MOLECULAR CLONING AND SEQUENCING OF HEPARINASE III FROM *Flavobacterium heparinum.*

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Flavobacterium heparinum is a gram-negative, nonpathogenic soil bacterium, which produces enzymes that degrade glycosaminoglycans through eliminative mechanism, leading to depolymerisation of the chains and formation of unsaturated fragments. Addition of heparin, heparan sulfate or their disaccharides to the medium leads to the induction of three enzymes, known as heparinase and heparitinases I and II, recently referred as heparinases I, III and II, respectively. The present work aims to clone the heparinase III (supposedly heparitinase I), that shows a unique specificity. It cleaves exclusively N-acetyl or N-sulfo-glucosaminide-glucuronic acid linkage on heparan sulfate. Total RNA was extracted from *F. heparinum* and mRNA was enriched by removal of rRNA with magnetic beads (Ambion). mRNA was converted to cDNA with reverse transcriptase, using random primers, and amplified by PCR with specific primers to heparinase III. A product of 1980 pb was detected and cloned in pET 26b (Novagen). The sequencing presented 100% identity with heparinase III, cloned in the literature. These results showed the efficiency of mRNA enrichment and indicate that we already have cloned the whole cDNA of this enzyme. Recombinant heparinase III will provide us with an important tool to compare with heparitinase I activity, and also to search for clinical applications, for example, on neovascularization (supported by CAPES, CNPq, Fapesp).