



## Mycologic Forum

## Sexual variability in *Histoplasma capsulatum* and its possible distribution: What is going on?



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## ARTICLE INFO

## Article history:

Received 27 August 2013

Accepted 1 October 2013

Available online 18 November 2013

## Keywords:

*Histoplasma capsulatum*

*MAT1* locus

Idiomorphs

Genetic diversity

Latin America

## ABSTRACT

*Histoplasma capsulatum* is a dimorphic fungal pathogen naturally found in the soil. Inhalation of conidia can result in pulmonary histoplasmosis and, in some cases, causes severe disseminated disease and death. This fungus is an ascomycete that has an anamorphic or asexual stage and a teleomorphic or sexual stage, known as *Ajellomyces capsulatus*, which results from (+) and (–) mating types. Sexual reproduction is regulated by a specialized genomic region known as the mating-type (*MAT1*) locus. The mating process in this heterothallic species is represented by isolates that contain only one of the two different *MAT1* locus idiomorphs (*MAT1-1* or *MAT1-2*) that have unrelated sequences encoding different transcription factors. In medically important dimorphic pathogens and in most ascomycete molds, one *MAT* locus idiomorph encodes a high-mobility-group (HMG) box-domain transcription factor, and the other idiomorph encodes an alpha-box domain transcription factor. There is scarce molecular information about *H. capsulatum* mating type although recombinant population structures have been reported that could occur in nature and this process has been documented in distinct models such as parasites and other fungi. In this review, we shall focus on published studies on *H. capsulatum* sexuality, and outline the distribution of the two *H. capsulatum* mating types in Latin America.

This manuscript is part of the series of works presented at the “V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi” (Oaxaca, Mexico, 2012).

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### Variabilidad sexual de *Histoplasma capsulatum* y su posible distribución: fundamento

## RESUMEN

*Histoplasma capsulatum* es un patógeno fúngico, dimórfico que habita en suelos ricos en materia orgánica. La inhalación de los conidios puede inducir histoplasmosis pulmonar y, en algunos casos, enfermedad diseminada grave y la muerte. Este ascomiceto caracterizado por un estadio anamórfico asexual y un estado teleomórfico o sexual, conocido como *Ajellomyces capsulatus*, que es consecuencia de los tipos de apareamiento (*MAT+* y *MAT–*) (*mating-type*, por sus siglas en inglés). La reproducción sexual está regulada por una región genómica especializada, conocida como *locus MAT1*. El proceso de apareamiento en esta especie heterotática (o autoincompatible) está representado por aislamientos que solo contienen uno de los 2 diferentes idiomorfos del locus *MAT1* (*MAT1-1* y *MAT1-2*), que tienen secuencias muy distintas que codifican diferentes factores de transcripción. En los patógenos dimórficos importantes desde un punto de vista médico y en la mayoría de los ascomicetos filamentosos, un idiomorfo del locus *MAT* codifica el dominio-caja HMG (*high-mobility-group*, por sus siglas en inglés) de un factor de transcripción, y el otro idiomorfo codifica el dominio-caja alfa de otro factor de transcripción. Apenas disponemos de información molecular sobre el *mating type* de *H. capsulatum*, aunque se ha descrito que en la naturaleza podrían estar presentes estructuras de población recombinante. Este proceso se ha documentado en distintos

## Palabras clave:

*Histoplasma capsulatum*

*MAT1* locus

Idiomorfos

Diversidad genética

Sudamérica

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modelos como parásitos y otros hongos. En esta revisión nos hemos centrado en los estudios publicados sobre la sexualidad de *H. capsulatum*, y hemos abordado la distribución de los 2 mating type de *H. capsulatum* en Sudamérica.

Este manuscrito forma parte de la serie de artículos presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

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Pleomorphism is a characteristic normally found in dimorphic fungal pathogens of the family Ajellomycetaceae represented by asexual and/or sexual reproduction.<sup>35,40</sup> Asexual reproduction in species of this fungal family is represented by hyphal fragmentation or conidia production in specialized hyphae or by budding in the yeast form. For the sexual cycle, the first step of mating in members of the Ajellomycetaceae family is ascocarps production by hyphal constriction of different mating type cells coiling to one another several times until reaching ascocarps formation. After hyphal aggregations, the cleistothecium is formed with ascus, harboring meiosporic spores.<sup>20</sup> Sexual reproduction, followed by meiosis and recombination, is a powerful event in eukaryotes in terms of evolution by increasing genetic diversity and, in some cases, generating novel genotypes with altered virulence.<sup>14,25,28</sup> Populations of the pathogenic fungus *Histoplasma capsulatum* have been shown to recombine in nature.<sup>4</sup> This process has been documented in other models, such as the parasite *Toxoplasma gondii*,<sup>13,34</sup> and may have occurred also in the fungus *Cryptococcus gattii* during the Vancouver Island outbreak.<sup>8</sup>

Understanding the structure of fungal mating type loci is important to clarify the evolutionary processes involving these genomic structures.<sup>3,10,16,38</sup> Sexual reproduction is regulated by a specialized genomic region known as the mating type (*MAT1*) locus that usually contains genes for one or more transcription factors with structural motifs.<sup>6,7,9,39</sup> The *MAT* locus has two idiomorphic regions; the *MAT1-1* idiomorph encodes an alpha-domain transcription factor, whereas the *MAT1-2* idiomorph encodes a high-mobility-group (HMG) domain transcription factor. These domains confer sexual identity to a haploid cell<sup>6,11</sup>; the alpha domain (*MAT1-1*) or HMG domain (*MAT1-2*) idiomorphs determine (+) and (–) strains, respectively.

Despite the available information regarding *MAT1* locus structures, there is scarce information regarding the Onygenales, one of the most medically important order of fungi, including a number of dimorphic primary pathogens, such as *H. capsulatum*, *Paracoccidioides brasiliensis*, *Paracoccidioides lutzii*, *Coccidioides immitis*, and *Coccidioides posadasii*, all capable of causing life-threatening systemic mycoses.<sup>5,41</sup> The idiomorphs *MAT1-1* and *MAT1-2* are responsible for fungal sexuality and control of mating in these species, which show syntenic organization.<sup>12,26</sup>

Based on molecular phylogenetic tools, *H. capsulatum* is thought to represent multiple cryptic species and vast genetic diversity has been detected among *H. capsulatum* isolates even from the same geographic region.<sup>17,29</sup> Recombination events have been shown within this species,<sup>2,18</sup> and could explain their high phenotypic and genotypic diversities. Recombination between two strains can result in a new strain. Populations of the pathogenic fungus *H. capsulatum* have been shown to recombine in nature.<sup>4</sup>

### State of the art of sexual reproduction of *H. capsulatum*

*H. capsulatum* is a heterothallic ascomycete that has an anamorphic or asexual stage, and a teleomorphic or sexual stage known as *Ajellomyces capsulatus*, which temporarily exhibits the dikaryotic and diploid phases that form haploid ascospores after meiotic reductions.<sup>23</sup> Thus, the species *H. capsulatum* and *A. capsulatus*

constitute the same holomorphic organism. The heterothallic bipolar mating system of *H. capsulatum* was first described in early the 1970s, with strains designated either (+) or (–),<sup>18,19</sup> representing one of the first discoveries of its sexual structures. Mating between freshly isolated (+) and (–) strains occurs in the mycelial phase and has been demonstrated in vitro<sup>20</sup>; however, in this environment the fertility is lost rapidly during continuous culture, suggesting that selective pressures may serve to maintain fecundity in their natural habitat.<sup>24</sup> It has been suggested that strains of each mating type are not equally represented among clinical isolates of *H. capsulatum*.<sup>23</sup> Environmental samples exhibit a 1:1 ratio of the two mating types; in clinical specimens a ratio of 7:1 (–/+) has been observed.<sup>20,24</sup> However, murine infection experiments revealed similar virulence potentials between isolates of opposite mating types, suggesting that (+) and (–) isolates may differ in the ability to produce infectious propagules due to an increased ability to convert from hypha to yeast.<sup>21,24</sup> How the mating type could participate in the regulation process of dimorphic transition and increase virulence is not clear yet.

The *H. capsulatum* mating type characterization was performed based on the similarities among the *MAT* locus of several ascomycetes, and this feature has allowed for the identification of a possible *MAT* locus in organisms whose genomes have been sequenced. The predicted *MAT1* locus, identified in *H. capsulatum*, shared common characteristics with the *MAT* locus in other ascomycete fungi.<sup>6,12,31,33,39</sup>

Based on *H. capsulatum* genomic sequence databases it was possible to perform the syntenic analysis of the *MAT1* locus. *MAT1-1* idiomorphic region was identified in *H. capsulatum* strains G217B and WU24, whereas *MAT1-2* idiomorphic region was identified in the strain G186AR allowing the development of a mating type-specific PCR assay. Two clinical isolates of opposite genotypic mating type, UH1 and VA1, were found to be compatible and also displayed mating-type-dependent regulation of the *MAT* transcription factors in response to extracts predicted to contain mating pheromones.<sup>2</sup> Microscopic observations of *H. capsulatum* mycelia revealed several structures consistent with mature mating events.

### Evolution of the *MAT* locus in dimorphic fungi

The mating type (*MAT*) locus controls sexual reproduction of fungi in a specialized region of the genome encoding key transcriptional regulators participating in regulatory networks to specify cell identity.<sup>9,10</sup> From an evolutionary perspective and considering the role of fungi as pathogens, the structure of the *MAT* locus of the order Onygenales was analyzed by Fraser et al.<sup>11</sup> Their findings provide molecular descriptions of the mating type locus for three thermally dimorphic fungal species primary pathogens of humans, *H. capsulatum*, *C. immitis*, and *C. posadasii* by combining bioinformatics analysis of genomes, direct sequence confirmation, PCR analysis of multiple isolates of each species, and comparative analyses with other fungi of the phylum Ascomycota. This report has revealed that all three species have canonical euascomycete bipolar *MAT* locus structures, encoding either an HMG domain or  $\alpha$ -box domain transcription factor at this locus.<sup>11</sup> A sexual cycle has been previously described only for *H. capsulatum*.<sup>18,19</sup>

The demonstration that the *MAT* locus of *C. immitis* and *C. posadasii* is arranged in a similar way permits to predict that the sexual cycle will also be heterothallic in these two pathogenic species. More recently, Torres et al.<sup>37</sup> and Li et al.<sup>27</sup> identified the presence and expression of *MAT1-1* and *MAT1-2* in the *P. brasiliensis* phylogenetic species complex and in *Blastomyces dermatitidis*, respectively. Also, a comparative sexual genomic analysis in the teleomorph *A. capsulatus* (anamorph *H. capsulatum*), *A. dermatitidis* (anamorph *B. dermatitidis*), and *Paracoccidioides* species was achieved *in silico*, supporting the existence of a sexual cycle in species of the genus *Paracoccidioides*.<sup>36</sup> The genomic identification of the mating components in these fungi suggests that the genetic apparatus for sexual reproduction has been conserved and vertically transferred among these species.<sup>36</sup> The studies revealing the molecular structure of the *MAT* locus, i.e. the two *MAT* idiomorphs encode either an HMG domain or an  $\alpha 1$ -box transcription factor similar to those of other eukaryotic fungal species, clarify the molecular nature of the *MAT* locus in dimorphic fungi, providing considerable insight into the evolution of mating type loci in fungi. Several types of genes have previously been found to be present in mating type loci, including those encoding key transcriptional regulators of two classes, the homeodomain proteins (as a pair of interacting proteins of HD1 and HD2 classes), and the HMG domain and  $\alpha 1$ -box proteins. However, the first hypothesis about sex determining regions of the genome was reported originally as autosomal genes that were incorporated into either a mating type locus or a sex chromosome, which then expanded.<sup>30</sup> Four genes are present as  $\alpha 1$  and  $\alpha 2$  in the *MTL $\alpha$*  locus and  $a 1$  and  $a 2$  in the *MTL $\alpha$*  locus in the *Candida albicans* model (hemiascomycetes).<sup>15,39</sup> Comparison using phylogenetic tools between *Saccharomyces cerevisiae* and *sensu stricto* lineages from a common ancestor in which all four genes were present demonstrated that the HMG domain protein  $a 2$  has been lost.<sup>3</sup>

Several important remarks have been made regarding that, in the eukaryotic lineage, HMG domain and  $\alpha 1$ -box domain genes are found in the two opposite *MAT* idiomorphs, but homeodomain genes are not present. In contrast, in the basidiomycete lineage, paired homeodomain genes are present in the *MAT* locus of all species analyzed up to now (*Ustilago maydis*, *Ustilago hordei*, *Schizophyllum commune*, *Coprinus cinereus*, *Cryptococcus neoformans*, and *C. gattii*), but HMG domain or  $\alpha 1$ -box genes are not found.<sup>1</sup> A very important hypothesis is that there are two ancestral fungal sex determinants (one is paired HD1/HD2 homeodomain genes and the other is the  $\alpha 1$ -box and HMG domain pair) and that one or the other or both have been lost from, or acquired by, the *MAT* locus in different lineages. In some cases, subsequent gene loss events have reduced the number from four to three of these genes, such as in *S. cerevisiae* and related species,<sup>3</sup> and as shown for non-*MAT* HMG genes in *U. maydis*.<sup>1</sup> Possibly, additional sex determining genes present in the *MAT* locus of some species still remain under the control of the *MAT* locus instead of being located elsewhere in the genome of other species. Thus, homeodomain genes essential for cell identity and sexual reproduction remain to be defined in eukaryotes, and HMG domain and  $\alpha 1$ -box proteins remain to be defined in members of the phylum Basidiomycota.<sup>1</sup>

## Mating type of *H. capsulatum*

*Distribution of the two mating types among H. capsulatum isolates obtained from an urban histoplasmosis outbreak*

In a previous study by Kwon-Chung et al.,<sup>22</sup> sexual compatibility was determined in 28 *H. capsulatum* isolates obtained from histoplasmosis outbreaks that occurred in Indianapolis, United States of America, in which the (–) sexual compatibility type predominated.

The overall ratio of (+) and (–) types was 1:2.5. However, the frequency and genetic diversity of the (+) and (–) mating types reported are not necessarily representative of other geographical areas, since *H. capsulatum* isolates exhibit a wide distribution and a significant genetic diversity, as has been documented in Latin America.<sup>17,29</sup>

## Frequency of *H. capsulatum* mating type between Brazil and Mexico

Our group, in a recent work about sexuality of *H. capsulatum* isolates from Mexico (North America) and Brazil (South America), determined preliminarily the frequency and genetic diversity of the sexual compatibility types of this fungus in these two distant geographical areas. The isolates were obtained from human clinical cases, naturally infected bats and other animals, and contaminated soils from the natural niche of *H. capsulatum*. PCR was applied to analyze the *MAT1-1* and *MAT1-2* idiomorphic regions of the two mating types, (+) and (–), associated with the *MAT1* locus of *H. capsulatum*. PCR products of each *MAT1* idiomorphic region were sequenced and aligned, using the sequences of the G-217B (+) and G-186AR (–) strains as references. Most *H. capsulatum* isolates from Mexico were of the *MAT1-2* genotype, and a totally different situation was observed for the Brazilian isolates with *MAT1-1* genotype predominance. *H. capsulatum* mating types were associated with the geographical origin of the isolates, and all isolates from Brazil correlated with their environmental sources.<sup>32</sup>

## Conclusion

The present mini-review highlights the main findings associated with the mating types in *H. capsulatum*, emphasizing their possible usefulness as a molecular geographic marker based on their significant polymorphism.

## Conflict of interests

The authors have no conflict of interests.

## Acknowledgments

This work was funded by the CNPq-Brazil (Proc: 490110/2009-6)-CONACYT-Mexico (Ref: B330/099/11), the Bilateral Collaboration Agreement, and by DGAPA-UNAM (PAPIIT IN204210), and FAPERJ E-26/111.532/2010-Rio de Janeiro, Brazil. RMZO is supported in part by CNPq 350338/2000-0. CNS is supported by INOVATEC FIOCRUZ/FAPERJ.

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