Tipo de Comunicación:
Póster: P_IB_49

Simposio:
INGENIERÍA BIOQUÍMICA: BIOCATÁLISIS, BIORREACTORES, DOWNSTREAM, BIOSENSORES E INTEGRACIÓN DE PROCESOS

Título:
Simultaneous production and recovery in situ of fibrinolytic protease from Mucor subtilissimus UCP 1262

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Palabras Clave:
fibrinolytic protease; Mucor subitilissimus; PEG/ammonium sulphate; ATPS; extractive fermentation

Comunicación:
Fibrinolytic proteases are enzymes that degrade fibrin and are a promising alternative for thrombolytic therapy. The aim of this study was to evaluate the optimum conditions for the integrated production and purification of fibrinolytic protease from Mucor subtilissimus UCP 1262. The integrated process of production and purification was carried out in a culture medium containing wheat bran and by adding polyethylene glycol (PEG) and $(NH_4)_2SO_4$ according to a 2^3 experimental design. A 2^3 full factorial design was used to investigate the influence of PEG molar mass, PEG concentration and salt concentration on the responses, namely partition coefficient (K), activity yield (Y) and purification factor (PF). The ATPS was composed of PEG (molar mass 400, 3350 and 8000g/mol) PEG (concentrations 15.0, 17.5 and 20.0%) and ammonium sulphate (concentrations 15, 20 and 25%). In all assays, the enzyme preferentially partitioned to the PEG phase (K>1), and the highest fibrinolytic activity was found in the PEG phase 23.15 U/mL in the trial 7 containing PEG 400 (20%) and Salt (25%). The fibrinolytic enzyme from Mucor subitilissimus UCP 1262 was pre-purified after extractive fermentation in PEG and ammonium sulphate ATPS, in which the fungal strain was able to grown even in high salt concentration, produced and extracted simultaneously to the PEG phase. The fibrinolytic Specific Activity in the PEG phase (SAPEG) of 135.51 U/mg was greater than in the crude extract 19.27 times-fold, since the Specific Activity of the crude extract of the homogeneous fermentation (SACE) was 7.03 U/mg. The main difference with this system composition, in comparison with other conventional systems, is the integration of the liquid-liquid extraction technique with fractional precipitation. The results indicate the use of a low-cost components and the integration of fermentation with an aqueous two-phase system extraction may be a promising alternative for the production and extraction of fibrinolytic proteases.