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Stimulation of Peroxidase Activity in *Inonotus radiatus* and *Phellinus pini* Fungi

Próby stymulacji aktywności peroksydazowej u grzybów *Inonotus radiatus* i *Phellinus pini*

Попытки стимулирования пероксидазовой активности у грибов *Inonotus radiatus и Phellinus pini*

INTRODUCTION

Fungi which belong to *Hymenomycetales* excrete some oxidoreductases into the medium (1, 2, 3, 5). Many species of fungi degrading wood such as *Pleurotus ostreatus*, *Pholiota mutabilis*, *Polyporus versicolor* and *Trametes versicolor* produce both exoenzymes — peroxidase and laccase (table 1).

There are, however, fungi which excrete into the medium only one of the enzymes mentioned e.g.: *Coriolus versicolor* excreting laccase and *Inonotus radiatus* as well as *Phellinus pini* excreting peroxidase. The above mentioned fungi fruit under natural conditions on trunks of deciduous and coniferous trees. Exoenzymes excreted by those fungi cooperate in creation of a proper habitat for the growth of mycelium and fruit bodies. Trojanowski's hypothesis (8, 9, 10) implies a role of peroxidase in degradation of lignin. Thus, the author of the present paper found it proper to choose those strains of fungi which excrete peroxidase into the medium and to investigate the influence of substances contained in wood meal on the level of peroxidase in filtrates from cultures of the fungi examined.

MATERIAL AND METHODS

The culture of *Inonotus radiatus* (Sow. ex. Fr. P. Karst.) — H. M. J. P. C. No 4335 as well as that of *Phellinus pini* (Thore ex. Fr. Pil.) — H. M. P. C. No 3377 were received from the Department of Forest Phytopathology, School of Agriculture, Cracow, by the courtesy of Prof. Dr S. Domański. The fungi were stored in agar medium. For the experiments cultures of fungi were inoculated into the liquid medium which consisted of 3% malt extract. Fungus mycelia were grown in Erlenmayer flasks containing 100 ml of medium. The experiments lasted 5 weeks. For each experiment 5 fungus cultures were used. Peroxidase activity in the filtrate of fungus culture was estimated by BAS test after Lyr (4) and by p-phenylenediamine method according to Aurand (6). In order to estimate the specific activity of peroxidase the total protein content was determined by the method of Lowry (7). Spekol colorimeter was used for colorimetric determination.

The following series of experiments were devised:

1. Control — basic medium, 3% malt extract.
2. Basic medium + beech wood meal. To 100 ml of basic medium 5 g of wood meal was added. The medium was sterilized at 100°C. Before being added to the medium the wood meal was sterilized at 80°C for 2 hrs.
3. Basic medium + wood meal extracted with ether. Dried wood meal was extracted with ether (100 g of wood meal per 150 ml of ether) and left for 24 hrs. Ethers was filtered, and the wood meal was dried at room temperature and added to the medium at the amount of 5 g per 100 ml of basic medium.
4. Basic medium + wood meal extracted with water. Dried wood meal was extracted with distilled water in boiling water bath under cover at the ratio of 5 g to 100 ml of water. The extract was filtered and after sterilization (3 times for 45 minutes) it was used in the 5th and 6th experiment. The wood meal was dried at room temperature and sterilized at 80°C for 2 hrs.
5. Basic medium + wood meal water extract. Water extract at the amount of 5 ml per 100 ml of medium was added just before inoculation of fungus mycelium. This amount corresponded to 0.5 mg of dry matter.
6. Basic medium + wood meal water extract added after a certain time of culture growth. Wood meal water extract was added to one-, two-, three week-fungus culture in basic medium.

The cultures of *Inonotus radiatus* or *Phellinus pini* were incubated at 25°C.

RESULTS AND DISCUSSION

In the culture of *Inonotus radiatus* and *Phellinus pini* grown in the basic medium enriched with wood meal (experiment 2), a stimulation of peroxidase activity was found (Fig. 1). The specific activity of peroxidase in the filtrate of 5-week culture of *Inonotus radiatus* was 36 times higher than in the control experiment. The filtrate of *Phellinus pini* culture showed a 10-fold increase of specific activity of peroxidase. Such remarkable increase of peroxidase activity in the medium was connected with simultaneous decrease of total protein content in it. Higher increase of peroxidase activity in *Inonotus radiatus* culture can be attributed to the occurrence of this fungus on trunks of deciduous trees under natural

conditions. *Phellinus pini*, however, lives on coniferous trees and hence a smaller influence of beech wood meal on the activity of peroxidase excreted by this fungus was observed.

Table 1. The activity of peroxidase and laccase in filtrates of two-week cultures of fungi. Medium — 3% malt extract + wood meal; 5 g/100 ml medium

Fungus species	Peroxidase		Laccase	
	BAS test (4) H_2O_2 A. U. × 10^{-4}	p-phenylenediamine + H_2O_2 (6) A. U. × 10^{-4}	BAS test A. U. × 10^{-4}	p-phenylenediamine A. U. × 10^{-4}
Pleurotus ostreatus	143	18	151	6
Pholiota mutabilis	738	356	732	23
Polyporus versicolor	66	16	66	2
Trametes versicolor	40	12.5	227	1.7
Coriolus versicolor	0	0	331	2
Inonotus radiatus	870	100	0	0
Phellinus pini	10	25	0	0

Abbrevation: A. U. — Activity units.

The increase of specific activity of peroxidase during mycelium growth of both fungus cultures grown in the medium supplemented with wood meal extracted with ether (experiment 3) was less intensive than that in experiment 2. Only after 5-week of growth — peroxidase activity in the filtrate reached the level of experiment 2 (Fig. 1). In the medium

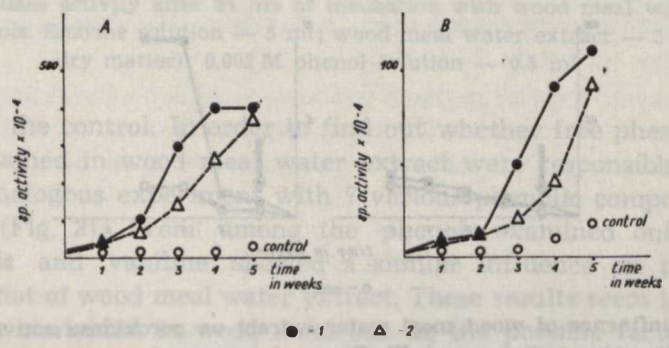

Fig. 1. The influence of wood meal on peroxidase activity in the filtrate of fungi culture: A — *Inonotus radiatus* and B— *Phellinus pini*; 1 — wood meal, 2 — wood meal extracted with ether. Peroxidase activity was determined by Lyr's method (4)

containing wood meal extracted with water (experiment 4) fungi did not grow. It could therefore be assumed that substances having a positive influence on mycelium growth and peroxidase production were present in wood meal water extract. Before the mycelium was inoculated an addition of wood meal water extract to the basic medium (experiment 5), caused the decrease of peroxidase activity in the filtrate of the fungus culture. This experiment was modified due to the assumption that substances being able to stimulate peroxidase activity may, at a higher concentration, have a destructive influence on small fungus fragments. An addition of wood meal water extract to one-week culture of *Inonotus radiatus* did not bring about any stimulation of peroxidase activity in the filtrate during four-week culture period. Wood meal water extract introduced into the medium of two-week culture caused stimulation of peroxidase activity two weeks later. But when the wood meal water extract

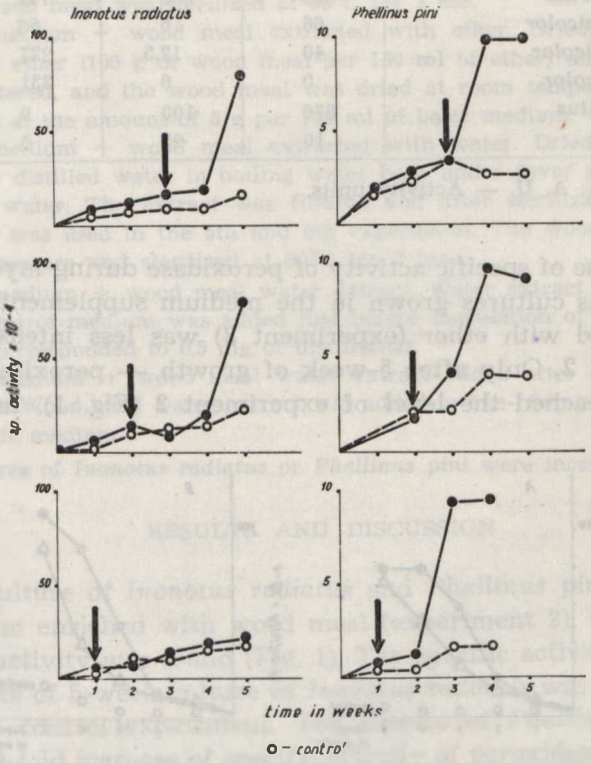

Fig. 2. The influence of wood meal water extract on peroxidase activity in liquid cultures of *Inonotus radiatus* and *Phellinus pini* fungi. Peroxidase activity in the filtrate of fungi culture was determined according to Lyr's method (4). Arrows denote the time of wood meal extract addition at the amount of 0.5 mg dry matter in 5 ml of water solution

was added after 3 weeks of growth, the stimulation effect occurred after one week. Peroxidase activity in the filtrate of fungus culture was about 3 times higher in experiments with *Inonotus radiatus* than that in the control (Fig. 2). Analogous experiments performed with the use of *Phellinus pini* culutres gave similar results (Fig. 2).

In the next experiment water extract from wood meal was added to the filtrate of the *Inonotus radiatus* culture, and that solution was incubated at room temperature for 24 hrs. After an hour of incubation peroxidase activity increased and remained on this level for 24 hrs (Fig. 3). The activity of peroxidase incubated with extract was 25% higher

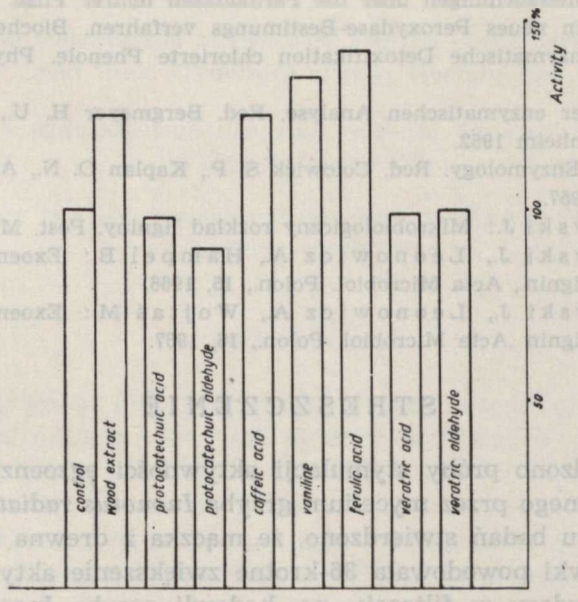

Fig. 3. Peroxidase activity after 24 hrs of incubation with wood meal water extract and free phenols. Enzyme solution — 5 ml; wood meal water extract — 5 ml (0.5 mg dry matter); 0.002 M phenol solution — 0.5 ml

than that of the control. In order to find out whether free phenolic compounds contained in wood meal water extract were responsible for that result, an analogous experiment with 7 various phenolic compounds was performed (Fig. 3). From among the phenols examined only caffeic, ferulic acids and vaniline showed a similar influence on peroxidase activity as that of wood meal water extract. These results seem to indicate that phenols contained in wood meal can be the possible factors which stabilize peroxidase in fungi. This is still more interesting because phenolic compounds are precursors of lignin. The experiments carried out showed that free phenolic compounds found in wood meal water

extract stimulated the peroxidase activity synthetised by mycelium of the *Inonotus radiatus* and *Phellinus* pini fungi.

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STRESZCZENIE

Przeprowadzono próby stymulacji aktywności egzoenzymu peroksydazy, wydzielonego przez mycelium grzyba *Inonotus radiatus* i *Phellinus pini*. W wyniku badań stwierdzono, że mączka z drewna bukowego dodana do pożywki powodowała 36-krotne zwiększenie aktywności specyficznej peroksydazy w filtracie po hodowli grzyba *Inonotus radiatus*. W analogicznych doświadczeniach z grzybem *Phellinus pini* aktywność peroksydazy wzrastała 10-krotnie.

W dalszych doświadczeniach próbowano bliżej scharakteryzować substancje zawarte w mączce drzewnej, wpływające na stymulację aktywności peroksydazy. Na podstawie przeprowadzonych badań przypuszcza się, że są to fenole.

РЕЗЮМЕ

Проведены попытки стимулирования активности экзоэнзима пероксидазы, выделяемого мицелием гриба *Inonotus radiatus* и *Phellinus pini*.

Мука букового дерева, добавленная в питательную среду, вызы-

вает 36-кратное увеличение специфической активности пероксидазы в фильтрате культуры гриба *Inonotus radiatus*. В аналогичных опытах с грибом *Phellinus pini* активность пероксидазы увеличивалась в 10 раз. В следующих опытах пробовали точнее охарактеризовать субстанции, содержащиеся в древесной муке, которые влияют на стимуляцию активности пероксидазы. На основе проведенных исследований допускается, что фенолы, содержащиеся в водном экстракте древесной муки, обладают стимулирующим влиянием на активность пероксидазы.

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