

Development of Specific Primers for the Molecular Detection of Bacterial Spot of Pepper and Tomato

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Abstract

Bacterial spot of tomato and pepper is an important seed-borne disease caused by a diverse group of bacteria currently classified as four species of *X. euvesicatoria* (*Xe*), *Xanthomonas vesicatoria* (*Xv*), *X. perforans* (*Xp*) and *Xanthomonas gardneri* (*Xg*).

The objective of this research was to develop PCR primer sets that allow the specific detection and/or identification of *Xe*, *Xv*, *Xp* and *Xg* strains, in particular in seed assays. AFLP® was used to identify markers specific for each of the four species. Marker fragments were sequenced and potential primer sets were evaluated in PCR for their specificity. For all species specific primers were obtained.

INTRODUCTION

Bacterial spot (BS) of pepper and tomato is a potential production constraint in regions with humid and warm weather conditions. Contaminated seeds of pepper and tomato can be the primary source of inoculum and therefore seeds are routinely tested in laboratories. Dilution plating on semi-selective media is widely used for the detection of xanthomonads from seeds. Additional identification is necessary to determine whether suspect colonies are pathogenic to pepper and/or tomato. A number of complementary techniques such as immunofluorescence (IF) microscopy, pathogenicity assays and PCR can be used for the identification of bacterial colonies. However the fast and reliable identification of suspected isolates from seeds of pepper and tomato is still hampered by poor reproducibility of pathogenicity assays, while currently available PCR and IF tools suffer from limited specificity. BS is caused by four different *Xanthomonas* species, *X. euvesicatoria* (*Xe*), *X. vesicatoria* (*Xv*), *X. perforans* (*Xp*) and *X. gardneri* (*Xg*) (Jones et al., 1984; Jones et al., 2004; 2006). The objective of this study was to develop primers specific for BS species using an AFLP® based approach.

MATERIALS AND METHODS

BS strains (52, 9, 6 and 3 of *Xe*, *Xv*, *Xp* and *Xg*, resp.) from various geographic regions and 8 outgroup strains (*Xanthomonas* spp. from pepper, *X. hortorum* and *X. campestris* pathovars) were collected. Forty strains were obtained from the collection of Jeffrey Jones and had been extensively characterised in previous studies. DNA was extracted from the strains and then subjected to AFLP and consecutive Jaccard analysis. DNA fragments that were exclusively present in all strains of one BS species, and not present in the outgroup strains, were selected and then sequenced. Based on this species-specific sequences PCR primers were designed. The primer sets were evaluated in PCR using various annealing temperatures with DNA from most strains of the BS species and outgroup strains.

RESULTS AND DISCUSSION

Jaccard analysis of the AFLP patterns showed the expected clustering of BS strains in four groups representing the different species (Fig. 1A-D). The 3 strains of *Xg* clustered together with 3 outgroup strains (Fig. 1D). Within a species there was polymorphism between strains. It should be noted that the similarity was relatively small among the *Xg* strains at one hand and the outgroup strains. A number of species-specific AFLP fragments were sequenced (data not shown) and several primer sets were evaluated. The specificity of the primer sets was evaluated in PCR using most of the strains. It appeared that some primer sets were not specific enough (data not shown) and four promising sets (Table 1) were further characterised in the study using additional out group xanthomonads (Fig. 2). As expected the annealing temperature of the PCR appeared to be critical. At 64°C the specificity of all sets was good although one primer, BS-XpR, needed adaptation by adding one nucleotide (G) at the 3'end to enhance the performance.

The specificity of the BS-Xv, BS-Xp and especially the BS-Xg primer sets was determined with a limited number of strains. Therefore additional validation of these primers on a larger set of isolates is under investigation. The BS-Xg primer set was recently tested on an additional 12 strains of a Russian collection of *X. gardneri* and all reacted with this primer set (A. Ignatov, personal communications).

CONCLUSIONS

BS specific primer sets, covering four species of xanthomonads, were developed using an AFLP based approach. The specificity of the primer sets was tested with a large number of BS strains from different geographic origins. All strains were identified by the four primer sets. Especially for *Xp* and *Xg* the number of strains studied was relatively limited and therefore additional validation of these primers on a larger set of characterized strains is necessary and under investigation. Also, the usefulness of all primer sets needs to be further established by testing more non-BS bacteria obtained from pepper and tomato seeds.

Literature Cited

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Tables

Table 1. Primer sets for the identification of *Xanthomonas euvesicatoria* (*Xe*), *Xanthomonas gardneri* (*Xg*), *Xanthomonas perforans* (*Xp*) and *Xanthomonas vesicatoria* (*Xv*). Note that after the presentation of the poster the primer sequence of Bs-XpR was adapted.

Primer	Sequence	Size of amplicon
Bs-XeF	CAT GAA GAA CTC GGC GTA TCG	
Bs-XeR	GTC GGA CAT AGT GGA CAC ATA C	173 bp
Bs-XvF	CCA TGT GCC GTT GAA ATA CTT G	
Bs-XvR	ACA AGA GAT GTT GCT ATG ATT TGC	138 bp
Bs-XgF	TCA GTG CTT AGT TCC TCA TTG TC	
Bs-XgR	TGA CCG ATA AAG ACT GCG AAA G	154 bp
Bs-XpF	GTC GTG TTG ATG GAG CGT TC	
Bs-XpR	GTG CGA GTC AAT TAT CAG AAT GTG G*	197 bp

Figures

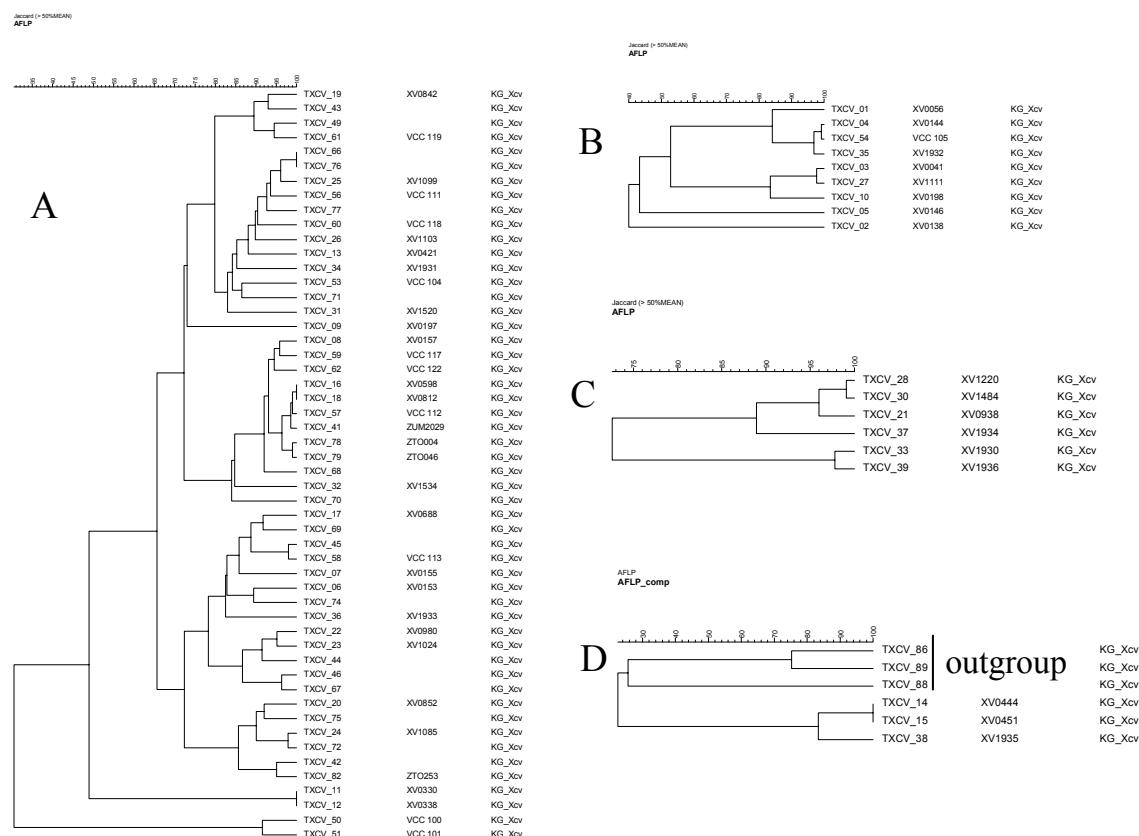


Fig. 1A-D. Dendrograms of BS strains based on Jaccard analysis of AFLP data. 52 strains of *Xanthomonas euvesicatoria* (A), 9 strains of *Xanthomonas vesicatoria* (B) and 6 strains of *Xanthomonas perforans* (C) were included in the study. Three non BS outgroup strains (a non-pathogenic strain of *Xanthomonas* spp. from pepper seed, *X. hortorum* pv. *pelargonii* and *X. h.* pv. *carotae*) were plotted with the 3 strains of *Xanthomonas gardneri* (D).

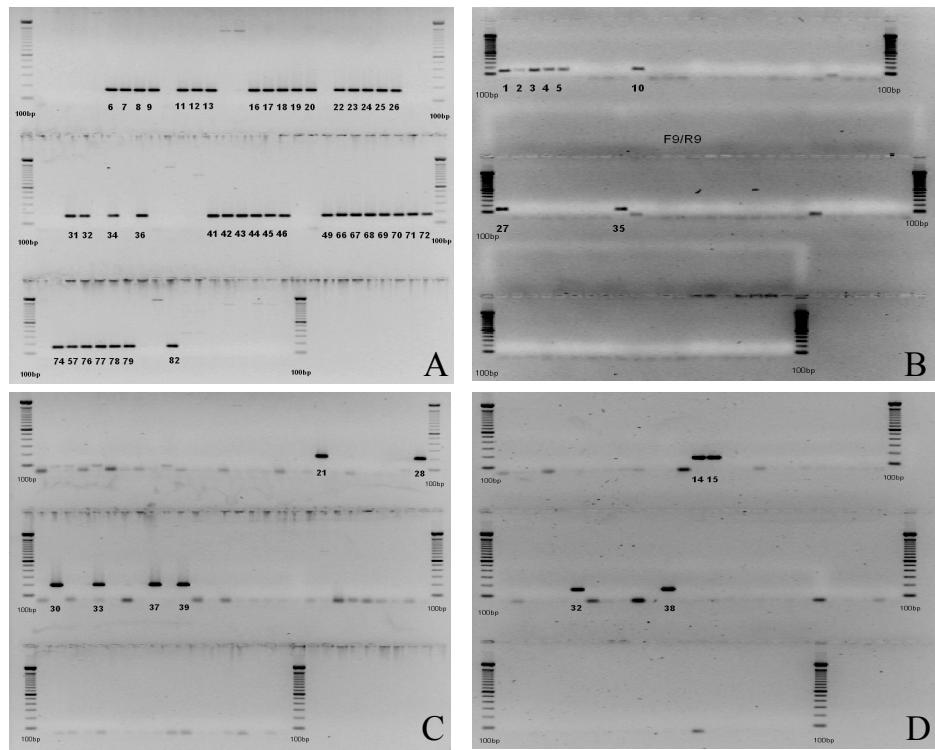


Fig. 2. Evaluation of BS-Xe (A), BS-Xv (B), BS-Xp (C) and BS-Xg (D) PCR primer sets with most strains that were included in the study.