

TITLE: IN SILICO ANALYSIS OF A PUTATIVE MEMBER OF THE CTR FAMILY IN *PARACOCCIDIOIDES SPP.*

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ABSTRACT:

Proteins of the CTR family (CTR1, CTR3, CTR4 and CTR5), copper pickups of high affinity, act in situations of deprivation of this metal. *Saccharomyces cerevisiae* has been used as a eukaryotic model in the characterization of these proteins. This study aims to identify and characterize sequences in *Paracoccidioides spp.* (isolates *Pb01* and *Pb18*) from proteins belonging to this family. In the first step, *in silico* analysis (BLAST, PFAM and I-Tasser) was carried out, followed by a qualitative and quantitative experiment using conventional PCR and Real Time PCR from *Paracoccidioides* members in copper-depleted medium. Higher homology was detected to the CTR3 protein of *S. cerevisiae*. Both isolates, *Pb01* and *Pb18*, have the necessary factors compatible with all members of the CTR family, as three transmembrane domain, one methionine 20 aminoacid upstream of the transmembrane domain 01 and one methionine motif on transmembrane domain 02 (TMD2). Some differences were found between hypothetical protein in isolates (*Pb01* and *Pb18*) and CTR3 protein in *S. cerevisiae*. In these isolates, a methionine motif was detected in an upstream region to transmembrane domain 01 (TMD1), but not in Ctr3 of *S. cerevisiae*. On the other hand, the amount of cysteine in CTR3 of *S. cerevisiae* is twice as much in relation to the amount found in the isolates *Pb01* e *Pb18*. While CTR3 of *S. cerevisiae* presents 11 cysteine residues, making up 4.50% of total aa, *Paracoccidioides* (*Pb01* and *Pb18*) have only 2.4%. Cysteine is an important residue in the uptake of copper. In the other members of the CTR family (CTR1, CTR4 and CTR5), the relative amount of cysteine is inversely proportional to the amount of methionine motifs, another important component in the uptake of the metal. *Pb01* and *Pb18* were grown in copper-depleted medium. After analysis of the cDNA by PCR, expression of the sequences homologous to CTR3 of *S. cerevisiae* was identified. Analysis by real-time PCR revealed that in *Pb01* and *Pb18* the expression of the *S. cerevisiae* CTR3 homologous grown in medium with absence of copper was higher 3 fold in both isolates than in those cultured in rich medium in this metal (*Pb01*, $p = 0.0007$, *Pb18*, $p = 0.0009$). The sequences studied have higher homology with CTR3 in *S. cerevisiae* and the characteristics necessary to include them as candidates for members of the CTR family, however, present specific characteristics that give them particularity.

Keywords: *Paracoccidioides spp.*, CTR, Copper stress, *in silico*

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