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Overexpression and characterization of strep-tagged thermostable organic solvent-stable lipase from *Bacillus* sp. strain 42

Raja Noor Zaliha Raja Abd. Rahman [1,2,*], Tengku Haziyamin Tengku Abdul Hamid [1], Mohamed Abdallah Eltaweel [1], Mahiran Basri1 [1,3], Abu Bakar Salleh [1,4]

[1] *Laboratory of Industrial Biotechnology, Institute of Bioscience, University Putra Malaysia 43400 Serdang, Selangor, Malaysia*
[2] *Department of Microbiology, Faculty of Biotechnology & Biomolecular Sciences, University Putra Malaysia 43400 Serdang, Selangor, Malaysia*
[3] *Department of Chemistry, Faculty of Science, Putra Malaysia 43400 Serdang, Selangor, Malaysia*
[4] *Department of Biochemistry, Faculty of Biotechnology & Biomolecular Sciences, University Putra Malaysia 43400 Serdang, Selangor, Malaysia*

E-mail address: rnzaliha@biotech.upm.edu.my (R.N.Z.R.Abd. Rahman).

In addition to stability at high temperatures, thermophilic enzymes also possess great resistance against proteolysis, detergents and chaotropic agents (Sellek and Chaudhuri, 1999). For this reasons, there was more attention to consider for their future use in organic solvent. *Bacillus* sp. strain 42 producing a solvent-stable thermostable lipase was isolated from Malaysian palm oil mill effluent. The 1.2 kb gene (AY 787835) code for lipase was amplified using consensus primers based on multiple sequence alignment with thermostable genes. The gene was cloned into pQE-30UA and pET51b expression vectors. An overexpression was achieved in heterologous system using pET51b vector with *Escherichia coli* host strain BL21(DE3)pLysS. The optimum expression was after 24 h incubation at 37 °C and lipase activity was at 80.0 U/ml culture (160.0 U/mg protein) after induction with 0.5 mM IPTG. Under strong T7 promoter system, expression using pET51b/BL21(DE3)pLysS host-vector system is 11.5 fold higher compared to pQE-30UA/M15(pREP4) host-vector system which achieved at 17 U/ml culture (34 U/mg protein). The fusion lipase in pET51b contained Strep-tag II affinity tag that in one step of purification, the lipase was purified to homogeneity using Strep-tag II agarose column with 1.3-fold purification factor and 70% recovery. Sodium-dodecyl sulphate polyachrylamide gel electrophoresis (SDS-phage) analysis showed that the molecular weight of fusion lipase was about 43 kDa. The purified lipase was most active at 70 °C and pH 8.0 and was stable in a broad pH range 7–10. The lipase showed high stability with half life of 315 min at 60 °C, for 125 min at 65 °C and 45 min at 70 °C. By 30 min incubation in 25% (v/v) solvents with shaking rate at 150 strokes per min, the solvent stability of the enzyme was different depending on solvents and temperatures. The lipase was more stable in polar organic solvent such as DMSO, DMF, acetone, methanol, heptanol and octanol. In this work, thermostable and organic solvent stable lipase gene from *Bacillus* sp. strain 42 was successfully identified and overexpressed into high expression vector pET51b using host BL21(DE3)pLysS, under the control of T7 expression mechanism. The fusion enzyme was successfully purified to homogeneity using Strep-tag affinity tag, and characterized. Stability of enzyme in organic solvent with low partition coefficient value (log *P*) will enable its future industrial use, for instance in biodiesel production.

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Preliminary purification of pectate lyase from *Paenibacillus polymyxa* N10

Phatthara Khomkamon [1,*], Pilanee Vaithanomsat [2], Vichien Kitpreechavanich [3], Sawitri Chuntranuluck [1]

[1] *Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Thailand*
[2] *Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Thailand*
[3] *Department of Microbiology, Faculty of Science, Kasetsart University, Thailand*

E-mail address: oplv@ku.ac.th (P. Khomkamon).

Apart from the ability in hydrolyzing the alpha-1,4-glycosidic bond of polygalacturonate, releasing unsaturated soluble oligogalacturonates, liberating water-soluble pectic substances by restricted hydrolysis of water-insoluble protopectin in plant tissue, pectate lyases from various microorganisms can remove the cuticle and wax layers together (Linhardt et al., 1986; Sakai et al., 1993). Pectinases are widely distributed in higher plants and microorganisms. They have been widely used in food production, detergent manufacture, etc. (Gummadi and Panda, 2003). Two types of pectate lyases: PAL1 and PAL2 from *Paenibacillus polymyxa* N10 (Sitthidilokratna et al., 2007) were purified to homogeneity by DEAE-Sepharose Fast Flow and Sephacryl S-100 High Resolution. The specific activities for pure PAL1 and PAL2 were shown as 275.00 and 124.67 units/mg protein, respectively. This corresponds to purification fold of 5.13 and 2.33 for PAL1 and PAL2, respectively. Both of them exhibited similar molecular weights about 50–75 kDa by SDS-PAGE.

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Silk degumming solution as substrate for protease production

Pilanee Vaithanomsat*, Taweesiri Malapant, Waraporn Apiwattanapiwat

Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, 50 Chatuchak, Bangkok 10900 Thailand

E-mail address: oplv@ku.ac.th (P. Vaithanomsat).

Silk manufacturing is one of the industrial sectors where intensive water consumption cannot be avoided therefore resulting in