

## **Abstract**

Thermostable lipolytic enzymes with the capability of withstanding harsh industrial settings are always in high demand and scientists are continuously exploring new environmental niches for discovering novel thermophilic and even hyper-thermophilic microbes. In this study, enhanced synthesis of a recombinant TnLip enzyme was carried out, which is encoded by a S9 prolyl oligopeptidase catalytic domain (Tnlip) previously cloned in a mesophilic host. Enhanced expression of TnLip was gained by employing various inducing/auto-inducing media, induction strategies and optimizing different cultural parameters such as pre-induction cell density, agitation rate, incubation time and temperature etc. TnLip activity was successfully enhanced **5.67 folds**, by using 4×ZB medium (induced with 150 mM lactose) instead of LB medium. Activity was improved by **5.56 folds**, when TnLip was synthesized in TB (auto-inducing) medium. Enhanced expression by lactose induction instead of IPTG, is also a positive aspect in this research because lactose is very cheap and easily available, as compared to IPTG. SDS-PAGE analysis was performed for assessing the induced expression of TnLip and for determining molecular weight, which was estimated as 35 kDa. Preliminary characterization with heat treated recombinant lipase was carried out and results indicated that TnLip had an optimum pH and temperature of 7 and 85 °C, respectively. This study describe the high increment of TnLip production in modified 4×ZB medium with lactose induction, a cheap and nontoxic inducer. Optimal fermentation approaches would be valuable aspect to increase the production and proper folding of recombinant protein. This work provides fundamental information to augment production of recombinant product.