Cloning and nucleotide sequence of a cDNA encoding the antifungal-protein of *Aspergillus giganteus* and preliminary characterization of the native gene

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A. giganteus secretes a 5.8 kDa protein which inhibits the growth of a variety of fungal species (1). In order to clone the cDNA encoding the antifungal-protein, a cDNA expression library was constructed in λZAP (Stratagene) using polyA$^+$RNA from induced mycelia as template for cDNA synthesis. A complete cDNA coding for the antifungal-protein was isolated by immunoscreening of the λZAP library with polyclonal antibodies. The cloned cDNA was used as a hybridization probe for the isolation of the native antifungal-protein gene from a λEMBL3 library of *A. giganteus*.

Sequencing of the antifungal-protein cDNA revealed an open reading frame (ORF) of 177 nucleotides (Fig. 1). The amino acid sequence deduced from the major part (nucleotide 28−177) of the ORF corresponds exactly to the entire sequence determined for the secreted protein (1). Additionally there is an N-terminal leader sequence of 9 amino acids which might be responsible for secretion. The functional identity of the leader peptide is currently under investigation using the transformation system recently established for *A. giganteus* (2). Northern analysis of total RNA from 24 and 48 h old mycelia cultivated in medium containing either starch or glucose as major carbon source indicates, that expression of the antifungal-protein gene is inhibited in the presence of glucose. While the amount of antifungal-protein mRNA increases with time in the presence of starch, only a weak signal was obtained with RNA from mycelia grown in glucose containing medium after 24 h (Fig. 2a). The apparent size of the antifungal-protein mRNA is around 520 bp which suggests that the ORF is flanked by relatively long untranslated regions. Unique restriction fragments of genomic DNA of 2.7 kb (*Eco*RI) and 13.5 kb (BamHI) hybridizing to the cDNA probe could be identified by Southern analysis (Fig. 2b). A restriction map of the 2.7 kb *Eco*RI fragment cloned from the λEMBL3 library shows the position of the antifungal-protein gene within this genomic region (Fig. 3). Preliminary sequencing data indicate the presence of a 55 bp long intron which is located 122 bp downstream of the beginning of the ORF.

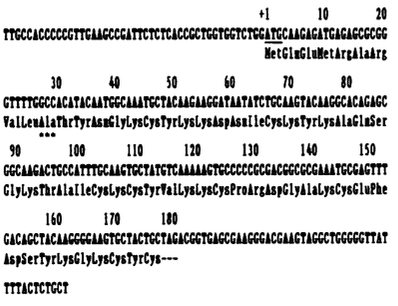

Figure 1. Complete nucleotide sequence of the *A. giganteus* antifungal-protein cDNA and amino acid sequence encoded by the ORF. The N-terminal amino acid of the mature protein (1) is marked by asterisks.

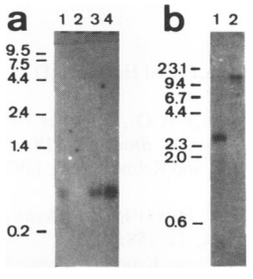

Figure 2 a: Northern blot of total RNA from 24 h (lanes 1 and 3) and 48 h (lanes 2 and 4) old *A. giganteus* mycelia grown in presence of glucose (lanes 1 and 2) or starch (lanes 3 and 4) probed with ^{32}P labelled antifungal-protein cDNA. The positions and sizes (kb) of standard RNAs are indicated at the left margin. **b:** Southern blot of genomic DNA digested with *Eco*RI (lane 1) or *Bam*HI (lane 2) and probed with ^{32}P-labelled antifungal-protein cDNA. λHindIII fragments were used as size marker (kb).

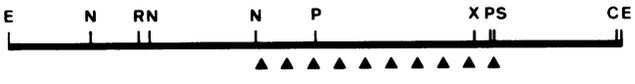

Figure 3. Restriction map of the 2.7 kb *Eco*RI fragment. E: *Eco*RI, C: *Cla*I, N: *Nru*I, P: *Pst*I, R: *Eco*RV, X: *Xho*I, S: *Sph*I. The region hybridizing to the antifungal-protein cDNA is represented by closed triangles.

ACKNOWLEDGEMENT

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