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Part Six

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A study of fungal complex development on wood

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The influence of the interspecific interaction during fungal development on wood was studied. The investigations showed that the release of some enzymes which were implicated in wood degradation (phenoloxidases and endoglucanase) and colony growth of one fungus could be changed by another individual in some cases.

Key words: fungi, interspecific relations, wood

INTRODUCTION

Most of natural substrates are decomposed by assemblages of microorganisms rather than by pure culture. Microfungi, early colonizers, and macrofungi (*Basidiomycetes)* take part in wood degradation [1]. The occupation of substrata depend not only on physical (temperature, light, relative humidity) and stochastic (the order in which individual spores arrive on the patch and their location on the patch) factors, but on interspecific interaction as well [2–3].

During interspecific interaction fungal colonies release metabolites, most of which are non-enzymatic in nature, however, some enzymes are secreted in response to the presence of another fungus. These metabolites can enhance their ability to capture previously colonized substrates or to defend their own substrate base [4]. Our studies were devoted to investigation of the development of the fungal complex and their pure cultures on wood. The aim was to estimate the influence of interspecific interactions on the production of extracellular enzymes which are implicated in cellulose (endoglucanase) and lignin (phenoloxidases) degradation and fix the changers of biological peculiarities.

MATERIALS AND METHODS

The following fungal strains, isolated from wood were used in this study: *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire 1-8, *Fusarium* sp. S-7, *Sporotrichum olivaceum* (Link et Fr,) Fr. 4KA and *Trichoderma harzianum* Rifai P1-1.

The international wood block testing standard ENV 807:1993 was used to determine the destructive ability of pure fungal cultures and their complex [5]. Wood (*Pinus* sp.) blocks 10 x 25 x 5 mm were infected with a spore suspension of solitary cultures or their mixture, incubated for 12 weeks at 25 °C, after which weight losses of blocks were recorded as a percentage of dry weight.

Enzyme assays were carried out on pure (control) and paired fungal cultures grown both on standard Czapek agar (CA) and low nutrition medium (LNM) designed to reflect the carbon : nitrogen ratio in wood. [6]. All plates were inoculated using 5 mm cores taken from stock culture plates, and interaction studies were carried out as described by Score et al. [4]. The confrontation plates were incubated until one fungus began to intermingle with the other colony. Three duplicate plates were prepared for each enzyme assay. For the endoglucanase assay, 0.5% of CMC (carboxymethylcellulose) was added to the substrate, and Congo Red was used for elucidation of enzyme activity. 0.5% of gallic acid was added to both media studied for phenoloxidase estimation [7].

RESULTS AND DISCUSSION

The results of wood block infection based on the ENV 807:1993 indicated that the fungal complex produced a significantly greater weight loss (1.27 ± 0.15) developing on wood than solitary strains and showed distinct differences in mean weight losses caused by infection of solitary cultures (Fig. 1). *Sporotrichum olivaceum* 4KA gave the greatest wood block weight loss (0.73 ± 0.02) among all solitary strains tested. The *Alternaria tenuissima* 1-8 strain showed no change in block weight, and at visual examination no micelium was noticed on these blocks. *Fusarium* sp. S-7 and *Trichoderma harzianum* P1-1 caused the wood block weight losses nearly of the same degree after 12 weeks of incubation.

Consequently, the superiority of the fungal complex in wood colonization was evident. In nature, the struggle for substrate leads to its degradation.

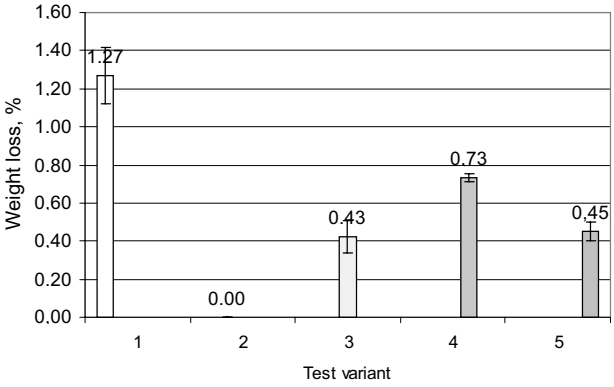

Fig. 1. Mean dry weight loss (%) of wood blocks infected with fungal complex (1) and pure cultures: *Alternaria tenuissima* 1-8 (2), *Fusarium* sp. S-7 (3), *Sporotrichum olivaceum* 4KA (4) and *Trichoderma harzianum* P1-1 (5)

On the presumption of the previous experiments that the strains tested didn't produce suppression metabolites in the competition for substrate, we studied the release of extracellular enzymes implicated in wood degradation during interspecific interaction.

Some facts of increase of phenoloxidase production in paired cultures were noticed in confrontation experiments. *Alternaria tenuissima* 1-8 released phenoloxidases in all studied pairings grown on CA, but on LNM no colored zones were formed around its colonies. The increase of phenoloxidase release (compared with solitary colonies) by *Sporotrichum olivaceum* 4KA in pairing with *Alternaria tenuissima* 1-8 (Fig. 2) and by *Fusarium* sp. S-7 in pairing with *Trichoderma harzianum* P1-1 were noticed on LNM.

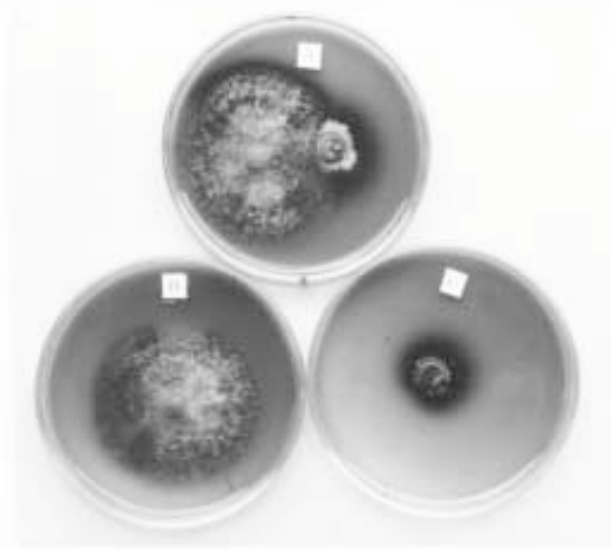

Fig. 2. The assessment of phenoloxidases activity of the paired cultures of *Alternaria tenuissima* 1-8 and *Sporotrichum olivaceum* 4KA (A), of the solitary culture *Alternaria tenuissima* 1-8 (B) and solitary culture *Sporotrichum olivaceum* 4KA (C) on the LNM

The same carbon source (CMC) in both media studied perhaps predetermined the similar results. Judging from pale orange zones round the colonies, all the strains studied released a similar amount of endoglucanase in pairings and growing alone. Only *Trichoderma harzianum* P1-1 caused some increase of endogluconase production at the confrontation sites in pairings; for *Alternaria tenuissima* 1-8 from 2 to 5 mm and for *Fusarium* sp. from 2 to 4 mm.

Some stimulation of growth was noticed in the tests of the interspecific relations. Some fungal colonies were larger in diameter in the pairing than growing alone: the *Alternaria tenuissima* 1-8 colonies were 50 ± 2 mm in pairing with *Fusarium* sp. S-7 (grown alone 44 ± 1 mm), and the diameter of the *Fusarium* sp. S-7 colonies was 16 ± 0 mm in pairing with *Sporotrichum olivaceum* 4KA (8 ± 2 mm grown alone) after 4 days of incubation on Czapek medium (Fig. 3).

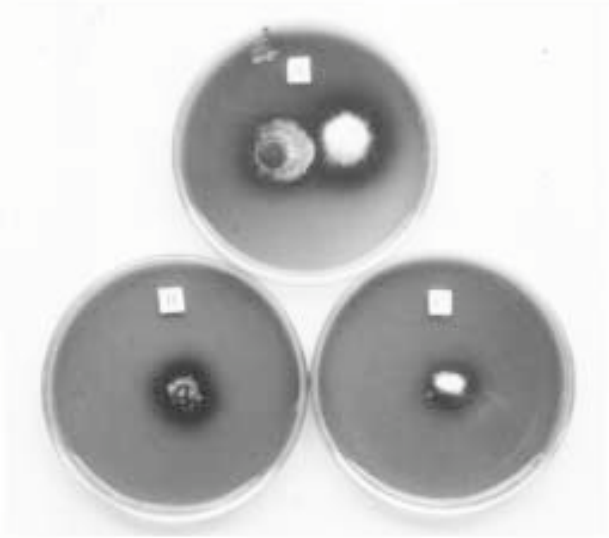

Fig. 3. The assessment of phenoloxidases activity of the paired cultures of *Sporotrichum olivaceum* 4KA and *Fusarium* sp. S-7 and (A), of the solitary culture *Sporotrichum olivaceum* 4KA (B) and solitary culture *Fusarium* sp. S-7 (C) on the Czapek medium

The results of the tests revealed that release of some extracellular enzymes such as phenoloxidases and endogluconase implicated in wood degradation and colony growth of one fungus could be changed by another individual in some cases. The enhancing of fungal activity in wood colonization by the complex action is evident. It is important, because early wood invaders predetermine the next step of wood degradation by *Basidiomycetes.* More precise studies are needed to elucidate the regularities of this phe-

nomenon. This fact must be taken into consideration when a fungal complex is used for practical purposes.

References

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MIKROMICETŲ KOMPLEKSO VYSTYMOSI ANT MEDIENOS TYRIMAI

S a n t r a u k a

Mikromicetų vystymasis ant medienos, kaip ir ant kitų substratų, priklauso ne tik nuo fizikinių bei stochastinių veiksnių, bet ir nuo tarprūšinių santykių su kitais individais, jau esančiais arba patenkančiais ant substrato. Mikromicetai, kaip pirminiai medienos pažeidėjai, lemia tolesnę medienos destrukcijos eigą.

Tyrimuose, panaudojus ne tik standartinę Čapeko, bet ir skurdžią mitybinę terpę, kurios cheminė sudėtis atitiko medienos C: N santykį, buvo pastebėti fermentų, dalyvaujančių medienos destrukcijoje (endogliukanazių ir fenoloksidazės), aktyvumo priklausomybė nuo konfrontuojančių individų. Mikromicetų kompleksas gerokai aktyvesnis (pastebėtas didesnis medienos masės netekimas ir apaugimas) už pavienes kultūras, vystantis ant medienos, tačiau šio reiškinio dėsningumams nustatyti reikia kruopščių tyrimų.