TITLE: AQUEOUS TWO-PHASE SYSTEM FOR LIPASE SEPARATION FROM *Candida viswanathii* STRAIN AND ENZYME BIOCHEMICAL PROPERTIES

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ABSTRACT:
Lipases (triacylglycerol acyl hydrolase, E.C 3.1-1.3) are important enzymes in industrial processes and used in various biochemical reactions. The fermentation process for lipase synthesis can also result in undesirable byproducts, which impair the enzyme utilization for industrial applications and so require further purification processes. Aqueous two-phase systems (ATPS) is considered low-cost process with a simple scaling-up. The aim of this work was to evaluate the lipase partition produced by *Candida viswanathii* under submerged conditions using ATPS composed of polyethylene glycol (PEG) and potassium phosphate. PEG of different molecular weight (1500, 4000, 6000) were evaluated. Biochemical properties of purified enzyme were determined by optimum activity in pH, temperature and organic solvent. Among PEG evaluated, PEG6000-phosphate presented the best selectivity (activity balance = 15.0%, partition coefficient, *K*=0.80, recovery = 78,12%). The best selectivity was reached using pH 4.0 and temperature of 40 ^{o}C (activity balance = 25.64, *K*=0.84, purification fold = 3.15, recovery = 90,1%). Optimum pH and temperature of enzyme activity were observed at 8.1 and 40.0 ^{o}C, respectively. The organic solvent effect on lipase activity was evaluated in glycerol, DMSO, methanol, ethanol, acetone, 1-propanol and hexane. The enzyme remained activated using ethanol, methanol, glycerol, DMSO, acetone, 1-propanol and hexane (357.0%, 338.0%, 288.0%, 185.0%, 176.0%, 132.0% and 107.0%, respectively). In conclusion, the lipase from *C. viswanathii* purified under ATPS using PEG6000-phosphate can be used in several industrial application at alkaline conditions and also in organic synthesis with high potential for lipid modification in food, cosmetic, biodiesel and pharmaceutical processes.

Keywords: lipase, protein purification, ATPS, biochemical properties, organic solvent activity