# EXPRESSION, PURIFICATION AND ENZYMATIC ACTIVITY OF RECOMBINANT HEPARINASE CLONED FROM *Flavobacterium heparinum*

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Heparinase, a heparin lyase produced by *Flabobacterium heparinum*, is used for the determination of the fine structure of heparin and heparan sulfates obtained from different sources. This enzyme can also be used to remove heparin in therapies, such as after extracorporeal circulation. The full heparinase DNA segment was obtained from *F. heparinum* by PCR reaction. The complete PCR product was inserted in a expression vector pET – 14b (Novagen). This heparinase was engineered to contain a His-Tag sequence (Ni-NTA Purification system, Invitrogen). The recombinant plasmid was used to transform *E. coli* pLys-S. In order to improve the expression a number of approaches have been tested during the induction process, including different concentrations of IPTG, temperature and period of induction. After the expression, recombinant heparinase was immobilized on his-bind resin. The purified heparinase was eluted from the resin and analyzed by SDS-PAGE. The recombinant enzyme when incubated with heparin yielded the same products as the native enzyme from *F. heparinum*, thus demonstrating the preserved activity of the purified recombinant heparinase. Some structural characteristics of heparinase were investigated using circular dichroism and the importance of specific aminoacids related to enzymatic activity determined. This new technology allows large scale production of heparinase, free of other lyases, sulfatases and glycuronidases. (Supported by FAPESP, CNPq and CAPES)