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Mating type (*MAT*) locus and possible sexuality of the opportunistic pathogen *Exophiala dermatitidis*

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Abstract

Sexual reproduction among the black yeasts is generally limited to environmental saprobic species and is rarely observed among opportunists in humans. To date, a complete sexual cycle has not been observed in *Exophiala dermatitidis*. In this study, we aimed to gain insight into the reproductive mode of *E. dermatitidis* by characterizing its mating type (*MAT*) locus, conducting *MAT* screening of environmental and clinical isolates, examining the expression of the *MAT* genes and analyzing the virulence of the isolates of different mating types. Similar to other members of the Pezizomycotina, the *E. dermatitidis* genome harbors a high mobility group (HMG) domain gene (*MAT1-2-1*) in the vicinity of the *SLA2* and *APN2* genes. The *MAT* loci of 74 *E. dermatitidis* isolates (11 clinical and 63 environmental) were screened by PCR, and the surrounding region was amplified using long-range PCR. Sequencing of the ~12-kb PCR product of a *MAT1-1* isolate revealed an α-box gene (*MAT1-1-1*). The *MAT1-1* idiomorph was 3544-bp long and harbored the *MAT1-1-1* and *MAT1-1-4* genes. The *MAT1-2* idiomorph was longer, 3771-bp, and harbored only the *MAT1-2-1* gene. This structure suggests a heterothallic reproduction mode. The distribution of *MAT* among 74 isolates was ~1:1 with a *MAT1-1*:*MAT1-2* ratio of 35:39. RT-PCR analysis indicated that the *MAT* genes are transcribed. No significant difference was detected in the virulence of isolates representing different mating types using a *Galleria mellonella* model ($P>0.05$). Collectively, *E. dermatitidis* is the first opportunistic black yeast in which both *MAT* idiomorphs have been characterized. The occurrence of isolates bearing both

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idiomorphs, their approximately equal distribution, and the expression of the *MAT* genes suggest that *E. dermatitidis* might reproduce sexually.

Keywords

Black yeasts; *Chaetothyriales*; ecology; *Galleria mellonella*; sexual reproduction; virulence

1. Introduction

The polyextremotolerant black yeast *Exophiala dermatitidis* is an emerging opportunistic pathogen of humans and animals (Seyedmousavi et al., 2014). The fungus causes cutaneous, subcutaneous, and systemic infections, the latter with high mortality rates, primarily in immunocompromised but also in apparently immunocompetent individuals (Matsumoto et al., 1993; Szaniszlo, 2006). The ability to grow at a wide range of temperatures, from 4°C to 47°C, is a primary virulence factor in the genus *Exophiala*, of which *E. dermatitidis* is the most successful opportunist (de Hoog et al., 2011). *Exophiala dermatitidis* is commonly present in a yeast form in nature, but multiple morphotypes are also observed, ranging from hyphae and pseudohyphae to sclerotic bodies (muriform cells) in the host tissue (Matsumoto et al., 1993; Szaniszlo, 2006). Morphological plasticity, melanin in the cell wall, the assimilation of aromatic hydrocarbons, and the production of extracellular polysaccharides are considered to be factors that potentially contribute to the virulence of this fungus (Boral et al., 2018; Sterflinger, 2006).

Because of its large range of temperature, pH, and water activity tolerance, *E. dermatitidis* can be isolated from a wide range of habitats, varying from glaciers (Vishniac, 2006) to tropical rainforests (Sudhadham et al., 2008). It is also prevalent in numerous anthropogenic environments, such as steam baths (Matos et al., 2002), bathtubs and household dishwashers (Döğen et al., 2013b; Gümral et al., 2016; Zalar et al., 2011), and railway sleepers contaminated with aromatic hydrocarbons (Döğen et al., 2013a; Gümral et al., 2014; Yazdanparast et al., 2017). Water is considered to be the primary route of *E. dermatitidis* transfer from natural to anthropogenic environments and may also be associated with infection, e.g., via contaminated medical equipment or via the inhalation of dishwasher aerosols (Novak-Babič et al., 2018; Zupančič et al., 2016).

Exophiala dermatitidis isolates are classified into three genotypes (A, B, and C) based on the internal transcribed spacer (ITS) sequences (Döğen et al., 2013b; Matos et al., 2003; Novak-Babič et al., 2018; Sudhadham et al., 2008). Genotype C is rare; genotype A is primarily comprised of clinical isolates, and genotype B is commonly detected in natural environments. In contrast, anthropogenic environments harbor both A and B genotypes (Machouart et al., 2011; Matos et al., 2003).

Sexual reproduction is common among eukaryotes. Although it is a costly process, sexual reproduction enables deleterious mutations to be purged from the genome and produces progeny with diverse genotypes with important implications for pathogenesis (Ene and Bennett, 2014; Heitman, 2010, 2015). In the Pezizomycotina subphylum of the Ascomycota, sexual reproduction and cell identity is controlled by a single mating type (*MAT*) locus with

divergent sequences, designated idiomorphs, in each mating type (Bennett and Turgeon, 2016; Fraser and Heitman, 2003; Turgeon and Yoder, 2000). These idiomorphs, designated *MAT1-1* and *MAT1-2*, harbor transcription factor-encoding genes as the key elements. In heterothallic fungi that require a compatible partner for mating to occur, one idiomorph (*MAT1-1*) encodes an α-box transcription factor, and the other idiomorph (*MAT1-2*) encodes a transcription factor with a high mobility group (HMG) domain. On the other hand, in homothallism both idiomorphs can be present in the genome enabling self-fertility (Turgeon and Yoder, 2000).

Exophiala dermatitidis is a member of the family Herpotrichiellaceae (Chaetothyriales, Pezizomycotina, Ascomycota), which contains a large diversity of opportunistic black yeasts (Teixeira et al., 2017). *Capronia* is a teleomorphic genus in the Herpotrichiellaceae (Haase et al., 1999), which is characterized by dark ascomata with setae (bristles) containing asci with eight (or more) spores (Müller et al., 1987; Untereiner, 1995). Although a few isolates of *E. dermatitidis* have been observed to produce immature ascomata without asci or ascospores, to date, sexual reproduction has not been reported for this fungus (de Hoog et al., 1994). On the other hand, the genome sequence of the *E. dermatitidis* CBS 525.76 (ATCC 34100) isolate indicated the presence of a *MAT1-2-1* (HMG) gene in the genome (Chen et al., 2014; Teixeira et al., 2017).

In this study, we aimed to gain insight into the reproductive characteristics of the opportunistic black yeast *E. dermatitidis*. We identified the *MAT1-1* locus of *E. dermatitidis* and characterized it by comparing the *MAT1-1* and *MAT1-2* idiomorphs. In addition, we determined the mating types of 74 isolates, revealing an approximately 1:1 distribution of *MAT1-1*:*MAT1-2*. In addition, we detected the transcription of the *MAT* genes. Overall, the results suggest that *E. dermatitidis* might be able to reproduce sexually, although mating was not observed under laboratory conditions. The assessment of virulence using a *Galleria mellonella* model indicated no difference between the mating types.

2. Materials and methods

2.1. Strains and media

In this study, 81 *E. dermatitidis* isolates were evaluated: 27 (16 environmental and 11 clinical) isolates from the CBS culture collection housed at the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands), four clinical isolates from the Molecular Genetics and Microbiology Department (Duke University, Durham, NC), and 50 environmental isolates from the Çukurova University Division of Mycology (Adana, Turkey) culture collection (Table 1; Döğen et al., 2013a, 2013b; Gümral et al., 2014, 2016). The *MAT* genotypes of 74 isolates (11 clinical and 63 environmental) were determined, and 75 isolates (12 clinical and 63 environmental) were tested in the *G. mellonella* virulence assay as shown in Table 1. The isolates were grown on either yeast extract peptone dextrose agar (YPDA; Sigma-Aldrich, St Louis, MO) or potato dextrose agar (PDA; Merck, Darmstadt, Germany), at 37°C and were stored at –80°C in YPD broth containing 20% (v/v) glycerol.

2.2. DNA extraction and PCR

Genomic DNA was extracted from the isolates as described by Turin et al. (2000). For PCR products smaller than 3 kb, the following PCR mix was used: 12.5 μL of ExPrime Taq of 2× ExPrime Taq premix (GenetBio, Daejeon, Korea), 1 μL of 10 mM forward primer, 1 μL of 10 mM reverse primer, 1.5 μL of DNA, and water up to a 25-μL reaction volume. A similar reaction mixture was prepared for multiplex PCR, but it included 1 μL of each (four) 10 mM-primer solution. The reactions were performed using a 96-well T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). The PCR conditions were as follows: initial denaturation at 94°C for 1 min, 34 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR primers are listed in Table S1. The primers for the *MAT1-2* locus were designed based on the genome sequence of *E. dermatitidis* CBS 525.76 (ATCC 34100) available at the GenBank. As a positive control, the universal fungal primers ITS1 and ITS4 (Table S1) were used to amplify the ITS region. Water was used instead of DNA as a negative control.

For long-range PCR, Takara LA Taq polymerase (Takara Mirus Bio Inc., Madison, WI) was used. The 50-μL reaction mixture contained 5 μL of 10× LA PCR buffer II (Mg^{2+} Plus), 8.0 μL of dNTP mix (2.5 mM), 0.5 μL of Takara La Taq (5 U/μL), 2 μL of 10 mM forward primer, 2 μL of 10 mM reverse primer, 3.0 μL of DNA, and 29.5 μL of water. Amplification was performed as follows: initial denaturation at 94°C for 1 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, extension at 68°C for 13 min, and a final extension at 72°C for 30 s.

The amplification products were resolved on 0.8–1% agarose gels with Red Safe DNA stain (Chembio, St Albans, UK) using the Blue Marine 200 electrophoresis unit (Serva, Heidelberg, Germany) in 1× TAE buffer. The PCR products were visualized using a Gel DocTM EZ Imager (Bio-Rad).

2.3. Sequencing of the ~12-kb PCR product

To sequence the ~12-kb long-range PCR product, the PCR product was first purified from an agarose gel using the reagents supplied in the TOPO® XL PCR cloning kit (ThermoFisher Scientific, Wilmington, DE) following the manufacturer's instructions. The PCR product was sequenced using an ABI3500 XL Genetic Analyzer (Applied Biosystems, Foster City, CA) using the primer walking approach. In that approach, the first sequencing reaction was conducted using the PCR primers as the sequencing primers, and new primers were designed based on the newly read sequences (Table S2).

The sequences generated have been deposited at GenBank under the accession number MH341450.

2.4. Sequence analysis

For regular-PCR primer design, Primer 3Plus web-based software was used (Untergasser et al., 2007). The sequencing primers were designed manually to anneal to the 3′-ends of the sequenced regions, and their GC content, formation of hairpin loops, and self-dimers was checked using OligoAnalyzer® 3.1 (Integrated DNA Technologies, Coralville, IA)

(Owczarzy et al., 2008). The sequences obtained by primer walking were assembled using the CLC Main Workbench 7 (Qiagen, Hilden, Germany). Once the entire sequence was obtained, the *MAT1-1* and *MAT1-2* loci were compared using the WebACT (Abbott et al., 2008). The genes in the sequenced region were identified using a BLAST search of the NCBI website.

2.5. RNA extraction and RT-PCR analysis

RNA extraction was performed using one *MAT1-1* (CBS 139118) and one *MAT1-2* (CBS 139108) isolate. First, the strains were inoculated into 5 mL of YPD broth and grown at 25°C overnight. One milliliter of the growing cells was added to 14 mL of YPD broth and grown for 5 h at 25°C. RNA extraction was conducted on the cells collected by centrifugation for 5 min at 12 000 × g using a GeneJET RNA Purification Kit (ThermoFisher Scientific) according to the manufacturer's instructions. RNA samples were quantified using a BioSpec-Nano spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD), and the integrity was checked by agarose gel electrophoresis as described in Section 2.2. The reverse transcription reaction was performed with 1 μg of each RNA sample using the QuantiTect Reverse Transcription (RT) Kit (Qiagen, Hilden, Germany) according to a manufacturer's instructions. For each sample, a control lacking RT was included to test for contaminating genomic DNA. The resulting cDNAs were used in the PCR reaction as described in section 2.2 except that the annealing temperature was chosen to be 59°C. The primers used are ACT1-F and ACT1-R that amplify the actin gene (NCBI locus ID: HMPREF1120_04671) as the constitutively expressed positive control, RT-PCR-ALFA-F and RT-PCR-ALFA-R for the *MAT1-1-1* gene, RT-PCR-MAT114-F and RT-PCR-MAT114-R for the *MAT1-1-4* gene, and RT-PCR-HMG-F and RT-PCR-HMG-R for the *MAT1-2-1* gene. The sequences of the primers are shown in Table S1.

2.6. Mating assays

Modified Leonian's agar [MLA; 0.625 g/L peptone, 1 g/L yeast extract, 6.25 g/L malt extract,1.25 g/L potassium phosphate, 0.625 g/L magnesium sulfate, 0.625 g/L peptone, and 20 g/L agar (all from Sigma-Aldrich), and 6.25 g/L maltose (Merck)], malt extract agar (MEA, Sigma-Aldrich), and oatmeal agar (OA, Fluka, Buchs, Switzerland) were used as the mating media (Untereiner, 1995). The compatible isolates were determined using the *MAT* locus PCR as described in section 2.2. The isolates were inoculated on the mating media plates mixed together or without a partner as the negative control. The plates were incubated under black blue light (320–390 nm) at room temperature (22°C–25°C) up to 6 months and examined for the production of ascomata.

An alternative protocol involving tomato stems was also employed (de Hoog et al., 1994). Briefly, the selected isolates were grown on PDA at 37°C for a week. They were used to inoculate 10 mL of the ML broth and incubated on a rotary shaker at 100 rpm at room temperature. After 3 d incubation, 3–4 pieces of 2-cm–long sterilized tomato stems were added to the media, and the incubation was continued for 3–21 d until sufficient growth on the stems was observed. The colonized tomato stems were transferred into Petri dishes containing filter paper moistened with sterile water. The Petri dishes were sealed with parafilm and incubated under black blue light (320–390 nm) at room temperature for up to 6

months (Untereiner, 1995). The Petri dishes were then examined under a microscope (YJ-2005B, Yujie, China) in order to investigate the production of dark ascomata with setae.

2.7. Galleria mellonella *virulence assay*

Galleria mellonella larvae were obtained from Vanderhorst Wholesale Inc. (St. Marys, OH). The larvae were inoculated within 3 d of receipt. Larvae weighing approximately 0.3–0.5 g with no cuticle discoloration were selected for experiments. The virulence of the fungal isolates listed in Table 1 was tested using 15 *G. mellonella* larvae per isolate. The fungal isolates were pregrown on YPDA for 24 h at 37°C (Gago et al., 2014). The fungi were harvested by gently scraping the colony surface with a plastic loop and washed three times using sterile phosphate-buffered-saline (PBS). Cell densities in the suspensions were determined using a hemocytometer, and adjusted to 10^6 cells/μL with sterile PBS. Four microliters of the adjusted suspensions were injected into the left rearmost proleg using a 100-μL Hamilton syringe with a dispenser. The syringe was rinsed several times with 70% ethanol followed by a PBS rinse prior to the injection of the larvae. The control group larvae were inoculated with sterile PBS. The inoculated larvae were incubated at 37°C, and the number of dead larvae was monitored daily for 3 weeks (Gago et al., 2014).

2.7. Statistical analysis

The Kaplan–Meier test (Minitab v.16.1, Cologne, Germany) was performed to assess the statistical significance of differences in survival between the larvae infected with different *E. dermatitidis* groups. The value of $P < 0.05$ was considered to indicate statistically significant differences.

3. Results

3.1. *The MAT loci of* E. dermatitidis *CBS 525.76 and closely related teleomorphic species*

Investigation of the *E. dermatitidis* CBS 525.76 (ATCC 34100) genome (Chen et al., 2014; Teixeira et al., 2017) revealed the presence of an HMG domain-encoding gene (*MAT-1–2-1*; Fig. 1a). As in other Pezizomycotina species, the genes *APN2* and *SLA2* were found flanking the *MAT* locus. In addition to the HMG gene, three additional genes were identified between *APN2* and *SLA2* encoding HMPREF 1120_08860, HMPREF 1120_08861, and 5S rRNA (Fig. 1a). The presence of the α-box gene was investigated by homology searches using the known α-box gene sequences, but the gene was not identified.

The homothallic species *Capronia epimyces* and *Capronia coronata* are the most closely phylogenetically related teleomorphic species to *E. dermatitidis*, whose genomes have been sequenced (de Hoog et al., 2003, 2006; Teixeira et al., 2017). As also reported by Teixeira et al. (2017), the *C. epimyces* and *C. coronata MAT* loci contain both an α-box gene (*MAT1-1-1*) and an HMG gene domain (*MAT1-2-1*) gene (Figs. 1b and 1c), consistent with their homothallic nature (Müller et al., 1987; Untereiner, 1995). In *C. coronata*, the *MAT* locus is closely linked to the *APN2* and *SLA2* genes (Fig. 1c). However, in *C. epimyces*, the *APN2* gene is located in the vicinity of the *MAT* locus, and *SLA2* is located 150 kb away (Fig. 1b). The *MAT* loci of both species harbor a gene ortholog encoding HMPREF 1120_08861 similar to *E. dermatitidis* and a *MAT1-1-4* gene, which is also found in or near

the *MAT* loci of some Pezizomycotina members, such as the dermatophytes and dimorphic fungi (Metin and Heitman, 2017).

3.2. *Amplification of the HMG gene (MAT1-2-1) of* E. dermatitidis *isolates*

The presence of the *MAT1-2-1* gene in the *E. dermatitidis* isolates was determined by PCR using primers designed based on the genome sequence of the isolate CBS 525.76 (ATCC 34100). A representative agarose gel is shown in Fig. 2a, and the complete isolate list is shown in Table 1. The binding sites for the primers are shown in Fig. 1a. Universal ITS primers ITS1 and ITS4 were used in positive-control PCR reactions. While the 600–650 bp ITS product was detected in all the isolates tested, the 225 bp *MAT1-2-1* gene product was only detected in some isolates (Fig. 2a).

3.3. *Long-range PCR amplification of the* E. dermatitidis *MAT1-1 locus*

To amplify the *MAT1-1* locus of the *E. dermatitidis* isolates that did not yield a *MAT1-2-1* product, a long-range PCR approach was used. Because the *MAT1-1* sequence is not known, primers were used that are specific to the *MAT1-2* locus but sufficiently distant from the *MAT1-2-1* gene to amplify a product from both *MAT1-1* and *MAT1-2* isolates. Thus, a forward primer complementary to the *APN2* gene (APN2-F) and several reverse primers [two complementary to sequences within the *SLA2* gene (SLA2-R1 and SLA2-R2), one complementary to a sequence within the HMPREF1120_08860 gene (8860-R), and another complementary to a sequence within the HMPREF1120_08861 (8861-R)] were used in long-range PCR. The binding sites of the primers are shown in Fig. 1a. Products from both *MAT1-1* and *MAT1-2* isolates were obtained when two primer pairs were used: APN2-F and SLA2-R1 produced an ~14-kb product, and APN2-F and 8860-R produced an ~12-kb product in the four isolates tested (two *MAT1-1* and two *MAT1-2* isolates; data not shown).

The ~12-kb product obtained from the *MAT1-1* isolate CBS 132752 was selected for sequencing. Sequence analysis revealed that the ~12-kb PCR product harbored *MAT1-1-4*, the α-box (*MAT1-1-1*) gene, the gene encoding HMPREF1120_08861, and a 5S rRNA gene between *APN2* and the gene encoding HMPREF1120_08860 (Fig. 3).

The *E. dermatitidis* CBS 132752 sequence obtained was compared with the *MAT* locus of *E. dermatitidis* CBS 525.76 using the Artemis comparison tool (Fig. 3). This approach revealed the presence of an internal dissimilar *MAT* region (similarity <50%) surrounded by flanking regions of high similarity (92%–98%). High sequence similarity was apparent upstream of the *MAT* locus (96%). This was followed by a dissimilar *MAT* region ending with a 116-bp region of the *MAT1-1-1* gene as a remnant in the *MAT1-2* locus. Downstream of the *MAT* locus, a highly similar region was observed (93%), but with insertions/deletions in the vicinity of the HMPREF1120_08861 gene, explaining why a long-range PCR product was not obtained using the 8861-R primer in the *MAT1-1* isolates.

The analyses revealed that while *E. dermatitidis* CBS 132752 harbored the *MAT1-1* locus, the isolate CBS 525.76 harbored the *MAT1-2* locus. In addition, the *E. dermatitidis MAT1-1* locus was 3544-bp long and harbored the *MAT1-1-1* (α-box) and *MAT1-1-4* genes, while the *MAT1-2* locus harbored only the *MAT1-2-1* (HMG domain) gene and was 3771-bp long (Figs. 1a and 3). The *MAT* locus of *E. dermatitidis* was located between *APN2* and the gene

encoding HMPREF1120_08861 (Fig. 1a). The structure of the *MAT* locus of *E. dermatitidis* indicated heterothallism.

3.4. *Isolate typing by the amplification of* MAT1-1 *and the intergenic region between the genes encoding HMPREF1120_08861 and 5S-rRNA*

After determining the *MAT1-1* locus sequence, the isolates were screened using primers APN2-F3 and Alfa-R4. These primers were complementary to *MAT1-1*, producing an 1812-bp PCR product. Their binding sites are shown in Fig. 1a. The 1812-bp PCR product was generated with the *MAT1-1* isolates used (Fig. 2b and Table 1). Among 74 isolates tested, 35 harbored the *MAT1-1* locus, and 39 harbored the *MAT1-2* locus (Table 2). Generally, the distribution of the idiomorphs was close to ~1:1. However, in clinical isolates, the distribution was skewed towards *MAT1-1* (~73%); similarly, ~85% of genotype C (11 out of 13) isolates harbored *MAT1-2* (Table 2). Greater numbers of isolates from the two populations should be screened to evaluate the mating-type distributions in these populations.

To investigate whether the insertions/deletions in the vicinity of the gene encoding HMPREF_08861 were linked to the *MAT* idiomorph, all the isolates typed for *MAT* were also screened using the ALFA-F6 and 8860-R6 primers (the binding sites are shown in Fig. 3). These primers amplified a 722-bp region in the *MAT1-1*-harboring the CBS 132752 isolate and a 561-bp region in the *MAT1-2*-harboring the CBS 525.76 isolate. While the *MAT1-1* idiomorph produced the longer 722-bp fragment in each of the 35 isolates tested; 21 out of 39 (54%) *MAT1-2* isolates yielded the 722-bp fragment, and 18 isolates (46%) yielded the 561-bp fragment (Table 1, Fig. 2c).

3.5. *Investigation of the transcription of the* MAT *genes*

To determine if the *MAT* genes are transcribed, RNA samples obtained from one *MAT1-1* (CBS 139118) and one *MAT1-2* (CBS 139108) isolate were used to obtain cDNA samples using reverse transcription. The resulting cDNAs were used to detect the expression of the *MAT* genes by PCR. The actin gene (NCBI locus ID: HMPREF1120_04671) was used as a constitutively expressed positive control. As observed in Fig. 4, actin gene expression is readily observed in both isolates as a positive control. The corresponding *MAT* genes are expressed in the *MAT1-1* and *MAT1-2* isolate although at lower levels than actin. No contaminating DNA is observed in the cDNA synthesis as can clearly be seen in the controls lacking RT (Fig. 4).

3.6. *Investigation of the sexual reproduction of* E. dermatitidis

After determining the mating type, selected isolates, including three that harbor *MAT1-1* (CBS 132752, CBS 115663, and CBS 149.90) and three that harbor *MAT1-2* (CBS 139122, CBS 120567, and CBS 424.67), were grown in solo culture, in combinations of two, and all together on the MLA, MEA, OA and tomato stem media, i.e., the media used for sexual reproduction experiments with the *Capronia* species (de Hoog et al., 1994; Untereiner, 1994, 1995). Although the incubation lasted more than 6 months, sexual reproduction was not observed.

3.7. Virulence assay

The virulence of 64 environmental and 12 clinical *E. dermatitidis* isolates (Table 1) in the *G. mellonella* larva model was compared using median survival times (Table S3). A significant difference was observed in the median survival time between the clinical (9 ± 0.32 d) and environmental (12 ± 0.28 d) *E. dermatitidis* isolates ($P < 0.001$). When the virulence of 32 *MAT1-1* and 37 *MAT1-2* isolates were analyzed (Table S4), no significant differences were observed in the survival times of the larvae infected with *MAT1-1* (11 ± 0.19 d) and *MAT1-2* (12 ± 0.16 d) isolates ($P > 0.05$; Fig. 5). The survival time of the fungus-infected larvae (*MAT1-1* and *MAT1-2)* was significantly different from that of the PBS-treated control group (20 ± 1.5 d) ($P < 0.001$; Fig. 5).

4. Discussion

As many as ~20% of fungi, including *E. dermatitidis*, have long been regarded as asexual (Dyer and O'Gorman, 2011). However, evidence accumulating with the development of molecular genetic analytical tools and the sequencing of additional fungal genomes appears to challenge this hypothesis. The identification of the presence of genes related to sexual reproduction, including the genomic *MAT* locus, the expression of these genes, population genetic analysis and a 1:1 distribution of mating types suggests the possible sexuality of such fungi. For example, in opportunistic *Aspergillus* species formerly accepted as asexual, such as *A. fumigatus*, *A. flavus*, and *A. terreus*, first the *MAT* genes and then the 1:1 mating type distribution were discovered (Eagle, 2009; Paoletti et al., 2005; Ramirez-Prado et al., 2008), which was followed by studies demonstrating the induction of sexual cycles under laboratory conditions (Arabatzis and Velegraki, 2013; Horn et al., 2009; O'Gorman et al., 2009). In this study, we characterized the *MAT* locus of *E. dermatitidis*, thought to be an asexual fungus, by identifying the *MAT1-1* and *MAT1-2* idiomorphs. We also demonstrated an approximately equal distribution of each idiomorph in 74 isolates that were analyzed. In addition, we showed that the *MAT* genes are transcribed. The data obtained suggest a heterothallic mode of sexual reproduction.

An important hallmark of sexual reproduction is the presence of mating/meiosis genes in the genome. When the *E. dermatitidis* genome was searched for the core set of eight meiotic genes defined (Schurko and Logston, 2008), three (*DMC1*, *MND1*, and *HOP2*) were found to be missing (Chen et al., 2014). However, these three genes are also missing from sexually reproducing organisms, such as *Drosophila melanogaster* and *Caenorhabditis elegans* (Schurko and Logston, 2008), from the heterothallic species *Candida lusitaniae* and *C. guilliermondii* (Butler et al., 2009), and more importantly, from the sexually reproducing *C. epimyces* and *C. coronata*, closely related to *E. dermatitidis*. Therefore, these genes may not always be required for meiosis. The pheromone receptor *STE3* gene is also present in the genome of *E. dermatitidis* annotated under the designation HMPREF1120_08176.

The genetic clues of sexual reproduction in the *E. dermatitidis* genome were apparent, but we were unable to induce mating under laboratory conditions. Indeed, for some fungi, evidence supporting sexual reproduction has been obtained, but a complete mating cycle has still not been observed in the laboratory. For instance, the dimorphic fungus *Paracoccidioides brasiliensis* harbors the mating type genes in the genome (Li et al., 2010)

with an approximately equal distribution of *MAT1-1* and *MAT1-2* idiomorphs (Torres et al., 2010) that provide evidence of recombination events (Matute et al., 2006; Teixeira et al., 2009), the expression of *MAT* genes, pheromone and pheromone receptor genes (Gomes-Rezende et al., 2012; Teixeira et al., 2013; Torres et al., 2010), and the formation of coiled hyphae indicative of ascomata initials (Teixeira et al., 2013). However, the formation of mature ascomata or ascospores has not yet been detected.

The difficulty in observing mating under laboratory conditions may be because the fungi might require specific conditions to mate. For example, for sexual reproduction to occur in the dermatophyte *Trichophyton onychocola*, the compatible strains should be incubated at 17°C instead of 25°C (the room temperature often used for mating assays) (Hubka et al., 2015). Similarly, *A. fumigatus* has to be incubated on OA medium at 30°C for at least 6 months for the ascomata to be observed (Kwon-Chung and Sugui, 2009; O'Gorman et al., 2009). Therefore, it might be difficult to identify the conditions for fungal mating. In this study, we tested three different media (MLA, MEA, and OA) and tomato stems, which successfully induce the sexual cycle of the teleomorphic species of *Capronia* that are closely related to *E. dermatitidis* (de Hoog et al., 1994; Untereiner, 1994, 1995). The plates were incubated at room temperature (22°C–25°C) with a selected group of isolates. Mating assays at different temperatures and with more isolates should be performed in future studies in an attempt to observe *E. dermatitidis* mating.

In a study analyzing 58 strains of *E. dermatitidis*, four isolates were suggested to be diploid, based on their large cell and nuclear sizes, and doubled DNA content as assessed by quantitative staining (Ohkusu et al., 1999). In fact, some artificially induced diploid strains are used in genetic analytical studies of *E. dermatitidis* (Cooper and Szaniszlo, 1993). We determined the cell size of the isolates analyzed in the current study, but we did not detect any significant differences between the isolates (data not shown).

Generally, mating is somewhat restricted in human pathogenic fungi (Ene and Bennett, 2014; Nielsen and Heitman, 2007). For example, for mating to occur, *Candida albicans* has to undergo a phenotypic change called the white/opaque transition (Miller and Johnson, 2002). In addition, instead of a sexual cycle with meiosis, a parasexual cycle involving concerted chromosome loss is observed (Bennett and Johnson, 2003). Another well-known fungal pathogen is the basidiomycetous yeast *Cryptococcus neoformans*. The mating system observed in the majority of basidiomycetes, including the closely related relatives of *C. neoformans*, is tetrapolar and involves two unlinked genomic loci that determine cell identity and govern the mating process (Findley et al., 2012; Metin et al., 2010). However, *C. neoformans* has evolved toward a bipolar mating system involving a single biallelic *MAT* locus (Fraser et al., 2004). Further, while the probability of mating within the progeny of a tetrapolar cross is 25%, that within a bipolar cross is 50%, suggesting enhanced inbreeding, which creates a lower degree of genetic diversity than outcrossing (Hsueh et al., 2008). In addition, *C. neoformans* occurs mostly as a single mating type in the environment (Kwon-Chung and Bennett, 1978). However, it retains the sexual reproduction capability by exhibiting a specific mode of homothallism and unisexual reproduction (i.e., mating in solo culture) in addition to the regular heterothallic mating system (Lin et al., 2005). Another example of sex restriction is observed in *A. fumigatus*, which requires very stringent mating

conditions, unlike its saprobic *Neosartorya* relatives, which produce abundant cleistothecia under a wider range of conditions (Kwon-Chung and Sugui, 2009). Similarly, while mating is ubiquitous in geophilic dermatophytes associated with soil, disease-associated anthropophilic dermatophytes generally occur as a single mating type with no defined sexual cycles (Metin and Heitman, 2017).

It has been proposed that asexual reproduction allows the pathogens to conserve well-adapted genomic arrangements and successfully proliferate within the host (Nielsen and Heitman, 2007). Pathogens may retain the sexual reproduction apparatus to adapt to stress conditions, such as antimicrobial therapy, but restrict the frequency of sex by a number of mechanisms (Nielsen and Heitman, 2007).

Similar observations were noted in black yeasts. While almost none of the black yeast pathogens possess known sexual cycles, closely related saprobic species from the *Capronia* genus reproduce sexually (Teixeira et al., 2017; Untereiner, 1994, 1995). The only exception might be *Capronia semiimersa*, which is an opportunist pathogen (Teixeira et al., 2017). *Capronia semiimersa* is accepted as the teleomorph of *Phialophora americana* (Untereiner and Naveau, 1999), which is closely related to the pathogenic black yeast *Phialophora verrucosa* (Li et al., 2017). However, according to recent studies, while *P. americana* is a predominantly environmental species, the clinical isolates are primarily *P. verrucosa* (Li et al., 2017).

Some *E. dermatitidis* isolates were previously reported to produce sterile ascomata-like bodies with setae, called “teleomorph initials” or “ascomatal initials” (de Hoog et al., 1994; Untereiner, 1995). For example, in one study, these sterile bodies were detected in six out of 30 isolates (de Hoog et al., 1994). These ascomatal initial-producing isolates might be rare diploid/aneuploid isolates, might harbor both idiomorphs, or might be isolates preparing for sexual reproduction but unable to complete the cycle, because there is no compatible mating type. In this study, we did not detect ascomatal initials in the isolates tested. Five of these teleomorph-initial-producing *E. dermatitidis* strains (IFM 4833, 4846, 5383, 41822, and 41826) were also used in the diploid study described above (Ohkusu et al., 1999), but their size and DNA content suggest that they are haploid (Ohkusu et al., 1999). Some *Paracoccidioides* isolates are also reported to form coiled hyphae and ascomatal initials. However, they do not harbor both idiomorphs (Teixeira et al., 2013). In addition to *E. dermatitidis*, the anamorphic species *Exophiala jeanselmei*, *E. pisciphila*, and *E. salmonis* were also reported to produce a type of immature ascomata (Untereiner, 1995). In addition to these species with no known sexual cycle, single spore isolates of the heterothallic species *Capronia dactylotricha* and *Capronia moravica* also produce ascomatal initials and form mature ascomata with asci and ascospores with a compatible mating type (Untereiner, 1995). Therefore, at least in sexually reproducing heterothallic species, these structures are formed in the absence of a compatible mating type. A population might contain isolates of varying degrees of sexual competence from completely sterile to highly fertile, and these structures might be formed only by the highly fertile fraction of the population (Dyer and Paoletti, 2005).

According to a phylogenetic analysis based on the small subunit (SSU) rDNA, the *Capronia* species that are most closely related to *E. dermatitidis* are *C. mansonii*, *C. munkii*, *C. coronata*, and *C. epimyces* (de Hoog et al., 2003). All of these species are homothallic as determined by studies performed with single-spore isolates (Müller et al., 1987; Untereiner, 1995). The presence of both the α-box and HMG domain transcription factor genes in the *C. coronata* and *C. epimyces* genomes confirms this (Fig. 1; Teixeira et al., 2017). The only known heterothallic *Capronia* species are *C. dactylotricha*, *C. moravica*, and *C. pulcherrima* (Untereiner, 1995, 1997), clustered together in a sister clade of the *E. dermatitidis* clade based on the SSU rDNA analysis (de Hoog et al., 2003). Phylogenetic analysis here suggests that the *E. dermatitidis* heterothallic mating system could have evolved from a homothallic ancestor. On the other hand, the *jeanselmei* clade, which is closely related to the *dermatitidis* clade, includes species, such as *Exophiala sideris*, *E. xenobiotica*, *E. spinifera*, and *E. oligosperma*, that are not known to have sexual cycles (Teixeira et al., 2017). These species have either the *MAT1-2-1* (HMG) gene (*E. spinifera* and *E. oligosperma*) or the *MAT1-1-1* (α) and *MAT1-1-4* gene combination (*E. xenobiotica* and *E. sideris*) in their genomes (Teixeira et al., 2017), and they do not appear to harbor orthologs encoding HMPREF 1120_08860 or HMPREF 1120_08861. Interestingly, *E. spinifera* and *E. oligosperma* also have a partial *MAT1-1-1* sequence next to the *MAT1-2-1* gene similar to *E. dermatitidis* (Figs. 1 and 3) and seems to be ancestral, as the same organization is observed in other, distantly related black yeasts. (Teixeira et al., 2017). This suggests the possible integration of *MAT1-1-1* into *MAT1-2* by unequal recombination, followed by degeneration through the deletion of the 5′-end of the α-box gene in a Chaetothyriales ancestor. Similar truncated *MAT* genes were also reported in other fungi (Rydholm et al., 2007; Seidl et al., 2009; Tsui et al., 2013).

Analysis of the polymorphic insertion/deletion region just outside the *MAT* locus revealed that while *MAT1-1* was linked to the insertion genotype in all 35 isolates examined, the *MAT1-2* isolates represented either genotype. This indicated possible recombination events in the vicinity of the *MAT* locus. The genetic diversity of *E. dermatitidis* isolates was recently investigated, and when the transcriptional elongation factor 1 (*TEF1*) and β-tubulin (*TUB*) gene variants were compared with the rDNA ITS haplotypes, a random variation of gene variants compared to haplotype associations was observed, representing diversity rather than different lineages (Song et al., 2017). Such high diversity might have resulted from sexual reproduction.

In human pathogenic fungi, mating type might be a virulence-determinant. For example, in *C. neoformans*, the α mating type has been shown to be more virulent than **a** in some genetic backgrounds (Kwon-Chung et al., 1992). Similarly, the (−) mating type of *Histoplasma capsulatum* was determined to be predominant among clinical isolates or among nondisseminated pulmonary cases (Kwon-Chung, 1973; Kwon-Chung et al., 1974; Kwon-Chung et al., 1984). Similarly, in the study by Alverez-Perez et al. (2010), the *MAT1-1* mating type of *Aspergillus fumigatus* was found to be associated with invasive aspergillosis cases. Consistent with that, the *A. fumigatus MAT1-1* mating type was found to be more virulent in a *Galleria mellonella* assay (Cheema and Christians, 2011).

In this study, we evaluated the virulence of isolates of different mating types of *E. dermatitidis* in the model host *G. mellonella*. Although the clinical *E. dermatitidis* isolates were more virulent than environmental isolates with median survival times of 9 and 12 d, respectively (20 d for the PBS-injected control), we have not detected a significant difference between the isolates of different mating types.

The data presented in this study demonstrated that *G. mellonella* larvae might be used as an alternative to mouse models to study the virulence of *E. dermatitidis* isolates. To further support the utility of the *G. mellonella* model, the virulence of specific isolates in mouse and *G. mellonella* models should be compared.

5. Conclusions

In this study, we identified the *MAT1-1* idiomorph and characterized the *MAT* locus of *E. dermatitidis*. In addition, the distribution of *MAT1-1* and *MAT1-2* isolates was approximately 1:1 in 74 screened isolates. Moreover, the *MAT* genes, *MAT1-1-1*, *MAT1-1-4* and *MAT1-2-1* were shown to be transcribed in the isolates of the corresponding mating type. The evidence obtained indicates the possible sexuality of *E. dermatitidis* consistent with a heterothallic mating system. In the *G. mellonella* model, the mating type was not found to be a virulence determinant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The *MAT1-1* idiomorph of *E. dermatitidis* harbors *MAT1-1-1* (α-box) and *MAT1-1-4* genes
- The *MAT1-2* idiomorph of *E. dermatitidis* harbors only the *MAT1-2-1* (HMG domain) gene
- The distribution of the *MAT* idiomorphs of *E. dermatitidis* is ~1:1
- The *MAT* genes of *E. dermatitidis* are expressed.
- Virulence is not mating type-dependent in the wax moth larva assay

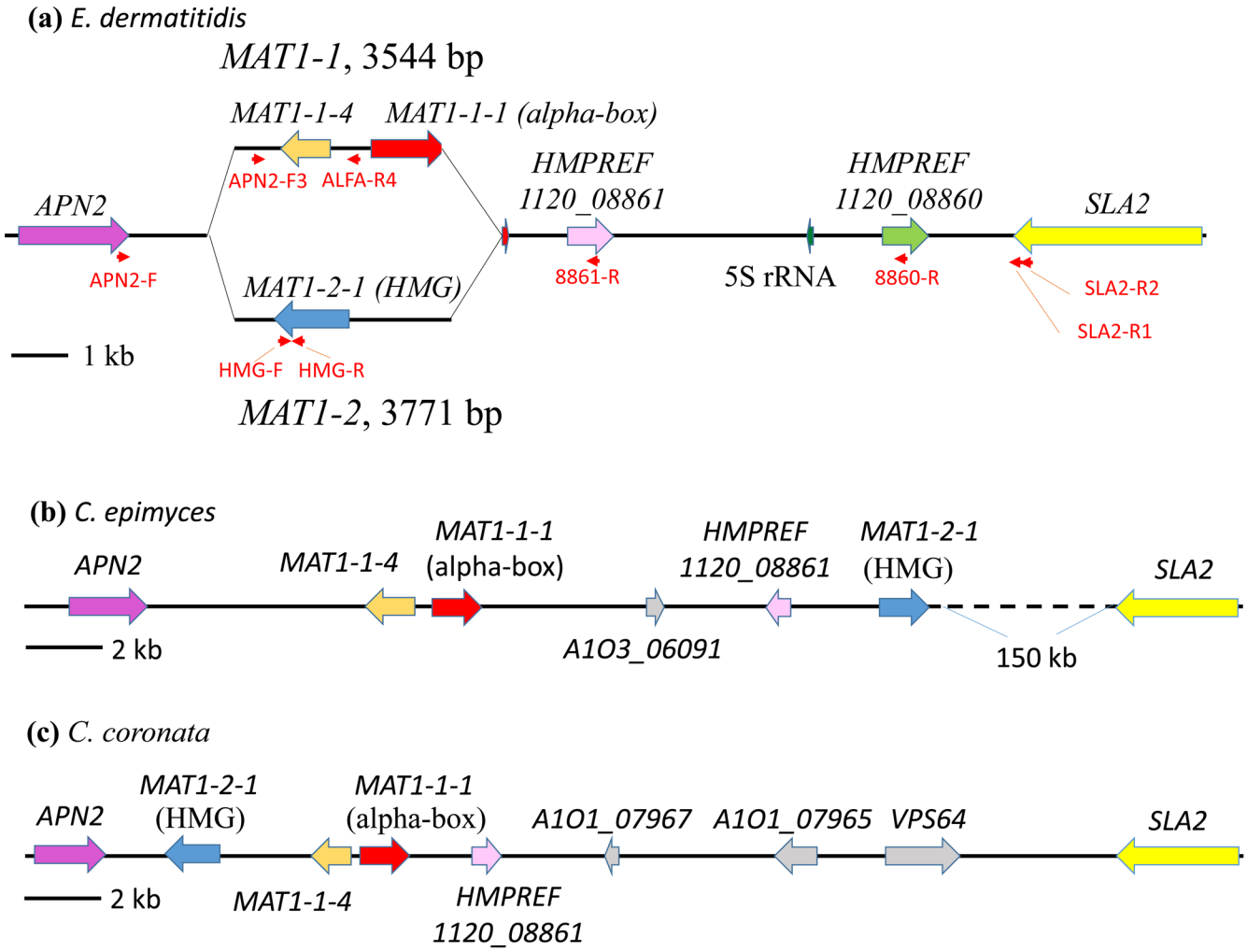

Fig. 1.

The *MAT* locus of *E. dermatitidis* **(a)**, and the *MAT* loci of the closely related teleomorphic species *C. epimyces* **(b)** and *C. coronata* **(c)**. **(a)** The *MAT* locus of the isolate *E. dermatitidis* CBS 525.76 (genome sequence available at GenBank) contains an HMG-domain gene (*MAT1-2-1*) in the vicinity of the *APN2* and *SLA2* genes, which generally lie on either side of the *MAT* loci in the Pezizomycotina members. Three additional genes are located between *APN2* and *SLA2*: the 5S rRNA gene and two genes encoding HMPREF1120_08860 and HMPREF1120_08861 (*E. dermatitidis* CBS 525.76 genome annotation). The *MAT1-1* sequence of the isolate CBS 132752 determined in this study has enabled the characterization of the *MAT* locus of *E. dermatitidis*. *MAT1-1* is 3544-bp long and harbors the *MAT1-1-1* (α-box) and *MAT1-1-4* genes. *MAT1-2* is slightly longer, 3771-bp, and contains a single gene, *MAT1-2-1* (HMG). The *MAT* locus is located between *APN2* and the gene encoding HMPREF1120_08861. *SLA2*, a gene commonly found in the vicinity of the *MAT* loci in Pezizomycotina fungi is located downstream of the 5S rRNA gene and the gene encoding HMPREF1120_08860. The binding sites of the primers are shown by small arrows. **(b)** *C. epimyces* and **(c)** *C. coronata* harbor both, the α-box gene and HMG-domain gene in their *MAT* loci, which supports their classification as homothallic. Orthologs

of the *E. dermatitidis* gene encoding HMPREF1120_08861 are also present in the *MAT* loci of *C. epimyces* and *C. coronata*. In *C. epimyces*, *SLA2* is located 150-kb from the *MAT* locus. The α-box genes of *C. epimyces* and *C. coronata* are located back-to-back with *MAT1-1-4*, a gene that is found in the *MAT* loci of dermatophytes and dimorphic fungi (Metin and Heitman, 2017).

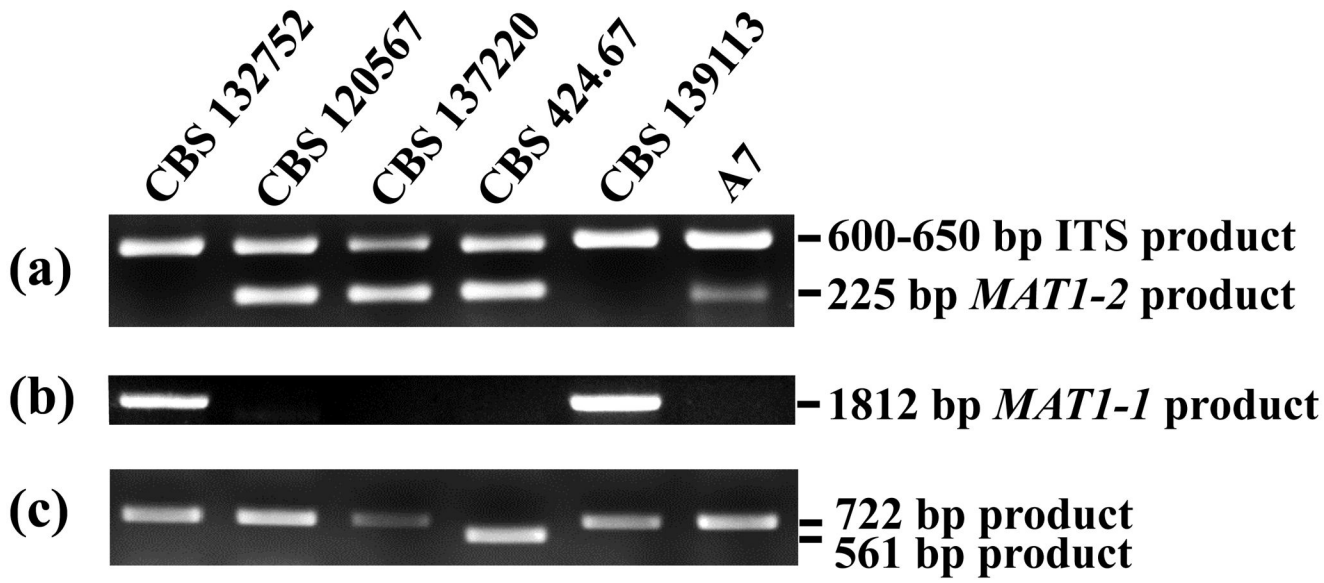

Fig. 2.

PCR screening of the selected *E. dermatitidis* isolates **(a)** for the presence of the HMG-domain gene, *MAT1-2-1*; a multiplex PCR was performed using ITS as a positive control; **(b)** for the presence of the *MAT1-1* idiomorph **c)** for the in/del region in the vicinity of the gene encoding HMPREF_008861.

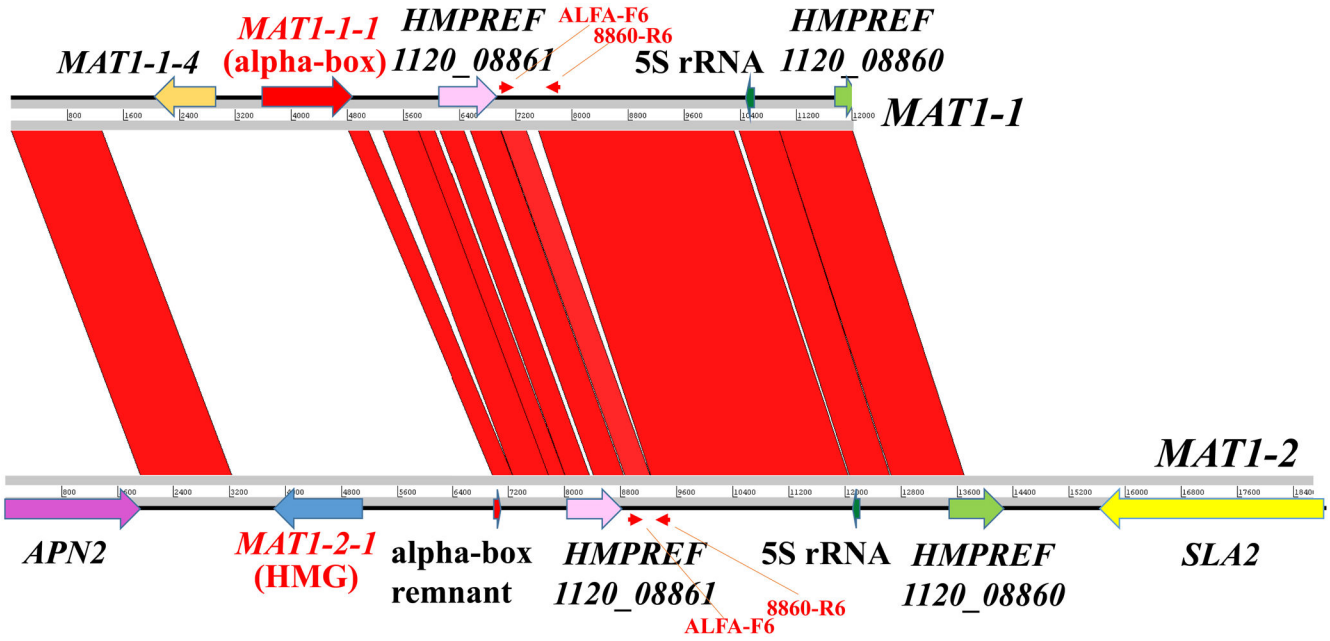

Fig. 3.

Comparison of the newly determined *MAT1-1* locus of *E. dermatitidis* CBS 132752 and the *MAT1-2* locus from the CBS 525.76 genome using the Artemis comparison tool. Sequencing of the long-range PCR product revealed the presence of the α-box gene and the *MAT1-1-4* gene in addition to the *5S rRNA* gene, and the genes encoding HMPREF1120_08860 and HMPREF1120_08861. The gene order was the same as in the sequenced genome of the *E. dermatitidis* isolate CBS 525.76. The *MAT* locus is located downstream of a high-similarity stretch spanning the downstream noncoding sequence of the *APN2* gene. The *MAT* locus ends at the 3′ terminus of the α-box (*MAT1-1-1*) gene, with a 116-bp remnant in the *MAT1-2* locus. Insertions/deletions were noted in the two isolates in the vicinity of the gene coding for HMPREF1120_08861. The binding sites for the *MAT1-1* screening primers APN2-F3 and ALFA-R4, and the insertion/deletion-typing primers ALFA-F6 and 8860-R6 are indicated by small arrows. The red blocks indicate stretches of high-similarity regions (92%–98%).

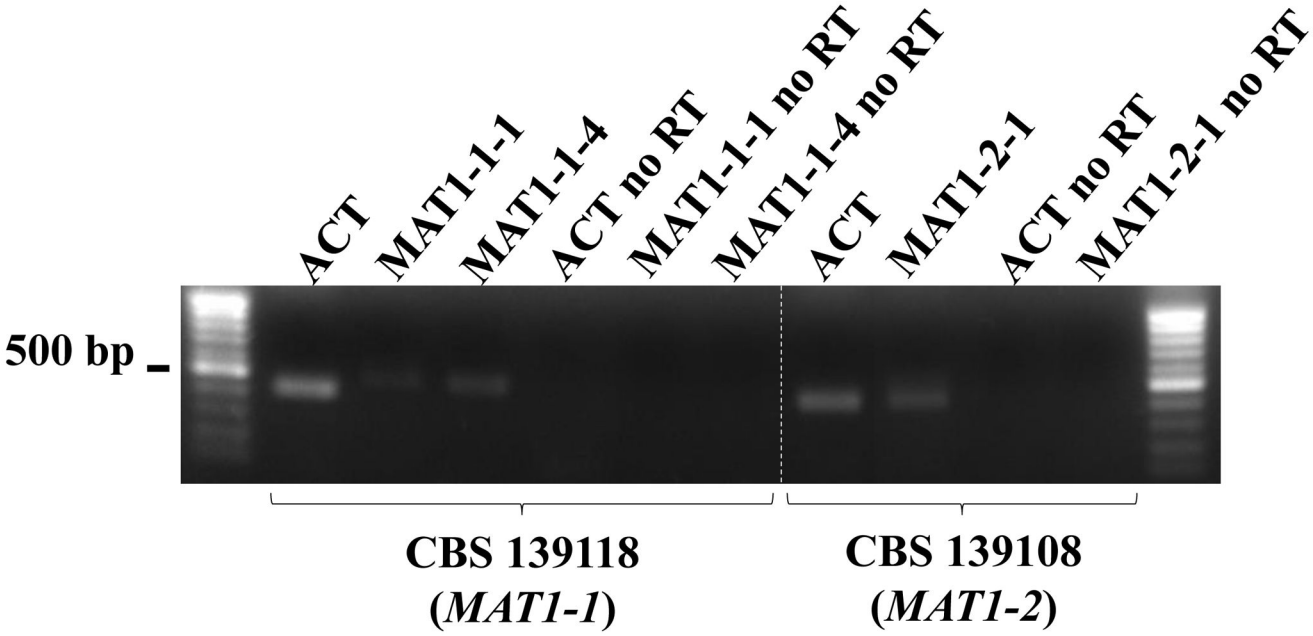

Fig. 4.

Expression analysis of the *MAT* genes using RT-PCR. A 415-bp actin (indicated as ACT) PCR product is observed in both isolates as the constitutively expressed positive control. The 455 bp-long *MAT1-1-1* and the 429 bp-long *MAT1-1-4* gene products can be observed with expression levels lower than actin in the *MAT1-1* isolate CBS 139118. Similarly, the *MAT1-2-1* gene product with a length of 408 bp is observed in the *MAT1-2* isolate CBS 139108. The absence of a product in the controls lacking RT indicates that the cDNA synthesis took place without any contaminating DNA.

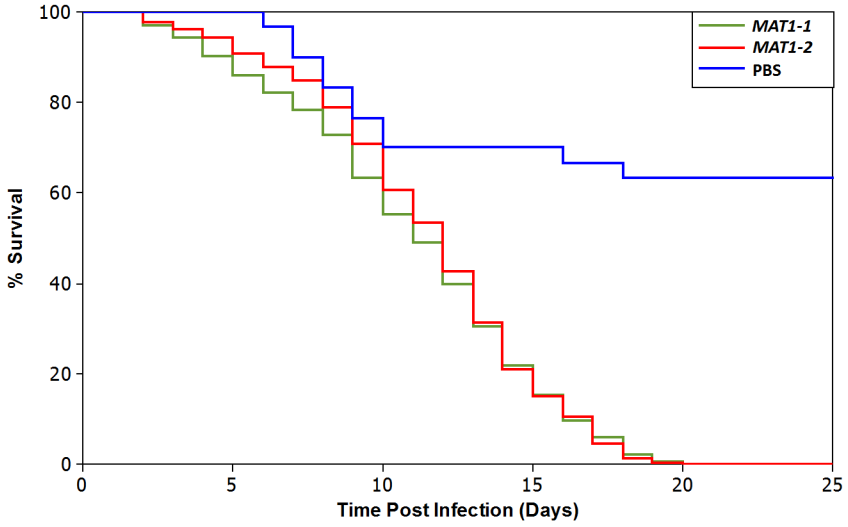

Fig. 5.

G. mellonella survival after larval infection with *E. dermatitidis*. A comparison of larval survival upon infection with different mating type isolates: *MAT1-1* (green) and *MAT1-2* (red). The PBS control is represented by blue color. The larvae were infected with 1×10^6 cells, and animal survival at 37°C was monitored.

Table 1.

E. dermatitidis isolates used in the study

Isolate	Gen.	Origin	Substrate of isolation	Country	*MAT* genotype	ins/del region, bp
CBS 132751*	A	Environmental	Bathroom wall	Turkey, Mersin	*MAT1-1*	722
CBS 132752*	A	Environmental	Dishwasher	Turkey, Mersin	*MAT1-1*	722
CBS 132753*	B	Environmental	Dishwasher	Turkey, Mersin	*MAT1-1*	722
CBS 132754*	B	Environmental	Bathtub	Turkey, Mersin	*MAT1-1*	722
CBS 132755	C	Environmental	Dishwasher	Turkey, Mersin	*MAT1-2*	561
CBS 137219	A	Environmental	Oak-wood	Turkey, Afyon	*MAT1-1*	722
CBS 137220*	A2	Environmental	Oak-wood	Turkey, Osmaniye	*MAT1-2*	722
CBS 139108*	A	Environmental	Dishwasher	Turkey, Erzurum	*MAT1-2*	561
CBS 139110*	A	Environmental	Dishwasher	Turkey, Osmaniye	*MAT1-1*	722
CBS 139113*	A2	Environmental	Dishwasher	Turkey, Mersin	*MAT1-1*	722
CBS 139114*	A3	Environmental	Dishwasher	Turkey, Izmir	*MAT1-1*	722
CBS 139116*	A3	Environmental	Dishwasher	Turkey, Sivas	*MAT1-2*	561
CBS 139118*	B	Environmental	Dishwasher	Turkey, Eskisehir	*MAT1-1*	722
CBS 139119*	B	Environmental	Dishwasher	Turkey, Sanliurfa	*MAT1-1*	722
CBS 139120*	C	Environmental	Dishwasher	Turkey, Adana	*MAT1-2*	561
CBS 139122*	C	Environmental	Dishwasher	Turkey, Sivas	*MAT1-2*	561
CBS 149.90*	A3	Clinical	Cystic fibrosis	Germany	*MAT1-1*	722
CBS 424.67*	A	Clinical	-	Germany	*MAT1-2*	561
CBS 639.96	A	Clinical	-	France	*MAT1-1*	722
CBS 686.92*	A	Clinical	Blood culture	Germany	*MAT1-1*	722
CBS 109154*	A	Clinical	Brain infection	S. Korea	*MAT1-1*	722
CBS 115663*	B	Clinical	Endotracheal asp.	Qatar	*MAT1-1*	722
CBS 120429*	A	Clinical	Cystic fibrosis	Finland	*MAT1-2*	561
CBS 120470	-	Clinical	Eye	USA	*MAT1-1*	722
CBS 120473*	A	Clinical	Brain infection	USA	*MAT1-1*	722
CBS 120562*	-	Clinical	Eye	USA	*MAT1-1*	722
CBS 120567	B	Clinical	Eye	USA	*MAT1-2*	722
152.02#*	-	Clinical	-	USA		
154.02#*	-	Clinical	-	USA		
170.02#*	-	Clinical	-	USA		
189.03#*	-	Clinical	-	USA		
A7*	A	Environmental	Dishwasher	Turkey, Adana	*MAT1-2*	722

Isolate	Gen.	Origin	Substrate of isolation	Country	*MAT* genotype	ins/del region, bp
A8*	A	Environmental	Dishwasher	Turkey, Ankara	*MAT1-1*	722
A9*	A	Environmental	Dishwasher	Turkey, Adana	*MAT1-2*	722
A10*	A	Environmental	Dishwasher	Turkey, Erzurum	*MAT1-2*	722
A11*	A	Environmental	Dishwasher	Turkey, Burdur	*MAT1-1*	722
A12*	A	Environmental	Dishwasher	Turkey, Mersin	*MAT1-2*	561
A13*	A	Environmental	Dishwasher	Turkey, Gaziantep	*MAT1-1*	722
A14*	A	Environmental	Dishwasher	Turkey, Mersin	*MAT1-1*	722
A15*	A	Environmental	Dishwasher	Turkey, Isparta	*MAT1-2*	722
A16*	A	Environmental	Dishwasher	Turkey, Osmaniye	*MAT1-2*	722
A2-17*	A2	Environmental	Dishwasher	Turkey, Mersin		
A2-18*	A2	Environmental	Dishwasher	Turkey, Mersin	*MAT1-1*	722
A2-19*	A2	Environmental	Dishwasher	Turkey, Ankara	*MAT1-1*	722
A2-20*	A2	Environmental	Dishwasher	Turkey, Sivas	*MAT1-2*	722
A2-21*	A2	Environmental	Dishwasher	Turkey, Erzurum	*MAT1-2*	561
A2-22*	A2	Environmental	Dishwasher	Turkey, K. Maras	*MAT1-2*	561
A2-23*	A2	Environmental	Oak-wood	Turkey, Antalya	*MAT1-1*	722
A2-25*	A2	Environmental	Oak-wood	Turkey, Istanbul	*MAT1-2*	561
A2-26*	A2	Environmental	Oak-wood	Turkey, Izmir	*MAT1-2*	561
A2-27*	A2	Environmental	Oak-wood	Turkey, Istanbul	*MAT1-1*	722
A3-33*	A3	Environmental	Dishwasher	Turkey, Sivas	*MAT1-1*	722
A3-34*	A3	Environmental	Dishwasher	Turkey, Kars	*MAT1-1*	722
A3-35*	A3	Environmental	Dishwasher	Turkey, Erzurum	*MAT1-2*	722
A3-36*	A3	Environmental	Dishwasher	Turkey, Sivas		
A3-37*	A3	Environmental	Dishwasher	Turkey, Mersin	*MAT1-2*	561
A3-38*	A3	Environmental	Oak-wood	Turkey, Mersin	*MAT1-1*	722
A3-39*	A3	Environmental	Oak-wood	Turkey, Adana	*MAT1-1*	722
A3-40*	A3	Environmental	Oak-wood	Turkey, Sivas	*MAT1-1*	722
A3-42*	A3	Environmental	Oak-wood	Turkey, Mersin	*MAT1-2*	722
A3-44*	A3	Environmental	Oak-wood	Turkey, Adana	*MAT1-2*	722
B-49*	B	Environmental	Dishwasher	Turkey, Burdur	*MAT1-1*	722
B-50*	B	Environmental	Dishwasher	Turkey, Eskisehir	*MAT1-1*	722
B-51*	B	Environmental	Dishwasher	Turkey, Ankara	*MAT1-2*	722
B-52*	B	Environmental	Dishwasher	Turkey, Antalya	*MAT1-2*	722

Isolate	Gen.	Origin	Substrate of isolation	Country	*MAT* genotype	ins/del region, bp
B-53*	B	Environmental	Dishwasher	Turkey, Erzurum	*MAT1-2*	722
B-55*	B	Environmental	Dishwasher	Turkey, Izmir	*MAT1-2*	722
B-56*	B	Environmental	Dishwasher	Turkey, Sanliurfa	*MAT1-2*	722
B-57*	B	Environmental	Dishwasher	Turkey, Sivas	*MAT1-2*	722
B-58*	B	Environmental	Dishwasher	Turkey, Mersin	*MAT1-2*	722
C-67*	C	Environmental	Dishwasher	Turkey, Adana	*MAT1-2*	722
C-68*	C	Environmental	Dishwasher	Turkey, Ankara	*MAT1-2*	561
C-69*	C	Environmental	Dishwasher	Turkey, Ankara	*MAT1-1*	722
C-71*	C	Environmental	Dishwasher	Turkey, Sivas	*MAT1-2*	722
C-72*	C	Environmental	Dishwasher	Turkey, Sivas	*MAT1-2*	722
C-73*	C	Environmental	Dishwasher	Turkey, Mersin	*MAT1-2*	561
C-75*	C	Environmental	Oak-wood	Turkey, Mersin	*MAT1-2*	561
C-76*	C	Environmental	Oak-wood	Turkey, Adana	*MAT1-1*	722
C-77*	C	Environmental	Oak-wood	Turkey, Afyon	*MAT1-2*	561
C-78*	C	Environmental	Oak-wood	Turkey, Afyon	*MAT1-2*	561

* used in *G. mellonella* virulence assay

Table 2.

Distribution of *MAT* idiomorphs among the *E. dermatitidis* isolates

	Total	*MAT1-1*		*MAT1-2*	
		#	%	#	%
Total isolates	74	35	47.3	39	52.7
Environmental isolates	63	27	42.9	36	57.1
Clinical isolates	11	8	72.7	3	27.3
Genotype A total (A+A2+A3)	44	24	54.5	20	45.5
Genotype B	15	7	46.7	8	53.3
Genotype C	13	2	15.4	11	84.6
Unknown genotype	2	2	100.0	0	0