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Thesis Abstract

Characterization and heterologous expression of isocitrate lyase of *Paracoccidioides brasiliensis*

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Paracoccidioides brasiliensis is a thermally dimorphic human pathogen that exists as a saprophytic filamentous hyphal form at 22°C and a pathogenic yeast form at 36°C. During infection, *P. brasiliensis* yeast cells are found intracellularly in macrophages. To cope with nutrient deprivation during the infection process, a number of pathogens employ the glyoxylate cycle (GC) to utilize fatty acids as carbon sources. The genes that constitute this pathway have been implicated in pathogenesis. The *P. brasiliensis* cDNA encoding isocitrate lyase (*Pb*ICL), a key GC enzyme, was cloned from a cDNA library derived from the mycelium to yeast transition. The cDNA sequence of 2136 bp contained an ORF of 1614 bp, and the deduced amino acid sequence comprised 537 residues. Phylogenetic analyses showed that *Pb*ICL and other fungal ICLs were grouped according to phylogenetic classification and that these proteins could have a similar evolutionary process, suggesting few differences in the functionality of the enzyme. The recombinant *Pb*ICL was obtained and found to have a high specific activity (3.850 U/mg) when compared to other ICLs, permitting further studies of kinetic parameters and inhibition. Experiments on the utilization of C2 compounds showed that *P. brasiliensis* ICL was overexpressed in the presence of potassium acetate, glycolic acid and ethanol and was repressed by glucose as the sole carbon source. To elucidate if the induction of *Pb*ICL protein expression depends on nutrients, *P. brasiliensis* yeast cells were grown in minimal medium containing glucose, glycolic acid, potassium acetate, and ethanol as sole carbon source for 24 h. The samples were blotted onto nylon membranes and analyzed by immunoblotting,

A high level of the *Pb*ICL protein (60 kDa) was found in cells growing in the presence of potassium acetate, followed by glycolic acid (91%), ethanol (85%) and glucose (62%). In the protein extracts, the higher specific activity was observed in the treatment with potassium acetate (0.250 U/mg) followed by glycolic acid (0.168 U/mg), ethanol (0.150 U/mg) and glucose (0.036 U/mg). We evaluated ICL by two-dimensional gel electrophoresis, using proteins from *P. brasiliensis* yeast cells grown for 24 h in the presence of potassium acetate, and pIs ranging from 5.8 to 6.2 were observed. Bioinformatics analyses of *Pb*ICL indicated that the pI of this protein could range from 5.5 to 6.5, possibly depending on the extent of phosphorylation of the protein.

Key words: *Paracoccidoides brasiliensis*; Isocitrate lyase; Glyoxylate cycle; Phylogenetic relationships; Recombinant protein; Enzymatic activity

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