Cloning and Characterization of Thermostable Esterase from *Archaeoglobus fulgidus*

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Abstract

New thermostable esterase from the hyperthermophilic archaeon *Archaeoglobus fulgidus* (EST-3) was cloned, functionally expressed in *Escherichia coli*XL1-blue strain and biochemically characterized. The sequence of esterases were got from GeneBank and size of esterase gene is 744bp. The EST-3 gene was amplified by PCR and the gene was cloned into pQE 30 at *Bam* HI and *Sal* I restriction site for expression and purification. Positive clones were selected by an in situ plate assay using a colony staining procedure with chromogenic substrate α -naphthyl acetate and fast blue RR salt(esterase activity staining). A plasmid library of *A. fulgidus* esterse gene was screened for colonies showing thermostable enzyme activity against α -naphthyl acetate. Positive colonies showed dark brown color after incubation at 75℃ in presence of the substrate. It was also easy to purify the eaterase by 75℃ heat treatment, which could denature proteins from *Escherichia coli.*

EST-3 is monomeric protein with a molecular weight of about 27.5 kDa. It had the pentapeptide GHSLG corresponding to the GXSXG motif, which is the characteristic sequence at active site of esterase. This esterase showed the best activity against ρ-nitrophenylbutyrate($pNPC_4$) among the ρ-nitropheny derivatives and the optimal temperature was 80℃. The enzyme is barely active at room temperature, displaying the maximal enzyme activity at about 75℃ and after 180 min incubation at 90℃ 20 % activity still remains.

References

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