Genetic variability among Alternaria solani isolates from potatoes in Southern Germany Based on RAPD-profiles.

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Abstract

One of the problems encountered when studying the population genetics of fungal plant pathogens is defining what constitutes a “population”. Potato early blight is a major disease of potatoes and other species of the Solanaceae. The causal agent is the ascomycete *Alternaria solani* (Sorauer), which infects leaves and stems of potato plants leading to premature defoliation. In this study, spatial genetic diversity within *A. solani* populations from potato leaves were investigated using RAPD markers in order to reveal the degree of homo- or heterogeneity. Analysis of RAPD profiles revealed distinct genetic diversity among isolates originating from the same field. In addition, pronounced genetic variability was found for isolates from different years. These results indicate a surprising genetic heterogeneity within the population of *A. solani*, which must be kept in mind when designing protective measures for agriculture.

Keywords early blight, intraspecific variation, pathogen population, *Solanum tuberosum*

Introduction

Species of the genus *Alternaria* are very common and abundant (Pscheidt ~~and~~ & Stevenson, 1986). *Alternaria* species in general, including *Alternaria solani* (Sorauer), have a world-wide geographic distribution (Rotem, 1994). The genus *Alternaria* includes saprophytic and pathogenic fungi. This paper deals with the pathogenic species *A. solani*, which is one of the best-known and economically most important members of this genus (Rotem, 1994). *A. solani* causes early blight of potatoes (*Solanum tuberosum* L.) and is also an important pathogen of tomatoes (*Lycopersicon esculentum* Mill.) and other members of the

Solanaceae (Rotem, 1994). The disease primarily affects aerial plant parts. Severe epidemics can lead to considerable defoliation and reduced yield within short periods of time~~s~~ (Batista et al., 2006). Early blight is widespread in most areas where potatoes are grown and occurs over a wide range of climatic conditions (Rotem, 1994). Genetic analysis of plant pathogen populations is important for understanding epidemiology and host-pathogen interactions as well as for the development of disease control strategies (Adachi et al., 1993; Leung ~~and~~ & Williams, 1986; McDonald ~~and~~ & Linde, 2002; Milgroom ~~and~~ & Fry, 1997; Morris et al., 2000).

A population is a group of organisms of the same species that occupies the same geographic region and exhibits reproductive continuity from generation to generation (Futuyma, 1986). It can consist of distinct individuals that remain genetically diverse, thus contributing to high genetic variability. According to Alexander et al. (1993), genetic variation in pathogen populations is common and thought to be a crucial factor influencing disease dynamics in plant systems. McDonald ~~and~~ & Linde (2002) suggested that the genetic structure of a population is determined by the evolutionary history of that population. They hypothesized that the evolution of (pathogen) populations was probably driven by mutation, selection and gene flow. There is evidence that these evolutionary processes have influenced *A. solani* populations (Martínez et al., 2004; Petrunak ~~and~~ & Christ, 1992; van der Waals et al., 2004; Weir et al., 1998). Therefore, the extent and distribution of genetic variation within and between populations of *A. solani* is of considerable interest in plant pathology, since it gives an indication of the potential for development of pathogenic specialization and fungicide resistance (Adachi et al., 1993; McDonald ~~and~~ & Linde, 2002).

Molecular genetics may be of interest to study populations of fungi (Lourenço et al., 2009), in particular of species lacking a known sexual life cycle. The absence of a sexual stage presents two problems for the traditional classification of species of the genus *Alternaria* (Cooke et al., 1998). In addition to the absence of sexual morphological features as taxonomic characters, it is also not possible to assess species boundaries on the basis of sexual incompatibility (Cooke et al., 1998). The analysis of gene products such as isozymes (Oudemans ~~and~~ & Coffey, 1991), or variation at the level of DNA itself, has been increasingly used to clarify the taxonomy of many groups of organisms (Meijer et al., 1994; Oudemans ~~and~~ & Coffey, 1991; Williams et al., 1990). In fungal systems, random amplified polymorphic DNA (RAPD)-PCR has been widely applied to characterize species and isolates (Schafer ~~and~~ & Wostemyer, 1992; Reeves ~~and~~ & Ball, 1991; Leal et al., 1994; Meijer et al., 1994).

Much work has been carried out on the diversity and phylogeny of species of the genus *Alternaria* (Adachi et al., 1993; Cooke et al., 1998; Petrunak ~~and~~ & Christ, 1992; van der Waals et al., 2004; Weir et al., 1998). Previous research has discovered the existence of physiological races of *A. solani* (Bonde, 1929; Henning ~~and~~ & Alexander, 1959). An early report by Bonde (1929) demonstrated that *Alternaria* isolates from potato tubers exhibited differences in colony morphology, sporulation capacity and growth rates on artificial media.

Henning ~~and~~ & Alexander (1959) obtained evidence for differences between *A. solani* isolates at a morphological, physiological and pathogenic level.

According to Milgroom (2001), investigations on population genetics are helpful tools which can provide greater insights into pathogen population biology and disease management. Population genetics aims to understand causes and consequences of the genetic structure of populations, i.e. distributions of genetic variants in space and time (Hoekstra, 1994). The objective of this study was to examine the variability of naturally occurring *Alternaria* populations, originating from the same location, in two different years. The amount of intraspecific variation among 45 isolates of *A. solani* was assessed. RAPD analysis was used to examine DNA polymorphism of *A. solani* isolates.

Material and Methods

Collection of *Alternaria solani* isolates

Early blight infected leaf samples from various regions and potato cultivars were collected from naturally infected fields during two growing seasons. The majority of *A. solani* isolates, 17 in 2006 and 20 in 2008, respectively, were obtained from one potato field located in Weihenstephan, Southern Bavaria. In addition, eight *A. solani* isolates were collected from different Bavarian and ~~German~~ Lower Saxon potato growing areas in 2008 (locations: Atting, Ehetal, Geltolfing, Straßmoos, Thonstetten, Uelzen, see Table 1). The geographical distribution of *A. solani* isolates collected in Germany is shown in Figure 1. Infected leaflets were sampled at random during early blight disease epidemics between July and September. Material was surface-sterilized in 5% NaOCl for 1 min and then washed in sterile, distilled water. Leaf pieces bearing a single lesion (0.5 to 1.0 cm in diameter) were cut from infected leaves. Necrotic leaf cuts were transferred to petri dishes containing synthetic low nutrient (SN) media (1 g KH_2PO_4; 1 g KNO_3; 0.5 g $MgSO_4x7H_2O$; 0.5 g KCl; 0.2 g Glucose; 0.2 g Saccharose; 0.6 ml 1 n NaOH; 20 g agar; dissolved in 1 l double distilled water). Agar plates were incubated for 3 days at 20°C under near ultraviolet light (Philips LTD 36W/80) with an alternating 12 h photoperiod. SN media favoured the production of mainly single-borne conidia. Only one isolate was collected per diseased plant in order to avoid collecting the same genetic individual. Single-spore isolations were carried out with a fine dissecting needle. Spores were streaked again on SN media and allowed to grow at 23°C. *Alternaria solani* cultures were first identified on the basis of morphological characteristics and spore size (Simmons, 2007).

PCR-based genetic discrimination of *Alternaria solani* isolates

In order to verify the identity of the sample isolates, PCR –based genetic discrimination was used. DNA of fungal strains was isolated from mycelia grown on malt extract as previously described (Bahnweg et al., 2005). As this method is widely used and standardized, DNA

extraction has been carried out only once. In addition to *A. solani,* the fungus *A. alternata* can also contribute to EB disease in potatoes, and so clear differentiation of isolates was necessary. Specific primers were developed with high sensitivity for the detection and identification of *Alternaria* species in order to clearly differentiate between species. *Alternaria* isolates were verified by the use of specific primer pairs, derived from internal transcribed spacer (ITS) sequences of *A. solani* CBS 105.51 (Centraalbureau voor Schimmelcultures, Utrecht, The Netherland), and *A. alternata* DSM 62006 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). They were cross-checked against a DNA library of about 90 fungal pathogens of potato and tomato. Neither *A. solani* nor *A. alternata* primers yielded PCR amplicons with DNA from 90 fungal strains (Bahnweg, not published). *A. solani* F (5´ to 3` CAC CAC AAG GAC CAA CCC A) and *A. solani* R (5´ to 3` TGG GGC TGG AAG AGA GCG), derived from base-sequences of the ITS of ribosomal RNA (rRNA) genes, were used to generate amplicons of 355 bp. The DNA fragment (443 bp) of *A. alternata* was amplified by the primer pair *A. alternata* F (5` to 3` GCG GGC TGG AAC CTC TC) and *A. alternata* R (5` to 3` AGA CCT TTG CTG ATA GAG AAG T). The amplified PCR products were loaded onto a 2% agarose gel, stained with ethidium bromide, and the bands were visualized by UV light.

RADPD analysis

Random 10 bp OP (Operon Technologies, Alameda, California) oligonucleotide primers were used to produce amplified DNA fragments (RAPD technique, Weir et al., 1998). The highly polymorphic nature of RAPD markers makes them particularly useful for the differentiation of clonal lineages of fungi that reproduce asexually. Preliminary tests were performed with five complete sets of OP random primers, OPA 1–20, OPC 1–20, OPD 1–20, OPE 1–20, and OPF 1–20. Most of these yielded either no amplicons or just one in all isolates and were thus of no diagnostic value. Random primers yielding amplicon patterns discriminating between isolates or groups of isolates were: OPC 5, OPC 6, OPC 9, OPC 10, OPC 11, OPC 14, OPC19, OPD 1, OPD 2 and OPD 11 (Table 2). The polymerase chain reaction was carried out in a 25 µl reaction volume containing approximately 25 ng of fungal DNA, 400 nM 10 bp primer, 1.5 mM $MgCl_2$, and 3.5 U Goldstar DNA polymerase (Eurogentech, Seraing, Belgium). DNA was amplified in a PTC-200 thermal cycler (MJ Research, Watertown, USA) with the following programme: 94°C for 3 min, 40°C for 1 min, 72°C for 2 min, followed by 35 cycles of 94°C for 1 min, 40°C for 45 s (annealing under highly stringent conditions, therefore repetitions of the RAPD assay were not necessary), 72°C for 2 min, followed by a final elongation step at 72°C for 5 min, and then cooled down to 8°C. Amplicons were separated on a 2–% agarose gel in TBE buffer (0.45 M TRIS-borate, 1 mM EDTA, pH 8.0). Gels were run at 2 V cm^{-1} for 4 h, stained with ethidium bromide and photographed on a UV light box. Amplicon size was calculated from a standard curve derived from DNA standards on each

156 gel [100 bp ladder GeneRuler™, Fermentas) and DNA Molecular Weight Marker IV (0.07-
157 19.3 kbp, Roche Diagnostics)].
158

RAPD data analysis and cluster analysis

Only bands that could be clearly distinguished were scored according to their presence (value = 1) or absence (value = 0). Similarity coefficients between all pairs of isolates were calculated by the Sokal & Michener coefficient: $sim_{x/y} = (a+d)/(a+b+c+d)$, where x and y are any two objects (two different isolates), a denotes the number of DNA bands present in both objects, d denotes the number absent in both, b denotes the number present in one but not in the other, and c the other way around. Data of all pairwise comparisons of isolates were collected in a two-way similarity matrix (not shown), which was then used to perform an average linkage cluster analysis (Sokal ~~and~~ & Michener, 1958; Chong-Hui et al., 2004). A binary matrix was generated to examine the distribution patterns of genetically distinct isolates. Branch support of the tree was assessed by bootstrapping using 100,000 replications (Shimodaira, 2002).

Results

DNA polymorphism-based discrimination of *Alternaria solani* isolates

A PCR-based method for the purpose of identification of *A. solani* was used to verify the identity of all isolates used in this study. No amplicons were observed when *A. alternata*-specific primers were applied to the *A. solani* DNA preparations (Fig. 2 A and B, upper gel rows) and vice versa. All isolates examined, which were primarily identified on the basis of morphological characteristics could be validated through PCR fingerprinting as *A. solani*. Thus, this PCR-based method allowed an easy and unambiguous identification of *A. solani* isolates. To further discriminate *A. solani* isolates, a RAPD analysis was performed. Ten RAPD primers were chosen from a preliminary screening of primers, based on their reproducible capacity to reveal polymorphisms between individuals. These primers were used to elucidate the DNA polymorphism-based diversity within 45 isolates of *A. solani*. A total of 90 different amplicons were generated by the 10 different RAPD primers (data not shown). Examples of differential band patterns are given for two RAPD primers on 28 out of the 45 *A. solani* isolates (Figs 3 & 4).

RAPD data analysis

A cluster analysis was performed in order to analyze the similarity between and among field isolates from different locations. Cluster analysis of all RAPD profiles revealed a pronounced diversity (Figure 5). For example, isolates 1 to 20, which were obtained from an identical geographic site (2008), formed different sub groups showing only distant similarity. Similarity of isolates ranged between 75 to 95%, indicating a high degree of heterogeneity. Similarly, isolates from the year 2006 showed high diversity in RAPD profiles. However, isolates from geographically distant fields were less divergent as compared to isolates sampled from the same location in 2008, in particular isolates 23, 24, 27 and 28, which were collected from

different fields far away from each other, showed only low RAPD polymorphism-based distances. Here, variability within non-local isolates (except isolate 25) was comparable or even less to isolates with identical geographic origin. Because of the high diversity of isolates with identical origin, a clustering according to geographic origin was not apparent.

In addition, differences between two sampling years were detected. Isolates collected in 2006 were clearly separated from isolates collected in 2008 (Figure 5). Isolates from the same site collected in 2006 and 2008 formed different sub clusters, showing a degree of diversity among *A. solani* populations. Similarity between isolates originating in 2006 and 2008 was 60%. According to these results, RAPD analysis revealed distinct genetic diversity among *A. solani* isolates, not only from different locations, but also from samples in the same field.

Discussion

The present study is the first to investigate DNA polymorphism-based diversity of *A. solani* isolates from potato plants in Germany. Analysis of RAPD profiles of 45 isolates, revealed pronounced variability. We found large differences among RAPD profiles of *A. solani* when comparing isolates derived from one particular geographic origin as well as from different years. Isolates which originated from the same field, showed high levels of heterogeneity. Genotyping using RAPD-PCR has previously been applied to *A. alternata* (Morris et al., 2000; Roberts et al., 2000) and to other fungi in order to investigate the DNA polymorphism-based relationships between populations of different geographic areas (Mclean et al., 1995; Guthrie et al., 1992).

Studies in India in tomato showed that even *A. solani* isolates from the same lesions can be genetically distinct, indicating the variable nature of this pathogen (Kumar *et al.*, 2008). Other authors (Lourenço et al., 2009; Martínez et al., 2004) described *A. solani* populations as highly variable by assessing genetic variation by RAPD, random amplified microsatellites and amplified length polymorphism (AFLP) markers. Petrunak ~~and~~ & Christ (1992) assumed that the high diversity among isolates might occur through the incidence of casual recombination, whether asexual or sexual. Nevertheless, reliable estimates of parameters related to recombination have not yet been obtained for *A. solani*.

Another cause for genetic variation may be natural mutation or large population size (Weir et al., 1998). There is general agreement that mutation is the main evolutionary mechanism that generates polymorphisms and its implications to disease management are noticeable (Alexander et al., 1993; Jolley et al., 2005). Leung ~~and~~ & Williams (1986) stated that mutations of the fungus *Magnaporthe grisea*, the causal agent of rice blast, produced significant isozyme variation, if isozymes are indeed neutral with respect to fitness. Former reports (Stall, 1958) discussed heterokaryosis as a driving force for variability among *A. solani* isolates. However, no evidence was found. Within *A. dauci*, genetic diversity was attributed to a high mutation rate, selection and genetic drift rather than recombination (Rogers, 2007). Lourenço et al. (2009) assumed that the population of *A. solani* was clonal, thus gene flow and mutation would be the main evolutionary processes shaping its genetic structure. According to Weber ~~and~~ & Halterman (2012) natural mutations may occur more commonly in asexually reproducing isolates compared to sexually reproducing isolates, which would support the hypothesis that a large number of mutations should be occurring within *A. solani,* thereby lead to a relatively high level of diversity.

This high level of variation in *A. solani* detected by RAPD fragment pattern comparison - in particular between two different years - is indicative of the presence of a genetically highly diverse population of this pathogenic fungus. Particular RAPD profiles have also been described by Weber ~~and~~ & Halterman (2012). According to them, this might be an indication that *A. solani* populations were constantly shifting, and selection for certain

genotypes was prevalent. Fungi are consistently exposed to hostile conditions, in which adverse factors are continuously changing their environment (Rotem, 1994). Provided that it is in balance with fungal fitness, a mutation phenotype may act as a driving force facilitating strain evolution.

Accordingly, environmental conditions and agricultural practices, which may vary considerably from year to year, would thereby support selection of particular, more or less adapted strains which are able to predominate a given growth period. Genetic diversity would therefore be essential for the adaptation of *A. solani* to different environmental conditions. Weir et al. (1998) assumed that high heterogeneity would contribute to pathogen specialization. Genome variability could therefore offer a selective advantage (van der Waals et al., 2004), enabling the adaptation to stress conditions exerted by the environment (e.g. host defence mechanisms). This selection of mutants, recombinants, or even immigrants leads to the evolution of pathogen populations which will then be optimally adapted to local environments (McDonald ~~and~~ & Linde, 2002).

Interestingly, some of the investigated isolates, which were sampled from widely separate regions (widest distance 650 km), displayed little genetic variation (isolates 23, 24, 27 and 28), indicating similar, if not identical, genotypes. These results indicate that the various geographical subpopulations are not genetically isolated. Previous studies on *A. solani* populations from the United States (Petrunak ~~and~~ & Christ, 1992) also did not support a geographic clustering of isolates. Brazilian investigations on *A. solani* from potato and tomato fields revealed that isolates from geographically distant fields were not more genetically divergent than isolates collected from one single field (Lourenço et al., 2009). This Brazilian populations of *A. solani* comprise haplotypes which are widely distributed throughout the main growing areas of potato and tomato. According to Martínez et al. (2004) and van der Waals et al. (2004), isolates from the same country are not distinctly separated by geographical origin due to the ability of *A. solani* spores to travel short or long distances. Lourenço et al. (2009) pointed out that few haplotypes are widely distributed. Our present results support these observations. Aerial dispersal and widespread distribution of host crops are likely to be the major factors that lead to intense inoculum movement and the lack of geographic subdivision regarding inferred haplotypes. *Alternaria solani* is capable of producing large numbers of spores in a short period of time (Leiminger, 2009) which can be dispersed over long distances. These large population sizes make it more likely that new mutants with higher fitness will emerge and be able to multiply within the infected host.

Knowledge about changes in pathogen population and its genetic structure gives insight into the evolutionary processes that shape a population. Palumbi (2001) pointed out that this knowledge may prove useful to optimize the management of resistance genes or fungicides to maximize their useful life expectancy and to minimize the losses that result from reduced efficacy of these control methods. According to McDonald ~~and~~ & Linde (2002),

pathogen populations with a high degree of gene flow and high genetic diversity, such as shown for *A. solani*, possess a greater risk of overcoming genetic resistance of the host. Van der Waals et al. (2004) inferred that this might be an explanation for the absence of varieties with complete resistance to early blight. Point mutations in the sequence of the *cytb* gene of *A. solani* resulted in loss of sensitivity to the fungicide Azoxystrobin (Pasche et al., 2005). New alleles introduced into the population might also increase the chances of cracking resistance genes in either potato or tomato cultivars. Mutation rates could potentially increase genomic variability and might therefore be a selective advantage to the strains possessing them, under certain stressful conditions (van der Waals et al., 2004).

Enhanced investigations about population diversity help to determine the genetic structure of pathogen populations and to better understand the evolutionary forces that shaped these populations. This knowledge of genetic structure offers insights into the future evolutionary potential of pathogen populations. Our results indicate the presence of surprising genetic heterogeneity within populations of *A. solani* originating from narrow geographic areas which must be kept in mind when designing protective measures for agriculture. A better understanding of the genetic diversity of *A. solani* on potato in Germany will thus help to improve control strategies against early blight.

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Figure Legends

Figure 1: Map of Germany showing locations for the isolation of *Alternaria solani*.

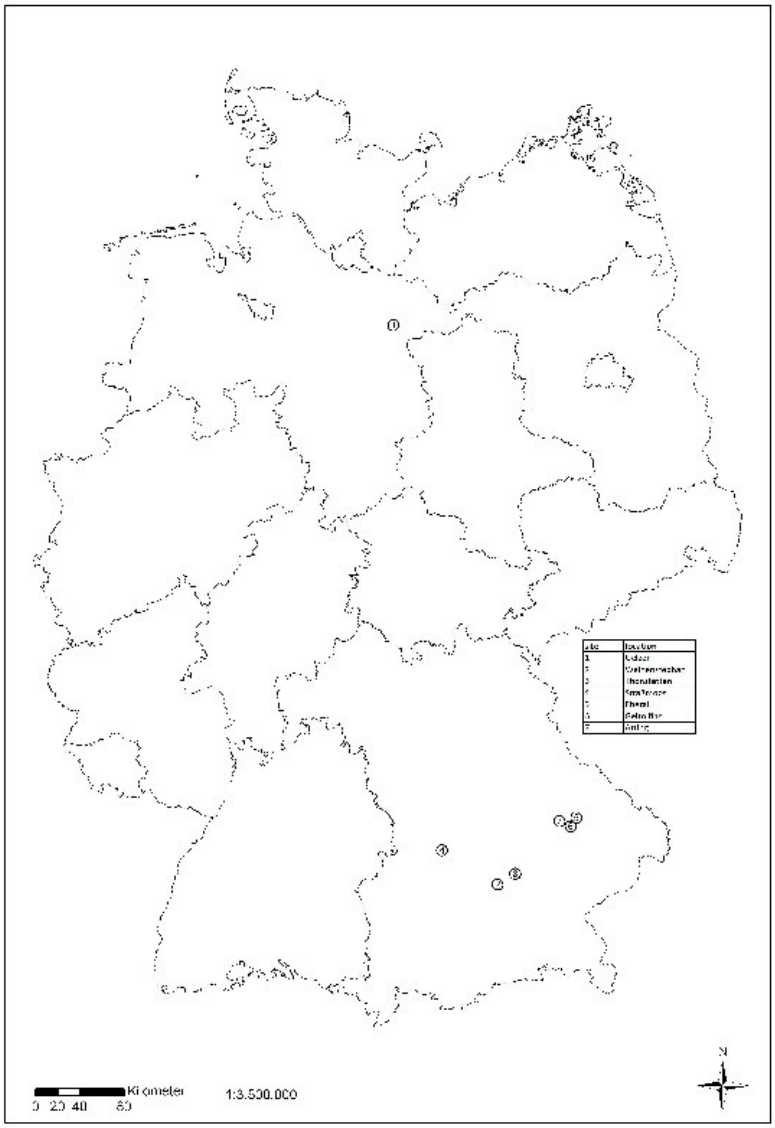
Figure 2: PCR analysis to differentiate *Alternaria* isolates. A) genetic discrimination of *Alternaria solani* isolates collected in 2006 and B) collected in 2008.

Figure 3: Characterization of *A. solani* isolated from infected potato leaves. Different band patterns were obtained by RAPD technique using primer OPD-14. All 28 investigated *A. solani* isolates were collected in 2008. Different band patterns of isolates collected from the same field are shown in lane 1 to 20 and those from different potato fields are in lanes 21 to 28. These band patterns were the basis for further cluster analysis.

Figure 4: Characterization of *A. solani* isolates amplified with RAPD-primer OPD-1. All 28 isolates of *A. solani* were collected in 2008. Different band patterns of isolates collected from the same field are shown in lane 1 to 20 and those from different potato fields are in lanes 21 to 28. Similarities between all pairs of isolates on the basis of any RAPD patterns were further analyzed. The resulting similarity matrix was then the basis for a cluster analysis.

Figure 5: Dendrogram of average linkage cluster analysis based on random amplified polymorphic DNA (RAPD) profiles showing the genetic relationship among 45 isolates of *A. solani*. Several distinct clusters of isolates can be seen, and in addition, there are also a number of only distantly related similar strains. 1a–18a: *A. solani* isolates collected in 2006, 1–28: *A. solani* isolates collected in 2008. *Alternaria solani* groups isolated from one location in 2006 and 2008 form different sub clusters showing high diversity within different years. Less variation was observed for isolates of foreign origin (23, 24, 26, 27, 28) showing only low genetic distances.

445 Figure 1



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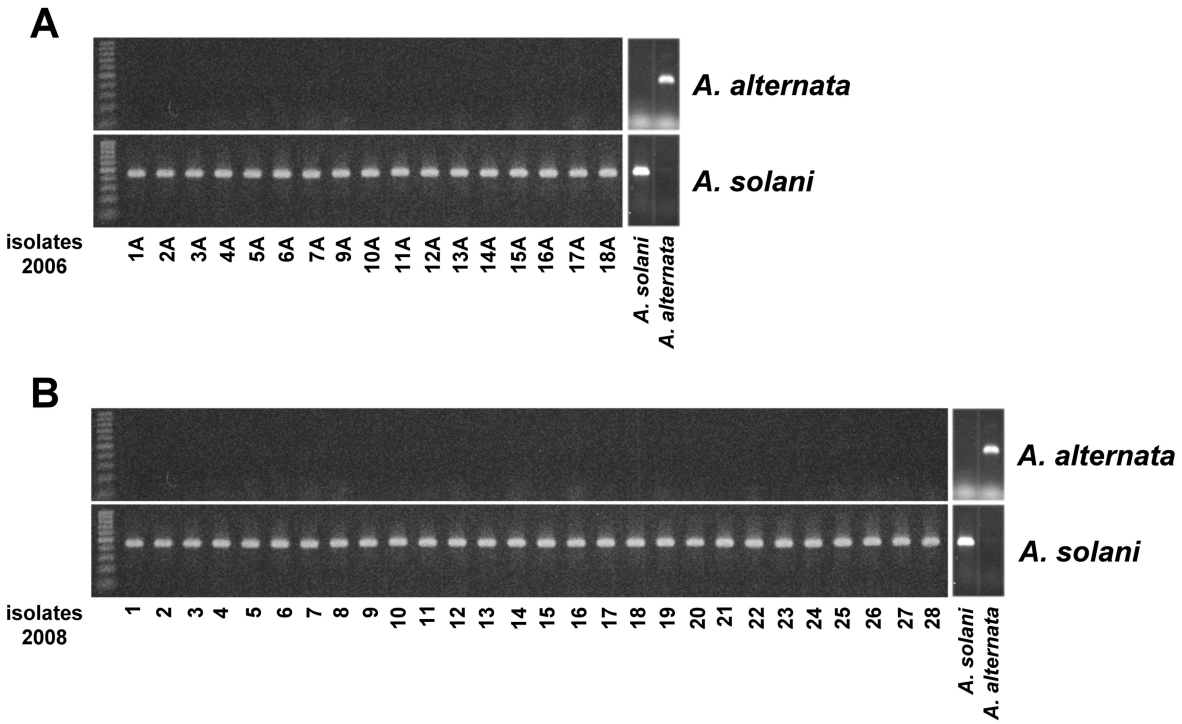
447 Figure 2



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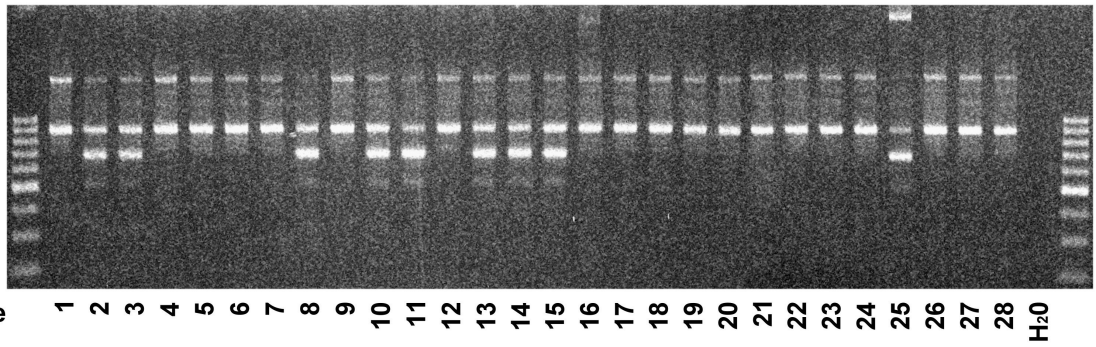
449 Figure 3



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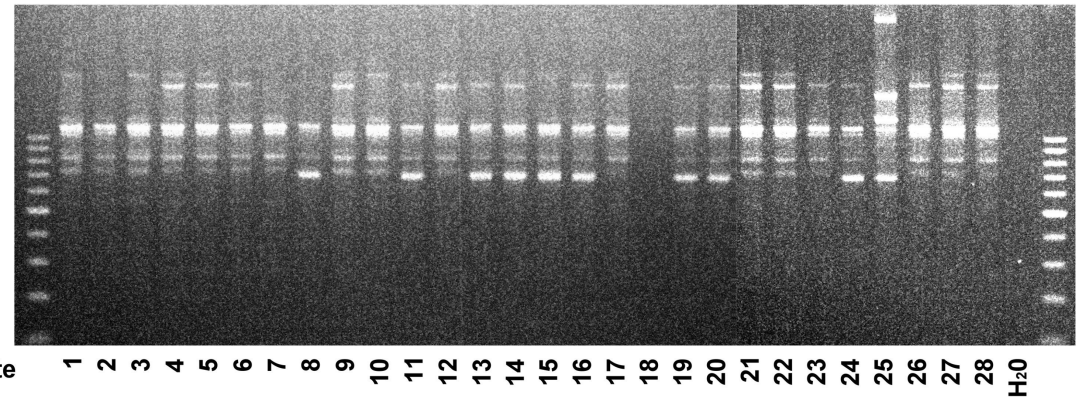
453 Figure 4



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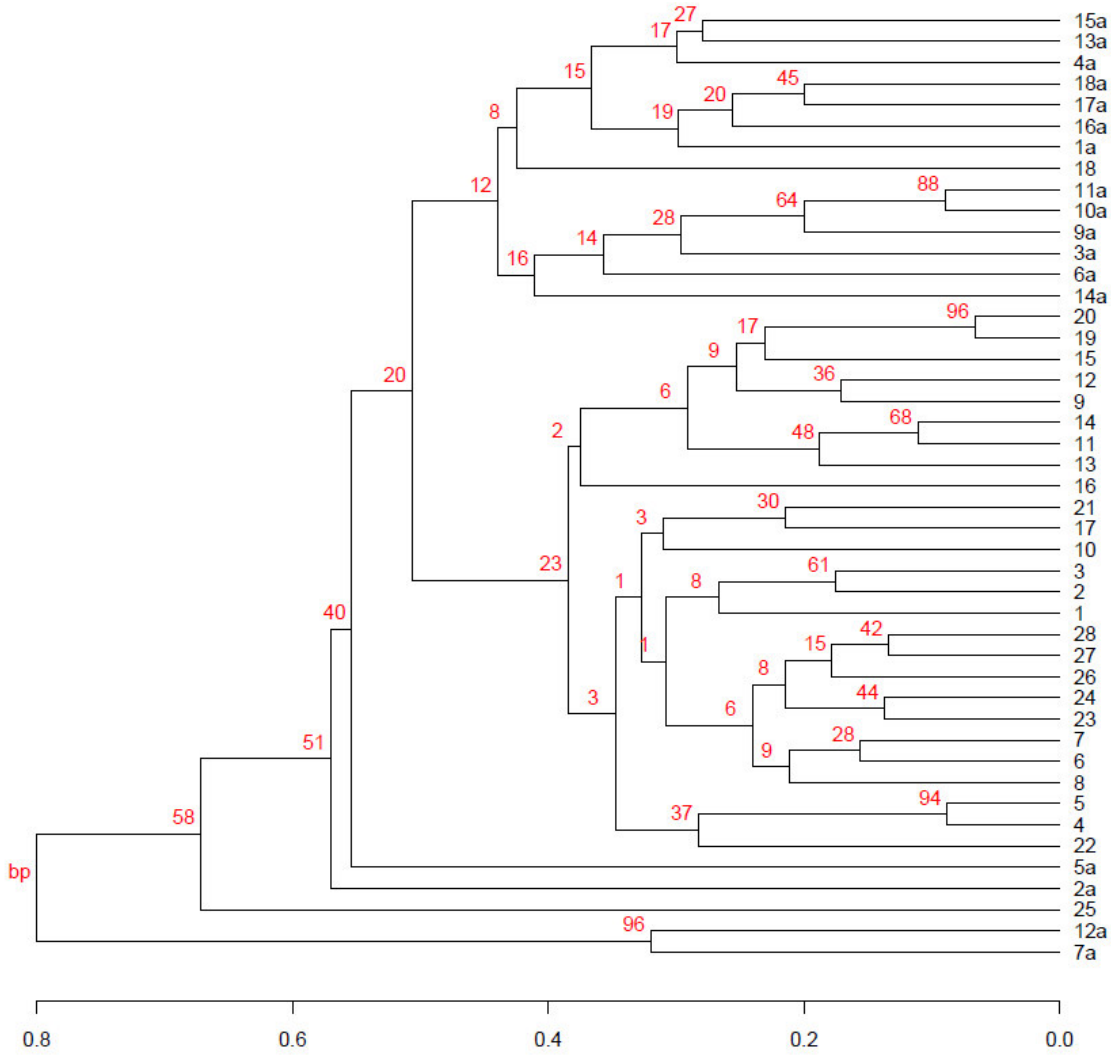
455 Figure 5



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457 Tables:

458 Table 1. Year of isolation and geographic origins of the *Alternaria solani* isolates, from early
459 blight infected potato *(Solanum tuberosum)* in Germany
460

Isolate code	Date of isolation	Isolated species	Host	Cultivar	Geographic origin
1a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
2a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
3a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
4a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
5a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
6a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
7a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
9a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
10a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
11a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
12a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
13a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
14a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
15a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
16a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
17a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
18a	Sep 2006	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
1	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
2	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
3	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
4	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
5	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
6	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
7	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
8	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
9	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
10	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
11	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
12	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
13	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
14	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
15	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
16	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
17	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
18	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
19	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
20	Sep 2008	*A. solani*	potato	Kuras	Weihenstephan, FS[1]
21	Jul 2008	*A. solani*	potato	Quarta	Straßmoos, ND[2]
22	Jul 2008	*A. solani*	potato	-	Thonstetten, FS[1]
23	Jul 2008	*A. solani*	potato	-	Thonstetten, FS[1]
24	Sep 2008	*A. solani*	potato	-	Ehetal, SR[3]
25	Sep 2008	*A. solani*	potato	-	Atting, SR[3]
26	Jul 2008	*A. solani*	potato	Lolita	Uelzen, UE[4]
27	Jul 2008	*A. solani*	potato	Kuras	Uelzen, UE[4]
28	Sep 2008	*A. solani*	potato	-	Geltolfing, SR[3]

461 FS[1] Freising, Upper Bavaria
462 ND[2] Neuburg-Schrobenhausen, Upper Bavaria
463 SR[3] Straubing, Lower Bavaria
464 UE[4] Uelzen, Lower Saxony
465
466
467 Table 2. RAPD primers used to identify and assess intraspecific genetic diversity among *A.*
468 *solani* isolates.
469

primer	sequence (5´to 3´)
OPC-5	GATGACCGCC
OPC-6	GAACGGACTC
OPC-9	CTCACCGCTC
OPC-10	TGTCTGGGTG
OPC-11	AAAGCTGCGG
OPC-14	TGCGTGCTTG
OPC-19	GTTGCCAGCC
OPD-1	ACCGCGAACC
OPD-2	GGACCCAACC
OPD-11	AGCGCCATTG

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