ABSTRACT

It is observed that enzymes derived from hyperthermophiles are better than the traditional catalysts because they can perform industrial processes even under harsh conditions, under which conventional proteins are completely denatured. Strain *Thermococcus kodakaraensis* KOD1 is an anaerobic hyperthermophilic archaeon who grows well on complex organic matter at 85°C. *Thermococcus kodakaraensis* KOD1 genomic sequence showed the presence of catalase like sequence (TK0377) which is not yet studied and confirmed by any researcher. So *TK0377* gene was amplified, cloned in pTZ57R/T and subcloned in pET22b+ expression vector. Positive clones were subjected to the expression analysis in *E. coli* BL21 CodonPlus cells. Small band of protein was observed after 2 hour, 4 hour, 6 hour and overnight. Different IPTG concentrations (0.1, 0.3, 0.5, 0.7, 0.9 mM) did not improve the expression of protein, so the gene was cloned in pET28a+ expression vector to enhance the expression of protein. A prominent band of 16 kDa was observed when induced with 0.5 mM IPTG at 37°C. Expression was optimized for time of incubation and IPTG concentration. Expression of TK0377 induced with 0.1 mM IPTG for 4 hours taken at 37 °C gave maximum protein band in pellet showing it insoluble. Expression taken at low temperature (16°C) made the protein soluble. Heating protein at high temperatures (60, 70, 80, 90°C) denatured the protein, purification was achieved at 0.5 mM imidazole and NaCl concentration by using nickel chromatography but no activity was observed when catalase assay was performed showing that protein was inactive.