Extended Abstract
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Proteases produced by micromycetes of genus Aspergillus with promising activity for diagnosing and treating the hemostasis system dysfunction diseases

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Statement of the Problem: Various thrombolytic agents, which are used in therapy, are proteolytic enzymes with the activity similar to the one of hemostasis system proteases. Such activity was also found in proteases present in venom of snakes and in cultures of microorganisms. The preparations obtained on their basis are used as a part of diagnostic kits and as therapeutic agents for the detection and treatment of such complications.In diagnosing and treating the hemostasis system dysfunction diseases preparations based on proteases of animal origin are used since they have a higher specificity compared to bacteria-synthesized enzymes; yet, their disadvantage comes at high cost. Therefore, searching for protease-producers active against the proteins of human hemostasis system and revealing their properties is challenging for modern biomedicine. Methodology & Theoretical Orientation: Here micromycetes of the genus Aspergillus were screened for ability to synthesize extracellular proteases with promising activity for designing anti-thrombotic preparations. For isolation, purification and study of physicochemical properties of proteases, standard methods were used (salting out, electrophoresis, isoelectrofocusing). Enzyme activity was measured spectrophotometrically using native proteins (fibrin, fibrinogen) and chromogenic peptide substrates (HD-Val-Leu-Lys-pNA, pGlu-Pro-Arg-pNA). Findings: As a result of the screening, 3 active producers were selected: Aspergillus alliaceus 7dN1, A. ustus 1, A. terreus 2. The studied extracellular proteases, produced by these micromycetes, had a molecular weight of 30, 33, 34 kDa and isoelectric point of 8.21, 5.10, 4.71, respectively. The protease of micromycete A. ustus 1 showed the greatest fibrinolytic activity (134.4 µm Tyr/ml*min), and the enzyme produced by A. alliaceus has the maximum fibrinogenolytic activity (184.0 µm Tyr/ml*min). The extracellular protease of A. terreus 2 possessed the highest plasmin-like activity (54.1 nmol pNA/ml*min), but the activity against native proteins was relatively low: fibrinolytic - 25.2 µm Tyr/ml*min, fibrinogenolytic - 85.9 µm Tyr/ml*min. However, the ability of A. terreus 2 proteases to activate the key factor of human anticoagulant hemostasis system — protein C (39.8 nmol pNA/ml*min) was shown. The strains were maintained in tubes with slant agar. Agar-plate cultivation of the fungi was carried out on Czapek's media with 1% casein or fibrin addition as the single source of nitrogen. First, on the medium containing wort, glucose, and pepton, and then, after two days of cultivation, the biomass was transferred into the fermentation media containing (g/L) glucose, 30.0; glycerol, 70.0; NaNO3, 2.0; fish flour 5.0; MgSO4 ⍰ 7H2O, 0.5; and KH2PO4, 0.5 (medium 1) or glucose, 35.0; starch, 1.2; peptone, 5.0; fish flour 5.0; NaCl, 2.0; MgSO4 ⍰ 7H2O, 0.5; and KH2PO4, 0.5 (medium 2). The activity of hemostatic proteases can be determined by the cleavage of sensitive chromogenic substrates, p-nitroanilides, after preliminary incubation in the presence of protein activators. An experimental scheme for testing the activity of proteases obtained from the fungal culture medium is based on this principal. Initially, micromycetes were selected according to the productivity of protease production, calculated from the enzymatic indices, as indicated. The testing was carried out using chromogenic peptide substrates cleaved by various proteins of the human hemostasis system: H-D-Val-Leu-Lys-pNA, a plasmin substrate; Bz-Ile-Glu(OR)-Gly-Arg-pNA and Z-D-Arg-

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Gly-Arg-pNA, human plasma Xa factor substrates; Glp-Pro-Arg-pNA, a substrate for activated protein C; Glp-Gly-Arg-pNA, an urokinase substrate; H-D-Phe-Pip-Arg-pNA and Tos-Gly-Pro-Arg-pNA, thrombin substrates. After this primary screening, cultures with higher EI for fibrin containing medium (values of EI more than 1.00 ± 0.05) and lower values for casein containing medium (values of EI less than 1.50 ± 0.1) were selected for further research. Conclusion & Significance: Thus, the proteases of micromycetes of the genus Aspergillus as components for enzyme antithrombotic preparations are promising. They were demonstrated to have a broad substrate specificity of extracellular proteases produced by various micromycetes, combined with economic advantage of cultivating these producers

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