ABSTRACT

Thermotoga petrophila contains the most thermo-reactive enzymes which fulfill most of the industrial demand. In present work, endo-1,4-beta-xylanase gene of 3.1 kb size from "*Thermotoga petrophila RKU-1*" was amplified by PCR. Two pairs of primers were designed to amplify xylanase gene into two halves. *NdeI* site was introduced at 5′ of forward primer of first pair to introduce this site in the start of gene. The product obtained after amplification by using this pair of primer was of 1.443 kb. Similarly, *Hind III* site was introduced in the reverse primer of second pair which gave amplified product of 1.658 kb with *HindIII* at the end of gene. Both the amplified products i.e. 1.443 and 1.658 kb contained *KpnI* at the end and start, respectively as this enzyme was single cutter of the gene of interest. 1.443 kb fragment was double digested with *NdeI* and *KpnI*, whereas, 1.658 kb fragment was restricted with *KpnI* and *HindIII*. Vector pET 28a (+) was double digested with *NdeI* and *HindIII*. Then both the double restricted inserts and expression vector were ligated by using T4 DNA ligase. The competent cells of BL21 Codon Plus were prepared by using $CaCl_2$ and ligated product was transformed into these cells. The cloning and transformation was confirmed through colony PCR. The positive recombinant plasmid was isolated by alkaline lysis method and confirmed after double digestion with *NdeI* and *HindIII*. SDS-PAGE was run to evaluate the expression of cloned gene and to determine the molecular mass of recombinant enzyme.