
PBRAGS1, AN α -1,3-GLUCAN SYNTHASE GENE FROM PARACOCCIDIOIDES BRASILIENSIS

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In the dimorphic fungus *Paracoccidioides brasiliensis*, α -1,3-glucan is the major neutral cell wall polysaccharide of the pathogenic yeastlike phase, organized as a sort of outer capsule [1]. It replaces almost entirely the β -1,3-glucan which comprises the neutral polysaccharide of the vegetative mycelial phase, and has been pointed out as virulence factor in *P. brasiliensis*, *Blastomyces dermatitidis* and *Histoplasma capsulatum* [1].

In order to clone the α -1,3-glucan synthase gene from *P. brasiliensis* (*PbrAGS1*), DNA was extracted as before [2]. From the alignment of the amino acid sequences from several fungal α -1,3 glucan synthases, high homology areas were determined on which primers were designed. For the isolation of the *PbrAGS1* gene, the 900 bp PCR product was used as a probe. From Southern analysis, a *Hind*III partial genomic library was constructed. Screening of this library by colony hybridization resulted in 4 positives clones containing inserts of 4.2 kb. One of the clones, plasmid pLBM1, was selected for further analysis. Nucleotide sequence analysis of the insert revealed the presence of an approximately 4 Kb open reading frame (ORF) for the 5' region of the gene, with high homology to fungal α -glucan synthase genes. However, it missed the 3' region of the gene, which represents about 40% of the gene, as compared with the *Aspergillus fumigatus AGS1* gene, the one with the highest homology to *PbrAGS1*. In order to identify and clone the missing region of the gene, a second Southern analysis of genomic DNA of *P. brasiliensis* was performed, using the 300 bp downstream *Bam*HI-*Hind*III fragment obtained from the pLBM1 insert. A *P. brasiliensis* partial genomic DNA library was constructed and the PCR amplified product used as probe to search for recombinant colonies carrying the fragment under study. A 4 Kb fragment was obtained, which is under sequencing for further studies. The gene is expressed in the yeast phase but not in the mycelial phase of *P. brasiliensis*, as deduced from RT-PCR, an information that matches the reported presence of α -1,3-glucan in the yeast cell wall of *P. brasiliensis* and its absence from the mycelial cell wall [1].

- 1.- San-Blas G. *et al.* 2002. *Med. Mycol.* 40: 225-242.
- 2.- Niño-Vega G.A. *et al.* 2000. *Med. Mycol.* 38: 31-39.