IN SILICO ANALYSIS OF MEMBER OF CTR FAMILY AND COPPER DEPENDENT TRANSCRIPTIONS FACTOR IN *Paracoccidioides spp*.

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INTRODUCTION

Copper is an essential cofactor for many enzymes that act on important metabolic processes in microorganisms, such as *Paracoccidioides spp*. The perfect balance of copper inside fungal cell is very important for survive, growth an virulence of that pathogen. The aim of this study was to find the sequence homology in *Paracoccidioides lutzzi* (isolate *Pb*01) and *Paracoccidioides brasiliensis* (isolate *Pb*18) with member of CTR family proteins and copper dependent transcription factor CUF1 as well as ACE1 and MAC1, and make a detailed structural and functional analysis between them.

MATERIALS AND METHODS

The study data were obtained by an analysis in silico using tools like BLAST (Basic Local Alignment Search toll), InterPro (Protein Sequence analysis and classification), Clustalx and I-Tasser. Was used sequence of *Aspergillus nidulans* (taxid:162425) and/or *Saccharomyces cerevisiae* (taxid:559292) how model for search homology sequence of CTR Family, Ace1 and Mac1 transcription fator on *Paracoccidioides lutzzi* (*Pb01*) (taxid:502779) and *Paracoccidioides brasiliensis* (*Pb*18) (taxid:502780).Table and figures were used to show the results, as well as statistics data (e-value, ID, others).

RESULTS

Table 1. Structural comparison between CTR3 protein in *Saccharomyces cerevisiae* and putative proteins in *Pb*01 (PAAG_05251) and *Pb*18 (PADG_05084).

Estrutura	CTR3 S. cerevisiae	CTR Pb01	CTR Pb18
Presence of 03 transmembrane domains (TDM)	Present	Present	Present
Presence of one metionine 20 aa upstream at the TDM1	Present	Present	Present
% of cysteine	4.50%	2.23%	2.20%
Mets motif upstream at the TDM1	None	MXXM Upstream TDM1	MXXMXXM Upstream TDM1
Mets motif on TDM2	MLLFM	MLLAM	MLLAM
N° of aa	241	179	182

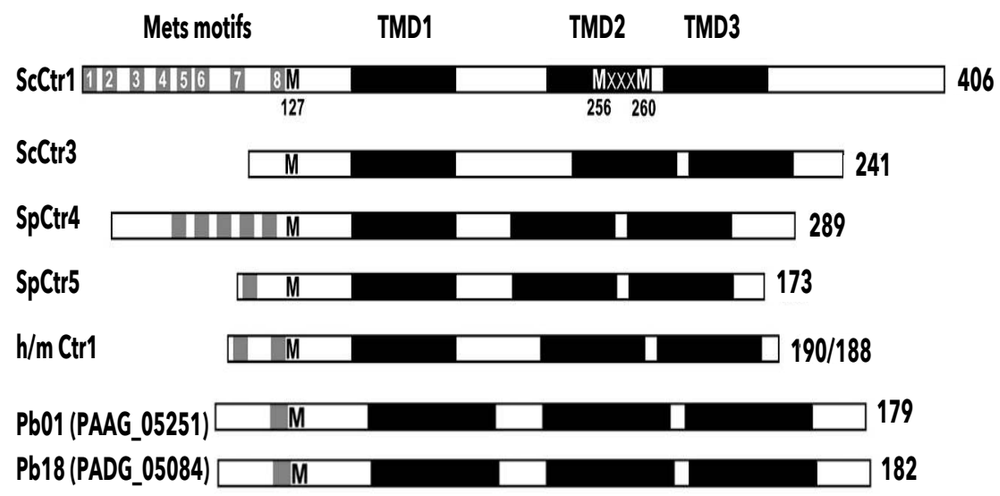
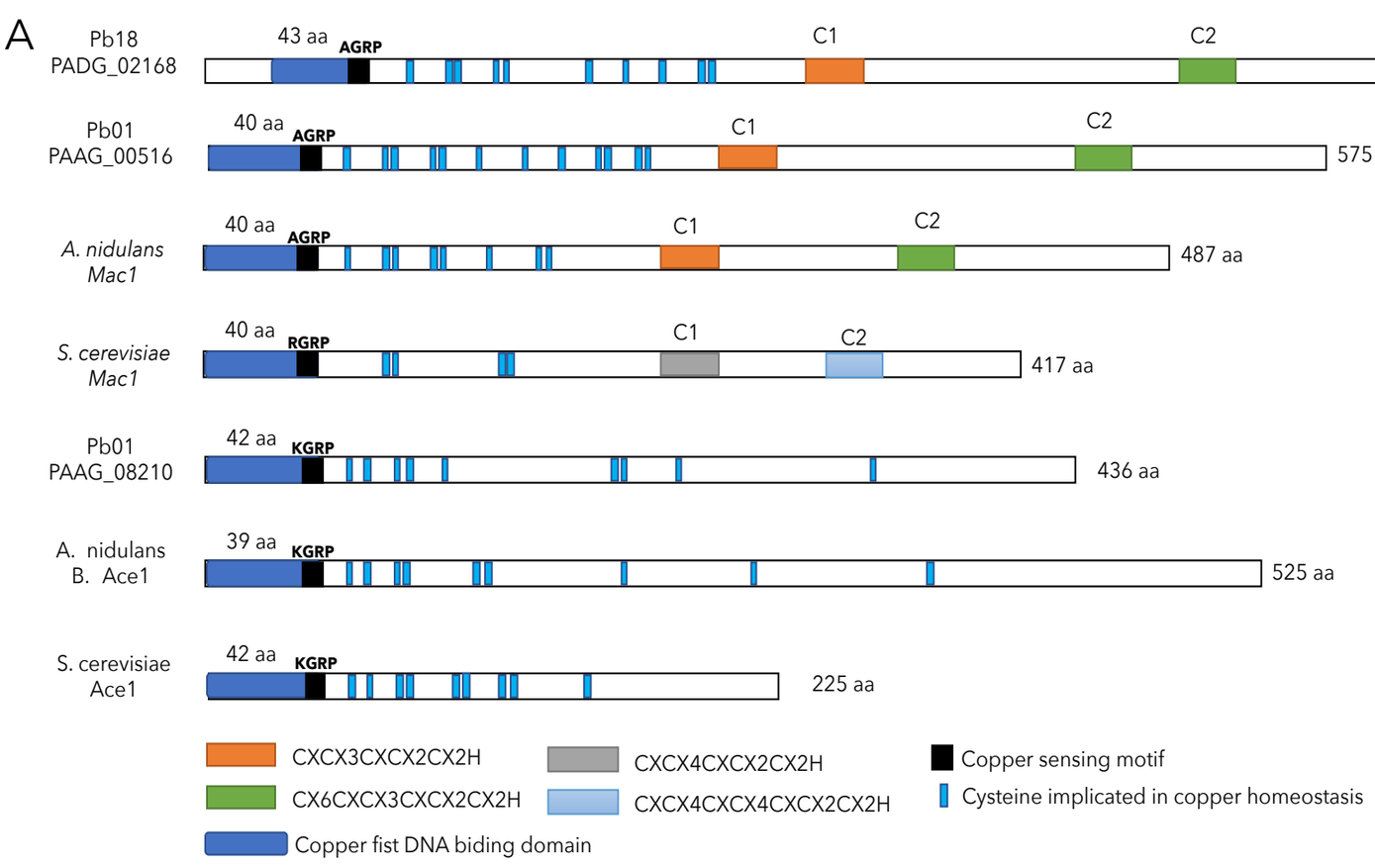


Figure 1. Comparasion between the primary structure model of CTR high affinity copper transport proteins proposed for CTR (CTR1, CTR3, CTR4 and CTR5) family in *Saccharomyces cerevisiae (Sc), Schizosaccharomyces pombe (Sp)* and human/mouse (h/m) and the model proposed for *Pb*01 and *Pb*18 after in silico analysis.



B

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PADG_02168_Pb18 384 CVCGDDCQCLGCASH 399
PAAG_00516_Pb01 354 CVCGDDCQCLGCASH 369
A.nidulans_Mac1 338 CSCGPQCSCLACPDH 353

PADG_02168_Pb18 515 CTNMTGTCQCGINCSCVGCLTH 537
PAAG_00516_Pb01 485 CTNMTGTCQCGINCSCVGCLTH 507
A.nidulans_Mac1 462 CAGEAGNCQCGPSCSCLGCLTH 484
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C

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PAAG_00516_Pb01      MPLDEEGAKWS----------------------------CEPCVRGHRSSKCQHFDRL 30
PADG_02168_Pb18]     MPLDEEGAKWSWYVSRPILCPYSIHYQQHEQPFDSQGNCLSSEPCVRGHRSSKCQHFDRL 60
A.nidulans_Mac1      MPFDENGVKWS----------------------------CEPCIRGHRSSKCAHYDRL 30
S.cerevisiae_Mac1    -MIIFNGNKYA----------------------------CASCIRGHRSSTCRHSHRM 29
                       :  :* *::                            . .*:******.* * .*:

PAAG_00516_Pb01      MMKVPKAGRPLAKCPHPKGTCSCQKLYAFMVRIPKGSSCLCRPLYQVPMVVTESGTSGKP 90
PADG_02168_Pb18]     MMKVPKAGRPLAKCPHPKGTCSCQKLYAFMVRIPKGSSCLCRPLYQVPMVVTESGTSVKP 120
A.nidulans_Mac1      MVSVGKAGRPLSKCPHVEGSCNCKKLGAFMVAIPKGSTCLCRPVYKMILGENGPTPAPLD 90
S.cerevisiae_Mac1    LIKVRTRGRP-----------------------------------------SPMA 43
                     ::.* . ***                                          .:
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D

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PAAG_08210_Pb01     -MLIKGEKWACEACIRGHRVSNCQHADRPLSHINKKGRPVSQCPHCRGLRKARTTHTKCE 59
A.nidulans_Ace1     -MLIDGEKWACEACVRGHRVSSCHHNDRPLTHINKKGRPVSQCPHCRGLRKSRTTHTKCE 59
S.cerevisiae_Ace1   MVVINGVKYACETCIRGHRAAQCTHTDGPLQMIRRKGRPSTTCGHCKELRRTKN------ 54
                      ::*.* *:***:*:****.::* * * **  *.:**** : * **: **::::.
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Figure 2. A. Compararion between the primary sequence structure model for trancription fator Mac1/Ace1 from *Aspergillus nidulans* (XP_658262/CBF85835.1) and *Saccharomyces cerevisiae* (NP_013734/NP_011349) with the putative sequence found on Pb01 and Pb18 . **B.** Cysteine rich motifs C1 and C2 on Pb01 (PAAG_00516), Pb18 (PADG_02168) and Aspergillus nidulans (Xp_658262). **C**. Copper-fist-DNA-biding domains on Pb01 (PAAG_00516), Pb18 (PADG_02168), Aspergillus nidulans (XP_658262) and Saccharomyces cerevisiae (NP_013734). **D.** Copper-fist-DNA-biding domains on Pb18 (PADG_02168), Aspergillus nidulans (CBF85835.1) and Saccharomyces cerevisiae (NP_011349).

CONCLUSION

Putative sequences were found for CTR3 on *Pb*01 (PAAG_05251) and *Pb*18 (PADG_05084) with all features necessary for a CTR protein family. Still, putative sequences for transcription factor Mac1 were found on both isolates, being PAAG_00516 for P*b*01 and PADG_02168 for *Pb*18. However, for transcription factor Ace1, only one sequence reveled homology, being present on Pb01 (PAAG_08210).

FINANCIAL SUPPORT: