

**TITLE:** PARTIAL PURIFICATION AND CHARACTERIZATION OF A MODERATELY THERMOPHILIC LIPASE FROM *Bacillus subtilis* STRAIN ISOLATED FROM MANGROVE SEDIMENTS

**AUTHORS:** ARAUJO, F. J.; PEREIRA, L. É. C.; PEREIRA, M.L.; HISSA, D. C.; MELO, V. M. M.

**INSTITUTION:** LABORATÓRIO DE ECOLOGIA MICROBIANA E BIOTECNOLOGIA-LEMBIOTECH, DEPARTAMENTO DE BIOLOGIA, UNIVERSIDADE FEDERAL DO CEARÁ (AV. HUMBERTO MONTE, 2977, CAMPUS DO PICI, BLOCO 909, CEP 60455-000, FORTALEZA, CE, BRAZIL).

Lipases constitute the most important group of biocatalysts being used in various biotechnological applications like detergents, cosmetics, biodiesel and leather processing. Microorganisms constitute the main source of enzymes and the species of the genus *Bacillus* are particularly well-known as extracellular enzymes producers, due to their great fermentation yield and non-toxic by-products. Mangroves are coastal ecosystems with extreme environmental conditions such as periodic flooding by tides and variations in salinity, oxygen, temperature and nutrients availability. Mangrove sediments are valuable sources for the discovery of novel extremophilic enzymes compatible to those harsh industrial conditions. Taking this into account, this study aimed the partial purification and characterization of a moderately thermophilic lipase produced by *Bacillus subtilis* strain TIM27 isolated from mangrove sediment of Timonha-CE, Brazil. For lipase production, TIM27 strain was grown in Nutrient broth supplemented with 0.2% of glycerol as carbon source at 30 °C, 150 rpm for 48 h. The cell-free supernatant was subjected to ammonium sulfate precipitation (0-40% and 40%-70% of saturation) at 10 °C. Enzymatic activity was evaluated by agar plate diffusion using 0.1% tributyrin and by spectrophotometric method using pNP-butyrate as the substrate. The optimum pH was determined by measuring the hydrolytic activity in the following buffers: Acetate pH 6; sodium phosphate pH 7; Tris-HCl pH 8; Borate pH 9 and Carbonate-bicarbonate pH 10). The effect of temperature was determined by measuring the hydrolytic activity from 20 to 60 °C. As results the strain TIM27 presented extracellular lipolytic activity after 48 h of culture confirmed by the appearance of clear halos in tributyrin agar plates. After partial purification, the activity was mainly observed in the 40 – 70% ammonium sulfate fraction, presenting better specific activity of 1.5 U/mL when compared to crude extract (0.3 U/mL). The enzyme presented optimal hydrolytic activity in pH 8 and 50 °C. These results are very encouraging and the next steps involve further assays and enzyme characterization of this new thermophilic lipase.

**Keywords:** *Bacillus subtilis*, Enzymes, Lipases, Mangroves

**Development Agency:** Fundação Cearense de apoio ao desenvolvimento científico e tecnológico (Funcap) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)