

Abstract

Culture-independent metagenomic technology is used to explore novel genes and metabolic pathways from uncultured microbes. Cellulose being the most abundant renewable resource in nature, enzyme cellulases has become the bottleneck of the efficient utilization of cellulose. In this study, a cellulase novel gene from metagenome has been cloned in *E. coli* using pET32a⁺ as a vector. The results showed over expression of the gene and production of inclusion body. Hence, protein expression was optimized by varying IPTG concentrations (0mM, 0.1mM, 0.5mM, 1mM) temperature (25°C and 37 °C) and time (1hr, 2hr,3hr and 4hr). After optimization, the SDS-PAGE analysis showed lower expression of protein expression at 25 °C. Protein expression was seen to increase with temperature, IPTG concentration and time. However, even after optimizing no soluble proteins were obtained. The inclusion was then dissolved in BugBuster Protein Extraction Reagent to refold the proteins and tested against 3 different substrates AZCL-Beta-Glucan, AZCL-Cellulose and AZCL-Xylan. Positive result was obtained in AZCL- Beta-Glucan only which suggested that this substrate was most suitable for the enzyme.