EXPRESSION IN ESCHERICHIA COLI AND PURIFICATION OF THE CDC12 SEPTIN FROM PARACOCCIDIODES BRASILIENSIS.

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In fungi and animals, septins constitute a conserved family of proteins that form hetero-oligomeric complexes that assemble into filaments in the cell periphery. Septin proteins are characterized by a distinct type of GTP-binding domain and a coiled-coil domain at the C-terminus. In yeast Sacharomyces cerevisiae they have a role in several processes as cytokinesis, sporulation, cell cycle and morphogenesis. To date, very little is known about septins and their roles in pathogenic fungi particularly Paracoccidoides brasiliensis. We searched a P. brasiliensis EST database and identified a cDNA clone with high homology to the Aspergillus fumigatus (91% identity) and S. cerevisiae (59% identity) CDC12 genes. After PCR amplification of the ORF (without the sequence coding for the coil-coiled domain) the fragment was cloned into the pQE plasmid (Qiagen) so as to attach His6 to its N-teminus. Bacterial strain M15 was transformed with the vector and protein expression was induced by addition of 1mM IPTG to liquid cultures. Cells were ruptured by sonication and the septin were purified over Ni²⁺agarose beads (Qiagen). The production of antibodies against *PbCDC12* septin will provide a valuable tool to study the pattern of septin expression and localization and help understand the cell biology of dimorphic transition in P. brasiliensis.

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