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NOTES AND COMMENTS

A multiplex PCR assay for determination of mating type in isolates of the honey bee fungal pathogen, *Ascosphaera apis*

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Detection of mating type (MAT) in fungi is very important for a better understanding of fungal biology, reproduction, and pathogenicity. Here we describe a new PCR-based identification of mating type in isolates of *Ascosphaera apis* (Maassen ex Claussen) (Spiltoir & Olive, 1955) the causative agent of chalkbrood disease in the honeybee (*Apis mellifera* L.). *A. apis* is a heterothallic filamentous fungus with two morphologically indistinguishable mating types (Mat-1 and Mat-2) (Aronstein & Murray, 2010; Aronstein, Murray, de Leon, Qin, & Weinstock, 2007) Mating-type assays have been previously used to identify opposite mating types in *A. apis*. This usually requires about five days and continuous propagation of the type strains (ARSF 7405 and ARSF 7406). The molecular identification method is a rapid and reliable option for detection of mating types of newly isolated fungal cultures.

A better understanding of the genomic organization of different *A. apis* isolates may shed light on the molecular basis of virulence, especially if crosses between isolates differing in virulence can be performed and the progeny analyzed for inheritance of traits associated with virulence. Furthermore, a better understanding of the molecular basis underlying differences in growth and reproductive ability of the fungus may have direct implications for disease control in honeybee colonies.

Multiplex PCR is routinely used for detection of a variety of microbial pathogens in research and clinical studies. We have previously described PCR-based detection to distinguish *A. apis Mat1-2* idiomorphs (Aronstein et al., 2007). However, for practical purposes it is desirable to achieve identification of both mating types simultaneously in a single reaction. Using a combination of genomic resources (NCBI and BeeBase http://BeeBase.org), we developed a multiplex PCR for rapid detection of *A. apis* mating types and tested this method using a variety of *A. apis* isolates collected in different states of the US and cultured in our laboratory.

A. apis strains used in this study were isolated from chalkbrood mummies or infected bee larvae collected from brood frames in four different US states (CA, WA, LA and TX) and followed by the isolation of single mating types in culture as described by (Aronstein & Murray, 2010). Two sequenced strains (ARSF 7405 and 7406) were used as controls in all experiments.

Fungal isolates were also grown in 15-ml Falcon tubes containing 5 ml of YGPSA liquid medium containing 100 mg/ml ampicillin and 12 mg/ml streptomycin, incubated for five days at 35° C with shaking at 125 r.p.m. Fresh mycelium from liquid cultures was used for genomic DNA extraction.

Genomic DNA was extracted using a modified SDS lysis method. Briefly, a mass of mycelia (~0.8 g wet weight) was transferred from the liquid cultures into a 1.5-ml microcentrifuge tube, centrifuged at 12,500*g* for 10 min and the remaining liquid media was removed. Fungal tissue was washed twice with 500 l of Solution A containing 0.1 M Tris-HCl of pH 7.5, 0.1 M NaCl and 50 mM EDTA. After centrifugation for 3 min at 12,500*g*, the washed solution was gently removed from the pellet. Fungal mycelia were hand homogenized with plastic disposable pestles and 0.1 mm Zirconia silica in 500 l of Solution A and 1% SDS added directly before use and incubated for 2 h at 70° C after homogenization. To remove SDS and SDS-bound proteins, 45 l of 8 M potassium acetate was added to each sample; tubes were inverted several times and incubated on ice for 30 min, followed by centrifugation at 12,500*g*, 4°C for 15 min. The supernatant of each sample was transferred to a new tube. Genomic DNA was then precipitated in 2.5 volumes of 200 proof ethanol (Sigma, St. Louis, MO), and washed in ice-cold 70% ethanol. DNA pellets were dried in a Vacufuge™ (Eppendorf North America, Inc.) for 2 min and re-dissolved in 50 l of nuclease-free water (Sigma, St. Louis, MO) and stored at -20 C until used.

The multiplex PCR was designed and tested on a number of different isolates. Primers were designed

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Table 1. List of primers used for DNA amplification in this study.

Primer name	Direction	Sequence 5′ to 3′	Gene	Notes
Mat1F-83	Forward	AGCAGACGCTAAAGAACTTG	*Mat1-1*	This study
Mat1R-562	Reverse	ATTGGGTGGAACAATGCCTA	*Mat1-1*	This study
Mat2scaf74F	Forward	AAA ATA CCA AGG CCA CCG A	*Mat1-2*	Aronstein et al. (2007)
Mat2scaf74R	Reverse	GGAGCATATTGGTAATTTGG	*Mat1-2*	Aronstein et al. (2007)

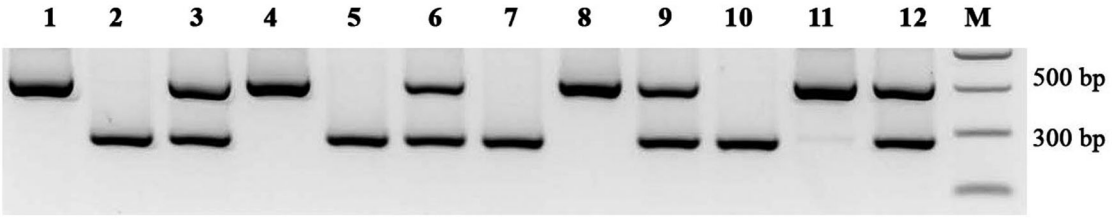

Figure 1. Multiplex PCR (lanes 1–12) showing amplification of DNA fragments specifically designed to identify *A. apis* mating type isolates. *Mat1-1* specific primers amplify a fragment of 478 bp (lanes 1, 4, 8, 11). *Mat1-2* specific primers amplify a single fragment of 212 bp (lanes 2, 5, 7, 10). Mixed idiomorphs produced two PCR bands (lanes 3, 6, 9, 12). Lane (M) showing Quick-Load PCR Marker (New England BioLabs, Inc).

based on the *Mat1-1* cDNA sequence (Cornman et al., 2012) and *Mat1-2* (GenBank, EF156413) gDNA of sequenced strains, ARSF 7405 and ARSF 7406, respectively. Primer design was facilitated by the MegAlign software (DNASTAR Inc., Madison, WI). All primers are listed in (Table 1). Amplification was performed in 30 l of final reaction volumes, containing 0.5 U of GoTaq Flexi DNA polymerase (Promega Co., Madison, WI, USA) with the colorless 5 GoTaq Flexi buffer, 0.2 mM dNTP mix, 2.5 mM MgCl2, 0.33 M of each of the four primers, and 1 l of gDNA. Reactions were run for 35 cycles at 95° C (30 s), 56° C annealing (30 s), extension at 72° C (30 s) after initial denaturing at 95° C for 3 min, and with a final extension of 72° C (2 min) on MJ Research (Watertown, MA) model PTC200 thermal cycler. PCR bands were separated on 1.8% agarose gels (Agarose-LE, Ambion) and documented using Gel Doc 2000™ imaging system and Quantity One software (Bio-Rad Laboratories, Hercules, CA).

(Figure 1) (lanes 1–12) shows results of a multiplex amplification of gDNA isolated from different *A. apis* isolates. *Mat1-1* specific primers amplify a fragment of 478 bp (lanes 1, 4, 8, 11); *Mat1-2* specific primers amplify a fragment of 212 bp (lanes 2, 5, 7, 10). Mixed idiomorphs produced two PCR bands (lanes 3, 6, 9, 12) of the respective sizes. Any single *A. apis* idiomorph should always have one of the mating-type genes present in the genome (both present in mixed mating-type gDNA), and therefore, one of them should be amplified in every PCR reaction, serving as an internal amplification control (IAC). No amplification indicates a failed reaction (not shown). Type strains (ARSF 7405 and ARSF 7406) were used as controls (Figure 1, lanes 4 and 5). All *A. apis* fungal isolates tested in this study produced results consistent with the two-idiomorph mating-type system.

Several factors may influence the efficiency of the multiplex PCR, including PCR reagents, reaction conditions, and DNA quality. The optimized conditions of this PCR method allow amplification of a wide range of DNA concentrations (160–1600 ng/l) from single or mixed isolates, providing 100% specificity. Reaction conditions were tested and confirmed by PCR amplification of *A. apis*-type strains (ARSF 7405 and 7406). The sensitivity of the new multiplex reaction is similar to that of a uniplex PCR.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Aronstein, K. A., Murray, K. D., de Leon, J., Qin, X., & Weinstock, G. (2007). High mobility group (HMG-box) genes in the honeybee fungal pathogen *Ascosphaera apis*. *Mycologia, 99*, 553–561. doi: 10.3852/mycologia.99.4.553

Aronstein, K. A., & Murray, K. D. (2010). Chalkbrood disease in honey bees. *Journal of Invertebrate Pathology, 103*, S20–S29. doi: 10.1016/j.jip.2009.06.018

Cornman, R. S., Bennett, A. K., Murray, K. D., Evans, J. D., Christine Elsik, C., & Aronstein, K. A. (2012). Transcriptome analysis of the honey bee fungal pathogen, *Ascosphaera apis*: Implications for host pathogenesis. *BMC Genomics, 13*, 285. doi: 10.1186/1471-2164-13-285

Spiltoir, C. F., & Olive, L. S. (1955). A reclassification of the genus pericystis betts. *Mycologia, 47*, 238–244. doi: 10.2307/3755414