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Introduction

Sexual reproduction in *C. tropicalis* → regulated by *MTL* loci.

Homocytotic *MTL*_a cells mate with homocytotic *MTL*_α cells forming *MTL*_{a/α} cells.

Candida biofilm facilitates the homocytotic opaque cell mating.



The aim of this study: to find a correlation between the mating type and biofilm-forming ability in 28 *C. tropicalis* clinical isolates.

Materials and Methods

Biofilm-forming ability, 37 °C, 24 and 96 h:

96-well microtiter polystyrene plate, RPMI-1640 medium.

OD_{490nm} and Slime Index (SI) (Biofilm OD_{492nm}/Growing culture OD_{492nm} %).



Filamentation level



Biofilm microscopic observations.

MTL genes:

RNA isolation: Illustra RNAs isolation Kit (GE Healthcare®)

cDNA formation: iScript cDNA Synthesis (BioRad®).

RTPCR: - 3 sec, 95°C
- 35 cycles:
30 sec, 95°C
30 sec, 55°C
45 sec, 72°C
- 10 min, 72°C

Statistic studies: SPSS11.5 for Windows; Mann-Whitney test for statistic significance.

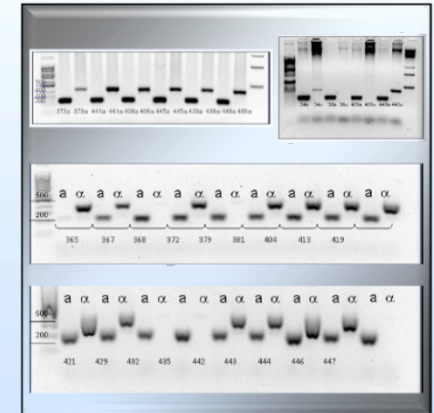
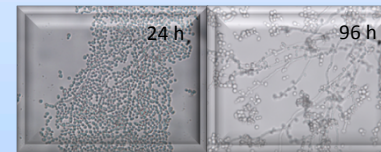
Results

(i) *MTL* genes in 28 clinical isolates:
72.4% *MTL*_{a/α}

27.6% { 87.5% *MTL*_{a/-}
12.5% *MTL*_{-/α}

(ii) Biofilm formation (OD₄₉₀) was lower in homozygotic cells (0.40 vs 0.49 for 24 h, p=0.53; 0.39 vs 0.48 for 96 h, p=0.26).

(iii) Slime Index was lower in homozygotic cells (44.34 vs 51.88 for 24 h, p=0.43; 38.49 vs 44.85 for 37 °C and 96 h, p=0.33).



(iv) The highest level of filamentation was reached mostly for 96 h.

Homozygotic cells presented lower level of filamentation than the heterozygotic ones.

Conclusions

Our study shows high presence of homozygotic-*MTL C. tropicalis* in clinical isolates.

Heterozygotic-*MTL* strains had higher level of filamentation and biofilm-forming ability than the homozygotic ones.

Once biofilm is formed by heterozygotic (non-mating competent) cells, a small homozygotic population can switch to opaque form, being mating possible, with formation of recombinant cells.

Future studies with more *C. tropicalis* strains must be done to investigate whether statistical significance can be achieved, and to assess clinical significance between *Candida* genetic interchange and the increased pathogenicity and resistance.

References

- Porman *et al.* MTL-independent phenotypic switching in *Candida tropicalis* and a dual role for Wor1 in regulating switching and filamentation. PLoS Genet. 2013; 9:e1003369. Epub 2013 Mar 21.
- Lockhart *et al.* Cell biology of mating in *Candida albicans*. Eukaryot Cell. 2003; 2: 49-61.
- Magee *et al.* Induction of mating in *Candida albicans* by construction of *MTL*_a and *MTL*_α strains. Science. 2000; 289: 310-313.

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