Isocitrate lyase protein-protein interaction assay of *Paracoccidioides* spp.

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The fungus *Paracoccidioides* spp. is the causative agent of paracoccidioidomycosis (PCM) which is a pulmonary fungal infection. The disease develops after inhalation of fungal propagules that reach the alveolar epithelium in the lungs and then they differentiate into the pathogenic yeast form. The isocitrate lyase (ICL) is a key enzyme for the glyoxylate cycle, it is present in the fungi but absent in human. Thus, ICL is an important target in the pursuit of inhibitors, since it would present little or no toxicity to humans. In fungi, it has been shown that ICL and the entire glyoxylate pathway enzymes are generally induced under conditions of low glucose and low oxygen tension and especially in the presence of acetate. In *Paracoccidioides* sp., our group has shown that the *Paracoccidioides* sp. isocitrate liase (*Pb*ICL) transcript and protein levels are the same for glucose and acetate. However, the ICL activity is higher in acetate than in glucose, being regulated by phosphorylation. The objective of this study is to identify and analyze the fungus proteins that are likely to bind to *Pb*ICL. It is well-known that protein interactions are intrinsic to cell processes, and it may be possible to infer the function of a protein through the identification of its ligands. Yeast, transition and mycelium protein crude extracts were obtained by disruption of cells in the presence of protease inhibitors. The mixture was centrifuged and the supernatant was used for further analysis of proteins by one-dimensional gel electrophoresis. Yeast cells were grown for 7 days in solid medium and mycelium was grown for 15 days also in solid medium. The purified recombinant *Pb*ICL was used to produce anti-*Pb*ICL polyclonal serum in mice. The investigation for interactions is performed through an in vitro assay. We identified more than 600 proteins that bind to *Pb*ICL in the yeast phase and more than 150 proteins that bind to ICL during mycelium and transition phases. We compared this results to the ones published in the scientific literature through String database and we realized that most of the proteins that bind to *Pb*ICL have not been identified before. In silico and docking analysis allied to virtual screening will be essential in order to continue the investigation of *Pb*ICL inhibitors, leading to the finding of potential antifungal with minimal side effects.

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