ABSTRACT

In current study cloning, expression, purification and characterization of xylanase gene was carried out from *Bacillus licheniformis* Atcc 14580 into *Escherichia coli*. During this course numerous procedures were followed including genomic DNA isolation, and amplified the xylanase gene by using a pair of primers under optimized conditions of PCR. By using T4 DNA ligase PCR product was ligated with a cloning vector pTz57R/T. Cloned *E. coli* was used as template in colony PCR. Then competent cells of DH5α were transformed with pTz57R/T. Cloned *E. coli* strain was used as template in colony PCR and alkaline method was used for extraction of recombinant plasmid. All results of these procedures were examined on agarose gel electrophoresis and bands on specified location confirmed the success of protocol followed. The size of PCR product was found to 1.38 kb and genomic DNA was located above the highest band of ladder. The recombinant plasmid was confirmed by its band position at 4.18 kb. Recombinant plasmid pTz57R/T was double digested by enzymes *HindIII* and *NdeI* and ligated into pET22b(+) by using T4 DNA ligase. After that this ligated product was transformed with competent cells of BL21. To evaluate the results the sample was run on SDS-PAGE. Quantitative analysis of xylanase shows the maximum activity 6.1 U/mi/min after 5 mints of incubation at 55°C and pH 7.0. Purification of xylanase was performed by ammonium sulphate precipitation and affinity chromatography. In this work enzyme was characterized and different inhibitors effect the enzyme activity . Maximum activity was observed by adding $FeCl_2$ (67.32% ±0.074) and 20% methanol (67.32% ±0.074). SDS and β-merceptoethanol inhibit the enzyme activity. Saccharification of plant biomass was performed and maximum sugar was released at 50°C, pH 6.0 after 6 hrs of incubation. Maximum activity of enzyme was observed at 120 rpm and concentration of substrate was 8%.