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Aspergillus micromycetes as Producers of Hemostatically Active Proteases

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Abstract : Micromycetes from Aspergillus genus can produce proteases capable of promoting proteolysis of hemostasis proteins or, along with hydrolytic activity, to show the ability to convert proenzymes of this system activating them into an active form. At the same time, practical medicine needs specific activators for quantitation of the level of some plasma enzymes, especially protein C and factor X, the lack of which leads to the development of thromboembolic diseases. Thus, some micromycetes of the genus Aspergillus were screened for the ability to synthesize extracellular proteases with promising activity for designing anti-thrombotic and diagnostic preparations. Such standard methods like salting out, electrophoresis, isoelectrofocusing were used for isolation, purification and study of physicochemical properties of proteases. Enzyme activity was measured spectrophotometrically fibrin as a substrate of the reaction and chromogenic peptide substrates of different proteases of the human hemostasis system. As a result of the screening, four active producers were selected: Aspergillus janus 301, A. flavus 1, A. terreus 2, and A. ochraceus L-1. The enzyme of A. janus 301 showed the greatest fibrinolytic activity (around 329.2 µmol Tyr/(ml × min)). The protease produced by A. terreus 2 had the highest plasmin-like activity (54.1 nmol pNA/(ml × min)), but fibrinolytic activity was lower than A. janus 301 demonstrated (25.2 µmol Tyr/(ml × min)). For extracellular protease of micromycete A. flavus a high plasmin-like activity was also shown (39.8 nmol pNA / (ml × min)). Moreover, according to our results proteases one of the fungi - A. terreus 2 were able to activate protein C of human plasma - the key factor of the human anticoagulant hemostasis system. This type of activity was 39.8 nmol pNA/(ml × min)). It was also shown that A. ochraceus L-1 could produce extracellular proteases with protein C and factor X activator activities (65.9 nmol pNA/(ml × min) and 34.6 nmol pNA/(ml × min) respectively). The maximum accumulation of the proteases falls on the 4th day of cultivation. Using isoelectrofocusing was demonstrated that the activation of both proenzymes might proceed via limited proteolysis induced by proteases of A. ochraceus L-1. The activatory activity of A. ochraceus L-1 proteases toward essential hemostatic proenzymes, protein C and X factor may be useful for practical needs. It is well known that similar enzymes, activators of protein C and X factor isolated from snake venom, South American copperhead Agkistrodon contortrix contortrix and Russell's viper Daboia russelli russeli, respectively, are used for the in vitro diagnostics of the functional state of these proteins in blood plasma. Thus, the proteases of Aspergillus genus can be used as cheap components for enzyme thrombolytic preparations.

Keywords : anti-trombotic drugs, fibrinolysis, diagnostics, proteases, micromycetes
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