

Proteolytic Enzymes of Basidiomycetes (Taxonomical and Ecological Aspects)

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The proteolytic activity (PA) of cultures and fruit bodies of Basidiomycetes belonging to various taxonomical and ecological groups was studied. The study involved a total of 700 strains and the fruit bodies of 400 species.

Fermentative activities of the mycelium and fruit bodies were revealed and estimated by means of its influence on a certain substrata, as in fibrin films (fibrinolytic activity, FA), human blood clots (thrombolytic activity, TA), milk (milk-clotting activity, MCA), and casein (caseinase activity, CA). A milk-clotting activity fairly often accompanies TA and FA. A caseinase activity should be considered as a nonspecific indicator of PA. Our main attention was paid to the study of ferments, which can execute the lysis of fibrin films and thrombi (FA and TA, respectively).

The current results allow the estimation of a certain level and character of proteolytic process for Basidiomycetes of various taxonomical groups as well as specific connections of certain types of

enzymatic activity with a taxonomical position and trophic preference of macromycetes under investigation. Besides, it becomes possible to determine some functions of fibrinolytic and thrombolytic proteinases in mushroom vitality and to estimate an opportunity of practical use of these ferments in certain branches of medicine.

Generally, it is possible to ascertain that the dominant type of activity within saprotrophs (together with xylothrophs) is CA, since it was observed among half of the studied species. The fibrinolytic activity was demonstrated only by representatives of 20–25% of total species. Within a symbiotrophic species the caseinase activity (as a nonspecific parameter of PA) was registered only at a quarter of investigated species, whereas FA is mentioned only at 12% of total species number.

It was established, within the aphyllorphoid fungi, the most expressed proteinase biosynthesis is characteristic to the families Polyporaceae and Schizophyllaceae, whereas within the agaricoid Ba-

sidiomycetes the families Pleurotaceae, Tricholomataceae, and Coprinaceae demonstrated the greatest activity. The active species of aphylophoroid fungi, in most cases, belong to the xylotrophs, which produce a white rot, no less than active agaricoid ones, which are the lignotrophs, decomposing the lignocellulose complexes of wood debris, forest litter, various composts, etc. The symbiotrophic species (families Amanitaceae, Cortinariaceae, Russulaceae, etc.) were characterized by a complete absence of fibrinolytic and thrombolytic activity (a few exceptions would include the genus *Tricholoma* sect. *Tricholoma* and some representatives of the order Boletales).

It seems the revealed pattern of distribution of proteolytic ferments of different substrate specificity (FA, TA, MCA, CA) in various taxonomical and ecological groups of Basidiomycetes certainly allows the prediction of the challenge of species with the mentioned biochemical activities and, besides, in some cases the ability to be useful taxonomically.

During the studies of fibrinolytic and thrombolytic activity of macromycetes cultures, the ability of Basidiomycetes to synthesize the proteinases of four types—namely, the aspartyl, the serine, the thiol, as well as the metalloproteinases—were confirmed. From the cultures of some representatives of the genus *Coprinus*, characterized by a high level of FA and TA, the proteinases of a serine type were allocated and described, and from the cultures of *Cerrena* and *Coprinus* species the metalloproteinases were received for the first time. The comparison of the biochemical and physicochemical characteristics of the proteinases received from the investigated basidiomycetes to those mentioned in the literature as bacterial, micromycetous, and vegetable proteinases allows us to conclude that there exists a principal similarity of these proteolytic enzymes.

It was also shown that Basidiomycetes of various trophic preferences synthesize the proteolytic fermentative complexes combining the proteinases of different classes. The metalloproteinases bring the basic contribution in the fibrinolytic activity among all the trophic groups. For example, the fibrinolytic activity of soil saprotroph *Coprinus domesticus* (Bolton) S.F.Gray is determined by the metalloproteinases on 47%, the serine proteinases on 30%, the thiol proteinases on 17%, and aspartyl only on 6%. The fibrinolytic activity of another soil saprotroph, *C. cinereus* (Schaeff.) S.F.Gray, is determined by these types of proteinases on 53, 21, 13, and 13%, respectively. Such type of activity for litter saprotroph *Clitocybe josserrandii* (Singer) Singer is caused by these types of proteinases in the ratio 40, 23, 28, and 9%. In the xylotrophic species, *Flammulina velutipes* (W.Curt.:Fr.) Singer and *Cerrena unicolor* (Bull.) Murrill, the contribution of metalloproteinases in FA is 70–90%, whereas in symbiotrophic *Tricholoma portentosum* (Fr.) Quéf. the fibrinolytic activity is caused by metalloproteinases completely (100%).

During the complex analysis of fibrinolytic and thrombolytic activity of this large group of fungi, the impression is created that there is a real dependence between the type of synthesized proteinases and the trophic preference of the fungi in question. High-level FA and TA in trophically specialized groups of saprotrophs and the occurrence of similar ferments in some symbiotrophic species can testify to an opportunity of transition of such taxa from obligate symbiotrophy to a facultative one. Such dependence of the type of synthesized proteinases and the trophic features of the fungi in question could be considered an additional criterion for estimation of the trophic amplitude of macromycetes in nature.