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A strain of *Pseudomonas syringae* which does not belong to pathovar *phaseolicola* produces phaseolotoxin

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Abstract

A bacterial strain, CFBP 3388, isolated from Vetch (*Vicia sativa*, L.) was identified as *P. s.* pv. *syringae* on the basis of nutritional and biochemical patterns which were obtained with classical tests and the Biolog™ system. It caused necrotic symptoms typical of *P. s.* pv.*syringae* on bean leaves and pods after artificial inoculation. However, the isolate caused a citrulline-reversible inhibition of *E. coli* in phaseolotoxin bioassay. Furthermore, with CFBP 3388 DNA as template a 1900 bp DNA fragment, specific for the phaseolotoxin DNA cluster of *P. s.* pv. *phaseolicola*, was amplified by PCR. This is the first demonstration that an isolate of *P. syringae* that is not pv. *phaseolicola* can produce phaseolotoxin

Abbreviations: bp = base pair; kb = kilobase; OCT = Ornithine Carbamoyl Transferase.

Introduction

Phaseolotoxin is produced by *Pseudomonas syringae* pathovar *phaseolicola*, the causal agent of halo blight of bean (*Phaseolus vulgaris* L.). It causes the typical halo of chlorosis in leaf tissues [Mitchell, 1976] and inhibits plant growth [Ferguson and Johnson, 1980; Rudolph, 1974; Rudolph and Stahmann, 1966]. At the molecular level, it inhibits ornithine carbamoyl transferase (OCT), an enzyme which catalyzes the conversion of ornithine to citrulline in the arginine biosynthesis pathway [Patil *et al.*, 1970; Turner and Mitchell, 1985]. The toxic action of phaseolotoxin is not specific to bean plants, and it also inhibits *Escherichia coli* OCT. This inhibition can be complemented *in vitro* [Staskawicz and Panopoulos, 1979] and *in planta* [Patil *et al.*, 1972] by the addition of L-citrulline. Genes involved in the production and those involved in the resistance to the phaseolotoxin were cloned in *E. coli*. They are clustered on a 25 kb DNA fragment [Peet *et al.*, 1986].

Until now, phaseolotoxin production has been strictly confined to *P. s.* pv. *phaseolicola,* and this specificity has been used as a basis for detection procedures using either microbiological assays for phaseolotoxin production [Jansing and Rudolph, 1990] or nucleic acid-based detection systems: DNA probes [Schaad *et al.*, 1989; Tourte and Manceau, 1991] or PCR [Manceau and Tourte, 1991; Prosen *et al.*, 1993].

We describe in this study a bacterial strain isolated from Vetch (*Vicia sativa*) which was characterized as *P. s.* pv. *syringae* according to its biochemical pattern and yet produces phaseolotoxin, a characteristic previously only ascribed to strains of *P. s.* pv. *phaseolicola.*

Materials and methods

Bacteria and media. The bacterial strains used in this study are listed in Table 1. Strain CFBP 3388 was isolated in May 1992 in the Angers area from epiphytic microflora of an apparently healthy Vetch (*Vicia sativa*, L) which was growing in an experimental field after two cultures of bean (*Phaseolus vulgaris*, L). Aerial portions of plants (100 g) were washed in 400 ml of sterile distilled water for 2 h at room temperature and the washing liquid was plated on MSP medium [Mohan and Schaad, 1987]. On this semi-selective medium, *P. syringae* forms yellow, smooth convex and fluorescent colonies surrounded by a yellow discolouration of the medium. *P. syringae* and *E. coli* were routinely cultivated on King's agar medium B [King *et al.*, 1954] and on Luria Bertani agar medium [Maniatis *et al.*, 1982], respectively. The strains were identified as *P. syringae* according to the determinative scheme (LOPAT) of Lelliott *et al.* [1966]. Three *Escherichia coli* strains were used to detect phaseolotoxin: in addition to strain N100 used by Jansing and Rudolph [1990], strain HB101 was used as phaseolotoxin-sensitive strain and strain HB101 (pRCP4) was used as phaseolotoxin resistante strain. pRCP4 is a recombinant plasmid carrying a DNA fragment encoding for phaseolotoxin insensitive OCT [Peet and Panopoulos, 1987].

Nutritional and enzymatic characterization. In addition to the LOPAT scheme, strains were assayed for their ability to use mannitol, sorbitol, inositol, erythritol, DL lactate, L+ tartrate and D-tartrate as the sole carbon source. These substances were filter-sterilized and were added at a final concentration of 5% (w/v) to sterile Ayers's medium (NH_4 H_2 PO_4, 1 g l^{-1}; KCl, 0.2 g l^{-1}; $MgSO_4$, $7H_2O$, 0.2 g l^{-1}, Bromothymol blue, 0.08 g l^{-1}; Agar, 3 g l^{-1}; pH 7). [Ayers *et al.*, 1919]. β-glucosidase activity was assayed by the hydrolysis of aesculin. The hydrolysis of gelatine was assayed as described by Dye [1962].

Metabolic fingerprints of strains using biolog™ GN system. Pure cultures of bacterial strains were isolated on NGA medium (nutrient agar 23 g l^{-1} [Difco, Detroit, MI]; glucose, 10 g l^{-1}; pH 6.8) Strains were then subcultured on Trypsic Soy Agar (TSA; Difco, Detroit, MI) as recommended by the manufacturer (Biolog Inc. Hayward, Ca., USA). TSA plates were incubated at 25 °C for 24 h. Cell suspensions in sterile saline (A600 = 0,3 ± 0.05) were used to inoculate Biolog™ GN microplates (150 µl per well). Biolog plates were read with a Dynatech MR 700 spectrophotometer (λ = 590 nm; Dynatech Laboratories Ltd, Billingshurst, UK).

Ice nucleation activity. Ice nucleation activity of strains was determined using 4.5 ml of bacterial suspension in distilled water (5×10^8 cfu ml^{-1}). Bacterial suspensions were preincubated at 0 °C for 2 h in an ethylene-glycol bath. The tempera-

Table 1. Bacterial strains used in this study

Strains	Plant host (disease)	Source and reference
Pseudomonas syringae pv. *syringae*		
2027-37	*Pyrus communis* (pear blast)	Yessad *et al.* (1992)
Dan 86-4	*Phaseolus vulgaris* (brown spot of bean)	D. Legard[a]
CFBP 3388	*Vicia sativa* (unknown)	This study
Pseudomonas syringae pv. *phaseolicola*		
SD68	*Phaseolus vulgaris* (halo blight)	R. Samson[b]
N7-6ar	*Phaseolus vulgaris* (halo blight)	Jansing and Rudolph (1990)
Escherichia coli		
N100		Jansing and Rudolph (1990)
HB 101		Hanachan (1983)
HB 101 (pRCP4)		Peet *et al.* (1986)

[a] NYSES, Geneva, N.Y., USA
[b] INRA, Beaucouzé, France

ture was then decreased by 1 °C step every 5 min. The temperature of freezing was recorded for each bacterial suspension.

Plant inoculation. Two types of plant material were used: (i) bean pods cultivar Belna and (ii) 14 day-old bean seedlings cultivar Michelet. Both were grown in a greenhouse prior to inoculation and are susceptible to halo blight. Bean pods were dipped into a solution of sodium hypochlorite (1% active hypochlorite; v/v) for 5 min to be surface sterilized. They were then rinsed three times in sterile distilled water. A bacterial suspension of 5×10^8 cfu ml^{-1} was injected subepidermially according to the procedure of Klement and Lovrekovich [1961]. For each bacterial strain, three pods were inoculated at five or six sites (approximately 1 cm apart) per pod. After inoculation, pods were laid on sterile filter paper in plastic boxes containing 5 ml of sterile distilled water and wrapped in a transparent plastic film.

Bean primary leaves were inoculated at two concentrations (5×10^8 cfu ml^{-1} and 5×10^6 cfu ml^{-1}) for each strain. Bacterial suspensions were injected in the foliar parenchym of the primary leaves of 14 day-old growing seedlings. The leaf tissues were pierced with a needle. A syringe without a needle was pressed on the leaf while the holes were sealed with a finger placed under the leaf. Then, 100 µl of bacterial suspension were injected into the leaf tissues under pressure. Inoculated pods and plants were incubated in a growth chamber (16 h light, 26.9 µmole m^{-2} s, 400–700 nm, 24 °C; 8 h of darkness, 20 °C. Symptoms were recorded daily for 7 days.

Detection of phaseolotoxin. Phaseolotoxin was detected by the agar diffusion test of Staskawicz and Panopoulos [1979] as modified and described by Jansing and Rudolph [1990].

PCR amplification. PCR amplifications were performed with a DNA thermal cycler (PTC100-60; MJ Research, MA, USA). Reactions were performed in a total volume of 50 µl containing 1 × PCR buffer (75 mM Tris-HCl [pH 8.8 at 25 °C], 20 mM $(NH_4)2\ SO_4$, 1.5 mM Mg Cl_2), 0.5 mM (each) dNTP, 2.5 U of Tth DNA polymerase (Eurogentec, Seraing, Belgium), 2 µM of each primer and template DNA. The primers used were those described by Prosen *et al.* [1993]. They are located in a 2.6 kb *Eco*R1 DNA fragment carrying genes involved in phaseolotoxin production. The distance between their hybridization sites was 1900 bp. Finally, two drops of mineral oil were laid over each reaction mixture. After 2 min at 92 °C, the reaction mixtures were cycled 35 times through phases of denaturation (94 °C for 1 min), annealing (60 °C for 1 min) and extension (72 °C for 1 min) with a final extension period of 10 min at 72 °C. Reaction mixtures (5 µl) were separated by electrophoresis in an agarose gel (1% Nuseive GTG, 1% Seakem GTG; FMC bioproducts, Rockland, ME, USA) for 1 h at 5 volts/cm in 1 × TAE buffer [Maniatis *et al.*, 1982]. Gels were stained with ethidium bromide and DNA bands were observed under UV light.

Results

Nutritional and enzymatic characterization. The five strains of *P. syringae* tested reacted similarly in the determinative scheme of Lelliott *et al.*, [1966] and showed all the characteristics of group Ia of phytopathogenic fluorescent pseudomonads. Strain CFBP 3388 hydrolysed aesculin and utilized mannitol, sorbitol, inositol and erythritol as the sole carbon source (Table 2). It did not use D-tartrate and L+ tartrate which are characteristics of *P. s.* pv. *syringae*. Furthermore, strain CFBP 3388 catalyzed the ice nucleation of water at –2 °C. However, it did not hydrolyse gelatine and it did not use DL lactate as sole carbon source which are characteristics of *P. s.* pv. *phaseolicola*.

Metabolic fingerprint analyses showed that carbon sources used by all the five strains tested were: tween 40, L-arabinose, D-fructose, D-galactose, 1-D-glucose, D-mannose, sucrose, D-psicose, methyl pyruvate, acetic acid, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-glucosaminic acid, 1-keto glutaric acid, quinic acid, D-saccharic acid, bromosuccinic acid, succinamic acid, D-alanine, L-alanine, L-aspargine, L-aspartic acid, L-glutamic acid, L-proline, L-serine, 3-amino-butyric acid, inosine, uridine, and glycerol.

Carbon sources used by none of the five strains tested were cyclodextrin, tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, cellobiose, L-fucose, gentobiose, 1-D-lactose, lac-

Table 2. Bacteriological properties of tested strains

Properties	*P. s.* pv. *syringae*			*P. s.* pv. *phaseolicola*	
	2027-37	Dan 86-4	CFBP 3388	N7-6ar	SD68
Hydrolysis of					
• aesculin	+	+	+	–	–
• gelatin	+	+	–	–	–
Use as sole carbon source					
• manitol	+	+	+	–	–
• sorbitol	+	+	+	–	–
• inositol	+	+	+	–	–
• erythritol	+	–	+	–	–
• DL lactate	+	+	–	–	–
• D–tartrate	–	–	+[1,a]	–	–
• L+ tartrate	–	–	–	–	+[1]
Ice nucleation temperature[b]	–2 °C	–2 °C	–2 °C	< –9 °C	< –9 °C

[a] Lighter and slower reaction than the positive check
[b] Temperature at which bacterial suspension (5×10^8 cfu/ml) froze

tulose, D-melibiose, 2-methyl-glucoside, L-rhamnose, turanose, xylitol, 1-hydroxybutyric acid, 2-hydroxybutyric acid, 3-hydroxybutyric acid, *p*-hydroxyphenyl acetic acid, itaconic acid, 1-keto valeric acid, sebacic acid, glucuronamide, glycyl-L-aspartic acid, L-hystidine, hydroxyl-L-proline, L-leucine, L-ornithine, L-phenyl alanine, L-pyroglutamic acid, L-threonine, D-L-carnitine, urocanic acid, thymidine, phenylethylamine, putrescine, 2-aminoethanol,2,3-butanediol, glucose-1-phosphate, and glucose-6-phosphate.

Variable reactions with other carbon sources are presented in Table 3. Most carbon sources were used either by both *P. s.* pv. *syringae* strains or by both *P. s.* pv. *phaseolicola* strains. Strain CFBP 3388 could not be assigned to *P. s.* pv. *phaseolicola* based on carbon source utilisation tests. It showed more similarity to carbon source utilization patterns of *P. s.* pv. *syringae* than *P. s.* pv. *phaseolicola.*

Pathogenicity tests. Comparative pathogenicity studies of the strains were performed on pods (Fig. 1) and leaves (Fig. 2). Bean pods inoculated with *P. s.* pv. *phaseolicola* developed water-soaked lesions 2–3 mm in diameter after four days of incubation. In moist incubation conditions, bacteria embedded in a white slime could be observed at some inoculation sites. Sunken brown lesions were observed at sites inoculated with the brown spot disease strain *P. s.* pv. *syringae* (Dan 86-4). Inoculations of the pear blast strain of *P. s.* pv. *syringae* (2027-37) and those of CFBP 3388 resulted in brownish necrotic lesions, typical of *P. s.* pv. *syringae* strains non-pathogenic on bean.

Inoculation of *P. s.* pv. *phaseolicola* strains into primary leaf tissues of a susceptible bean cultivar caused water soaked lesions (Fig. 2). These lesions appeared only 2 days after inoculation. When high bacterial concentration (5×10^8cfu ml^{-1}) were infiltrated, leaf tissues collapsed in the infiltrated area. The necrotic areas were surrounded by a green edge and a typical yellow halo 5 days after inoculation. When lower concentrations (5×10^6 cfu ml^{-1}) were infiltrated, water soaked lesions were observed 3 days after inoculation.

Inoculations of strains CFBP 3388, 2027-37 and Dan 86-4 into primary leaf tissues resulted in hypersensitive brown necrosis as early as 24 h after inoculation when a high concentration (5×10^8 cfu ml^{-1}) was infiltrated. A light yellow discolouration was observed in the infiltrated area when a low concentration (5×10^6 cfu ml^{-1}) was used (Fig. 2).

Toxin related tests. In the *E. coli* bioassay for phaseolotoxin production (Fig. 3) *P. s.* pv. *phaseolicola* strain SD68 and N7-6ar and *P. syringae* CFBP 3388 produced clear inhibition zones of *E. coli* strain N100. Identical results were obtained

Table 3. Variable utilization of carbon sources as determined using the Biolog™ system

Carbon source	*P. s.* pv. *syringae*			*P. s.* pv. *phaseolicola*	
	2027-37	Dan 86-4	CFBP 3388	N7-6ar	SD68
• D-arabitol	+	+	+	–	–
• *i*–erythritol	+	+	+	–	–
• *m*-inositol	+	+	+	–	–
• D-mannitol	+	+	+	–	–
• D-sorbitol	+	+	+	–	–
• D-galacturonic acid	+	+	+	–	–
• D-gluconic acid	+	+	+	–	–
• D-glucuronic acid	+	+	+	–	–
• Dextrine	–	–	–	+	+
• Glycogen	–	–	–	+	+
• Maltose	–	–	–	+	+
• D-raffinose	–	–	–	+	+
• D-trehalose	–	–	–	+	+
• 1-ketobutyric acid	–	–	–	+	+
• Propionic acid	–	–	–	+	+
• Alaninamide	–	–	–	+	+
• D-serine	–	–	–	+	+
• Mono-methyl succinate	+	+	+	–	+
• Succinic acid	+	+	+	–	+
• D-L-1-glycerol phosphate	+	+	+	–	+
• D-L-lactic acid	+	+	–	+	+
• L-alanine glycine	+	+	–	+	+
• Glycyl-L-glutamic acid	+	+	–	+	+
• Formic acid	+	–	–	+	+
• Malonic acid	+	–	–	+	+

Fig. 1. Pathogenicity test on bean pods. Green water-soaked lesions developed 5 days after inoculation in pods (cv. Belna) inoculated with *P. s.* pv. *phaseolicola* strains: N7- ar (A) and SD 68 (B). Sunken brownish lesions developed in pods inoculated with *P. s.* pv. *syringae* strains: CFBP 3388 (C), Dan 86-4 (D) and 2027-37 (E). Pods inoculated with distilled water (F) did not show any symptoms.

with *E. coli* strain HB101. However, no inhibition were recordered with HB101 (pRCP4) strain which contained a recombinant plasmid carrying DNA fragment encoding for a phaseolotoxin insensitive OTC (Table 4). These inhibitions were removed by addition of citrulline. No inhibition zones resulted when strains of *P. s.* pv. *syringae* Dan 86-4 and 2027-37 were tested under these conditions.

The amplification by PCR analysis of a 1900 bp DNA fragment, using phaseolotoxin-specific primers occurred with DNA template isolated from *P. s.* pv. *phaseolicola* strains and from *P. syringae* strain CFBP 3388. Non-specific PCR-amplifications occurred with *P. s.* pv. *syringae* strains visualized by smaller DNA fragments on the agarose gel (Fig. 4). These non-specific amplications may be due to PCR conditions used in this study which were different from those described by Prosen *et al.* [1993]: A Tth DNA polymerase were used in a $(NH_4)_2\ SO_4$ reaction buffer instead of a Taq DNA polymerase in a KCl buffer.

Nevertheless, the reaction displayed the expected specificity taking in account the size of the amplified fragments.

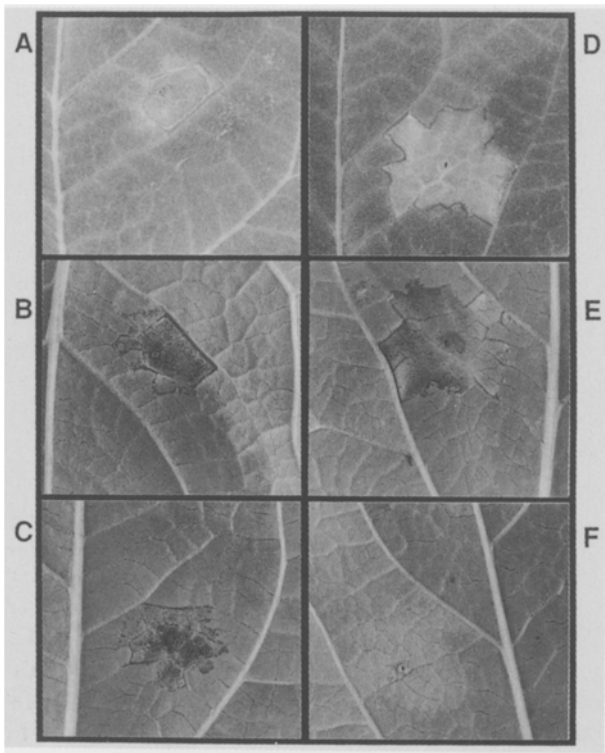

Fig. 2. Pathogenicity test on bean primary leaves cv. "Michelet". Necrosis surrounded by a typical halo on adaxial face of leaf 7 days after inoculation of *P. s.* pv. *phaseolicola* strain N7-6ar at 5×10^8 cfu/ml (A). Water soaked necrosis on abaxial face of bean leaf after inoculation of *P. s.* pv. *phaseolicola* at 5×10^8 cfu/ml (B) and 5×10^6 cfu/ml (C). Dry necrotic responses on adaxial (D) and abaxial (E) bean leaf after inoculation of *P. s.* pv. *syringae* strain CFBP 3388 at 5×10^8 cfu/ml. Light discolouration of infiltrated leaf area after inoculation of 5×10^6 cfu/ml of *P. s.* pv. *syringae* strain CFBP 3388 (F).

Discussion and conclusion

The strain CFBP 3388 cannot be identified as *P. s.* pv. *phaseolicola*. Although some strains of *P. s.* pv. *phaseolicola* were able to use D-mannitol as sole carbon source [Schroth *et al.*, 1971] none were also able to hydrolyze aesculin and to catalyze ice nucleation of water at –3 °C. Furthermore, most biochemical and nutritional characteristics of the strain CFBP 3388 fit in with those of *P. s.* pv. *syringae*. CFBP 3388 has some traits characteristic of *P. s.* pv. *syringae* including use of sorbitol, inositol, erythritol, and L+ tartrate.

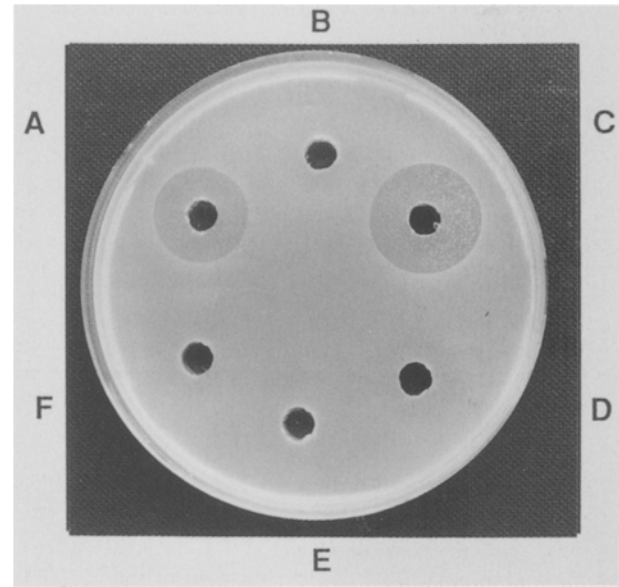

Fig. 3. Bioassay for phaseolotoxin production using inhibition of *E. coli* strain N100. Autoclaved cultures of *P. s.* pv. *phaseolicola* strain SD68 (A) and of *P. syringae* strain CFBP 3388 (C) inhibited the growth of *E. coli*. The inhibitions were removed when citrulline was added to cultures of SD68 (B) and CFBP 3388 (D). No inhibition was produced by culture of *P. s.* pv. *syringae* strain Dan 86-4 with (F) or without citrulline (E).

Table 4. E. coli inhibition zones (in mm) caused by autoclaved culture of *Pseudomonas* strains

E. coli strain	*P. s.* pv. *syringae*			*P. s.* pv. *phaseolicola*	
	2027-37	Dan 86-4	CFBP 3388	N7-6ar	SD68
N100	0	0	25	23	25
HB101	0	0	18	17	17
HB101 (pRCP4)	0	0	0	0	0

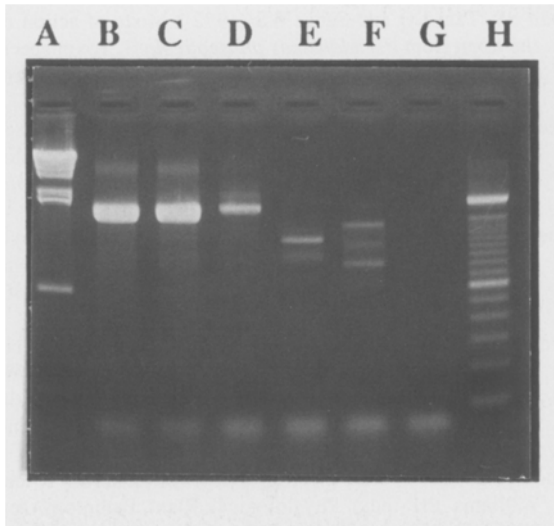

Fig. 4. Analysis of amplified products after ethidium bromide staining on 2% agarose gel electrophoresis. Lane A: molecular-weight marker λ-Hind III with DNA fragments ranged from 500 bp to2300 bp. Lanes B, C and D show a 1900 bp fragment obtained with DNA of *P. s.* pv. *phaseolicola* strains SD68 and N7-6ar, and *P. s. syringae* strain CFBP 3388 respectively. Lanes E and F do not show typical 1900 bp PCR DNA fragments when *P. s.* pv. *syringae* strain Dan 86-4 and 2027-37 were utilized respectively. Not specific signals appear lower in the gel. Lane G is a negative control (no DNA in PCR reaction). Lane H: molecular-weight marker (100 bp DNA ladder, GIBCO-BRL) with DNA fragments ranged from 100 bp to 2072 bp.

This strain did not use lactate as sole carbon source, which is rarely reported for *P. s.* pv. *syringae*. Yet *P. s.* pv. *syringae* is well known for its wide variability. Yessad-Carreau *et al.* [1994] reported that 3% of 81 strains of *P. s.* pv. *syringae* isolated from various plants did not use lactate. Fingerprints obtained with the Biolog™ system confirmed the similarity of the biochemical pattern of CFBP 3388 to that of *P. s.* pv. *syringae*: CFBP 3388 shared 91 out of 96 characters with both tested strains of *P. s.* pv. *syringae*, and 93 out of 96 with at least one of them. Conversely, only three characters were identical to those of one strain of *P. s. pv. phaseolicola*, and none to both tested strains. Strain CFBP 3388 can be assigned to pathovar *syringae* according to Young [1991] who suggested that the basonym *P. s.* pv. *syringae* should be reserved for pathogens sharing hosts in common with the organisms originally isolated from lilac. Pathogenicity tests confirmed the identification of CFBP 3388 to *P. s.* pv. *syringae*. The inoculation of this strain caused a typical hypersensitive reaction on leaf and pod tissues, when inoculated at high concentration. However, a light discolouration was observed on leaves, when a low inoculum concentration was used (Fig. 1F). This discolouration, which was not observed when typical *P. s.* pv. *syringae* strains were inoculated (data not shown), could be induced by phaseolotoxin. Although hypersensitive reaction can be induce by avirulent strains of *P. s.* pv. *phaseolicola* in resistant bean cultivars, the strain CFBP 3388 can not be assigned to an avirulent strain of this pathovar *phaseolicola* because it showed too many differences in biochemical patterns.

The strain CFBP 3388 produced phaseolotoxin. It caused a citrulline-dependent growth inhibition of *E. coli*, and allowed the amplification of the phaseolotoxin-specific DNA fragment of *P. s.* pv. *phaseolicola*. This is the first report of the production of phaseolotoxin by a strain which does not belong to *P. s.* pv. *phaseolicola*.

Two hypotheses could explain the production of phaseolotoxin by a strain of *P. s.* pv. *syringae*: (i) production of phaseolotoxin is a characteristic which is shared by *P. s.* pv. *phaseolicola* and a sub-group of *P. s.* pv. *syringae* or (ii) genetic transfer from *P. s.* pv. *phaseolicola* to an epiphytic strain of *P. s.* pv. *syringae*. Genetic transfer might have occurred in the field, because the CFBP 3388 strain was isolated from a plant of *Vicia sativa* which was growing in a field where beans highly infected by *P. s.* pv. *phaseolicola* were grown during the previous season.

Phaseolotoxin has been specifically associated with the pathovar *phaseolicola* previously. Several authors proposed tests for the identification of *P. s.* pv. *phaseolicola* based on the detection of the activity of this toxin [Jansing and Rudolph, 1990] or the detection of genomic sequences encoding for the toxin [Manceau and Tourte,1991; Prosen *et al.* 1993; Schaad *et al.*, 1989; Tourte and Manceau, 1991]. Although the CFBP 3388 genotype is probably rare and may not be associated with bean plants, the occurrence of the phaseolotoxin production in other than *P. s.* pv. *phaseolicola* strains should be further investigated in order to assess the reliability of such identification tests.

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References

Ayers SH, Rupp P and Johnson WT (1919) A study of alkali forming bacteria found in milk. Bulletin of the U.S. Department of Agriculture 782

Dye DW (1962) The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. N. Z. J. Sci. 5 4: 393–416

Ferguson AR and Johnson JS (1980) Phaseolotoxin: chlorosis, ornithine accumulation and inhibition of ornihine carbamoyltransferase from different plants. Physiol. Plant Pathol. 16: 269–275

Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. Journal of Molecular Biology 166: 557–580

Jansing H and Rudolph K (1990) A sensitive and quick test for determination of bean seed infestation by *Pseudomonas syringae* pv.*phaseolicola*. Journal of Plant Diseases and Protection 97: 42–55

King EO, Ward MK, and Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescein. Journal of Laboratory and Clinical Medicine 44: 301–307

Klement Z and Lovrekovich L (1961) Defence reactions induced by phythopathogenic bacteria in bean pods. Phytopathologisches Zeitschrift 3: 217–227

Lelliott RA, Billing E, and Hayward AC (1966) A determinative scheme for the fluorescent plant pathogenic pseudomonads. Journal of Applied Bacteriology 29: 470–489

Manceau C and Tourte C (1991) Détection directe de *Pseudomonas syringae* pathovar *phaseolicola* par amplification génique en chaîne (PCR). Phytoma – La Défense des Végétaux 430: 36

Maniatis T, Fritsch EF and Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Mitchell RE (1976) Bean halo-blight toxin. Nature 260: 75–76

Mohan SK and Schaad NW (1987) An improved agar plating assay for detecting *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *phaseolicola* in contaminated bean seed. Phytopathology 77: 1390–1395

Patil SS, Kolattukudy PE and Diamond AE (1970) Inhibition of carbamoyltransferase from bean plants by the toxin of *Pseudomonas phaseolicola*. Plant Physiology 46: 752–753

Patil SS, Tam LQ and Sakai WS (1972) Mode of action of the toxin from *Pseudomonas phaseolicola*. I. Toxin specificity, chlorosis and ornithine accumulation. Plant Physiology 49: 803–807

Peet RC, Lindgren PB, Willis DK, and Panopoulos NJ (1986) Identification and cloning of genes involved in phaseolotoxin production by *Pseudomonas syringae* pv. *phaseolicola*. Journal of Bacteriology 166: 1096–1105

Peet RC and Panopoulos NJ (1987) Ornithine carbamoyltransferase genes and phaseolotoxin immunity in *Pseudomonas syringae* pv. *phaseolicola*. The EMBO Journal 6: 3585–3591

Prosen D, Hatziloukas E, Schaad NW and Panopoulos NJ (1993). Specific detection of *Pseudomonas syringae* pv. *phaseolicola* DNA in bean seed by polymerase chain reaction-based amplification of a phaseolotoxin gene region. Phytopathology 83: 965–970

Rudolph K (1974) Non-specific toxins. In: Heitefuss R and Williams PH (eds) Physiological Plant Pathology (pp 270–315) Springer-Verlag, Berlin

Rudolph K and Stahmann MA (1966) The accumulation of L-ornithine in halo-blight infected plants (*Phaseolus vulgaris* L.) induced by the toxin of the pathogen *Pseudomonas phaseolicola* (Burkh.). Phytopathology 57: 29–46

Schaad NW, Azad H, Peet RC and Panopoulos NJ (1989) Identification of *Pseudomonas syringae* pv. *phaseolicola* by a DNA hybridization probe. Phytopathology 79: 903–907

Schroth MN, Vitanza VB and Hildebrand DC (1971) Pathogenic and nutritional variation in the halo blight group of fluorescent pseudomonads of bean. Phytopathology 67: 852–857

Staskawicz BJ and Panopoulos NJ (1979) A rapid and sensitive microbiological assay for phaseolotoxin. Phytopathology 69: 663–666

Tourte C and Manceau C (1991) Construction et utilisation de sondes froides pour l'identification de *Pseudomonas syringae* pathovar *phaseolicola*, agent de la graisse à halo du haricot. Phytoma – La Défense des Végétaux 430: 40

Turner JG and Mitchell RE (1985) Association between symptom development and inhibition of ornithine transcarbamoylase in bean leaves treated with phaseolotoxin. Plant Physiology 79: 468–473

Yessad S, Manceau C and Luisetti J (1992) A detached leaf assay to evaluate virulence and pathogenicity of strain of *Pseudomonas syringae* pv; *syringae* on pear. Plant Disease 76: 370–373

Yessad-Carreau S, Manceau C and Luisetti J (1994) Occurence of specific reactions induced by *Pseudomonas syringae* pv. *syringae* on bean pods, lilac and pear plants. Plant Pathology 43: 528–536

Young JM (1991) Pathogenicity and identification of the lilac pathogen, *Pseudomonas syringae* pv. *syringae* van Hall 1902. Annals of Applied Biology 118: 283–298