

Abstract

An esterase gene XD57_0650 from Thermotoga naphthophila was cloned and expressed in E. coli BL 21(DE3). The gene length was 921bp which encode a protein comprising of 306 amino acids. The cloned gene was confirmed by colony PCR, single and double restriction of recombinant plasmid. The recombinant plasmid pET21a (+)/esterase gene was used for expression of esterase enzyme in E. coli under the regulatory control of T7 promotor. The recombinant esterase was found to be partially purified at 70% of ammonium sulphate concentration as maximum precipitation was achieved at 70% saturation level of ammonium sulphate. The enzymatic activity was carried out by performing titrimetric method using olive oil as substrate. The esterase exhibited maximum activity at pH 7 and 80°C which was found to be 65±1.0 units/ml/min. At 90°C and above, the enzyme stability was considerably decreased. In summary, the esterase enzyme from Thermotoga naphthophila is a heat stable enzyme which is expressed and characterized for first time in E. coli BL 21(DE3) having molecular mass of 35 kDa which was analyzed by utilizing 12% SDS-PAGE. The recombinant thermostable esterase was established to have resistance against most of the metal ions, organic solvents and inhibitors making esterase a promising candidate for use in industrial biotechnology.