent, AV = avirulent; virulence as described by Verdier et al. (1998), Khatri-Chhetri (1999) and Wydra et al. (2003).
[2]Blight = isolated from blight symptom.
[3]Serial number of strains in this study.
[4]Strain number from Göttinger Sammlung Phytopathogener Bakterien (GSPB), Göttingen, Germany.
[5]Pustule + blight = isolated from pustule, but blight was also present on the leaf.
[6]Blight + pustule = isolated from blight, but pustules were also present on the leaf.
[7]Pustule = isolated from pustule. FNPB1 and FNPB5 obtained from Y. P. Rao, Maputo, Mozambique.
[8]Reference strains originating from Thailand and USA were obtained from N. Furuya, Japan Culture Collection (MAFF), Kyushu University, Japan and J. Janse, Wageningen, Netherlands, respectively.
[9]NCPPB = National Collection of Plant Pathogenic Bacteria, Hatching Green, Harpenden, UK; ICMP = International Collection of Micro-Organisms from Plants, Auckland, New Zealand.

To increase the distinguishing capacity of the system for diversity studies, colour reactions were categorized (different to the manufacturer's instructions) which propose three categories, into five scores: strongly positive (class 3), moderately positive (class 2), weakly positive (class 1), border line (b) and negative (−). To determine the variability of results, readings from washed and unwashed bacteria (Fessehaie, 1997) of three *X. campestris* pv. *vignicola* strains (nos 3, 23 and 34) were compared. Strains nos 8, 15, 24, 33, 34 and 54 were repeated twice to test the reproducibility of the system.

Data were analysed using the programme for bacterial identification (Biolog's Micrologᵀᴹ1, Release 3.50). A similarity index (S.I.) of $\geqslant 0.5$ meant a correct identification. For characterization of their diversity and grouping, the metabolic patterns of 55 *X. campestris* pv. *vignicola* strains were subjected to cluster analysis (Genstat 5, Release 3.2).

Results

Standardization of the test system

Generally, three unwashed *X. campestris* pv. *vignicola* strains used more substrates and showed stronger colour reactions than washed bacteria (data not shown). Therefore, for subsequent studies, bacteria were washed. Improvements in identification of *X. campestris* pv. *vignicola* by the database were not obtained by the washing step. Two replications of five strains generally differed only slightly in the metabolism of those substrates, which were only weakly metabolized by the 55 *X. campestris* pv. *vignicola* strains (data not shown).

Metabolism of carbon sources

The 55 *X. campestris* pv. *vignicola* strains varied considerably in the utilization of carbon sources, based on an evaluation of colour reaction in five classes. Nineteen substrates (cellobiose, D-fructose, L-fucose, α-D-glucose, D-mannose, D-psicose, sucrose, D-trehalose, methylpyruvate, mono-methylsuccinate, α-keto-glutaric acid, succinic acid, bromo succinic acid, alanin-amide, D-alanine, L-alanine, L-alanine-glycine, L-glutamic acid and L-serine) were used by all the strains, 47 not by any strain and 29 were only used by some strains (dextrin, glycogen, tween 40, tween 80, N-acetyl-D-glucosamin, D-galactose, gentiobiose, maltose, D-melibiose, β-methyl-D-glucoside, acetic acid, *cis*-aconotic acid, citric acid, α-hydoxy-butyric acid, itaconic acid, α-keto-butyric acid, D,L lactic acid, malonic acid, propionic acid, D-saccharic acid, succinamic acid, L-aspartic acid, glycyl-L-glutamic acid, L-proline, L-threonine, glycerol, D,L-α-glycerol-phosphate, glucose-1-phosphate and glucose-6-phosphate). Among the 29 variably used substrates, eight substrates were metabolised by 93–96%, and 2 substrates were not used by 91–93% of the strains.

Differences according to geographic origin

Clear differences between strains originating from Benin, Thailand and Uganda and those from other countries were observed in the use of 10 carbon sources (Table 2). The African strains were further be divided into three groups: (a) from Benin and Uganda, (b) from Mozambique (see below) and (c) from other countries. Cluster analysis resulted in two major groups (Figure 1), with (1) strains from Cameroon, Nigeria, Niger, Venezuela, Brazil, USA, Sudan and one strain from Mozambique, and (2) strains from Benin, Thailand and Uganda and one strain from Mozambique. This grouping corresponds to the grouping in a two-dimensional plot of axis 1 *versus* axis 2, where it was also shown that group (2) is more homogeneous than group (1) (data not shown). Most of the strains of group 2 were correctly identified by the database as *X. campestris* pv. *vignicola*. Strain no. 9 from Nigeria was classified separately from the two groups. In a 2-dimensional plot of axis 1 *versus* axis 3, three relatively distinct groups were formed, differentiating strains of the former group 1: group I with strains originating from Benin and Thailand, group II with strains from Venezuela, Brazil and Mozambique and group III with strains from other countries, with strain no. 45 from Uganda being separate from the groups (Figure 2). Considering the variations in substrate utilization and clustering patterns (Table 2, Figures 1 and 2), strains were be finally categorized into four groups: (i) those from Cameroon, Nigeria, Niger, Sudan and USA, (ii) from Benin, Thailand and Uganda, (iii) from Venezuela and Brazil and (iv) from Mozambique. Only the grouping pattern of strains from Benin and Thailand correlated with a nearly 100% correct identification as *X. campestris* pv. *vignicola* by the database. The database, therefore, proves not to be representative of the diversity of the pathogen.

Differences between strains isolated from blight and pustule symptoms

When 41 'blight' strains isolated from blight symptoms and 12 strains from pustule symptoms (Table 1), which after inoculation produced both blight and pustules, irrespective if they were isolated from blight or pustule symptoms (Khatri-Chhetri, 1999), were compared, striking differences were not detected (data not shown). Only the two strains isolated from pustule symptoms which did not show any blight, originating

Table 2. Differences between *X. campestris* pv. *vignicola* strains from Benin, Thailand and Uganda and those from other countries in metabolism of variably used carbon sources

Origin	No. of strains	Use of carbon sources									
		A3[1]	A4	A8	B10	C1	E7	F2	H9	H11	H12
Benin	9	−[2]	−	+	−[3]	−[4]	+[5]	−	−[6]	−	−
Uganda	1	−	−	+	b	−	+	−	+	−	−
Thailand	2	−	−	+	−	−	+	−	−	−	−
Cameroon	8	+	+	−	+	+	−	+	+	+[3]	+[3]
Nigeria	22	+	+	−	+	+	-	+	+	+[6]	+[3]
Niger	5	+	+	−	+	+	−	+	+	+[7]	+[8]
Venezuela	3	+	+	−	+	+	−	+[5]	+	+	+
Sudan	1	+	+	+	+	+	−	+	+	+	+
Brazil	1	+	+	+	+	+	−	+	+	+	+
USA	1	+	b	−	+	+	−	+	+	+	+
Mozambique[5]	2	+	+	+	+	−	−	+	−	+	+

[1]A3 = dextrin; A4 = glycogen; A8 = N-acetyl-D-glucosamine; B10 = maltose; C1 = D-melibiose; E7 = malonic acid; F2 = succinamic acid; H9 = glycerol; H11 = glucose-1-phosphate; H12 = glucose-6-phosphate.
[2]Evaluation: + = used by all strains if not differently indicated; b = border line evaluation; − = not used by any strain.
[3]Used by percentage of strains: 50%; [4]80%; [5]66%; [6]77%; [7]60%; [8]20%.
[5]Additionally, strains from Mozambique used lactulose, while others did not, and did not use gentiobiose and D,L-lactic acid, while other strains used these C-sources.

from Mozambique, varied considerably from the other 53 strains: gentiobiose, D-melibiose, D,L-lactic acid and glycerol were not used by the strains from Mozambique, but by all the others (100%, 96%, 90%, and 87% of the other strains, respectively), while lactulose was used by the strains from Mozambique, but not by the others.

Comparison according to virulence classes

Comparing strains belonging to three virulence classes (Table 1), considerable differences in the use of carbon sources were not observed. However, D-saccharic acid was not used by highly virulent strains, but by 40% and 30% of the medium and lowly virulent strains, respectively, whereas glycyl-L-glutamic acid and D,L-α-glycerol phosphate were used by all highly virulent strains, but not by 10% and 25% of the medium and 11% and 42% of the weakly virulent strains, respectively (data not shown).

Differences between X. campestris *pv.* vignicola *and two* X. campestris *pv.* phaseoli *strains*

Two strains of *X. campestris* pv. *phaseoli* differed slightly from 55 *X. campestris* pv. *vignicola* strains: dextrin, not or weakly used by the pv. *vignicola* strains, was strongly used by both pv. *phaseoli* strains. The pv. *vignicola* strains showed no metabolism of i-erythritol, turanose, glycyl-L-aspartic acid and urocanic acid, whereas these were used by the strain of *X. campestris* pv. *phaseoli* var. *fuscans*, though not by the strain of *X. campestris* pv. *phaseoli*. The substrates D,L lactic acid, D,L-α-glycerol phosphate and glucose-1-phosphate, which were oxidized by most of the *X. campestris* pv. *vignicola* strains, were not used by both the pv. *phaseoli* strains.

Identification by the Biolog database

The Biolog database identified all the strains as *X. campestris*, belonging to eight different pathovars: *carotae* (38% of the strains), *manihotis* (20%), *vignicola* (18%), *alfalfae* (9%), *campestris* (7%), *vesicatoria* (4%), *phaseoli* (2%) and *begoniae* (2%) (Figure 1). Only the strains originating from Benin (except strain no. 42) and Thailand were identified as *X. campestris* pv. *vignicola* with high similarity indices of 0.42–0.93 for strains from Benin, and 0.28 and 0.76 for strains from Thailand.

For breeding purposes, inoculation with a set of strains representing the diversity in metabolic patterns

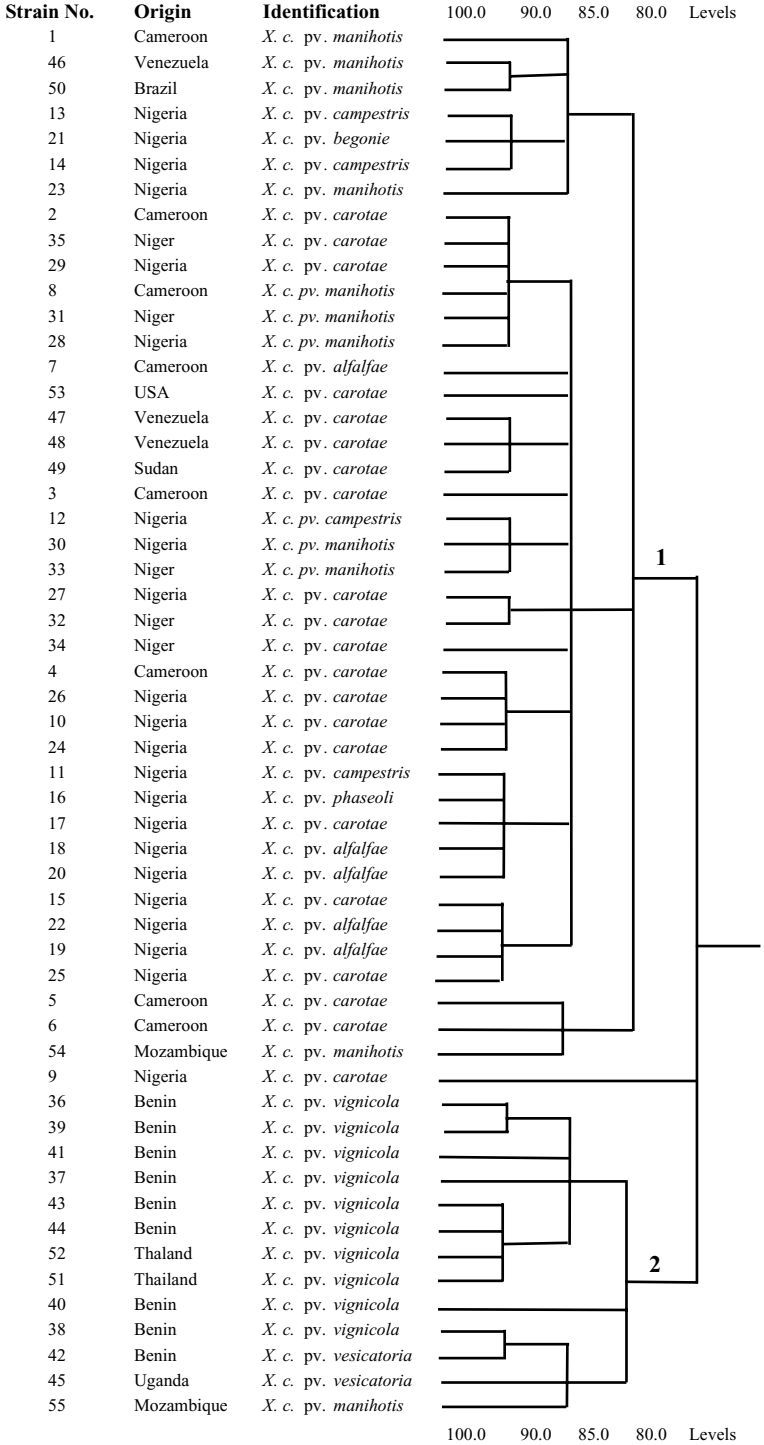

Figure 1. Grouping of 55 *X. campestris* pv. *vignicola* strains based on metabolism of 95 carbon sources and mostly 'incorrect' identifications by the Biolog database.

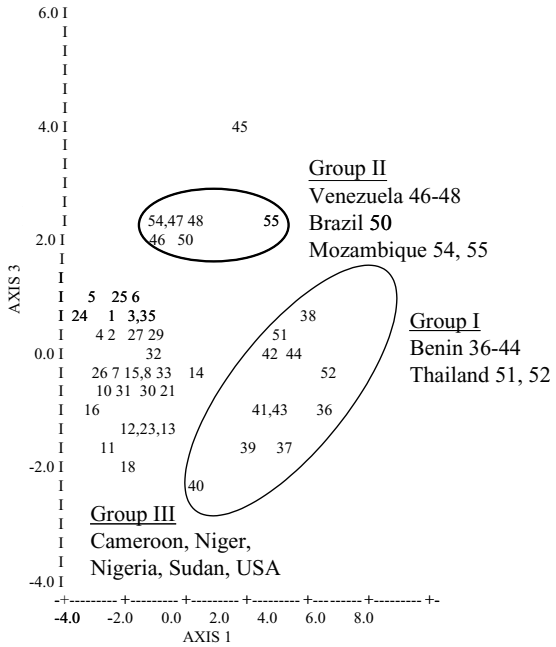

Figure 2. Cluster analysis of 55 *X. campestris* pv. *vignicola* strains based on metabolism of 95 carbon sources (axis 1 × axis 3). Coinciding strains: 9 and 49 with 7, 17 with 6, 19 and 22 with 15, 20 with 18, 28 and 34 with 21 and 53 with 5. Strain no. 40 was included in group II, because it was close to this group of strains from Benin in the axis 1 × axis 2 graphic. Strain 45 (ungrouped) originated from Uganda.

(groups i–iv), virulence (L, M, H), geographic origin and molecular diversity (nine haplotypes in four clusters in RFLP analysis) (Verdier et al., 1998), is proposed: strains 8 (4b1) (i, H, Cameroon, cluster 1, respectively), 11 (7b1, 2405) (i, H, Nigeria, cluster 2), 31 (44a, 2414) (i, H, Niger, cluster 1), 32 (47a) (i, M, Niger, cluster 4), 41 (16g, 2517) (ii, M, Benin, no RFLP data), 47 (536a1g) (iii, H, Venezuela, cluster 3) and 54 (FNPB1, 2417) (iv, L, Mozambique, no RFLP data).

Discussion

Differences between strains from various origins

The metabolism patterns based on an extended evaluation system of five colour degrees allowed a grouping of strains according to geographic origin, among which the groups of strains from Thailand, Benin and Uganda, strains from Venezuela and Brazil, and strains from Mozambique were most prominent. Also biochemical characterization of the fatty acid composition of most of the here tested strains grouped the strains from Venezuela apart from the other strains, and strains from Mozambique on one side of the main cluster, while strains from Benin and Thailand did not group separately (Khatri-Chhetri, 1999). Generally, the tested strains were identified as *X. campestris* pv. *vignicola* by an improved fatty acid database (40 of the strains of this study analysed) (Khatri-Chhetri, 1999), and by ribotyping (36 of the strains analysed) (Verdier et al., 1998). The nutritional variations may be due to differences in climate, host type and other factors in various geographical areas, which may have led to the development of slightly different growth requirements of the bacteria. On the other hand, the similarity of strains from Benin and Thailand may be due to the distribution of seeds carrying the seedborne pathogen between these two areas and a limited further distribution from those regions. The similarity of strains from Venezuela, Brazil and Mozambique may also indicate dissemination of the pathogen via contaminated seeds. Additional RFLP analysis (Verdier et al., 1998) of the strains from Benin, Thailand and Mozambique would be useful to check the grouping and could give further insight into pathogen migration. The high values for correct identification of the strains from Benin and Thailand by the database was also confirmed by Sikirou (1999), with six of the *X. campestris* pv. *vignicola* strains tested here from Benin.

Comparison of strains of different virulence and isolated from blight and pustule symptoms

Differences in virulence classes were not correlated to metabolic grouping. Also Verdier et al. (Verdier et al., 1998), analysing 36 of the strains of the present study by RFLP, did not find differences in virulence (phenotype) being linked to the nine observed haplotypes, which fell into four clusters. The Biolog system did not reveal considerable differences in carbon source metabolization between 'blight' and 'pustule' strains originating from Cameroon, Nigeria, Niger and Benin. However, the strains did not differ in pathological, biochemical (fatty acid patterns (Khatri-Chhetri, 1999)) or genetic characters (Verdier et al., 1998). Only two 'pustule' strains originating from Mozambique behaved different from all the other *X. campestris* pv. *vignicola* strains in the Biolog tests. However, from this small number of strains tested, it could not be decided whether these reactions were typical for all 'pustule' strains originating from Mozambique or East Africa. Further tests of the 'pustule' strains from Mozambique

(Khatri-Chhetri, 1999) indicated that these strains may be atypical strains of *X. campestris* pv. *vignicola* with very low virulence. Future studies with more 'pustule' strains from this area could allow an answer to this question. Thus, the present data generally confirm that designation of a separate pv. *vignaeunguiculatae* (Patel and Jindal, 1982) for pustule-forming strains is not justified.

Metabolization of carbon sources

Variations in metabolization of organic substrates by the same bacterial pathovar were often observed (Schaad, 1988) and may be due to different methods applied for metabolization tests such as variations in nutrient media, temperature, incubation time, and differences between strains (Hildebrand et al., 1993). In the present studies, none of the *X. campestris* pv. *vignicola* strains used arabinose, lactose, mannitol and raffinose and only 9% used galactose. The inability to use arabinose was also observed by Stapp (1956) and Hildebrand et al. (1993), while other authors (Jindal and Patel, 1984; Jainkittivong et al., 1989; El-Sadek, 1990) reported that arabinose was used by *Xanthomonas* strains isolated from cowpea and other vegetables. Lactose was also not used (Hildebrand et al., 1993; Jainkittivong et al., 1989), or used (Stapp, 1956; El-Sadek, 1990) in tests of other authors. reported the use of this C-source. The metabolization of mannitol was also not found (Jainkittivong et al., 1989; Stapp, 1956) or found (Hildebrand et al., 1993; El-Sadek, 1990) in other studies. Maltose, used by 93% of the strains in this study, was also observed as positive (Stapp, 1956; Hildebrand et al., 1993; El-Sadek, 1990) or not used by 48 *X. campestris* strains (Jainkittivong et al., 1989). Contrary to our observations, raffinose was used in the tests of Stapp (1956) and El-Sadek (1990), and the use of galactose was reported by all other authors (Stapp, 1956; Jindal and Patel, 1984; Jainkittivong et al. 1989; El-Sadek, 1990; Hildebrand et al., 1993). No severe contradictions were found comparing the present results with the metabolism tests of the large and heterogeneous DNA hybridization group 9, to which *X. campestris* pv. *vignicola* belongs (Vauterin et al., 1995). Among strains from group 9, 15% used arabinose, 14% lactose, 12% mannitol, 37% raffinose, 87% galactose and 94% maltose. The results of our tests contributed to develop a semi-selective medium for the quick and easy isolation of *X. campestris* pv. v *ignicola* (Khatri-Chhetri et al., 1998b).

Standardization of the test system

The use of more substrates by unwashed than by washed bacteria may be due to the presence of residual constituents of the growth medium or of bacterial slime which allowed an initial growth of the bacteria and may have enabled them to metabolize also the partially available substrates. Nevertheless, washing did not improve a correct identification by the Biolog System. The reason may be a great divergence between a limited number of strains of low diversity in the Biolog database and the high diversity of the tested strains, which could not be overcome by improving the system by washing the bacteria. Thus, the unsatisfactory representation of the database does not contradict the positive effect achieved by the washing step, since, contrary to our results, Fessehaie (1997) found a considerable reduction in the number of substrates used and a parallel an increase from 19% to 75% in the number of correctly identified strains of *X. campestris* pv. *manihotis* after including a washing step.

In some cases the data obtained by the Biolog procedure were not completely reproducible, however, variation in metabolism of most of the substrates between two replications was small. The differences between replications may be due to an insufficient incubation period (24 h), variations in cell numbers per well, differences in substrate quantity and quality between different microplates or differences in assessments of degree of metabolization between two replications. Also Fessehaie (1997) found only repetitions of three from eight strains completely reproducible after overnight incubation, and Harris-Baldwin and Gudmestad (1996) reported variations between two replications in the identification of phytopathogenic coryneform bacteria, while a reproducibility of 94% was observed by Vauterin et al. (1995) between two repetitions of 10 *Xanthomonas* strains 48 h after incubation.

Differences between strains according to 'identification' by the Biolog database

According to the 'identification' by the Biolog database, the strains were categorized into two groups: those 'identified' as the pathovars *vignicola* and *vesicatoria* and the rest of the strains. The strains had

formerly been identified as *X. campestris* pv. *vignicola* by genetic methods (Verdier et al., 1998) and fatty acid and virulence analysis (Khatri-Chhetri, 1999). The low number of strains identified as *X. campestris* pv. *vignicola* by the Biolog datbase may be due to a limited number of strains included in the database from a limited area and representing few pathogenic groups of strains, a high variability among the pv. *vignicola* strains, and high similarities between pathovars of *X. campestris*. Many xanthomonads are very similar in morphological and physiological characters which can be differentiated only by the host range (Rudolph, 1993). Poor identification by the database was also observed by Jainkittivong et al. (1989) using strains of *X. campestris* pvs. *vignicola*, *glycines* and *phaseoli*, and by Jones et al. (1993) for four strains of *X. fragariae*. Black and Sweetmore (1994) observed a wide variation in the identification of 90 isolates from six bacterial genera by the Biolog System (GN, version 3.00). Especially the pathovars of *X. campestris* were rarely correctly identified, while Jones et al. (1993) found a high degree of relatedness between various pathovars. The metabolization pattern of 83 strains of *X. campestris* pv. *manihotis* was also neither correlated to origin nor virulence (Fessehaie, 1997).

In conclusion, the metabolic patterns allowed a characterization, clustering and geographic differentiation of *X. campestris* pv. *vignicola* strains. The results indicate that the strains isolated from blight and pustule symptoms originating from Africa (except Mozambique) belong to the same pathovar *vignicola*. In most cases, the Biolog database identified *X. campestris* pv. *vignicola* strains only up to species level. It is suggested, therefore, that the metabolic pattern of additional *X. campestris* pv. *vignicola* strains originating from different geographical areas be added to the database to allow a more specific identification by the Biolog System. Although no correlation was observed between metabolic patterns and virulence, despite a correlation with geographic origin, representative strains of metabolic pattern groups are included in the recommended set of representative strains for screening for resistant cowpea cultivars, based on metabolic and molecular diversity, virulence and geographic origin.

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