Studies on the biochemical and physiological variations among strains of *Xanthomonas campestris* pv. *citri*, the causal agent of citrus bacterial canker disease.

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STUDIES ON THE BIOCHEMICAL AND PHYSIOLOGICAL VARIATIONS AMONG STRAINS OF *XANTHOMONAS CAMPESTRIS* pv. *CITRI*, THE CAUSAL AGENT OF CITRUS BACTERIAL CANKER DISEASE.

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ABSTRACT - 22 strains of *Xanthomonas campestris* pv. *citri*, associated with A, B, C, D and E [now called citrus bacterial spot disease (CBSD)] forms of citrus bacterial canker disease (CBCD) were studied for their biochemical and physiological characteristics and their metabolic profiles for the use of 147 substrates as sole carbon source. The MEVAG medium tested revealed a substantial oxidative metabolism of glucose, giving more distinct results than with the Hugh and Leifson medium generally used. The strains were classified into three groups by means of the hydrolysis of gelatin, casein and tolerance to NaCl. A and CBS Strains associated with CBCD - A and CBSD can be separated from strains associated with CBCD - B, - C and - D by theit ability to use maltose, strach and glycogen. The B and D strains had very similar metabolic profiles. C strains were unique from all the other strains based on their ability to use D.α alanine and L. serine.

Key words : citrus canker, citrus bacterial spot, variability, carbohydrates.

INTRODUCTION

Citrus bacterial canker disease (CBCD) is a bacterial disease caused by *Xanthomonas campestris* pv. *citri* (Hasse 1915) Dye 1978 (DYE *et al.*, 1980). It affects most *Citrus* species (*Rutaceae* family) and wild *Rutaceae* (LEE, 1918). *X. campestris* pv. *citri* present in most tropical citrus - producing countries where considerable damage may be caused. It is widespread in Asia, where it is endemic. It has not yet been detected in the Mediterranean basin (CALAVAN, 1956 ; BRUN, 1971 ; ROSSETTI, 1977 ; CIVEROLO, 1981 ; CIVEROLO, 1984 ; KOIZUMI, 1985). The disease has been reported recently for the first time in the Maldive Islands where it causes serious decline of the Mexican lime (ROISTACHER and CIVEROLO, 1989).

Several forms or pathotypes were initially described according to their host range and geographical distribution :

- pathotype A or Asiatic canker has the broadest host range and is found in most countries affected by the disease. It was first observed in Japan at the end of the 19th century (CIVEROLO, 1984), although it probably originated in India (FAWCETT and JENKINS, 1933).

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- pathotype B, causal agent of South American canker, has been known since 1923. It occurs in Argentina and Uruguay and probably in Paraguay (ROSSETTI, 1977). It first appeared on *Citrus limon* and mainly affects lemon. It is pathogenic to Mexican lime after artificial inoculation.

- pathotype C, the canker agent on Mexican lime (*Citrus aurantifolia*), was discovered in Brazil in 1963. It appears to be limited to Brazil and to this *Citrus* species (NAMEKATA and OLIVEIRA, 1972 ; ROSSETTI, 1977). According to KOIZUMI (1985), this form is weakly aggressive on Persian lime and on 'Eureka' and Sicilian lemon.

- pathotype D was observed in 1981 on Mexican lime in Mexico. It does not seem to induce any symptoms on fruits (SANCHEZ and LOAIZA, 1983 ; SANCHEZ-ANGUIANO and FELIX-CASTRO, 1984). Another citrus pathogen was sought because of the difficulty of consistently isolating *Xanthomonas* and reproducing the symptoms of the disease (named Citrus Leaf Spot). A fungus, *Alternaria* sp., was consistently isolated and Koch's postulates were verified. Most of the *Xanthomonas* strains were probably opportunistic epiphytes (BECERRA *et al.*, 1988). A single strain of *X. c.* pv. *citri*, whose pathogenicity has been confirmed, was isolated in Mexico (E.L. Civerolo's Xc 90).

- pathotype E is the agent of citrus bacterial spot disease (CBSD). This appeared in Florida in 1984 on several *Citrus* species, but most frequently on swingle citrumelo (*Poncirus trifoliata x Citrus paradisi* cv. Swingle). The symptomatology of the disease was described in detail by SCHOULTIES *et al.* (1985). The disease is distinguished from citrus canker by the absence of typical erumpent pustules on plant organs. Strains associated with this disease are henceforth referred to as CBS strains.

The above host specificity combined with the variations in virulence (NAMEKATA and BALLMER, 1977 ; STALL *et al.*, 1982 ; GRAHAM and GOTTWALD, 1990) make necessary the development of a rapid and reliable characterization procedure for the various strains and pathotypes of xanthomonads from citrus. Different techniques are available.

A, B and C types can be differentiated by serological techniques using polyclonal antibodies, but these methods do not clearly discriminate B and C types (NAMEKATA and OLIVEIRA, 1972 ; GOTO *et al.*, 1980 ; CIVEROLO and FAN, 1982). The use of monoclonal antibodies (MCAs) has shown some similarity between strain Xc 90 (D type) and B and C types. MCAs also distinguish CBS strains from all strains of *X. c.* pv. *citri*. Furthermore, serological variations were found among strains respectively belonging to B and CBS types (ALVAREZ *et al.*, 1987 ; PERMAR and GOTTWALD, 1989).

Analyses of bacterial DNAs using molecular biology techniques provide another way of determining *X. c.* pv. *citri* variation. CIVEROLO (1985) separated strains belonging to A, B and C types by visualizing unrestricted plasmids. Restriction fragments of total DNA (HARTUNG and CIVEROLO, 1987) and analysis of RFLP polymorphism (GABRIEL *et al.*, 1988 ; HARTUNG and CIVEROLO, 1989) make it possible to discriminate A and non - A (i.e. B, C and D) strains, the latter displaying a greater homogeneity between each other than with the A group. The genomic DNA profile of the CBS strains is clearly different to that of the other strains. Some variation occurs among the CBS strains, whereas pathotype A and pathotypes B, C and D form two distinct, homogeneous groups. GABRIEL *et al.* (1989) proposed that the type A strains should be reclassified in the species *Xanthomonas citri* (ex. Hasse) and B, C and CBS type strains should be classified as two new pathovars called *X. campestris* pv. *aurantifolii* pv. nov. and *X. campestris* pv. *citrumelo* pv. nov. This proposal was contested by VAUTERIN *et al.* (1990) and by YOUNG *et al.* (1990).

Isozyme analysis revealed many similar features in pv. *citri* (types A, B, C and D) and showed a certain degree of heterogeneity of CBS type strains (KUBICEK *et al.*, 1989).

Several biochemical and physiological tests also distinguished the pathotypes (GOTO *et al.*, 1980 ; LAVILLE, 1985 ; MIZUNO *et al.*, 1988) or revealed intrapathotype variability (GOTO, 1962 ; ALCARAZ, 1977 ; ALCARAZ, 1980 ; WU *et al.*, 1986).

The purpose of the present work was to use simple biochemical and physiological tests to characterize the above pathotypes in order to achieve rapid identification likely to exhibit interpathotype and intrapathotype variation within pathovar *citri*.

MATERIAL AND METHODS

The strains were obtained from freeze - dried cultures (Table 1). All tests were carried out using 24 hour - old cultures incubated at 28°C on YPDAC medium (yeast extract 7 g, bactopeptone 7 g, dextrose 7 g, agar 15 g, distilled water 1000 ml, pH 7.2, cycloheximide 50 mg).

MEVAG medium ($NH_4H_2PO_4$ 1 g, KCl 0.2 g ; yeast extract 1 g, agar 3 g, glucose 10 g, bromocresol purple 0.015 g, distilled water 1000 ml, pH 7.1) was compared to HUGH and LEIFSON's medium (1953) for determination of the metabolic type. The medium used for the study of the hydrolysis of casein contained 3.2 g/l sterile skim milk and 25 g/l of agar (pH 7.0). The other tests were performed as shown in Table 2. The responses in the tests described above and in Table 2 were recorded for 14 days.

Metabolic profiles for the use of 147 substrates as sole carbon source (carbohydrates, organic acids and amino acids) was carried out using API strips (API 50 CH, LRA 50 AO and LRA 50 AA respectively). The API CHE medium (recommended for gram - negative rods), containing approximately 10^6 c. f. u./ml, was used to inoculate the strips (API system, La Balme - Les Grottes - 38390 Montalieu-Verdieu, France). Growth was observed after 3, 6 and 9 days of incubation. Two replications were performed for each strain - substrate combination. The incubation temperature was 28°C in all the tests.

RESULTS

Seventeen out of the 21 isolates expressed their oxidative metabolism in less than 7 days on MEVAG medium.

TABLE 1 - References of the strains of *Xanthomonas campestris* pv. *citri* studied.

Strain number	Pathotype	Host	Origin	Year of isolation	Other numbers
CFBP[a] 2525[b]	A	*Citrus limon* (L.) Burm. F.	New Zealand	1956	NCPPB[c] 409
CFBP 2900	A	*Citrus* sp.	Japan	?	NCPPB 3234, Xc 62 (E.L. Civerolo)
CFBP 2865	A	*C. aurantifolia* (Chr.) Sw.	Brazil	?	NCPPB 3232, Xc 59 (E.L. Civerolo)
CFBP 2909	A	*C. aurantifolia* (Chr.) Sw.	Brazil	1982	J R 416 (J.R. Neto)
JJ 157	A	*C. limon* (L.) Burm. F.	Argentina	1981	Xc 91 (E.L. Civerolo)
CFBP 1814	A	*C. paradisi*	Reunion Island	1978	
JJ 9.5	A	*C. limon* (L.) Burm. F.	Mauritius Island	1985	CC 37 (S.P. Benimadhu)
JJ 10.5	A	*C. aurantifolia* (Chr.) Sw.	Rodrigues Island	1985	CCR 13 (S.P. Benamidhu)
CFBP 2868	B	*C. limon* (L.) Burm. F.	Argentina	?	NCPPB 3237, Xc 69 (E.L. Civerolo)
CFBP 2901	B	*C. limon* (L.) Burm. F.	Argentina	?	Xc 64 (E.L. Civerolo)
CFBP 2902	B	*C. limon* (L.) Burm. F.	Argentina	?	Xc 93 (E.L. Civerolo)
CFBP 2903	B	*C. limon* (L.) Burm. F.	Argentina	?	Xc 94 (E.L. Civerolo)
CFBP 2904	B	*C. limon* (L.) Burm. F.	Argentina	?	Xc 96 (E.L. Civerolo)
JJ 159	B	*C. limon* (L.) Burm. F.	Argentina	?	Xc 148 (E.L. Civerolo)
JJ 160	B	*C. limon* (L.) Burm. F.	Argentina	?	Xc 80 (E.L. Civerolo)
JJ 161	B	*C. limon* (L.) Burm. F.	Argentina	?	Xc 84 (E.L. Civerolo)
CFBP 2866	C	*C. aurantifolia* (Chr.) Sw.	Brazil	?	NCPPB 3233, Xc 70 (E.L. Civerolo)
CFBP 2905	C	*C. aurantifolia* (Chr.) Sw.	Brazil	1981	JR 380 (J.R. Neto)
CFBP 2906	C	*C. aurantifolia* (Chr.) Sw.	Brazil	1981	PDDCC[d] 2432, JR 381 (J.R. Neto)
JJ 164	D	*C. aurantifolia* (Chr.) Sw.	Mexico	?	Xc 90 (E.L. Civerolo)
CFBP 2910	E[e]	Citrumelo	USA (Florida)	1984	F6 (E.L. Civerolo)
CFBP 3238	E	Citrumelo	USA (Florida)	1984	F1 (E.L. Civerolo)

a : CFBP : Collection Française de Bactéries Phytopathogènes - INRA - Station de Pathologie Végétale - 49070 BEAUCOUZE (France)

b : Souche de référence internationale.

c : NCPPB : National Collection of Plant Pathogenic Bacteria - Plant Pathology Laboratory, Ministry of Agriculture, Fisheries and Food - HARPENDEN - Hertfordshire - U.K.

d : PDDCC : Culture Collection of Plant Diseases Division - DSIR Mt Albert Research Center, Private Bag - AUCKLAND - New Zealand.

e : Souche induisant le «Citrus Bacterial Spot Disease» (CBSD).

A clear change in the colour of the pH indicator from mauve to yellow (indicating acidification of the medium) in the tube occured under aerobic conditions. The oxidation of glucose induced by *X. c.* pv. *citri* was extremely difficult to observe (even after three weeks of incubation) when HUGH and LEIFSON's medium was used. A clear change in the colour of the pH indicator under aerobic conditions occured on MEVAG medium but not on HUGH and LEIFSON's medium for the neopathotype strains of *Xanthomonas campestris* pvs. *begoniae,, campestris, corylina, juglandis, malvacearum, mangiferaeindicae, manihotis, oryzae, pelargonii* and *vesicatoria*. The MEVAG medium should, therefore, be preferred to HUGH and LEIFSON's medium, which is classically used in the study of the glucose metabolism of *Xanthomonas*.

The responses to various biochemical and physiological tests clearly showed that some variation exists between the different pathotypes (Table 2). Hydrolysis of gelatin, hydrolysis of casein and growth in the presence of NaCl was used to classify the strains in three different biochemical groups :

- the first group consisted of strains associated with CBCD - A and CBSD. These hydrolysed gelatin and casein and were able to grow in the presence of 2% NaCl.

- the second group consisted of strains associated with CBCD - B and a single strain associated with CBCD - D (Xc 90) whose behaviour was identical. These strains did not hydrolyse gelatin and casein and their growth was inhibited by a 1% concentration of NaCl.

- the third group consisted of C strains, which could hydrolyse casein but not gelatin and were able to grow in the presence of 1% NaCl. A 2% NaCl concentration strongly slowed their growth but did not inhibit it totally.

The ability of the strains to use sole carbon sources was based on the reactions on 147 substrates. The pattern of utilization was homogeneous for 116 (78.9%) substrates for all the *Xanthomonas campestris* pv. *citri* (Table 3). Variability between the pathotypes and within pathotypes occured for 31 other substrates (Table 4). The assimilation profile of these substrates did not clearly differentiate strains associated with CBCD - A and CBSD. The CBCD -

TABLE 2 - Biocheminal and physiological characteristics of strains of *Xanthomonas campestris* pv. *citri* belonging to different pathotypes.

Character studied	Pathotype A[a]	B[b]	C[c]	D[d]	E (CBS)[e]
Gram (SUSLOW *et al.*, 1982)	-	-	-	-	-
O/F metabolism on MEVAG medium[f]	ox	ox	ox	ox	ox
Cytochrome C oxidase (KOVACS, 1956)	-	-	-	-	-
Nitrate reductase (GARDAN et LUISETTI, 1982)	-	-	-	-	-
Urease (Anonymous, 1981).	-	-	-	-	-
Indole production (Anonymous, 1981)	-	-	-	-	-
Production of fluorescent pigments (KING *et al.*, 1951)	-	-	-	-	-
Hydrolysis of :					
- starch (GARDAN et LUISETTI, 1982)	+	+	+	+	+
- esculin (GARDAN et LUISETTI, 1982)	+	+	+	+	+
- gelatine (FRAZIER, 1926)	+	-	-	-	+
- gelatine (LELLIOTT *et al.*, 1966)	+	-	-	-	+
- casein	+	-	+	-	+
- cellulose (OSHIRO *et al.*, 1964)	+	+	+	+	+
Tween esterase (SIERRA, 1957)	+	(+)	(+)	(+)	+
Production of H_2S from cysteine (DYE, 1962)	+	+	+	+	+
Pectinolytic activity (PRUNIER et KAISER, 1964)	v	-	-	-	v
Pectinolytic activity (HILDEBRAND, 1971)					
- pH 5,0	v	-	-	-	v
- pH 7.0	v	-	-	-	v
- pH 8,5	v	-	-	-	v
Hypersensitivity on tomato (LELLIOTT et STEAD, 1987)	+	(+)	+	(+)	+
Growth on YPDA+ NaCl 1%	+	-	+	-	+
Growth on YPDA+ NaCl 2%	+	-	(+)	-	+
Growth on YPDA+ NaCl 5%	-	-	-	-	-
Arginine dihydrolase (THORNLEY, 1960)	-	-	-	-	-

+ : positive reaction (+) : weakly positive reaction - : negative reaction
v : reaction variable according to strains of the same pathotype ox : oxidative
a : 8 strains studied b : 8 strains studied c : 3 strains studied
d : 1 strain studied e : 2 strains studied
f : no change in the colour of the pH indicator after 14 days for the strains CFBP 2865 and 2900 (pathotype A) and strains CFBP 2868 and 2901 (pathotype B).

A/CBSD group exhibited a clearcut difference when compared to the other groups (CBCD - B, - C, - D) for the assimilation of maltose, starch and glycogen. The strains associated with CBCD - B and D had very similar metabolic profiles. They were differentiated only by the use of L. histidine, which is utilized only by strain XC 90. The pathotype C strains stand out from all the other types based on D. α alanine (not used by C strains) and L. serine (used by the type C strains only) utilization.

DISCUSSION AND CONCLUSION

The results of the biochemical characterization obtained in this study for the pathotype B strains are different to those of GOTO *et al.* (1980) for pectinolytic activity, tolerance to NaCl and hydrolysis of gelatin (Table 5). Although it is possible that the variations obtained may be caused by the use of different media, it is fairly surprising to note that according to GOTO *et al.* (1980), the biochemical profiles of the A and B strains are almost identical. Characteristic lysis in the presence of phage CP3 also confirmed that all the strains described by these authors belong to the B type. All the B strains in the present study were also sensitive to the citriphage CP3. In addition, these strains reacted in ELISA with one of the three monoclonal antibodies (B1, B2 and B3) produced against this type, but did not react with the monoclonal antibodies A1 and C1 produced against A and C types, respectively (ALVAREZ *et al.*, 1987 ; ALVAREZ and BENEDICT, 1990 ; ALVAREZ and PRUVOST, unpublished data).

The utilization of carbon substrates seems to vary for the same substrate according to the method used (Table 5). However, it appeared that the API system, which enables a good reproducibility of working conditions, is fairly

TABLE 3 - Carbon substrates utilized or not by all the strains of *Xanthomonas campestris* pv. *citri* (recorded after 9 days of incubation).

Substrates utilized	Substrates not utilized				
glycerol	erythritol	D. fucose	suberic acid	Isophtalic acid	D.L. 3. aminobutyric acid
galactose[a]	L. arabinose	D. arabitol	azelic acid	terophtalic acid	D.L. 4. aminobutyric acid[e]
D. glucose	ribose	L. arabitol	sebacic acid	glycine	D.L. 5. aminovaleric acid
D. fructose	L. xylose	gluconic acid	glycolic acid	L. leucine	D.L. 2. aminobenzoic acid
D. mannose	adonitol	2. ketogluconic acid	D.L. 3. hydroxybutiric acid	L. norleucine[d]	D.L. 3. aminobenzoic acid
N. acetylglucosamine	α methylglucoside	5. ketogluconic acid	D. malic acid	D.L. norvaline	D.L. 4. aminobenzoic acid
esculin	L. sorbose	butyric acid	D. tartaric acid	D.L. 2. aminobutyric acid	urea
D. cellobiose	rhamnose	N. valeric acid	L. tartaric acid	L. methionine	acetamide
sucrose	dulcitol	isovaleric acid	mesotartaric acid	L. phenylalanine	sarcosine
trehalose[b]	inositol	N. caproic acid	levulinic acid	D. tryptophane	ethylamine
β gentiobiose	sorbitol	heptanoic acid	citraconic acid	L. tryptophane	butylamine
succinic acid	β methylxyloside	caprylic acid	itaconic acid	trigonelline	amylamine
fumaric acid	α methylmannoside	pelargonic acid	mesaconic acid	L. ornithine	ethanolamine
D. L. glyceric acid	arbutin	capric acid	phenylacetic acid	L. lysine	benzylamine
L. malic acid	salicin	oxalic acid	benzoic acid	L. citruline	diaminobutane
pyruvic acid	inulin	malonic acid	o. hydroxybenzoic acid	L. arginine	spermine
2. cetoglutaric acid	D. melezitose	maleic acid	m. hydrobenzoic acid	D.L. kynurenine	histamine
p. hydroxybenzoic acid[c]	xylitol	glutaric acid	D. mandelic acid	betaine	tryptamine
	D. turanose	adipic acid	L. mandelic acid	creatine	
18 substrates utilized (12.2%)	D. tagatose	pimelic acid	Phtalic acid	β alanine	98 substrates not utilized (67.7%)

[a] with the exception of CFBP 2900
[b] with the exception of CFBP 2866
[c] growth and colour changes are interpreted as positive reactions
[d] except for CFBP 3238
[e] except for CFBP 2525

TABLE 4 - Differential utilization of carbon substrates by *Xanthomonas campestris* pv. *citri* (recorded after 9 days of incubation).

Substrates	Pathotype				
	A[a]	B[b]	C[c]	D[d]	E (CBS)[e]
D. xylose	+	v	+	+	+
D. arabinose	-	-	-	-	v
mannitol	v	-	-	-	-
amygdalin	v	-	-	-	v
lactose	v	-	+	-	v
maltose	+	-	-	-	+
D. melibiose	v	v	+	-	+
D. raffinose	v	-	-	-	+
starch	+	-	-	-	+
glycogen	+	-	..	-	+
D. lyxose	+	v	-	+	+
L. fucose	+	v	-	+	+
acetic acid	v	v	-	+	+
propionic acid	+	v	+	-	+
isobutyric acid	v	-	-	-	+
D.L. lactic acid	v	v	-	+	+
aconitic acid	v	v	-	-	+
citric acid	+	v	+	-	+
D. α alanine	+	+	-	+	+
L. α alanine	+	v	-	+	+
L. isoleucine	v	-	+	-	v
L. valine	v	-	-	-	-
L. serine	-	-	+	-	-
L. threonine	+	v	-	-	+
L. cysteine	-	-	-	-	+
L. tyrosine	-	-	-	-	v
L. histidine	v	-	-	+	+
L. aspartic acid	v	v	-	+	v
L. glutamic acid	+	v	+	+	+
L. proline	v	v	-	+	+
glucosamine	-	v	-	+	-

+ : substrate utilized - : substrate not utilized v : variable reaction according to the strains investigated
a : 8 strains studied b : 8 strains studied c : 3 strains studied d : 1 strain studied
e : 2 strains studied

well - suited to this type of study. Any suspicion of growth after 9 days of incubation could easily be resolved after plating the culture and checking its purity.

The behaviour of *Xanthomonas campestris* pv. *citri* strains screened biochemically and physiologically confirmed the occurence of variation between the pathotypes as they have been described, and revealed intrapathotype variations. It was difficult to differentiate strains associated with CBCD - A and CBSD on the basis of their biochemical profiles and metabolic fingerprints. However, the fundamental differences in their respective pathogenicity, together with serological studies and investigation of their DNA, show that they each belong to different groups (HARTUNG and CIVEROLO, 1987 ; GABRIEL *et al.*, 1988 ; HARTUNG and CIVEROLO, 1989 ; PERMAR and GOTTWALD, 1989). Likewise, these investigations revealed metabolic and physiological heterogeneity within the Florida strains associated with CBSD. This strong internal variability of the CBS type is in accordance with the hypothesis of the existence of several origins for CBSD. GRAHAM *et al.* (1990) mentionned that certain *Xanthomonas campestris* pathovars not isolated from *Citrus* expressed a similar pathogenic reaction against citrumelo cv. Swingle to that of the CBS strains belonging to the weakly aggressive group. The apparent existence of a large number of hosts for these strains leads to questioning the pathovar concept for this species.

The biochemical profiles and the metabolic fingerprints of the strains associated with CBCD - B and - D are similar. Only utilization of L. histidine allows differentiation of strain Xc 90 from the B strains. A larger number of strains of each pathotype should be studied to ascertain whether the uptake of L. histidine is truly a discriminant character. Similarities in their antigenic structure (ALVAREZ *et al.*, 1987), genomic fingerprints (HARTUNG and CIVEROLO, 1987), profile after analysis of the polymorphism of restriction fragments (RFLP) (GABRIEL *et al.*, 1988 ; HARTUNG and CIVEROLO, 1989) and their common sensitivity to citriphage CP3 make it practically impossible to distinguish between these two types. Separation of these strains into two pathotypes based simply on geographic origin and different isolation hosts no longer appears

TABLE 5 - Biochemical characteristics and utilization of carbon substrates by *Xanthomonas campestris* pv. *citri*, according to data published in the literature.

Character studied	Pathotypes described										
	A[1]	A[2]	B[2]	A[3]	A[4]	CBS[4]	A[5]	B[5]	C[5]	A[6]	B[6]
pectinolytic activity	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	+
growth on NaCl 3%	NR	NR	NR	v	+	+	NR	NR	NR	+	+
growth on NaCl 4%	NR	NR	NR	NR	-	-	NR	NR	NR	-	-
casein hydrolysis	NR	NR	NR	+	NR	NR	NR	NR	NR	+	+
gelatine hydrolysis	NR	+	v	+	+	+	NR	NR	NR	+	+
utilization of carbon substrates	Ac, As	Ac	Ac	Ac	As	As	As	As	As	As	As
maltose	+	+	-	NR	NR	NR	+	-	+	+	-
lactose	v	+	-	+	-	v	v	-	+	+	-
starch	+	NR	NR	NR	NR	NR	v	-	+	+	+
malic acid	+	NR	NR	NR	NR	NR	v	-	+	+	+
arabinose	NR	NR	NR	+	-	+	v	-	-	-	-
xylose	+	NR	NR	NR	NR	NR	v	+	+	+	+
fructose	+	+	-	NR	+	+	v	+	+	+	+
mannose	v	NR	NR	+	v	+	+	+	+	+	+
sucrose	NR	+	-	NR	+	+	v	+	+	-	-
glycerol	+	NR	NR	NR	+	+	v	+	+	+	+
mannitol	v	+	-	+	NR	NR	v	+	+	v	+
malonic acid	NR	NR	NR	NR	+	+	v	-	-	+	v
citric acid	+	NR	NR	+	+	+	-	-	-	+	+

+ : positive reaction - : negative reaction v : reaction variable according to strain
NR : test not performed Ac : substrate utilization recorded by acidification of the medium
As : substrate utilization recorded by growth of the studied strain on the medium
[1] According to GOTO (1962) [2] According to ALCARAZ (1977) [3] According to WU *et al.* (1986)
[4] According to MIZUNO *et al.* (1988) [5] According to LAVILLE (1985) [6] According to GOTO *et al.* (1980)

to be well - founded. However, no final conclusion can be made, because of the small number of available B and D strains.

The main pathotypes described (A, B and C) can easily be characterised by the use of biochemical tests which give a rapid identification and are less expensive than either monoclonal antibodies or RFLP. The latter methods are generally too sophisticated and expensive for routine application in developing countries in which citrus canker is present. The division of the *citri* pathovar into pathotypes was confirmed by the existence of specific traits.

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