

Cloning, Expression of Hirudin Gene from Leech (*Hirudo orientalis*) in BL21(DE3) Strain

Abstract

The use of leeches in bloodletting therapy is one of the most important techniques in ancient medicine from Greek time up to now. Leeches saliva contain anticoagulant hirudin which facilitate blood flow, this is one reason for using leeches to save thousands of severed fingers, noses and ears in recent years. In this study, hirudin gene successfully cloning and expressed in BL21(DE3) strain. Leech type was detected as *H. orientalis* by using two types of genes 18SrDNA and CO-I genes, Hirudin gene was amplified by specific primers from *H. orientalis* cDNA. Furthermore, Hirudin gene was expressed in BL21(DE3) strain under the control of T7 promoter in pET-16b vector, constructed vector pET-16-HR vector was extracted and used to amplify hirudin gene by specific primers, then hirudin gene band was appeared after digestion of extracted plasmid with Hind III and NdeI restriction enzyme. Hirudin expression was established by Real-time PCR. Production of hirudin established in LB medium and purified by IMAC column, DEAE Sepharose and SP Sepharose. Concentration of produced hirudin within its solution was measured by ELISA kit which reached to 1.35ng, thrombin titration method was used to determine hirudin activity which showed Hirudin protein required 360 μ l from thrombin for clot formation.

Keyword: Hirudin; *Hirudo orientalis*; Real-Time PCR; DEAE Sepharose; SP Sepharose.