Science and Cultivation of Edible Fungi, Elliott (ed.) © 1995 Balkema, Rotterdam. ISBN 90 5410 570 4

Effects of *Bacillus macerans* Fr. on growth of *Pleurotus ostreatus* (Jacq.:Fr.) Kumm.

Nina A. Bis'ko & Victor T. Bilay
N.G. Kholodny Institute of Botany, National Academy of Sciences, Kiev, Ukraine

ABSTRACT: Biotic effect of *Bacillus macerans* strains on growth of *Pleurotus ostreatus* (Jacq.:Fr.) Kumm.were studied. It was shown that there was a positive influence of filtrates of *B.macerans* strains, extracts of the pine, alder, poplar or willow bark (0,5 and 0,005%) and vitamins on mycelial biomass of *P.ostreatus*. Mutual growth of *B.macerans* with *P.ostreatus* increase the cellulose and lignin decomposition of wheat straw in comparison with *P.ostreatus* monoculture.

1 INTRODUCTION

Biotic factors play an important role in cultivation of higher edible Basidiomycetes. It was shown that productivity of cultivated mushrooms is greatly dependent on microorganisms which inhabit substrates or casing soil (Stanek 1972; Gyurko 1979; Cochet *et al.* 1992). By substrate component composition fermentation conditions it is possible to affect the formation of microflora selective for cultivation of *Pleurotus ostreatus* (Jacq.:Fr.) Kumm. (Stanek, Bis'ko 1982). It has been found that the substrate selectivity during fermentation depends on the activities of thermophilic bacteria of genus *Bacillus*: *B.macerans*, *B.subtilis*, *B.mesentericus*, *B.polymyxa* (Mrazkova *et al.* 1976, Flick 1982). We have studied the effect of *B.macerans* strains during growth of *P.ostreatus* on wheat straw and glucose-asparagine medium.

2 MATERIALS AND METHODS

2.1 *Strains*

B.macerans (7 strains) were isolated from wheat straw after fermentation of this substrate for 48 h at 50-55°C. *P.ostreatus* strain 517 was isolated from fruit bodies collected from *Fagus silvatica* L. in the Lvov district, Ukraine.

2.2 *Influence of* B.macerans *on destruction of wheat straw by* P.ostreatus

B.macerans strains were cultivated on sterile wheat straw during 14 days at the temperature 50°C. *P.ostreatus* 517 was cultivated on sterile wheat straw for 14 days

at 28°C. Influence of *B.macerans* on degradation of wheat straw by *P.ostreatus* was studied by cultivation of *P.ostreatus* on wheat straw after growth of *B.macerans*. The content of cellulose (Pleshkov 1976) and lignin (Obolenskaja *et al.* 1955) was determined during growth of pure cultures of *B.macerans* (average for 7 strains), *P.ostreatus* and their growth in co-culture.

2.3 *Influence of* B.macerans *filtrates on growth of* P.ostreatus

Mycelium of *P.ostreatus* was grown on glucose-asparagine medium (GAM) (g/l:glucose 10, asparagine 0.4, KH_2PO_4 1, $MgSO_4$.7H2O 0.5) for 3 days at 28°C. The effect of bark from different trees, vitamins and filtrates of *B.macerans* strains on growth of *P.ostreatus* was studied by their adding to the GAM. Extracts of alder, pine, poplar or willow bark (0.5 or 0.005%) were used. Extracts were prepared by boiling 5 g of bark for 15 min in 100 ml of distilled water. Vitamins (ppm/10 ml) B_1-100 or mixture of vitamins B_1-100, B_6-10, pantoyl-β-alanine-10, niacin-10, mesoinositol-10, biotin-0.1 were used. *B.macerans* were grown on GAM for 3 days at 50°C. Then its cultural liquid was filtered through the bacterial filter. Bacterial filtrates were used at a concentration of 0.05%.

2.4 *Scanning electron microscopy observation*

Samples of wheat straw after mutual growth of *B.macerans* and *P.ostreatus* were fixed in a vapour of 2% OsO_4 for 48 h. The specimens were sputter coated with gold and examined in a JEOL JSM 35-C Scanning Electron Microscope(SEM).

3 RESULTS AND DISCUSSION

The main components which *P.ostreatus* used during growth on substrates are cellulose and lignin. We studied the ability of *B.macerans* strains to decompose this complex of natural polymers. Out of seven strains studied, seven had a high cellulolytic activity and grew on filter paper as a sole source of carbon. Investigated strains of *B.macerans* destroyed both cellulose and lignin during growth on sterile wheat straw (Table 1). Addition of *B.macerans* to sterile wheat straw followed by its inoculation with *P.ostreatus* intensified cellulose decomposition by 30.8% and lignin by 37.5% as compared to *P.ostreatus* monoculture (Table 1). Our previous investigations showed the ability of the *B.macerans* strains to grow on nitrogen-free medium using glucose or filter paper as a source of carbon. It was proved that *B.macerans* produces, during

Table 1. Influence of *B.macerans* on degradation of sterile wheat straw (SWS) by *P.ostreatus*

Variant of experiment	Cellulose		Lignin	
	content, % a.d.m.	losses, %	content, % a.d.m.	losses, %
SWS inoculated by *Bac.macerans*	40,0	11,4	18,0	4,7
SWS inoculated by *P.ostreatus*	39,0	14,4	17,5	7,4
SWS inoculated by *P.ostreatus* after the growth of *Bac.macerans*	36,0	20,8	17,0	10,2

the fermentation of wheat straw, water-soluble polysacharides, various vitamins, heteroauxins and other biologically active substances in the substrate(Mrazkova *et al.* 1979; Stanek, Bis'ko 1982). It has been shown that by adding *B.macerans* P_9 to substrates before their fermentation results in a decrease of negative influence of contaminating moulds (for example *Trichoderma* spp.) and an increase in the yields of *P.ostreatus* by 30 - 100% (Stanek, Bis'ko 1982).

We studied the effect of the filtrate of *B.macerans* on growth of *P.ostreatus* on GAM in comparison with different substances. Minimal positive influence on growth of *P.ostreatus* was noted for extracts of pine bark - the quantity of mycelium was increased by 5-11% depending on concentration. Maximal positive effect was shown for alder and willow bark on the growth of mycelium of *P.ostreatus* - by 65%. The increased content of all studied tree extracts bark from 0.005-0.5% influenced the increase of *P.ostreatus* biomass by 5-42% and 11-65% respectively. The biomass of *P.ostreatus* was increased by adding vitamin B_1 or a mixture of vitamins by 65 and 52% respectively. The most mycelial biomass occurred when the filtrate of *B.macerans* strain P_9 was added - up to 85% in comparison with biomass on GAM.

Studies of growth of *B.macerans* and *P.ostreatus* on wheat straw by using SEM showed the concentration of bacterial cells near mycelium of *P.ostreatus* and their close attachment to each other (Figure 1a,b). The same formation of a hyphosphere of *Agaricus bisporus* with bacteria in mushroom compost was described by Stanek (1974). Our results confirm the important role of *B.macerans* in the formation of selective substrates for the cultivation of *P.ostreatus*.

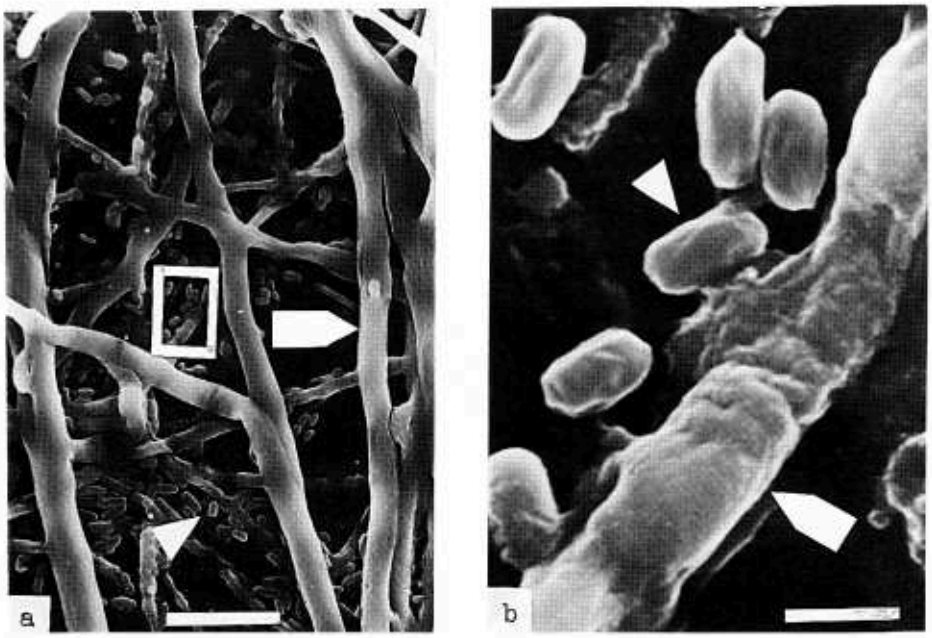

Figure 1. a, mycelial growth of *P.ostreatus* (arrow) on fermented wheat straw by *B.macerans* P_9 (arrowhead) - bar mark = 10 µm; b, part of Figure 1a - bar mark = 1 µm.

4 REFERENCES

Cochet, N., Gillman, A. & Lebeault, J.M. (1992). Some biological characteristics of the casing soil and their effect during *Agaricus bisporus* fructification. *Acta biotechnol.* 5,411-419.

Flick, M. (1982). Biologie und Biotechnologie der Substratefermentation beim Austernpilz (*Pleurotus* sp.). *Der Champignon* 245,29-30.

Gyurko, P. (1979). Importance of bacterial preparation for substrate of *Pleurotus ostreatus*. *3rd Int.Symp. Physiology, Ecology and Cultivation of Edible Fungi. Prague*: pp19-20. Mrazkova,L., M.Dobra,

Kriz, J. & Stanek, M. (1979). Microorganisms in fermented nutrient substrate for *Pleurotus ostreatus* - a review of results. *3rd Int.Symp. Physiology, Ecology and Cultivation of Edible Fungi. Prague* pp 143-148.

Pleshkov, B.P. (1976). *Methods of biochemical analysis* pp 15-17. Moscow:Science. Obolenskaja,A.V.,

Tchegolev, V.P. & Akim, G.L. (1955). *Practical work on chemistry of wood and cellulose.* Moscow:Lesnaya Promyshlennost.

Stanek, M. (1972). Microorganisms inhabiting mushroom compost during fermentation. *Mush.Sci.VIII*,797-811.

Stanek, M. (1974). Bacteria associated with mushroom mycelium, *Agaricus bisporus* (J.Lge)Imbach., in hyphosphere. *Mush.Sci.IX*,197-207.

Stanek, M. & Bis'ko, N.A. (1982). Regulation of microbiological processes in substrate for the cultivation *Pleurotus ostreatus*. *Zahradnictvi*. 3,221-233.