CHARACTERIZATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE AND UREASE OF *Paracoccidioides brasiliensis* DURING NITROGEN STARVATION

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INTRODUCTION

Nitrogen participates in the synthesis of proteins, nucleic acids and others biomolecules, and its uptake and metabolism are essencial to growth and fungal establishment in host milieu (HUERGO et al, 2006; BOLTON, THOMMA, 2008). In this regard, this study aims to evaluate the role of nitrogen in *Paracoccidioides brasiliensis* (*Pb*18) pathogenesis, a human pathogenic fungi, through characterization of two proteins, gamma glutamil transpeptidase and urease, that are related to nitrogen metabolism regulation in pathogenic fungi. To reach this objective we expressed the proteins in *Escherichia coli* heterologous system and started gene silencing through antisense RNA technology.

MATERIALS AND METHODS

Heterologous expression ⇨ Cloning of *Pb*18*ggt* (PADG_07986) and *Pb*18*ure* (PADG_03871) in pET32a and *E. coli* pLysS cells transformation. ⇨ Induction of *Pb*18Ure and *Pb*18Ggt with IPTG and identification by Nano UPLC-MSE. ⇨ Enzymatic activity performing, policlonal antibody production and Western-blot

Mutants construction ⇨ Amplification of fragments by PCR ⇨ Cloning in primary vector pCR35 and *E. coli* Stellar transformation. ⇨ Cloning in binary vector pUR5750 and *E. coli* Stellar cells transformation. ⇨ *Agrobacterium tumefaciens* LB1100 cells transformation with pUR5750-*ggt*-aRNA

RESULTS

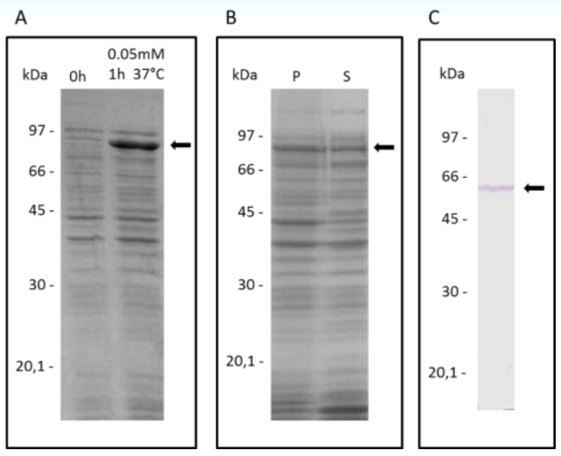

FIGURE 1: Representative figure of r*Pb*18Ggt and policlonal antiboby α-Ggt. A. Induction of r*Pb*18Ggt. (arrow) B. Solubilization of r*Pb*18Ggt (arrow). P: Pellet. S: Supernadant. C. Western-blot of policlonal antiboby α-Ggt with *Pb*01 secretome.

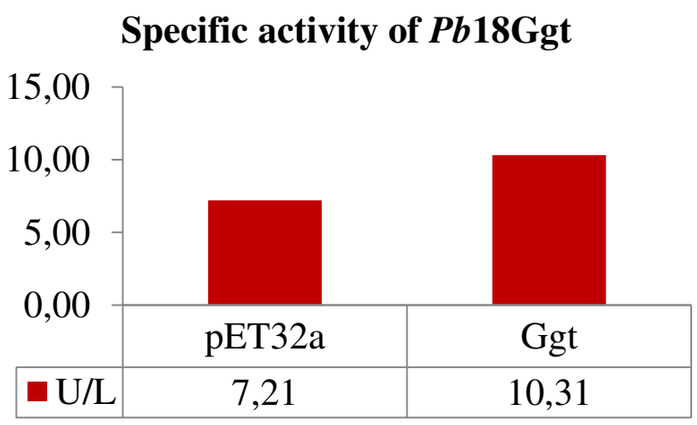

FIGURE 2: Analysis of *Pb*18Ggt recombinant protein enzimatic activity. pET32a: Enzimatic activity in µmol/mg/min of gamma-GT in pET32a induction. Ggt: Enzimatic activity in µmol/mg/min of gamma-GT in *Pb*18Ure induction.

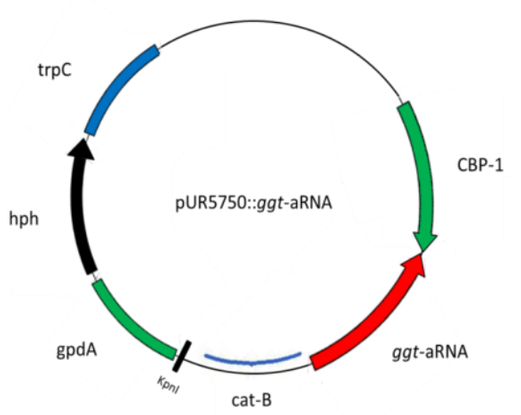

FIGURE 3: pUR5750*::Pbggt*-aRNA. Antisense *ggt* cloned in the binary plasmid pUR5750. Red: Antisense *ggt*. Green: Promoters CBP-1 and gpdA. Blue: Terminator cat-B and trpC. Black: Resistence gene to Hygromycin (hph). KpnI restriction site.

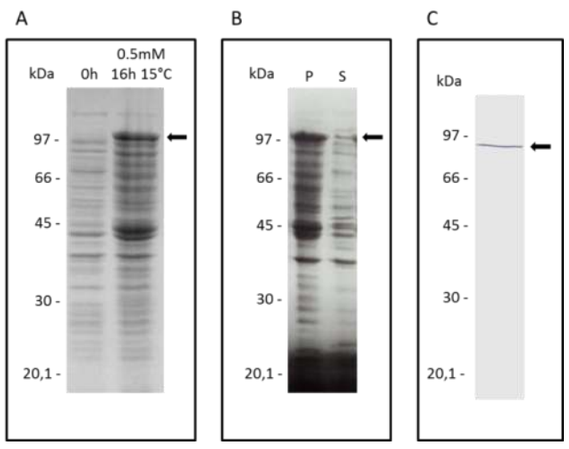

FIGURE 4: Representative image of r*Pb*18Ure and policlonal antiboby α-Ure. A. Induction of r*Pb*18Ure (arrow). B. Solubilization of r*Pb*18Ure (arrow). P: Pellet. S: Supernadant. C. Western-blot of policlonal antiboby α-Ure with *Pb*01 citplasmatic proteome.

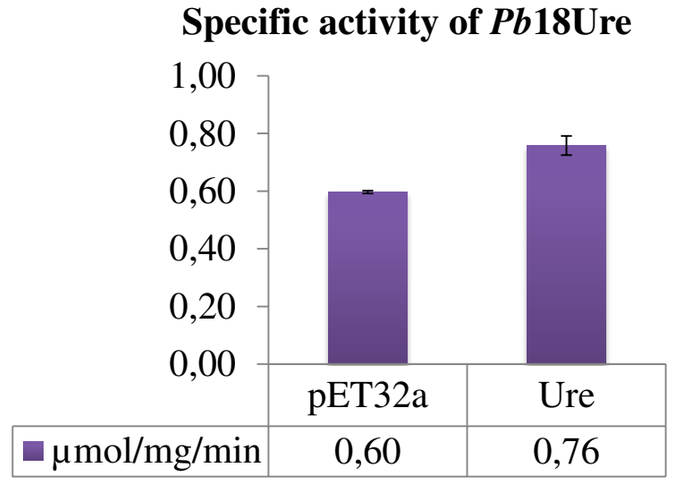

FIGURE 5: Analysis of *Pb*18Ure recombinant protein enzimatic activity. pET32a: Enzimatic activity in µmol/mg/min of Urease in pET32a induction. Ure: Enzimatic activity in µmol/mg/min of Urease in *Pb*18Ure induction. Error bar represents SD of biologic duplicate.

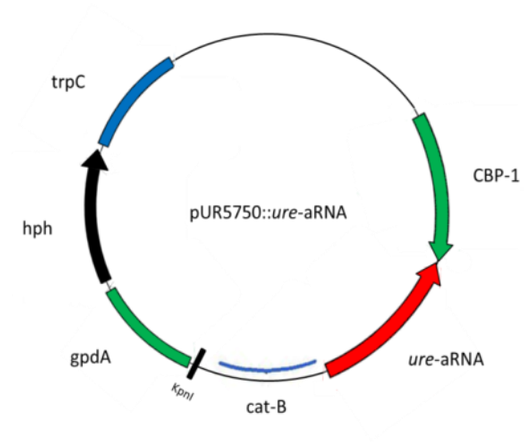

FIGURE 6: pUR5750*::Pbure*-aRNA. Antisense *ure* cloned in the binary plasmid pUR5750. Red: Antisense *ure*. Green: Promoters CBP-1 and gpdA. Blue: Terminator cat-B and trpC. Black: Resistence gene to Hygromycin (hph). KpnI restriction site.

CONCLUSION

- The proteins gamma-glutamiltranspeptidade and urease were successfully expressed in bacterial heterologous system and demonstrated to be biologically active.
- Still is necessary more studies to ensure if these targets are involved in the uptake of secondary sources of nitrogen in *Pb*18 during nitrogen starvation.
- As a perspective, it is intended to purify the recombinant proteins, and cellular location assays. As well as mice infection and growth on different nitrogen sources of the mutants, in order to inscrease to the data obtained in this work.

FINANCIAL SUPPORT: