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Development of a DNA array to discriminate the cucumber pathogens *Fusarium oxysporum* f. sp. *cucumerinum* and f. sp. *radicis-cucumerinum*

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***Fusarium oxysporum* is a ubiquitous soilborne fungus that harbours pathogenic as well as non-pathogenic or even beneficial strains. Pathogenic strains are characterized by a high degree of host specificity and strains that infect the same host range are organized in so-called formae speciales. Therefore, identification below the species level is highly desired. Currently, identification of pathogenic *F. oxysporum* isolates is mainly based on time-consuming and laborious bioassays. Increasingly, attempts are made to replace these methods by straight-forward, culture-independent molecular identification techniques. Ideally, molecular identification of pathogenic strains is based on the detection of genetic targets that are linked to pathogenicity. However, so far the genetic basis of host specificity and virulence in *F. oxysporum* is unknown. In this study, a random amplified polymorphic DNA (RAPD) marker-based assay was developed to specifically detect and identify the economically important cucumber pathogens *F. oxysporum* f. sp. *cucumerinum* (Fusarium wilt) and *F. oxysporum* f. sp. *radicis-cucumerinum* (Fusarium root and stem rot). Highly reliable markers were implemented in a DNA macro-array with multiple levels of phylogenetic specificity. The array was optimized for specific, sensitive and reliable detection of the target organisms in diverse environmental samples.**

Introduction

In general molecular identification of plant pathogenic fungi is based on the detection of polymorphisms in ubiquitously conserved genes. The use of universal primers that anneal to conserved sequences flanking variable domains within these genes offers the possibility of simultaneous detection and identification of multiple plant pathogens in a single assay, for instance by DNA array hybridization [1,2]. This strategy has proven to be successful for species identification, even when different species can only be discriminated by a single nucleotide polymorphism [3]. However, housekeeping genes do not always reflect sufficient sequence variation for the discrimination below the species level, such as for example for *Fusarium oxysporum* formae speciales [1]. The overall objective of this study was to implement robust DNA markers for the genetically different and economically important formae speciales *cucumerinum* (Foc) and *radicis-cucumerinum* (Forc) in a previously designed DNA array-based detection procedure [1]. Results presented in this poster will be published in Environ. Microbiol. (*in press*)

Identification of specific RAPD markers

RAPD analyses were performed using a strategy of several elimination rounds. Eventually, the most discriminative oligonucleotides were tested on a collection of 273 *Fusarium* strains, encompassing all known vegetative compatibility groups (VCGs) of the target formae speciales as well as representative strains of other *F. oxysporum* formae speciales. Of the initially tested primers, oligonucleotide OPB-07 produced a clear diagnostic band of 277 bp for the tested Forc strains, while oligonucleotide OPZ-12 was found to produce a diagnostic band of 865 bp for all Foc strains.

	OPZ-12 (865 bp)	OPB-07 (277 bp)
# 46 Foc	+	-
# 28 Forc	-	+
# 1 Forc (strain 37)	+	-
# 2 Forc strains isolated from soil (strains 10 and 11)	-	-
# 3 avirulent strains isolated from cucumber	-	-
# 183 isolates from other formae speciales	-	-
F. ox. f. sp. *conglutinans* 81-4	+	-
F. ox. f. sp. *dianthi* NRRL 26960	+	-
F. ox. f. sp. *gladioli* NRRL 26993	+	-
F. ox. f. sp. *lilii* NRRL 26955	+	-
F. ox. f. sp. *lycopersici* MUCL 15159 and NRRL 22544	+	-
# 4 isolates from 2 other *Fusarium* sp.	-	-

Exceptions were tested for their ability to cause Fusarium wilt and Fusarium root and stem rot: Forc 37 appeared to be Foc, while strains 10 and 11 appeared to be non-pathogenic. The other exceptions were not able to infect cucumber. Nevertheless, based on EF-1α sequences these isolates grouped together with Foc isolates (Fig. 1), illustrating their close genetic relationship.

Development of robust SCAR markers

In order to develop a robust diagnostic assay, the selected RAPD markers were used to develop reliable sequence characterized amplified region (SCAR) primers. As a result, the number of cross-reactions decreased to two cross-reactions, i.e. for *F. oxysporum* f. sp. *conglutinans* 81-4 and f. sp. *gladioli* NRRL 26993.

Design of a DNA array

In order to further increase the specificity and sensitivity of the developed assay, as well as to implement the developed SCAR markers in a previously designed internal transcribed spacer (ITS)-based DNA array enabling parallel detection of multiple pathogens and targets in a single assay [1], multiple oligonucleotide detectors were designed based on the sequence of the selected markers and integrated in the array. Labeled amplicons from the different PCR reactions were combined and hybridized to the array, resulting in a DNA array with multiple levels of phylogenetic specificity.

Analyzing environmental samples

The DNA array was validated using complex samples from different origins. Artificially inoculated plant and soil samples as well as water, root or foot samples collected at commercial cucumber production greenhouse settings were successfully analyzed with the array (Fig. 2.), illustrating the power of the novel assay for routine analysis of environmental samples.

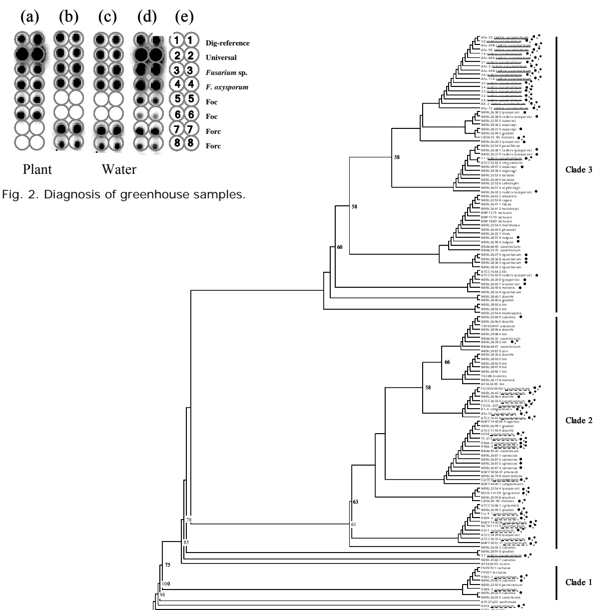

Fig. 2. Diagnosis of greenhouse samples.

Fig. 1. Genetic organization of Foc and Forc within the *F. oxysporum* species complex based on EF-1α seqences. The cladogram displays the different clades within the *F. oxysporum* species complex as proposed by O'Donnell *et al.* 1998, PNAS 95: 2044-2049.

References

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