Assessment of genetic diversity of *Xanthomonas campestris* pv. *campestris* isolates from Israel by various DNA fingerprinting techniques

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Three molecular typing methods were used to investigate genetic diversity among *Xanthomonas campestris* pv. *campestris* isolates obtained in Israel and others previously obtained from different geographical locations (collection isolates). Using pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) and repetitive sequence-based PCR (rep-PCR), 22 different isolates were divided into 11, 12 and 13 differentiated genotypes, respectively. All collection isolates yielded different genotypes and, among the isolates from Israel, several new genotypes were found. These findings not only support the observed heterogeneity within *X. campestris* pv. *campestris*, but also suggest that variability at the genomic level in this pathovar is higher than previously estimated. Moreover, while previous studies suggested that PCR patterns obtained with integron-specific primers are conserved in most *X. campestris* pathovars, PCR patterns of this element yielded four different types among the *X. campestris* pv. *campestris* isolates tested, thus supporting the relatively high diversity in this pathovar. Although differences in pathogenicity were observed among isolates, assays using cauliflower and radish did not indicate a correlation between pathogenicity and genotype.

Introduction

Based on DNA-DNA homology values, the *Xanthomonas* genus comprises at least 20 different species (Vauterin et al., 1995). *Xanthomonas campestris* belongs to DNA group 15 and is one of the most dominant species within the genus. It consists of several pathovars identified by classical taxonomic methods (Dye et al., 1980), which, in most cases, can be differentiated on the basis of their interactions with their corresponding plant hosts (Vauterin et al., 1995). One of the most economically important pathogens belonging to this species is *X. campestris* pv. *campestris*, the causal agent of black rot of cruciferous plants. This is the most serious disease of cultivated brassicas and radishes worldwide (Williams, 1980). Based on the interaction of various isolates with different *Brassica* species, a total of six pathogenic races of *X. campestris* pv. *campestris* were determined (Vicente et al., 2001).

Besides conventional pathogenicity assays and morphological and biochemical tests, a great variety of genotyping methods have been used to examine the biodiversity of members within the *Xanthomonas* genus. These include, among others, DNA-DNA hybridization studies (Vauterin et al., 1995), rRNA gene analysis (Moore et al., 1997; Goncalves & Rosato, 2002), repetitive sequence-based PCR (rep-PCR) (Louws et al., 1994, 1999; Rademaker et al., 2000) and amplified fragment length polymorphism (AFLP) (Vos et al., 1995; Rademaker et al., 2000). DNA-DNA homology values are generally considered the ‘gold standard’ for defining bacterial species (Stackebrandt & Goebel, 1994). However, this technique is time-consuming when large collections of bacterial isolates are being considered. In a comparative study carried out by Rademaker et al. (2000), it was demonstrated that rep-PCR and AFLP genomic fingerprints of diverse *Xanthomonas* isolates correlate strongly with pairwise DNA-DNA hybridization studies, and can be used to determine the taxonomic diversity of bacterial populations.

In addition to the aforementioned genotyping methods, the use of rare-cutting restriction endonucleases in conjunction with fragment resolution using pulsed-field gel electrophoresis (PFGE) was shown to be a powerful tool for differentiating closely related isolates (Quezado-Duval et al., 2004). Within the *X. campestris* species, PFGE has been used to generate physical maps of several pathovars, including pv. *campestris* (Tseng et al., 1999), and to determine 16S rRNA copy number (Lin & Tseng, 1997), but only in a few studies has PFGE been used to determine variability among different *Xanthomonas* isolates (Quezado-Duval et al., 2004).
Integrons are horizontal gene transfer systems containing elements necessary for site-specific recombination and expression of foreign DNA (Rowe-Magnus et al., 2001). Recently, a PCR analysis using integron-based primers was performed with 32 isolates belonging to 12 pathovars of *X. campestris* and *X. axonopodis* species (Gillings et al., 2003). Because one of those primers, AJH60, has multiple binding sites in a cassette array, this assay results in multiple PCR products for each isolate. Twelve different complex banding patterns corresponding to the different pathovars were obtained, suggesting that different isolates of a pathovar share the same integron-based PCR banding pattern (Gillings et al., 2005).

The aims of the present study were: (i) to analyse the genetic diversity among *X. campestris* pv. *campestris* isolates from Israel and other geographic locations, representing the six known races of this pathogen; (ii) to compare the resolution capability of three classical fingerprinting methods, PFGE, AFLP and rep-PCR, for studying the genetic diversity of this widespread plant pathogen, and assess whether any correlation exists between pathogenicity and haplotype affiliation of the isolates; and (iii) to determine whether *X. campestris* pv. *campestris* isolates display integron gene cassette PCR profiles identical to those previously observed for other pathovars of this species (Gillings et al., 2005).

### Materials and methods

**Bacterial isolates and their characterization**

Twenty-two *X. campestris* pv. *campestris* isolates were used in this study. Their origins, hosts and years of isolation are listed in Table 1. *Xanthomonas campestris* pv. *vesicatoria* isolate 97-2 (Asta-Monge et al., 2000) was included as the outgroup control. Isolates originated in this study were obtained as described by Massomo et al. (1990) with minor modifications. Briefly, diseased leaves were collected from plants with symptoms, and leaf tissue segments of ∼2 × 3–4 mm were excised from lesion margins. The leaf segments were placed into drops of 1% sodium hypochlorite for 2 min, rinsed twice with 0.85% saline solution and left to stand in the same solution for 5 min in a laminar flow chamber. Leafpulps of the saline suspensions were streaked on SX semiselective media (Schaad & White, 1974). Plates were inspected for the presence of characteristic convex mucoid colonies after 2 days of incubation at 28°C. Bacteria were stored at

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**Table 1** Bacterial isolates used in this study

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Host</th>
<th>Year of isolation</th>
<th>Origin</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRI 3811 (PHW1205; race 1)</td>
<td>Brassica oleracea</td>
<td>USA</td>
<td>P. Williams</td>
<td></td>
</tr>
<tr>
<td>HRI 3849A (2D520; race 2)</td>
<td>B. oleracea var. botrytis</td>
<td>USA</td>
<td>C. I. Williams</td>
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<td>HRI 5212&quot; (ATCC 33913”; race 3)</td>
<td>B. oleracea var. gemmifera</td>
<td>UK</td>
<td>NCPPB</td>
<td></td>
</tr>
<tr>
<td>HRI 6412 (1713; race 3)</td>
<td>B. oleracea var. botrytis</td>
<td>France</td>
<td>D. Silué</td>
<td></td>
</tr>
<tr>
<td>HRI 1279A&quot; (race 4)</td>
<td>B. oleracea var. capitata</td>
<td>UK</td>
<td>HRI¹</td>
<td></td>
</tr>
<tr>
<td>HRI 3880° (2086; race 5)</td>
<td>B. oleracea var. capitata</td>
<td>Australia</td>
<td>NCPPB</td>
<td></td>
</tr>
<tr>
<td>HRI 6181° (Xcc551; race 6)</td>
<td>Brassica rapa</td>
<td>Portugal</td>
<td>J. Vicente</td>
<td></td>
</tr>
<tr>
<td>B100 (DSM 1526)</td>
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<td>Italy</td>
<td>Hotte et al. (1990)</td>
<td></td>
</tr>
<tr>
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<td>Israel</td>
<td>S. Manulis</td>
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<td>B. oleracea var. botrytis</td>
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<td>S. Manulis</td>
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<td>S. Manulis</td>
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</tr>
<tr>
<td>TH33</td>
<td>B. oleracea var. botrytis</td>
<td>2004</td>
<td>Israel</td>
<td>This study</td>
</tr>
</tbody>
</table>

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ATCC, American Type Culture Collection, Manassas, VA, USA; NCPPB, National Collection of Plant Pathogenic Bacteria, Sand Hutton, York, UK; HRI, Warwick HRI, Wellesbourne, UK; DSM, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.

¹Proposed race type isolates (Vicente et al., 2001).

²All HRI isolates except HRI 5212 were purchased from HRI. Isolate HRI 5212 was purchased from ATCC. Isolate B100 was kindly given by K. Niehaus (Universität Bielefeld, Germany) and SM isolates were kindly given by S. Manulis (Volcani Center, Israel).
–80°C in 20% glycerol and were routinely grown in nutrient broth (NB; Difco) or peptone-sucrose broth (PSB) containing 10 g peptone, 10 g sucrose and 0·1 g glutamic acid L⁻¹, pH 7·2 (Tsuchiya et al., 1982), at 28°C. For solid media [peptone-sucrose agar (PSA) and nutrient agar (NA)], agar was added at 16 g L⁻¹. Isolates obtained from Israel were tested for viscosity of bacterial suspension (Pierce et al., 1990), Gram reaction, Kovacs’ oxidase reaction, starch hydrolysis, oxidative metabolism of glucose and hypersensitive reaction on pepper plants (Leliott & Stead, 1987). Amplification with Amplicon enrichment of enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR), primers REP1R-I and REP2-I (for repetitive extragenic palindromic-PCR; REP-PCR), ERIC1R and ERIC2 (for enterobacterial repetitive intergenic consensus-PCR; ERIC-PCR) and BOXA1R (for BOX elements-PCR; BOX-PCR) were as previously described by Louws et al. (1994) and purchased from Integrated DNA Technologies. PCR mixtures (25 µL) contained: 1 U of Red Taq Polymerase (Sigma), 2·5 µL of 10× reaction buffer (Sigma), 2·5 µL of 25 m M MgCl₂, 0·2 µM of each primer (0·4 µM of BOXA1R in BOX-PCR), 0·2 mM of each dNTP (Promega), 2·5 µL BSA (Roche) and 20 ng of template DNA. DNA amplifications were performed in an Eppendorf Thermal Cycler with an initial denaturation cycle of 7 min at 95°C, followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 40, 42 or 53°C (REP-, ERIC- and BOX-PCR, respectively), and elongation at 65°C for 2 min. A final extension step was performed at 65°C for 15 min. Samples of 12–15 µL from each reaction were separated by electrophoresis in 1·5% agarose gels for 2 h at 6 V cm⁻¹. The gels were stained and photographed with transmitted UV light at 295 nm.

**DNA isolation**

Genomic DNA was isolated for all applications (with the exception of PFGE; see below) with the GenElute Bacterial Genomic DNA kit (Sigma) according to the manufacturer’s instructions. DNA was quantified using a Helius Gamma spectrophotometer (Thermo Electron Corporation) by measuring absorbance at 260 nm, and preparations were stored at −20°C until use.

**DNA digestions with SpeI and PFGE**

Bacterial growth, preparation of agarose plugs and DNA digestion with SpeI (New England Biolabs) were as described by Burdman et al. (2005). PFGE was carried out in 15 × 15 cm 1% Seakem Gold agarose gels using a CHEF DR-II apparatus (Bio-Rad). The gels were run in 0·5 × TBE buffer (Sambrook et al., 1989) at 14°C. The SpeI-digested gels were resolved using a 0–3 to 18 s switching pulse over a ramp period of 30 h at 6 V cm⁻¹. Concatemeric lambda DNA (New England Biolabs) was used as a marker. Following electrophoresis, gels were stained in an ethidium bromide solution (0·5 µg mL⁻¹) for 30 min and photographed with transmitted UV light at 295 nm.

**Amplified fragment length polymorphism**

PstI and TaqI adapters were made by mixing equimolar amounts of complementary oligonucleotides purchased from Integrated DNA Technologies: 5′-CTCGTAGACTGGTAGCTACGCA-3′ and 5′-TGTTAGCTAGCTAC-3′ for PstI adaptor, and 5′-GACGATCCTGCTACTA-3′ and 5′-CGGTCAGACTCAT-3′ for TaqI adaptor. The restriction-ligation procedure was carried out in two steps. First, 50–100 ng of genomic DNA were incubated with 5 U of TaqI (New England Biolabs) and 2 µL of 0·1% BSA in a total volume of 20 µL of 1 × reaction buffer, for 2 h at 65°C. In the second step, the total reaction volume was transferred to a new tube containing 10 U of PstI (New England Biolabs), 1 U of T4 DNA ligase (New England Biolabs), 50 pmol of TaqI adapter, 10 pmol of PstI adapter, 8 µL of 0·5 M NaCl, 4 µL of 0·1% BSA and 4 µL of 10 × T4 buffer. The volume was brought to 40 µL with ultrapure water and incubated for 3 h at 37°C. A 4 µL aliquot of the resulting restriction-ligation mixture was used for preselective amplification using an Eppendorf Thermal Cycler under the following conditions: 1 pmol of PstI and TaqI primers (5′-CTCGTAGACTGGTGCTACGCA-3′ and 5′-GACGATCCTGCTACTA-3′, respectively), 12·5 µL of REDTaq PCR ReadyMix (Sigma) and 6·5 µL of ultrapure water. The cycling parameters were as follows: an initial step of 94°C for 5 min; 20 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 1 min; and a final step of 72°C for 10 min. The reaction mixture was diluted 10-fold and 4 µL were subsequently used in a second selective amplification with primers 5′-PstI + GC-3′ and 5′-PstI + GC-3′ primers, performed with the same reagents as in the preselective amplification. The cycling parameters were as follows: an initial step of 94°C for 5 min; 13 cycles of initially 94°C for 30 s, 65°C decreasing 0·7°C per cycle for 30 s and 72°C for 1 min; 23 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min; and a final step of 72°C for 10 min. Amplified products were separated on a 5% denaturing polyacrylamide gel run at 80 W for 90 min, and stained with the Silver Sequence DNA Sequencing System (Promega) according to the manufacturer’s instructions.

**Repetitive PCR fingerprinting**

Primers REP1R-I and REP2-I (for repetitive extragenic palindromic-PCR; REP-PCR), ERIC1R and ERIC2 (for enterobacterial repetitive intergenic consensus-PCR; ERIC-PCR) and BOXA1R (for BOX elements-PCR; BOX-PCR) were as previously described by Louws et al. (1994) and purchased from Integrated DNA Technologies. PCR mixtures (25 µL) contained: 1 U of Red Taq Polymerase (Sigma), 2·5 µL of 10× reaction buffer (Sigma), 2·5 µL of 25 m M MgCl₂, 0·2 µM of each primer (0·4 µM of BOXA1R in BOX-PCR), 0·2 mM of each dNTP (Promega), 2·75 µM BSA (Roche) and 20 ng of template DNA. DNA amplifications were performed in an Eppendorf Thermal Cycler with an initial denaturation cycle of 7 min at 95°C, followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 40, 42 or 53°C (REP-, ERIC- and BOX-PCR, respectively), and elongation at 65°C for 2 min. A final extension step was performed at 65°C for 15 min. Samples of 12–15 µL from each reaction were separated by electrophoresis in 1·5% agarose gels for 2 h at 6 V cm⁻¹. The gels were stained and developed as described above for PFGE.

**Data analysis of DNA-fingerprinting techniques**

Pulsed-field gel electrophoresis, rep-PCR and AFLP data were analysed using the Fingerprinting II Informatix Software (Bio-Rad). Fragment products from the three different sets of rep-PCR primers (BOX, ERIC and REP) were linearly combined resulting in a BER profile (Rademaker et al., 2005). PFGE was carried out as described above for PFGE.

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to a final concentration of approximately 10<sup>8</sup> cfu mL<sup>-1</sup> after germination. Bacteria were grown on PSA plates for 48 h at 28°C. After 2 weeks, emerging plants were used for inoculation after they developed three fully expanded leaves (4–5 weeks). Plants were kept in the same glasshouse. Plants were inoculated using the leaf clipping method, in which fully expanded leaves were cut with scissors that had been dipped in the bacterial suspension (Tang et al., 2005). Lesion length data were subjected to one-way analysis of variance (ANOVA) and multiple range tests, using NisuvWin version 4-0 (software written and distributed by Professor Avishalom Marani, Hebrew University of Jerusalem; http://departments.agri.huji.ac.il/plantscience/nisuy/).

**Results**

**Characterization of Israeli isolates**

All Israeli isolates used in this study were isolated from field-cultivated crucifer plants showing typical black rot symptoms. They were all capable of growing on SX semi-selective media and developed typical mucoid, convex, yellow colonies on PSA. All isolates were Gram-negative and showed typical *X. campestris* features, such as oxidative utilization of glucose, starch hydrolysis, viscosity and hypersensitive reaction on pepper. All isolates were confirmed as belonging to the *Xanthomonas* genus by GC-FAME analysis; however, this approach did not allow formal conclusions on species allocation of the isolates, probably because of the relatively low taxonomic resolution of this method for *Xanthomonas* (Rademaker et al., 2005). Amplification with selective primers 804F and 87R (Tsengkova et al., 2004) yielded an expected, single fragment of about 630 bp for all isolates. In contrast, this PCR product was not obtained for DNA of the *X. campestris* pv. *vesicatoria* isolate (data not shown).

**DNA fingerprinting by PFGE**

Despite the fact that it is a six-cutter endonuclease, SpeI is suitable for DNA fingerprinting of *X. campestris* pv. *campestris*, which possesses a relatively GC-rich genome (Da Silva et al., 2002). PFGE analysis displayed a good resolution for fragments in the range 40–340 kb, yielding 12–21 discriminate bands for the different isolates. In reproducibility testing, the banding pattern of the reference isolate remained constant. However, because of differences in the baseline, the level of similarity between three different replicates was 88%. Therefore, a cutoff value of 88% was used to define a PFGE type. By this criterion, a total of 11 types were identified among the collection isolates (Fig. 1a; Table 2). Four PFGE types comprised more than one isolate, while seven types were composed of single isolates. All collection isolates used in this study (HRI isolates and B100; Table 1) showed different fingerprint patterns, and thus belonged to unique PFGE types. Two groups of Israeli isolates showed PFGE types identical to some of the collection isolates: ANB06 and TH33 showed a type identical to some of the collection isolates used in this study (HRI isolates and B100; Table 1). This indicated that the group of Israeli isolates used in this study (HRI isolates and B100; Table 1) showed different fingerprint patterns, and thus belonged to unique PFGE types. Two groups of Israeli isolates showed PFGE types identical to some of the collection isolates: ANB06 and TH33 showed a type identical to isolate HRI 1279A, whereas SM57, SM96 and SM144 corresponded to the type of isolate B100 (Fig. 1a, Table 2). A PFGE fingerprint for isolate SM75 could not be obtained.

**DNA fingerprinting by AFLP**

Amplified fragment length polymorphism analysis of the isolates generated a total of 25–33 scorable fragments, ranging between 100 and 500 bp. In replicated
experiments, the banding pattern of the isolates remained constant. However, despite identical patterns, the isolates showed 93% or higher similarity between three different replicates because of small baseline differences. Therefore, a cutoff value of 93% was used to define an AFLP type. A total of 12 AFLP types were identified (Fig. 1b; Table 2). AFLP results were in complete agreement with those of the PFGE analysis and revealed that isolate SM75, which could not be resolved by PFGE, consisted of a unique type (Fig. 1b; Table 2). All tested collection isolates showed different AFLP patterns, and as for PFGE, the same two sets of Israeli isolates showed AFLP types identical to those of two of the collection isolates (see above; Table 2).

Repetitive PCR
BER analysis yielded a complex fingerprint pattern formed by 29–41 bands ranging between 200 and 3000 bp. As a result of small differences in baseline and reproducibility, a cutoff value of 85% was used to define a BER type. A total of 13 BER types were identified (Fig. 1c; Table 2). BER results were in complete agreement with those of the PFGE analysis and revealed that isolate SM75, which could not be resolved by PFGE, consisted of a unique type (Fig. 1c; Table 2). All tested collection isolates showed different AFLP patterns, and as for PFGE, the same two sets of Israeli isolates showed AFLP types identical to those of two of the collection isolates (see above; Table 2).

Pathogenicity assays
In preliminary inoculation experiments, all isolates were found to be pathogenic on all tested crucifer species (cauliflower, radish, cabbage and broccoli), inducing typical V-shaped lesions on inoculated leaves (not shown). Cauliflower and radish were selected for further experiments because in the preliminary assays trends of differential pathogenicity among isolates were more clearly observed in these hosts than in cabbage and broccoli.
Figure 1 Cluster analyses of Xanthomonas campestris pv. campestris isolates: pulsed-field gel electrophoresis (PFGE) (a), amplified fragment length polymorphism (AFLP) (b) and BER-fingerprints (c). Unweighted paired group mathematical average (UPGMA) dendrograms were generated using the Dice similarity coefficient. The significance of each branch is indicated by the bootstrap percentage calculated for 1000 subsets (only values greater than 50% are shown). Dashed lines show cutoff similarity values. Molecular weight positions are indicated (kb for PFGE and bp for AFLP and BER).
These results were in agreement with those of Vicente et al. (2001), who showed that diverse isolates from the six X. campestris pv. campestris races determined to date were similarly pathogenic on both cabbage and broccoli, thus not allowing these plants to be used as differentials for race determination.

Three further experiments were carried out with cauliflower and radish. Because similar differences in pathogenicity between isolates were observed in these and in the preliminary experiments, data from a representative experiment are shown (Table 2). As expected, plants treated with negative controls (X. campestris pv. vesicatoria and water) did not show disease symptoms (not shown). Isolates SM144 and TH33 showed consistently high levels of pathogenicity in cauliflower and differed significantly \((P = 0.05)\) from several other isolates. In contrast, the race-2 isolate, HRI 3849A, showed significantly \((P = 0.05)\) reduced pathogenicity relative to the other isolates on this host, as did isolates HRI 6181 (race 6) and B100 on cauliflower.

In contrast, isolate HRI 6181 showed consistently longer lesions in radish than did other isolates, although the difference was not always significant \((P = 0.05)\). Race 5, 3 and 1 isolates (HRI 3880, HRI 5212 and HRI 3811), as well as isolate B100, were consistently less pathogenic on radish than other isolates (Table 2).

Differences in pathogenicity were found between isolates belonging to the same haplotype. For instance, within BER type XI, isolates TH30 and TH26 did not differ significantly between each other on both hosts; however, isolate TH30 consistently induced longer lesions than isolate TH26 in all experiments with cauliflower (not shown). Within BER type V, isolate SM144 consistently induced longer lesions than isolates SM96 and SM57 on cauliflower, although differences were not statistically significant. On both hosts, these three isolates were significantly \((P = 0.05)\) more aggressive than isolate B100, with which they clustered according to PFGE and AFLP (Table 2). Lastly, within BER type VI, no significant differences were found between isolates HRI1279A and TH33; however, a consistent tendency was observed by which the former isolate showed higher levels of pathogenicity than the latter on radish, whereas the opposite was observed on cauliflower.

**Discussion**

In the present study AFLP, rep-PCR and PFGE were used to examine genetic diversity among X. campestris pv. campestris isolates. For that purpose, in addition to 14 isolates obtained in Israel, six isolates representing the six known races of this pathogen and two recently sequenced isolates were included (ATCC 33913\(^2\) and B100).

Cluster analysis of PFGE and AFLP fingerprints showed a high level of correlation, leading to the detection of 11 and 12 types, respectively. The only exception was Israeli isolate SM75, for which a PFGE profile could not be obtained, probably because of DNA degradation during sample preparation (Klaassen et al., 2002).

The linearly combined rep-patterns led to the detection of 13 BER types that were basically in agreement with AFLP and PFGE cluster analyses. The only difference was that BER analysis distinguished isolate B100 from isolates SM57, SM96 and SM144, whereas these four isolates clustered together according to AFLP and PFGE. The high correlation found between AFLP and BER analyses was in agreement with results from a previous study with different Xanthomonas species and pathovars (Rademaker et al., 2000).

Vorholter et al. (2003) and Qian et al. (2005) compared two sequenced genomes of X. campestris pv. campestris (B100/ATCC 33913\(^2\) and 8004/ATCC 33913\(^2\), respectively) and revealed significant differences in their gene composition. In a study aimed at determining the extent of genetic variation within X. campestris pv. campestris in Tanzania, Massomo et al. (2003) detected seven BOX-PCR types among 108 tested isolates, and five REP-PCR types among 71 tested isolates. Likewise, Rademaker et al. (2005) distinguished five BER groups within 23 X. campestris pv. campestris isolates. The present study not only supports genetic heterogeneity among X. campestris pv. campestris isolates, but also suggests that diversity within this pathovar is higher than previously estimated. The high variation found in this study (13 BER types within 22 isolates) may be explained, at least in part, by the utilization of several collection isolates from different geographic locations. Indeed, each of the eight collection

![Figure 2](image-url)
isolates tested (HRI isolates and isolate B100) gave unique PFGE, AFLP and BER types. However, relatively high diversity was also observed among the Israeli isolates (five BER types within 14 isolates).

Interestingly, the B100 and HRI 1279A types (isolated in Italy and the UK, respectively) were found in Israeli isolates. Whilst, the presence of B100 and HRI 1279A types in Israel could suggest great capability of dissemination and/or adaptability of these genotypes in comparison with other X. campestris pv. camppestris isolates, the fact that a great number of isolates showed unique fingerprint patterns might be the result of adaptation of these isolates to particular habitats. Linkage of different genotypes to geographical origin was recently reported by Massomo et al. (2003) and Scortichini et al. (2001) in X. campestris pv. camppestris and X. arboricola pv. arboricola, respectively. Selective pressure of the host plant and environmental conditions might play a central role in selecting different populations of this pathogen.

Integrons and gene cassettes are frequent components of bacterial genomes (Nemer gut et al., 2004). In a study performed with 32 Xanthomonas isolates from 12 pathovars, Gillings et al. (2005) showed that integron array PCRs were identical within each of the pathovars begoniae, oryzae, pruni, pelargoni, vitians, citri and vesicatoria. Consequently, it was suggested that different Xanthomonas species and pathovars differ in their integron array profiles, but the profiles are conserved among isolates within each pathovar. In that study, however, only one isolate of pathovar camppestris was used (DAR30538). Assuming the possibility that genetic diversity within pathovar camppestris is higher than in most other X. campestris pathovars, a certain level of diversity in integron array profiling may be found among isolates of this pathovar. Indeed, in support of the high level of integron pattern conservancy found within pathovars by Gillings et al. (2005), significantly lower variation was found among the tested isolates using this technique than with PFGE, AFLP and rep-PCR. However, in the case of X. campestris pv. camppestris, at least four different integron array patterns existed, three of which (types II, III and IV) were new. Interestingly, one of the new reported types (type IV) was found to be the most dominant among the tested isolates. No correlation between cluster analyses obtained with rep-PCR, PFGE and AFLP, and the four different integron array patterns could be inferred, a fact that could be explained by the horizontal transfer of these elements.

An important question regarding diagnosis and evaluation of potential damage caused by particular isolates on different crops is whether race affiliation correlates with DNA-fingerprint typing. The present study showed not only that the two race-3 isolates, HRI 5212 and HRI 6412, clustered in different PFGE, AFLP and BER types, but also that the types in which these isolates clustered were relatively distant, particularly in BER profiling (Fig. 1c). This finding indicates that race affiliation cannot be inferred by DNA fingerprinting, which could be explained by the fact that single mutations occurring in bacterial avirulence (avr) genes, which are often responsible for the generation of new races or for race switch, are barely detectable by DNA-fingerprint techniques. In support of the above, a clear correlation between pathogenicity and genotype affiliation of the different isolates could not be detected.

This appears to be one of the first studies in which PFGE, rep-PCR and AFLP have been compared for their potential to assess isolate diversity of a phytopathogenic bacterium, and the first study in which PFGE has been evaluated for its potential to assess genetic diversity of X. campestris pv. camppestris. The observed results showed that these techniques possess comparable resolution capabilities to assess the diversity within this pathogen. The advantage of PFGE and AFLP techniques resides in their relatively high reproducibility, whereas rep-PCR is faster, cheaper and usually more discriminative.

This is the first study in which integron gene cassette PCR banding patterns of various X. campestris pv. camppestris isolates have been compared, and results support the relatively high genomic diversity of this pathogen. The low variability of integron amplified patterns relative to that observed with the other approaches could be exploited for diagnostic purposes for the detection of this bacterium.

Acknowledgements

We thank Dr Shulamit Manulis and Professor Karsten Niehaus for kindly providing isolates for this study, and Dr Joana Vicente for her suggestions and help in obtaining the HRI isolates. We also thank Professor Yaakov Okon, Dr Edouard Jurkevitch and Mrs Dafna Tamir for their valuable comments during the preparation of this manuscript. This study was carried out as part of a project that is being funded by grant 499/03 from the Israel Science Foundation. The work of Angel Valverde was supported by a postdoctoral fellowship from the Spanish Government.

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