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Purification and characterization of a novel esterase Est03 derived from an activated sludge metagenome (1)

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Abstract

The purpose of this research was to perform sub-cloning, expression, purification, and characterization of the novel esterase gene *est03* selected from the activated sludge metagenome for subsequent research and application. The *est03* gene is 762 bp in length and can be translated into an esterase, namely Est03, which contains 253 amino acids with a molecular mass of about 28.8 kDa. The *est03* gene was sub-cloned on the expression vector pPAL7 and transformed into the expression host *Escherichia* coli BL21 (DE3). After the transformant was induced by IPTG to express a large number of target proteins, the expressed esterase was purified and excised using the purification kit to become a purified enzyme with the same amino acid sequence of the original esterase. It was found that Est03 had a higher preference for short-chain fatty acid substrates, and particularly the two-carbon substrate (C2) activity was significantly higher than others ($P < 0.001$). The temperature test showed that the esterase expressed higher activity between 35 - 50℃, with the optimal temperature of 45℃. The specific activity and k_{cat}/K_m of Est03 was 12.15 unit/mg and 1.58×10^{-3} $s^{-1} \cdot \mu M^{-1}$, respectively. Est03 had greater than 50% activity between pH 6.5 and pH 9.0, with the highest activity at pH 8.0. At the same time, Est03 was active in common types and concentrations of organic solvents, metal ions, and surfactants. In summary, Est03 has noteworthy biochemical properties and the potential for development in the applications of the biotechnology industry

Key words: Activated sludge metagenome, Novel esterase, Characterization.

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