Partitioning of Fibrinolytic Protease from *Bacillus* sp. UFPEDA 485 by Aqueous Two-Phase Systems using PEG/Sodium Sulfate

Souza, F.A.S.D.¹; Nascimento, J.C.S.¹; Sales, A.E.¹; Silva, N.M. ²; Marcos, J.C. ²; Porto, T.S¹; Teixeira, J.A.C³; Porto, A.L.F¹.

¹ Department of Animal Physiology and Morphology (DMFA), Universidade Federal Rural de Pernambuco, Recife, Brazil.
² Department of Chemistry (DC), University of Minho, Braga, Portugal.
³ Institute of Biotechnology and Bioengineering (IBB) / Department of Biological Engineering (DEB), University of Minho, Braga, Portugal.

The fibrinolytic protease produced by bacteria of the genus *Bacillus* has attracted large interest in the pharmaceutical industry as a promising alternative in thrombolytic therapy due to their effectiveness in degrading fibrin, its production requiring the development of an efficient recovery process. Aqueous two-phase system (ATPS) have been recognized as an efficient and economical process for recovering enzymes due to their relative ease and low cost. The purpose of this work was to study the partition of fibrinolytic protease produced by *Bacillus* sp. UFPEDA 485 in a ATPS composed by Polyethylene glycol (PEG) and sodium sulfate using factorial design. The fibrinolytic protease production occurred in liquid culture medium containing 2% soy flour, pH 7.2, 150 rpm at 37 °C for 48 hours. To study the partitioning, was used a $2^3$ full factorial design with four replicates at the central with the purpose of evaluating the effects and interactions of the independent variables: PEG molar mass (MM$_{PEG}$), PEG concentration (C$_{PEG}$) and sodium sulfate concentration (C$_{Na_2SO_4}$) on the response variable: partition coefficient (K) of the fibrinolytic enzyme. In all the runs the enzyme partitioned to the top phase, indicating a significant interaction between the protein and the PEG. The best result was obtained at the central point, using MM$_{PEG}$ 6000 g/mol, C$_{PEG}$ 24 % and C$_{Na_2SO_4}$ 11.6 %. The partitions coefficients ranged between K = 327.63 and 2879.38. According to the results, the ATPS composed of PEG/sodium sulfate proved to be a promising method to extraction fibrinolytic protease.

**Word Keys:** ATPS, PEG/Sodium Sulfate, Fibrinolytic protease, Bacillus

**Supported by:** CAPES e CNPq.