ABSTRACT

A novel 4-phytase gene of *Thermotoga naphthophila* RKU-10 was cloned and expressed into *E. coli* BL21(DE3). pET21a (+) was used as cloning and expression vector. An ORF of 1845bp from 4-phytase gene was used for amplification. Amplification was carried out at annealing temperature of 58°C. Following amplification, double restricted amplified gene was ligated with double restricted pET21a (+). The ligated product was transformed into *E. coli*BL21(DE3). After successful transformation, expression was studied of phytase gene by addition 0.5mM IPTG. The recombinant phytase enzyme was partially purified by using heat treatment method. The molecular mass of protein was estimated to be approximately 71 kDa by SDS-PAGE analysis. The maximum activity of the enzyme was calculated to be 9 U/ml/min at 80°C, pH 4.0. This phytase was found to be stable in pH ranging from 3.5-5.5. The maximum activity of recombinant phytase (9.5± 0.12 U/ml/min) was obtained after 25 minutes of incubation. The maximum enzyme activity was observed at 10mM substrate concentration which was calculated to be 8.5 ± 0.06 U/ml/min. Metal ions effect along with EDTA was studied by incubating 5mM concentration of bivalents ions e.g. Mg^{+2}, K^{+2}, Na^{+2} and Fe^{+2}. Relative activity was observed around 80% in presence of Ca^{+2}, Mg^{+2}, K^{+2}, while in presence of iron ions, enzyme showed 30% less activity. Phytase lost its 50% relative activity in presence of Cu^{+2} and Zn^{+2}. 5mM EDTA increased residual enzyme activity around 95%. The results obtained from current study indicate that thermophilic 4-phytase enzyme from *Thermotoga naphthophila* as an important candidate for industrial use.