

Purification and characterization of lipase produced by *Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC 8293 using an Aqueous Two-Phase System (ATPS) composed of Triton X-100 and maltitol

ABSTRACT

Purification of lipase produced by *L. mesenteroides* subsp. *mesenteroides* ATCC 8293 was conducted for the first time using a novel aqueous two-phase system (ATPS) composed of Triton X-100 and maltitol. The partitioning of lipase was optimized according to several parameters including pH, temperature, and crude load. Results showed that lipase preferentially migrated to the Triton X-100 rich phase and optimum lipase partitioning was achieved in ATPS at TLL of 46.4% and crude load of 20% at 30 °C and pH 8, resulting in high lipase purification factor of 17.28 and yield of 94.7%. The purified lipase showed a prominent band on SDS-PAGE with an estimated molecular weight of 50 kDa. The lipase was stable at the temperature range of 30-60 °C and pH range of 6-11, however, it revealed its optimum activity at the temperature of 37 °C and pH 8. Moreover, lipase exhibited enhanced activity in the presence of non-ionic surfactants with increased activity up to 40%. Furthermore, results exhibited that metals ions such as Na⁺, Mg²⁺, K⁺ and Ca²⁺ stimulated lipase activity. This study demonstrated that this novel system could be potentially used as an alternative to traditional ATPS for the purification and recovery of enzymes since the purified lipase still possesses good process characteristics after undergoing the purification process.

Keyword: *Leuconostoc mesenteroides*; Triton X-100; Aqueous two phase system; Characterization; Lipase; Maltitol