Purification and characterization of the molecular target KRE2 of *P. lutzii* aiming at development of new antifungal therapies

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The increasing number of cases of systemic infections has been the cause of great concern worldwide. The therapeutic options currently available are limited, and another problem is the pathogens resistance to the classical antifungal agents resulting in the requirement for the development of new antifungal agents. Preliminary results obtained by our group using computational tools such as comparative genomics, molecular modeling by homology and docking identified possible target genes and compounds of low molecular weight (small molecules) that potentially have the ability for to inhibit these targets. *Kre2 or Mnt1,* a gene highly conserved in human pathogenic fungi which encodes a protein of approximately 49 kDa, the α - 1,2 mannosyltransferase. It is an important protein for cell viability and virulence of the pathogen within the host. The molecule inhibitory of KRE2 was called molecule 3.Reasonable amounts of protein were produced heterologously, allowing the tests to determine the kinetic parameters and enzyme inhibition tests using the molecule 3 against the target KRE2 *P. lutzii*. Using labeled substrate [14C] in the reactions, apparent KM of KRE2 of *P. lutzii* was determined, which reached 3.7 pM, demonstrating the high affinity of the enzyme for the substrate in comparison to data in the literature. Similarly, the enzymatic inhibition tests used the radioactive isotope and the molecule 3 was used against enzymes KRE2 of *P. lutzii* and MNT1 of *Candida albicans*. The results obtained for KRE2 of *P. lutzii* demonstrated that in the presence of the inhibitor molecule, was observed a reduction in the enzymatic activity by 60%. However, reduction of the enzymatic activity of MNT1 of *C. albicans* was 20%. Recently the experiments of purification of the recombinant protein by chromatography ion exchange demonstrated a high degree of purity which enables the execution of other steps, such as physical – chemical test, and crystallography experiments. These dates will contribute to the knowledge of the structural and functional characteristics of this protein. The results presented in this work represents an advancement in the development of new therapies for the treatment of fungal infections of global relevance.