Título: Fibrinolytic Proteases from Filamentous Fungi Belonging to the Fungi Collection of Amazonia-CFAM / FIOCRUZ-AMAZONIA

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ABSTRACT

The mining of naturally occurring biological resources, such as microorganisms, has driven many important developments in the bioindustry. In this study we have selected 50 fungal strains presently maintained in the "*Coleção de Fungos da Amazônia"* and investigated their fibrinolytic protease activity. Fibrinolytic proteases of microbial origin can be used in the treatment of thrombosis and this study was therefore designed to identify new fibrinolytic protease sources, which might later help with the development of new anti-thrombolytic treatments. Fungal samples were activated in Agar Malt Extract (MEA) and incubated in a BOD incubator at 28°C before being tested for proteolytic activity on Milk Agar plates. The samples positive for protease production were subjected to submerged fermentation. Each fermented enzyme extract was then used to quantify the proteolytic activity using a spectrophotometer. The same proteolytic-positive fungal extracts were also used to inoculate fibrin plates to detect fibrinolytic activity. From these initial enzymatic activities, a single strain of fungi (with the highest level of proteolytic activity and fibrinolytic action) was selected for two-phase-aqueous enzyme purification. Of the 50 samples tested, 84% showed protease activity. Especially high levels of activity were recorded for the sample *Penicillium implicatum* (CFAM 0521). In determining the fibrinolytic action, 76% of the samples were positive in the fibrin plate. Test results ranged from: 0.5 to 2.0 cm in halo size. *Penicillium implicatum* CFAM 0521 had halo size of 1.9 cm, and *A. flavus* CFAM 0523 had a halo size of 2.0 cm. During the purification of *Penicillium implicatum* (CFAM 0521), it was observed that the greater number of proteases remained in the top phase (rich in PEG), but proteases with fibrinolytic action remained in the bottom phase (rich in salt). This study highlights the potential of Brazilian biodiversity for the production of fibrinolytic proteases from filamentous fungi. However, further investigations are needed before the developments reported here can be harnessed for the treatment of thrombosis and other diseases.

Palavras-chaves: Fibrinolytic Proteases, Amazon Collection, Filamentous Fungi.

Agência de Fomento: FAPEAM, CNPq.