An spectrophotometric assay in 96-well microplate for rapid detection of lipase synthetic activity in organic solvent

Authors: Romano, I. P., Pereira, S. W., Machado, J. Q., Zanotto, S. P.

Institutionals: UEA - Universidade do Estado do Amazonas - Av. Carvalho Leal, 1777, Cachoerinha, Manaus AM ; UFAM – Av. General Rodrigo Otávio, 3000, Coroado, Manaus-AM; IFAM CPIN – Estrada Odovaldo Novo, s/n, Comunidade Aninga, Parintins AM)

Lipases with synthetic activity in organic solvents are used as biocatalysts in various industrial activities, such as fine chemical or drugs production. Several research groups perform screening programs of microorganisms demand for new lipases with features especially useful for these processes. The development of assays that allow quick and accurate synthetic activity detection lipases in organic solvents is critical to the success of these programs. Teng and Xu proposed in 2007 by spectrophotometric assay based on the release of p-nitrophenol resulting from the transesterification of p-nitrophenyl palmitate in ethyl palmitate for lipase activity detection in heptane. They quantify the release of p-nitrophenol by absorbance at 410 nm measured in a 1 ml cuvette common UV-Vis spectrophotometer. In this study, the test proposed by Xu Teng and was adapted for use in a spectrophotometer with 96-well microplate reader. Two commercial lipase (Amano Lipase PS and Sigma-Aldrich acrylic resin from *Candida antarctica*) and crude mycelial preparations from three fungi (*Picnoporus sanguineus*, *Phomopsis* sp. and *Trichoderma* sp) were used in this study. The colorimetric assays were carried out in 2-mL Eppendorf tubes. For commercial lipase was used the weight in milligrams equivalent to 300 U. For crude mycelial preparations were used 10 mg. In both cases, biomass was mixed with 0.5 ml stock solution (10 mM pNPP in n-hexane). To start the reaction, 30 µL ethanol (1 M) was added to the reaction mixture. The mixture was incubated at 40 °C with a shaking speed of 200 rpm for 30 min. After settling the lipase in the reaction mixture for 30 s, 25 µL of the clear supernatant was taken and then mixed with 1 ml of 0.1 M NaOH in Eppendorf tubes. The pNP liberated was extracted by the aqueous alkaline phase, and then 200 µL of each tube was taken and added in a well of the microplate. The extraction was detected at 410 nm against a blank without enzyme using a UV–visible spectrophotometer SpectraMax 384 Molecular Devices. The molar extinction coefficient of pNP was estimated at 1.97 · 10[4] M[–1] cm[–1] by using standard solutions of pNP in NaOH. In the reaction conditional described above, the best results were obtained, respectively, with the lipases of Amano and Sigma Aldrich (44,8 µM and 2,33 µM). This assay adapted from Teng and Xu proposal has shown potential to be used in synthetic lipase activity screening programs.

Key- Words: lipase assay, organic solvents, synthetic activity, 96-well microplate, Amazonian fungi

Agency: FAPEAM