MOLECULAR STUDY OF THE ASPARTYL PROTEASE (eap-1) FROM *Sporisorium reilianum*.

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Introduction. The extracellular aspartyl proteases produced by phytopathogen have been associated in infection and colonization process (1). The fungus *Sporisorium reilianum* is a phytopathogen from soil that causes the head smut disease on corn, this basidiomycete produces an extracellular aspartyl protease called *eap-1,* which was purified and characterized biochemically. The protein has high similarity with the access number sr11394 than is a gene encoding to an aspartyl protease located in the chromosome 1 of the fungus (2). In this work was made the cloning of gene encoded the protease *eap-1* in order to determine their role in pathogenesis processes by molecular methods.

Methods. The theoretical analysis of the promoter region of gen *eap-1* was performed with the program online MatInspector. 4687 bp promoter regions upstream of the start codon (ATG) were analyzed, by comparing the sequence with the target promoter regions previously known in fungi and applying a "core similarity" of 0.75.
A pair of primers was designed to amplify the gene encoded to aspartyl protease *eap-1*. The PCR product was cloned into the plasmid pGEM-T (Promega). The construction was confirmed by restriction profile with the enzymes *SalI* and *ApaI*.

Results. The analysis of the promoter sequence using MatInspector software, allowed determine 434 specific sites DNA-binding for transcription factors, including XBP1, MSN2, SKN7 y MGA1 associated with stress, PACC, CAT8 Y NIT2 binding sites for transcription factors dependents of pH, carbon and nitrogen regulation, respectively. Also found sites related with the sexual complementation MATAa1 and MATALPHA2 and other related with the asexual reproduction MBP1.
The primers designed to amplify the gene *aep*-1 were EapF and EapR (5´ATGCAACTCAAGCTCTCGTTTGTTGC3´ and 5´TTAGGCCTTGTTGGCGAAGCCG3´**)** which allowed the amplification of complete gene *eap-1* (1182 pb) (Fig. 1).

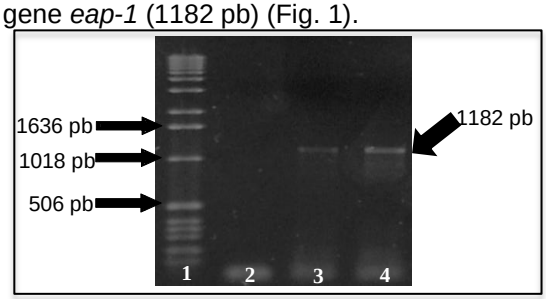

Fig. 1. Amplification of aspartyl protease *eap-1* gene from *S. reilianum*. 1. Molecular weight marker. 2. Negative control. 3. Positive control and 4. Amplification ofeap-1

The construction was confirmed by restriction profile (Fig. 2). The sequence of cloned fragment corresponds to the sequence of the gene *eap-1*.

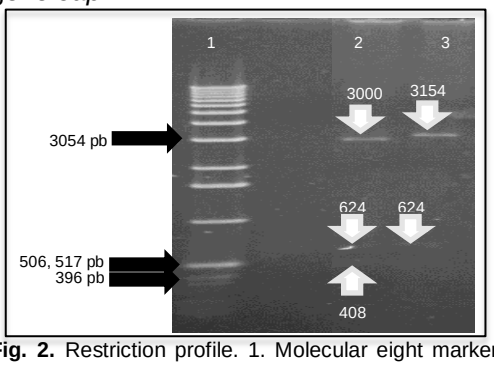

Fig. 2. Restriction profile. 1. Molecular eight marker. 2. Restriction profile with *SalI* and *ApaI*. 3. Restriction profile with *SalI*.

Conclusions. The analysis of promoter region has suggested that gene expression of the aspartyl protease is related with sexual complementation and reproduction, also with stress, carbon source and pH regulation. The cloned gene that encoded to the aspartyl protease from *S. reilianum* was performed.

References.

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