Detection of *Phytophthora nicotianae* by PCR*

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Phytophthora nicotianae is an important soil-borne pathogen. The classical determination based on morphological or physiological characters is time and labour-consuming and is taxonomically unsure. Two specific primers designed from the spacer regions ITS1 and ITS2 were used to detect *P. nicotianae* by polymerase chain reaction (PCR). A PCR fragment of 737 bp was obtained from all *P. nicotianae* isolates assayed, but not from other *Phytophthora* spp. or other genera. Thus, the PCR characterization was species-specific. Polyclonal antisera against mycelium and zoospores were used for comparison and were only genus-specific.

Introduction

Phytophthora nicotianae has a wide host range of over 70 genera of plants (Hickmann, 1958). The pathogen causes stem and root rot thereby inducing water deficiency symptoms. Healthy nursery plants and chemical and/or biological control are basic elements in disease control. Early detection and diagnosis of this pathogen either in plants, soil, water or especially in recirculating nutrient solutions of hydroponic cultures are critical for efficient control. Classical determination based on morphological or physiological characters is time and labour consuming and requires considerable knowledge of the genus. It is in any case taxonomically unsure. Serological techniques and DNA probes have been developed for *Phytophthora* detection (Jones & Shew, 1988; McDonald *et al.*, 1990; Richter & Gabler, 1991; Grote & Gabler, 1999; Goodwin *et al.*, 1990). Polymerase chain reaction (PCR) allows the amplification of a species-specific sequence by using specific primers (Bruce *et al.*, 1992; Picard *et al.*, 1992; Ersek *et al.*, 1994; White *et al.*, 1990). In this article, the specificity of the two detection methods for *P. nicotianae* is presented.

Polymerase chain reaction

Two specific primers from internal transcribed spacer regions ITS1 and ITS2 of ribosomal DNA were designed: the 20-mer P_{NIC} upper 5′-CAA TAG TTG GGG GTC TTA TT-3′ and the 22-mer P_{NIC} lower 5′-GTA TAC CGA AGT ACA CAT TAA G-3′. The reaction mix (25 μL) consisted of common concentrations of all components, with additional 2% formamide. The amplification was carried out in a Perkin Elmer 9600 cycler. The origin, host plants, PCR reaction and ELISA response from isolates presented in this work are listed in Table 1 for *P. nicotianae* isolates and in Table 2 for other *Phytophthora* species and other genera.

Fungal DNA was extracted according to a conventional phenol extraction method (Lee & Taylor, 1990) with some modifications. A dilution in distilled water containing 50–100 ng DNA was used for PCR. An amplified PCR fragment 737 bp in size was obtained with DNA from all *P. nicotianae* isolates assayed including two *P. nicotianae/cactorum* hybrids, but not with DNA from other *Phytophthora* species or other genera (Tables 1, 2). The results show the specificity of the designed primers and the reliability of the reaction.

Many *Phytophthora* isolates from hydroponically grown cultures of *Spathiphyllum*, *Capsicum* and tomato were identified as *P. nicotianae*. The new, specific PCR method described was easily adapted for the detection of *P. nicotianae* and its zoospores in plant material or in the nutrient solution of hydroponic systems.

Indirect ELISA

After four immunizations of rabbits with 10 mg mL^{-1} mycelium and 10^7 zoospores per mL, the respective antisera were obtained following the usual protocols and tested against *P. nicotianae* (Harlow & Lane, 1988). The results of the specificity test of the mycelium antiserum are given in Tables 1 and 2. All *P. nicotianae* isolates gave a very high or high positive response by indirect ELISA. A high positive response was also obtained for the two hybrids (Table 1). The serological response was also positive for other *Phytophthora* species, however, though mostly at lower intensity than for *P. nicotianae*. Only one isolate which was not *P. nicotianae* showed a very high positive response. All non-*Phytophthora* species gave negative responses (Table 2).

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Table 1 *Phytophthora nicotianae* isolates used in this study, their origin and reaction with specific PCR primers and ELISA

Number	Country of origin	Year of isolation	Host/part of plant	Institution where held*	ELISA	PCR
310.62	IN		*Nicotiana tabacum*	CBS	+++	+
10664	NL	1998	*Spatiphyllum* sp.	PD	+++	+
Pn5 (R)	DE	1988	*Lycopersicon esculentum* (fruit)	IGZ	++	+
Pnp2 (R)	DE		*N. tabacum*	BBA/IGZ	+++	+
1432 (R)	DE		*Euphorbia pulcherrima*	BBA		+
Pn1	DE	1989	*L. esculentum*	IGZ	+++	+
Pn2	DE	1998	*L. esculentum* (rockwool slab)	IGZ	+++	+
IVIA-P1	ES		Unknown	IVIA	++	+
249	BE	1999	*L. esculentum* (rockwool slab)	IGZ	++	+
309	BE	1999	*L. esculentum* (stem base)	IGZ	+++	+
374	BE	1999	*Capsicum* spp.	IGZ	++	+
AN 96/4	NL	1996	*Spathiphyllum* sp.	PBG	+++	+
AN 97/28	NL	1997	*L. esculentum*	PBG	+++	+
AN 99/3†	NL	1999	*Cyclamen* sp.	PBG	++	+
IVIA-P2†	ES	1991	*Pyrus communis*	IVIA	++	+

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† *P. nicotianae* × *P. cactorum* hybrid.

Conclusions

P. nicotianae was frequently isolated from hydroponic cultures of *Spathiphyllum*, *Capsicum* and tomato plants. The PCR detection method was species specific and the indirect ELISA genus specific. The great agronomic impact of the pathogen requires early and species-specific detection. This new specific PCR method could easily be adapted for detection in plant material and nutrient solution. ELISA can be used for quantitative description of pathogenesis in plant material or distribution potential in a nutrient solution. Two *P. nicotianae/P. cactorum* hybrids were recognized as *P. nicotianae* in the ITS target region.

Table 2 *Phytophthora* and other species used in this study, their origin and reaction with specific PCR primers and ELISA

Species	Number	Country of origin	Year of isolation	Host/part of plant	Institution where held*	ELISA	PCR
Phytophthora citricola	1817 (R)	NL		*Medicago sativa*	PD	++	–
Phytophthora megasperma	118 (R)	NL	1994	*Rubus idaeus*	PD	++	–
Phytophthora citrophthora	IVIA-P3	ES		*Citrus* sp.	IVIA	++	–
Phytophthora syringae	4292 (R)	NL	1997	*Malus* sp.	PD	++	–
Phytophthora cryptogea	307.62 (R)	ZA		*Godetia* sp.	CBS		–
Phytophthora cryptogea	63779 (R)			Unknown	BBA		–
Phytophthora cambivora	21/95-K II (R)		1995	*Chamaecyparis lawsoniana*	BBA		–
Phytophthora palmivora	64972 (R)			Unknown	BBA		–
Phytophthora cinnamomi	IVIA-P4	ES		*Quercus ilex* var. *rotundifolia*	IVIA	++	–
Phytophthora cactorum	IVIA-P5	ES		*Fragaria* sp.	IVIA	++	–
Phytophthora capsici	IVIA-P6	ES		*Capsicum* sp.	IVIA	+	–
Phytophthora infestans	Naumann	DE	1998	*Lycopersicon esculentum*	BAZ	+	–
Phytophthora sp.	P3	BE	1998	*L. esculentum* (roots on rockwool slab)	IGZ	+++	–
Pythium aphanidermatum	Pythium-2	DE		*L. esculentum*	IGZ		–
Fusarium oxysporum	IVIA-F2	ES	1999	*Phoenix dactylifera*	IVIA		–
Fusarium oxysporum	IVIA-F3	ES	1999	*Citrus* sp.	IVIA		–
Fusarium sp.	IVIA-F1	ES	1999	*P. dactylifera*	IVIA		–
Alternaria alternata f. sp. *citri*	IVIA-A1	ES	1999	*Citrus* sp.	IVIA		–
Botrytis cinerea	IVIA-B1	ES	1999	*Allium cepa*	IVIA		–

* See Table 1.

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Détection de *Phytophthora nicotianae* par PCR

Phytophthora nicotianae est un pathogène important transmis par le sol. La détermination classique qui utilise des caractères morphologiques ou physiologiques demande beaucoup de temps et de travail, et n'est pas sûre du point de vue taxonomique. Deux amorces spécifiques conçues à partir des espaceurs ITS1 et ITS2 ont été utilisées pour détecter *P. nicotianae* par PCR. Un fragment de 737 pb a été obtenu pour tous les isolats de *P. nicotianae* testés, mais pas pour les autres *Phytophthora* spp. ou les autres genres. Ainsi, la caractérisation obtenue par la PCR est spécifique à l'espèce. Les antisérums polyclonaux contre le mycélium et les zoospores ont été utilisés à titre de comparaison et étaient seulement spécifiques au genre.

Выявление *Phytophthora nicotianae* методом PCR

Phytophthora nicotianae – серьезный патоген, передающийся через почву. Классическое его определение основано на морфологических или на физиологических признаках, оно занимает много времени, трудоемко и ненадежно с точки зрения таксономии. Два специфичных праймера были разработаны из областей спейсеров ITS1 и ITS2 и использовались для выявления *P. nicotianae* с помощью PCR. Фрагмент PCR 737bp был получен от всех тестировавшихся изолятов *P. nicotianae*, но ни от других *Phytophthora* spp., ни от каких-либо других родов. Таким образом, характеризация PCR была видоспецифичной. Поликлональные антисыворотки против мицелия и зооспор использовались в порядке сравнения и были только родо-специфичными.

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