

Development of a PCR assay for amplification of mating-type loci of *Alternaria* spp. and related fungi

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A large number of ascomycete anamorphs including *Alternaria* spp. are considered asexual, but carry structurally conserved genes in a mating-type locus (*MATI*) with two alternate idiomorphs, *MATI-1* and *MATI-2*. Since the locus has lost its main function in many species, different directions of natural selection acting on the idiomorphs can be observed. Thus identification of mating types can be used in population biology studies. Also analysis of *MATI* locus sequences is needed for evolution and phylogeny reconstruction. Eighteen primers from previous works and this study were assessed for their ability to amplify *MATI* in 25 *Alternaria* and 7 *Ulocladium*, *Embellisia* and *Alternariaster* species. The most suitable primer pairs for 6 sections of *Alternaria* and several *Ulocladium* species were revealed.

Key words: population genetics, recombination, *Ulocladium*.

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Mnohé anamorfy askomycetů včetně druhů rodu *Alternaria* jsou považovány za asexuální, ale nesou strukturně konzervované geny v lokusu párovacích typů (*MATI*), a sice ve dvou alternativních idiomorfách, *MATI-1* and *MATI-2*. Jelikož tento lokus ztratil u mnoha druhů svou hlavní funkci, můžeme pozorovat rozdílný směr přirozené selekce těchto idiomorf. V důsledku toho může být identifikace párovacího typu použita v populačně-biologických studiích. Analýza sekvencí lokusu *MATI* je také potřebná pro evoluční a fylogenetické rekonstrukce. Osmnáct primerů použitých v předchozích pracích a naší studii bylo hodnoceno z hlediska schopnosti amplifikovat *MATI* u 25 druhů rodu *Alternaria* a 7 druhů rodů *Ulocladium*, *Embellisia* a *Alternariaster*. Byly zjištěny vhodné páry primerů u 6 sekcí rodu *Alternaria* a několika druhů rodu *Ulocladium*.

INTRODUCTION

Alternaria is a fungal genus including approximately 280 species of haploid ascomycetous anamorphs. Many *Alternaria* species are causal agents of plant diseases, toxigenic contaminants of food and feed, and common allergens. A number of species are causes of harmful diseases with worldwide distribution. While

several *Alternaria* species have a sexual stage belonging to the genus *Lewia* (Simmons 1986), most *Alternaria* species are considered to be mitosporic.

Many studied filamentous ascomycetes have structurally conserved specific “sexual” genes in their genomes. The mating type in this group of fungi is determined by genes found at a single *MAT1* locus, which contains either a *MAT1-1* or *MAT1-2* idiomorph highly divergent in sequence (Turgeon & Yoder 2000). Mating-type genes normally regulate sexual compatibility and sexual reproduction in fungi. Some asexual Dothideomycetes were proved to have a mating-type locus which is structurally similar to those of heterothallic sexually reproducing relatives. If *MAT* loci have lost their main function, they cannot have been under balancing selection. In that case aberrant ratios of *MAT* idiomorphs in asexual populations can be observed due to genetic drift.

Hypothetically, some species derived from a single asexual clone must have only one *MAT1* idiomorph. Another reason of the presence of a single *MAT1* idiomorph in a population is the occupation of a new place by one clone, which is not a hypothetical example. Mating type A2 of a very common phytopathogenic Oomycete, *Phytophthora infestans*, was not known until 1958. In Europe mating type A1 was dominant until the 1980s. The first report of occurrence of mating type A2 came from Switzerland (Hohl & Iselin 1984). Thus mating-type loci are the tool to study population biology, mating and evolution of fungi. Availability of mating-type sequences allows for an investigation of key issues concerning genetic and phylogenetic analyses of fungal species (Pöggeler 2001).

Mating-type loci have been used in recent studies of the population biology of several ascomycetous fungi, e.g. *Mycosphaerella graminicola* (Zhan et al. 2002), *Aspergillus fumigatus* (Paoletti et al. 2005), and *Leptosphaeria maculans* (Hayden & Howlett 2005). The *MAT1* locus has been studied in a few *Alternaria* species, thus some primers for its amplification have already been designed. Arie et al. (2000) have identified the structure of *A. alternata* mating-type loci. Berbee et al. (2003) have used mating-type loci together with ITS regions of rDNA of *A. tenuissima*, *A. brassicicola* and *A. brassicae* to study the recombination history of these species. Stewart et al. (2011) have investigated the purifying selection and codon usage at the *MAT1* locus in *Alternaria* spp. (the same species as in previous works, *A. solani*, and 3 species closely related to *A. alternata*).

For frequency analysis as well as for sequencing, mating-type loci have to be amplified first. The goal of this work is to choose PCR primers for reliable amplification of the mating-type locus *MAT1* of species from different phylogroups of *Alternaria* and closely related mitosporic ascomycetes.

MATERIAL AND METHODS

All 20 primers published by Arie et al. (2000) and Berbee et al. (2003) for *Alternaria* were checked with BLAST (Altschul et al. 1990) for universality in 4 *Alternaria* species with a completely sequenced *MAT1* locus. After analysis the 12 most universal primers that allow amplifying targets approximately 100–1000 bp long in different parts of the *MAT1-1* and *MAT1-2* regions were chosen for subsequent study. Three primers, BPHO4, BPHO4, and MCHMG1, were modified by removal of 3 or 4 nucleotides from the 3' end. To design novel primers, all available sequences of *Alternaria MAT1* were downloaded from GenBank and conservative regions were then analysed. Primers were designed using the Primer3Plus online software (Untergasser et al. 2007). Sequences of all primers used in this study are listed in Tab. 1.

Twelve pair combinations of primers were assessed on 117 isolates of 25 *Alternaria*, five *Ulocladium*, one *Embellisia*, and one *Alternariaster* species (Tab. 2). *Alternaria* species represented 7 of 8 sections which were established based on the genus phylogeny (Gannibal 2012; Lawrence et al., in press). All isolates are kept in the All-Russian Institute of Plant Protection (St. Petersburg).

Genomic DNA of fungi was isolated from fungal cultures as described by Doyle & Doyle (1987). Identification of the *MAT1* idiomorph was performed by PCR amplification on a C-1000 (Bio-Rad, USA). Each reaction (25 µL) contained 1 U of *Taq* DNA polymerase (Helicon, Russia), 200 µM of each dNTP, 0.5 µM of each primer, 1× reaction buffer (67 mM Tris-HCl, 16.6 mM (NH₄)₂SO₄, 2.5 mM of MgCl₂, 0.1% Tween-20; Helicon, Russia) and 1–10 ng of template DNA. After a 3-min. denaturation step at 94 °C, samples were processed in 30 cycles, consisting of 50 s at 92 °C, 50 s at *Ta* (Tab. 2) and 60 s at 72 °C. A final 10 min. elongation step was performed at 72 °C. PCR products were separated by electrophoresis on agarose gel in a 0.5× TBE buffer using a 100-bp ladder (Fermentas, Lithuania) as size reference, and stained with ethidium bromide.

RESULTS AND DISCUSSION

Five primers to amplify the *MAT1* locus in *Alternaria* were designed during this study. Three primer sets were utilised to amplify *MAT1-1* while one primer was designed to amplify *MAT1-2*. Primer sites are presented in a diagram (Fig. 1).

Different primers amplified mating-type loci of different groups of species with different efficacy. No universal primers were found to amplify the DNA of all isolates of each mating type. Results of the identification of *MAT1* idiomorphs as most efficient primers are presented in Tab. 3.

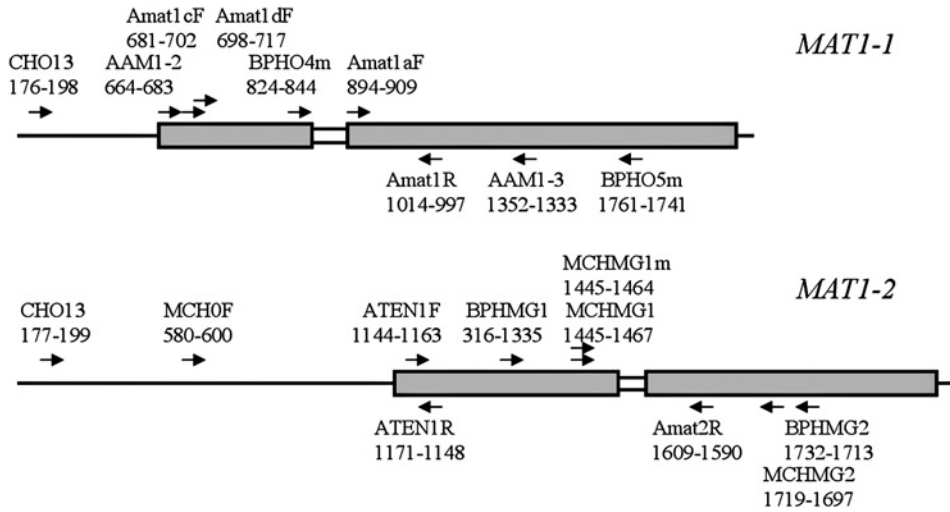


Fig. 1. Diagram of the primers sites and their nucleotide positions, relative to two *Alternaria alternata* mating-type idiomorphs, *MATI-1* (GenBank accession no. AB009451) and *MATI-2* (GenBank accession no. AB009452). Shaded boxes represent coding regions, blank boxes represent introns.

Tab. 1. List of primers used in this study.

Primer name	Sequence (5'-3')	Citation
<i>MATI-1</i>		
AAM1-2	TCC AAA CTC GCA GTG GCA AG	Arie et al., 2000
AAM1-3	GAT TAC TCT TCT CCG CAG TG	Arie et al., 2000
CHO13	ATT GCA GAT TGG AAA GGC CAA GT	Berbee et al., 2003
BPHO4m	TGC CTT TGT TGG ATT TCG GTG	Berbee et al., 2003; modified
BPHO5m	CCG GAG CGG AAA GCA TCG AAG	Berbee et al., 2003; modified
Amat1aF	ACT ACA TCT CAA TCC C	This study
Amat1cF	AAG CAR ATG GTT CAR TTG TTC C	This study
Amat1dF	GTT CCG TTT GAT WMC AGA GC	This study
Amat1R	GAC CAG GCT TTC GYC ATC	This study
<i>MATI-2</i>		
MCH0F	GGC TAT TTC CTT TGT CTC ATA	Berbee et al., 2003
ATEN1F	AGC CCT TCT CAC TTG CAC TG	Berbee et al., 2003
ATEN1R	CCA GCC GTC AGT GCA AGT GAG AAG	Berbee et al., 2003
BPHMG1	CAC ACT CTT GTA AAG ATG CC	Berbee et al., 2003
BPHMG1m	AAG GCT CCT CGA CCG ATG AA	Berbee et al., 2003; modified
BPHMG2	CTC GCC AGG TTT CCT GGG AG	Berbee et al., 2003
MCHMG1	AAG GCT CCT CGA CCG ATG AAC TG	Berbee et al., 2003
MCHMG2	CTG GGR GTR TAC TTG TAG TCR GG	Berbee et al., 2003
Amat2R	GGA GCA GCG TGT AGC TAT GA	This study

Tab. 2. Isolates of alternarioid hyphomycetes studied.

Species	Isolate ID	Substratum/host-plant	Geographic origin	MAT1 idio-morph
<i>Alternaria acalyphae</i>	MF-P111-011	<i>Acalypha australis</i>	Russia, Khabarovskiy kray	1
<i>A. alternata</i>	EGS 34-016 (CBS 916.96)	<i>Arachis hypogaea</i>	India	1
<i>A. arborescens</i>	EGS 39-128 (CBS 102605)	<i>Lycopersicon esculentum</i>	USA, California	1
	MF-P106-021	<i>Lycopersicon esculentum</i>	Russia, Primorskiy kray	2
	MF-P106-031	<i>Lycopersicon esculentum</i>	Russia, Primorskiy kray	2
	MF-P138-011	<i>Lycopersicon esculentum</i>	Russia, Khabarovskiy kray	1
	MF-P138-031	<i>Lycopersicon esculentum</i>	Russia, Khabarovskiy kray	1
	MF-P138-021	<i>Lycopersicon esculentum</i>	Russia, Khabarovskiy kray	2
	MF-P138-051	<i>Lycopersicon esculentum</i>	Russia, Khabarovskiy kray	2
	MF-P141-031	<i>Lycopersicon esculentum</i>	Kirgystan	1
<i>A. avenicola</i>	EGS 50-185 (IMI 387429)	<i>Avena sativa</i>	Norway	1/2
	MF-P037-011	<i>Triticum aestivum</i>	Russia, Orlovskaya oblast	1/2
	MF-P059-031	<i>Hordeum vulgare</i>	Russia, Leningradskaya oblast	1/2
	MF-P067-011	<i>Solanum tuberosum</i>	Russia, Leningradskaya oblast	1/2
	MF-P071-011	<i>Hordeum vulgare</i>	Russia, Leningradskaya oblast	1/2
	MF-P080-021	<i>Triticum aestivum</i>	Russia, Tambovskaya oblast	1/2
	MF-P088-011	<i>Triticum aestivum</i>	Russia, Orlovskaya oblast	1/2
	MF-P089-011	<i>Triticum aestivum</i>	Russia, Sverdlovskaya oblast	1/2
	MF-P094-011	<i>Triticum aestivum</i>	Russia, Leningradskaya oblast	1/2
	MF-P457-071	<i>Avena sativa</i>	Russia, Leningradskaya oblast	1/2
<i>A. brassicae</i>	MF-P152-011	<i>Brassica napus</i>	Belarus	2
	MF-P165-011	<i>Armoracia rusticana</i>	Russia, Republic of Adygeya	2
	MF-P203-021	<i>Brassica oleracea</i>	Russia, Leningradskaya oblast	2
	MF-P265-011	<i>Brassica napus</i>	Russia, Permskiy kray	1
<i>A. brassicicola</i>	MF-P055-011	<i>Brassica oleracea</i>	Russia, Primorskiy kray	2
	MF-P156-011	<i>Brassica oleracea</i>	Russia, Republic of Adygeya	1
	MF-P156-021	<i>Brassica oleracea</i>	Russia, Republic of Adygeya	1
	MF-P216-011	<i>Brassica oleracea</i>	Russia, Khabarovskiy kray	1
	MF-P270-041	<i>Brassica oleracea</i>	Ukraine	2
<i>A. dauci</i>	EGS 46-152 (CBS 109156)	<i>Daucus carota</i>	New Zealand	2
	MF-P182-011	<i>Daucus sativus</i>	Russia, Moskovskaya oblast	1
	MF-P182-021	<i>Daucus sativus</i>	Russia, Moskovskaya oblast	1
	MF-P187-011	<i>Daucus sativus</i>	Russia, Moskovskaya oblast	1
	MF-P190-011	<i>Daucus sativus</i>	Belarus	2
	MF-P190-021	<i>Daucus sativus</i>	Belarus	1
	MF-P191-011	<i>Daucus sativus</i>	Belarus	1
	MF-P214-011	<i>Daucus sativus</i>	Russia, Primorskiy kray	2
	MF-P291-011	<i>Daucus sativus</i>	Russia, Permskiy kray	2
<i>A. eryngii</i>	EGS 41-005	<i>Eryngium</i> sp.	New Zealand, Auckland	2
<i>A. helianthiinficiens</i>	MF-P061-021	<i>Helianthus annuus</i>	Russia, Krasnodarskiy kray	1
<i>A. infectoria</i>	EGS 27-193 (CBS 210.86)	<i>Triticum</i> sp.	UK, Suffolk	?

Species	Isolate ID	Substratum/host-plant	Geographic origin	MAT1 idio-morph
<i>A. japonica</i>	MF-P181-011	<i>Raphanus sativus</i>	Russia, Moskovskaya oblast	1
	MF-P239-011	<i>Raphanus sativus</i>	Russia	1
	MF-P245-031	<i>Raphanus sativus</i>	Russia, Republic of Dagestan	1
	MF-P247-011	<i>Raphanus sativus</i>	Russia, Republic of Dagestan	2
	MF-P247-051	<i>Raphanus sativus</i>	Russia, Republic of Dagestan	2
MF-P253-011	<i>Raphanus sativus</i>	Russia, Republic of Dagestan	2	
<i>A. mali</i>	EGS 38-029 (CBS 106.24, ATCC 13963)	<i>Malus domestica</i>	USA	2
<i>A. metachromatica</i>	EGS 38-132 (CBS 553.94, IMI 295682)	<i>Triticum aestivum</i>	Australia	?
<i>A. oregonensis</i>	EGS 29-194 (CBS 542.94)	<i>Triticum aestivum</i>	USA, Oregon	?
<i>A. photistica</i>	EGS 35-174	<i>Digitalis purpurea</i>	UK	1/2
	MF-P036-011	<i>Hordeum</i> sp.	Russia, Leningradskaya oblast	1/2
<i>A. saponariae</i>	MF-P107-011	<i>Saponaria officinalis</i>	Russia, Lipetskaya oblast	2
	MF-P144-011	<i>Gypsophila</i> sp.	Russia, Primorskiy kray	2
	MF-P201-011	<i>Caryophyllaceae</i>	Russia, Irkutskaya oblast	2
	MF-P294-011	<i>Saponaria officinalis</i>	Russia, Permskiy kray	1
<i>A. silybi</i>	MF-P050-011 (VKM F-4109)	<i>Silybum marianum</i>	Russia, Primorskiy kray	1
<i>A. simmonsii</i>	MF-P024-011 (VKM F-4110)	<i>Sonchus</i> sp.	Russia, Voronezhskaya oblast	1
<i>A. solani</i>	EGS 44-098 (CBS 109157)	<i>Solanum tuberosum</i>	USA, Washington	1
	MF-P045-011	<i>Solanum tuberosum</i>	Russia, Primorskiy kray	1
	MF-P048-011	<i>Solanum tuberosum</i>	Russia, Khabarovskiy kray	1
	MF-P228-011	<i>Solanum tuberosum</i>	Russia, Moskovskaya oblast	1
	MF-P535-011	<i>Lycopersicon esculentum</i>	Russia, Leningradskaya oblast	2
	MF-P580-181	<i>Lycopersicon esculentum</i>	Russia, Irkutskaya oblast	2
	MF-P580-191	<i>Lycopersicon esculentum</i>	Russia, Irkutskaya oblast	2
	MF-P580-211	<i>Lycopersicon esculentum</i>	Russia, Irkutskaya oblast	2
	MF-P583-011	<i>Lycopersicon esculentum</i>	Russia, Irkutskaya oblast	2
	MF-P583-031	<i>Lycopersicon esculentum</i>	Russia, Republic of Adygeya	1
	MF-P601-011	<i>Solanum tuberosum</i>	Russia, Omskaya oblast	1
	MF-P601-021	<i>Solanum tuberosum</i>	Russia, Omskaya oblast	1
	MF-P649-011	<i>Lycopersicon esculentum</i>	Russia, Republic of Adygeya	2
	MF-P752-011	<i>Solanum tuberosum</i>	Russia, Pskovskaya oblast	1
	MF-P754-121	<i>Lycopersicon esculentum</i>	Russia, Kamchatskiy kray	2
<i>A. sonchi</i>	MF-P005-021	<i>Sonchus</i> sp.	Italy	1
	MF-P007-011	<i>Sonchus oleraceus</i>	Russia, Leningradskaya oblast	1
	MF-P031-020	<i>Sonchus</i> sp.	Russia, Krasnodarskiy kray	1
	MF-P031-030	<i>Sonchus</i> sp.	Russia, Krasnodarskiy kray	1
	MF-P032-010	<i>Sonchus</i> sp.	Russia, Republic of Adygeya	2
	MF-P032-020	<i>Sonchus</i> sp.	Russia, Republic of Adygeya	2
	MF-P033-010	<i>Sonchus</i> sp.	Italy	1
	MF-P034-010	<i>Sonchus</i> sp.	Italy	1
	MF-P035-021	<i>Sonchus</i> sp.	Russia, Vologodskaya oblast	2

Species	Isolate ID	Substratum/host-plant	Geographic origin	MAT1 idiomorph
	MF-P035-031	<i>Sonchus sp.</i>	Russia, Vologodskaya oblast	2
	MF-P284-011	<i>Sonchus sp.</i>	Russia, Republic of Dagestan	2
	MF-P286-011	<i>Sonchus sp.</i>	Russia, Irkutskaya oblast	1
	MF-P287-011	<i>Sonchus sp.</i>	Russia, Irkutskaya oblast	1
<i>A. tenuissima</i>	EGS 34-015 (IMI 255532)	<i>Dianthus sp.</i>	UK	2
	MF-P138-041	<i>Lycopersicon esculentum</i>	Russia, Khabarovskiy kray	1
	MF-P478-031	<i>Triticum aestivum</i>	Russia, Krasnodarskiy kray	2
	MF-P480-011	<i>Triticum aestivum</i>	Russia, Primorskiy kray	1
<i>A. triticimaculans</i>	EGS 41-050 (CBS 578.94)	<i>Triticum aestivum</i>	Argentina	?
<i>A. triticina</i>	EGS 17-061 (ATCC 36205)	<i>Triticum aestivum</i>	India, New Delhi	?
<i>A. zinniae</i>	MF-P139-011	<i>Zinnia elegans</i>	Russia, Tomskaya oblast	2
	MF-P212-011	<i>Zinnia elegans</i>	Russia, Republic of Adygeya	1
	MF-P213-011	<i>Zinnia elegans</i>	Belarus	1
<i>Alternaria sp.</i>	MF-P122-011	<i>Dianthus barbatus</i>	Russia, Kamchatskiy kray	1
	MF-P122-021	<i>Dianthus barbatus</i>	Russia, Kamchatskiy kray	1
<i>Alternariaster helianthi</i>	MF-P049-011	<i>Helianthus annuus</i>	Russia, Primorskiy kray	?
<i>A. helianthi</i>	MF-P135-011	<i>Helianthus annuus</i>	Russia, Belgorodskaya oblast	?
<i>A. helianthi</i>	MF-P204-011	<i>Helianthus annuus</i>	Russia, Republic of Adygeya	?
<i>Embellisa chlamydospora</i>	MF-P427-011	<i>Triticum aestivum</i>	Russia, Krasnodarskiy kray	1?
<i>Ulocladium alternariae</i>	MF-P529-051	<i>Triticum aestivum</i>	Russia, Republic of North Ossetia	1
<i>U. atrum</i>	MF-P047-011	<i>Solanum tuberosum</i>	Russia, Leningradskaya oblast	2
	MF-P171-021	<i>Brassica oleracea</i>	Russia, Republic of Buryatiya	2
	MF-P503-051	<i>Triticum aestivum</i>	Russia, Leningradskaya oblast	2
<i>U. botrytis</i>	MF-P057-011	<i>Solanum tuberosum</i>	Russia, Leningradskaya oblast	2
	MF-P363-332	<i>Hordeum distichon</i>	Russia, Leningradskaya oblast	2
	MF-P388-021	<i>Bromopsis inermis</i>	Russia, Omskaya oblast	1
	MF-P425-011	<i>Cucumis sativus</i>	Russia, Leningradskaya oblast	1
<i>U. chartarum</i>	MF-P143-011	<i>Ascophyllum nodosum</i>	Russia, Murmanskaya oblast	1
	MF-P242-011	<i>Brassica oleracea</i>	Russia, ?	1
	MF-P262-011	<i>Daucus sativus</i>	Russia, ?	1
	MF-P443-021	wall paper	Russia, St. Petersburg	2
	MF-P450-011	<i>Malus domestica</i>	Russia, Krasnodarskiy kray	2
<i>U. consortiale</i>	MF-P013-021	<i>Malus domestica</i>	Russia, Krasnodarskiy kray	2
	MF-P220-011	<i>Leucanthemum maximum</i>	Russia, Leningradskaya oblast	2
	MF-P477-011	<i>Capsicum annuum</i>	?	1

Notes. List of strain collection acronyms: ATCC – American Type Culture Collection (Manassas, VA, USA); CBS – Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre (Utrecht, The Netherlands); EGS – private collection of Emory G. Simmons (Crawfordsville, IN, USA); IMI – CABI Bioscience (Egham, UK); MF – Laboratory of Mycology and Phytopathology of the All-Russian Institute of Plant Protection (St. Petersburg, Russia); VKM – All-Russian Collection of Microorganisms (Moscow, Russia). During this study MAT1 idiomorphs were identified.

Tab. 3. Identification of mating-type idiomorphs in several groups of alternarioid hyphomycetes using PCR with different primers.

Alternaria section*	Species	Number of isolates which mating-type loci amplified with different primers																
		MATI-1 idiomorph						MATI-2 idiomorph										
		Total	AAMI-2	AAMI-3	Amat1aF	Amat1aR	Amat1aF	Amat1cF	Amat1cR	Amat1cF	Amat1cR	Amat1dF	Amat1dR	Total	ATEN1R	MCHMG1	ATEN1F	MCHMG2
Heterothallic isolates																		
Alternaria	<i>A. alternata</i>	1	1	1	1	1	1	1	1	1	1	1	1	0	–	–	–	–
	<i>A. arborescens</i>	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	4
	<i>A. mali</i>	0	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	1
Brassicicola	<i>A. tenuissima</i>	2	2	2	1	2	1	2	1	2	1	2	1	2	1	2	2	2
	<i>A. brassicicola</i>	3	3	2	0	2	3	3	2	3	3	2	3	2	1	2	2	2
	<i>A. japonica</i>	3	3	3	0	2	3	3	3	3	3	3	3	3	3	2	3	3
Gyposphiala	<i>A. saponariae</i>	1	1	0	0	0	1	0	1	0	3	0	3	0	3	0	3	1
	<i>Alternaria</i> sp.	2	2	2	0	2	1	0	2	1	0	0	2	0	–	–	–	–
Panax	<i>A. eryngii</i>	0	–	–	–	–	–	–	–	–	–	–	–	1	0	1	0	0
Porri	<i>A. solani</i>	8	1	8	0	8	7	8	7	6	7	0	7	0	7	7	7	7
	<i>A. dauci</i>	5	0	5	0	5	3	5	3	5	4	4	4	4	4	4	4	4
Porri?	<i>A. zinniae</i>	2	0	2	0	1	1	1	1	1	1	1	1	1	1	0	1	1
	<i>A. silgbi</i>	1	0	1	0	0	0	1	0	1	0	0	1	0	–	–	–	–
	<i>A. helianthifaciens</i>	1	0	1	0	1	0	1	0	1	0	1	0	0	–	–	–	–
Sonchi	<i>A. acalypthae</i>	1	0	1	0	1	0	1	0	1	0	1	0	0	–	–	–	–
	<i>A. simmonsii</i>	1	0	0	0	1	1	1	1	1	1	0	0	0	–	–	–	–
	<i>A. brassicae</i>	1	1	1	0	1	1	1	1	1	1	3	3	3	3	3	3	3
Genus Ulocladium	<i>A. sonchi</i>	8	8	0	8	8	8	8	8	4	5	0	5	0	5	5	5	5
	<i>U. alternariae</i>	1	1	0	1	1	1	1	1	1	1	0	1	0	–	–	–	–
Ulocladium	<i>U. atrum</i>	0	–	–	–	–	–	–	–	–	–	–	–	3	3	3	3	3
	<i>U. botrytis</i>	2	1	0	0	1	2	2	2	2	2	1	2	1	1	1	2	2
	<i>U. charitatum</i>	3	0	0	0	3	3	3	3	3	3	2	2	2	2	2	2	2
Homothallic isolates	<i>U. consortiale</i>	1	0	0	0	1	1	1	1	1	1	2	2	2	2	2	2	2
	Homothallic isolates																	
Incertae sedis	<i>A. avenicola</i>	10	0	8	0	10	10	10	10	10	10	10	10	10	0	9	10	10
	<i>A. photostica</i>	2	0	2	0	2	2	2	2	2	2	2	2	2	0	2	2	2
Number of heterothallic isolates		51	28	33	15	46	43	40	45	45	45	26	40	42	40	42	40	42
Number of homothallic isolates		12	0	10	0	12	12	12	12	12	12	0	11	12	0	11	12	12
Approximate product size, bp			690	120	940	800	350	350	350	350	570	280	580	580	570	280	580	580
Annealing temperature (T _a), °C			55	48	55	55	54	52	53	53	53	60	55	60	53	60	55	55

*sections are according to Gannibal (2012) and Lawrence et al. (2013). Cases of the primer pair being efficient for all tested isolates are marked in bold.

The *MAT1-1* idiomorph of many strains was better amplified by primer pairs CHO13/Amat1R and Amat1cF/Amat1R. However the *MAT1-1* of all *Gypsophilae* section isolates was amplified only by primers AAM1-2/AAM1-3 which were not appropriate for other sections. All first mating-type *Ulocladium* isolates were revealed by using primer sets Amat1cF/Amat1R and Amat1dF/Amat1R.

Primers MCHMG1/MCHMG2 and ATEN1F/MCHMG2 were most efficient in amplification of *MAT1-2*. However no universal oligonucleotides were found. Both pairs need to be combined to reveal the mating type of isolates. Primers BPHMG1/BPHMG2 and BPHMG1m/Amat2R were not efficient. They amplified only a few *Alternaria* isolates.

A PCR product of expected size was obtained for the sole isolate of *Embellisia chlamydospora* when primers Amat1cF/Amat1R (*MAT1-1*) were used. No amplicons were obtained for 5 isolates of five members of the *A. infectoria* species-group (*A. infectoria*, *A. metachromatica*, *A. oregonensis*, *A. triticimaculans*, and *A. triticina*) and 3 isolates of *Alternariaster helianthi* with any primer sets.

Thus, in order to identify mating-type idiomorphs in many alternarioid hyphomycetes, we can recommend to choose primer sets from Tab. 3 in accordance with taxonomic affiliation. When the section is not identified or not known, the primer sets CHO13/Amat1R or Amat1cF/Amat1R and MCHMG1/MCHMG2 or ATEN1F/MCHMG2 can be used first to amplify *MAT1-1* and *MAT1-2*, respectively. Mating-type loci of the *A. infectoria* species-group obviously have undergone serious rearrangement and should not be studied by means of known PCR assays.

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