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INVESTIGATIONS ON MACROFUNGI PRODUCTION EFFECTED BY FUNGISTASIS AT PILISSZENTKERESZT (HUNGARY)

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Fungistasis of bacterial origin was studied by fungal production in *Bacillus subtilis* treated and control sites among natural conditions over three years. It was established that in the treated site the primary decomposer and the mycorrhizal fungi show no considerable difference in the number of fruit-bodies compared to the controls. However, the numbers of species and fruit-bodies greatly increased. According to one of the possible reasons *Bacillus subtilis* starts the decomposing process, makes nutrients more easily available for secondary decomposers. Another possibility that *Bacillus subtilis* produces such fungistatic substances which inhibit or slow down the growth of the competitor organisms, while this effect is weaker on macrofungi.

Key words: *Bacillus subtilis*, fungistasis, fruit-body, macrofungi, production

INTRODUCTION

Under natural conditions macrofungi are in continuous interaction with several other groups of living organisms. This fact is clearly demonstrated by the changes of several developing parameters of a macrofungi which was taken into sterile culture. Growth is usually accelerated, since nutrient sources are more abundant in the lack of competition with other species. The external conditions (temperature, humidity, etc.) are also possible to be optimalised. In case of fruit-body formation the correlation is not so clear. Most of the wood decaying fungi are able to produce fruit-bodies also under sterile conditions. In the case of mycorrhizal species the growth of mycelium is extremely slow and fruit-bodies have never formed so far. Neither terricolous saprotrophic fungi are able to produce fruit-bodies in culture, even if the growth of the mycelium remains at an acceptable level (VASAS *et al.* 1997).

During the establishment and development of the mycological culture collection of the Hungarian Natural History Museum several thousands isolates were originated mainly from fruit-bodies (VASAS *et al.* 1998). Bacterial infections obviously started from the pieces of fruit-bodies were detected most of all at cultures isolated from secondarily saprotrophic and mycorrhizal species. The overwhelming majority of these infections was due to the presence of *Bacillus* and *Pseudomonas* species (CS. LOCSMÁNDI pers. comm.). These species presumably effect pro-

cesses of growth and development in a positive way and they have fungistatic effect on the competitor microfungi species also among natural conditions. Fungistatic processes of bacterial origin were detected several times under laboratory conditions (PARK and AGNIHOTRI 1969, HUBBARD *et al.* 1983, DIX and WEBSTER 1994, LOCSMÁNDI and VASAS 2001). However, a several year long investigation, aiming at the issue of biological mass production of macrofungi under bacterium infected otherwise natural conditions, has not been carried out. Our aim was to find out if there was a difference between the amount of fruit-bodies and the species composition in infected sampling plots compared to control areas. *Bacillus subtilis* was applied as infecting agent, because it was easy to keep it in cultures having moderate requirements.

MATERIAL AND METHODS

Our investigation was started on 22 May 2000 in a mixed deciduous forest (dominated by *Fagus*, *Carpinus* and *Quercus*) at the right side of the road leading from Pilisszentkereszt to Pilisszántó. Two quadrates of 15 m × 15 m were selected and marked. One of them was sprayed by 20 litre *Bacillus subtilis* cultures of 10^6 cells/ml concentration monthly from May to November, while the other quadrate served as a control. In 2001, further two quadrates (treated and control) were selected in the nearby spruce forest. The sampling sites were visited 6 times during the year 2000, 7 times in 2001, and 8 times in 2002. All fruit-bodies were collected in both the treated and control quadrates, then the bacterium cultures were sprayed at the treated sites.

Attributes of fungal coenology – such as variation of species composition and abundance compared to control – were investigated in 21 occasions within 3 years on 4 sampling sites.

RESULTS

The results of the fungal production in treated and control sites of the mixed forest (Table 1), and in treated and control sites from the spruce forest (Table 2) are summarised in Table 3.

At the sampling site in the mixed deciduous forest (treated by *Bacillus subtilis*) 31 terricolous saprotrophic (S) species with 952 fruit-bodies, 15 lignicolous wood decaying (X) species with 279 fruit-bodies, 16 mycorrhizal (M) species with 163 fruit-bodies were detected.

At the control site in the mixed forest 23 terricolous saprotrophic (S) species with 201 fruit-bodies, 7 lignicolous wood decaying (X) species with 74 fruit-bodies, 17 mycorrhizal (M) species with 177 fruit-bodies were recorded.

At the sampling site in the spruce forest (treated by *Bacillus subtilis*) 23 terricolous saprotrophic (S) species with 784 fruit-bodies, 209 of this is fruit-body

Table 1. Fungal production in treated (*Bacillus subtilis*) and control sampling site in the mixed forest at Pilisszentkereszt between 2000–2002.

Name of species	Number of fruit-bodies		Nutrition
	Treated site	Control site	type
Agaricus arvensis (Moell.) Pil.	46	12	S
Agaricus essettei M. Bon	60	17	S
Agaricus langei (Moell.) Moell.	47	16	S
Agaricus praeclaresquamosus Freeman	5	–	S
Agaricus semotus Fr.	36	16	S
Agaricus xanthoderma Genev.	68	22	S
Agrocybe praecox (Pers.) Fay.	8	–	S
Amanita citrina (Schaeff.) S. F. Gray	–	3	M
Amanita phalloides (Fr.) Link	2	8	M
Amanita rubescens (Pers.) S. F. Gray	48	52	M
Amanita vaginata (Bull.) Vitt.	6	5	M
Armillaria mellea (Vahl.) Kumm.	29	7	X
Auricularia auricula-judae (Fr.) J. Schröt.	8	–	X
Boletus impolitus Fr.	2	–	M
Clitocybe geotropa (Bull.) Quél.	2	–	S
Clitocybe gibba (Pers.) Kumm.	68	4	S
Clitocybe inornata (Sow.) Gill.	8	–	S
Clitocybe nebularis (Batsch) Kumm.	31	4	S
Clitocybe odora (Bull.) Kumm	3	3	S
Collybia butyracea (Bull.) Kumm.	29	3	S
Collybia dryophila (Bull.) Kumm.	186	42	S
Collybia fusipes (Bull.) Quél.	33	11	X
Collybia hariolorum (DC.) Quél.	17	–	S
Collybia peronata (Bolt.) Sing.	41	6	S
Cortinarius (*Tel.*) *hinnuleus* (Sow.) Fr.	–	2	M
Cortinarius (*Phl.*) *infractus* (Pers.) Fr.	4	5	M
Cortinarius (*Tel.*) *duracinus* Fr.	3	–	M
Cortinarius (*Myx.*) *trivialis* J. Lge.	–	2	M
Entoloma rhodopolium (Fr.) Kumm.	12	–	S
Gyroporus castaneus (Bull.) Quél.	1	–	M
Hypholoma fasciculare (Huds.) Kumm.	79	34	X
Hypholoma sublatericium (Fr.) Quél.	5	–	X
Inocybe rimosa (Bull.) Kumm.	3	–	M
Lactarius piperatus (L.) S. F. Gray (ss. Mos. 1983)	4	6	M
Lactarius quietus (Fr.) Fr.	7	9	M
Lactarius serifluus (DC.) Fr.	–	5	M
Lepiota clypeolaria (Bull.) Kumm.	8	8	S
Lepiota cristata (Bolt.) Kumm.	29	–	S
Lepista flaccida (Sow.) Pat.	44	11	S
Lepista nuda (Bull.) Cke.	18	2	S
Lepista sordida (Schum.) Sing.	16	2	S
Lycoperdon perlatum Pers.	69	6	S
Lycoperdon pyriforme Schaeff.	8	–	X
Macrolepiota procera (Scop.) Sing.	27	7	S

Table 1 (continued)

Name of species	Number of fruit-bodies		Nutrition
	Treated site	Control site	type
Macrolepiota rhacodes (Vitt.) Sing	15	2	S
Marasmius wynnei Berk. et Br.	18	5	S
Megacollybia platyphylla (Pers.) Kotl. et Pouz.	3	2	S
Mycena galericulata (Scop.) S. F. Gray	23	2	X
Mycena inclinata (Fr.) Quél.	7	–	X
Mycena pura (Pers.) Kumm.	15	4	S
Mycena renati Quél.	8	–	X
Mycena rosea (Bull.) Gramberg	–	4	S
Panellus stypticus (Bull.) P. Karst.	5	–	X
Paxillus involutus (Batsch) Fr.	8	2	M
Pholiota cerifera (P. Karst.) P. Karst.	3	–	X
Pholiota lenta (Pers.) Sing.	5	–	S
Pleurotus ostreatus (Jacq.) Kumm.	8	–	X
Pluteus cervinus (Schaeff.) Kumm.	27	14	X
Pluteus nanus (Pers.) Kumm.	21	–	X
Psathyrella candolleana (Fr.) R. Mre.	11	–	S
Russula atropurpurea (Krbh.) Britz. non Peck	10	14	M
Russula cyanoxantha (Schaeff.) Fr.	–	3	M
Russula heterophylla (Fr.) Fr.	34	24	M
Russula vesca Fr.	8	21	M
Russula virescens (Schaeff.) Fr.	–	2	M
Stropharia aeruginosa (Curtis) Quél.	7	3	S
Trametes versicolor (L.) Pil.	–	3	X
Xerocomus chrysenteron (Bull.) Quél.	17	14	M
Xerocomus rubellus (Krbh.) Quél.	6	–	M
Xerula radicata (Relhan) Doerf.	15	3	X
Total number of species	1394	452	70

of eudominant species, 575 is fruit-body of subdominant species, 2 lignicolous wood decaying (X) species with 31 fruit-bodies, 14 mycorrhizal (M) species with 135 fruit-bodies were collected.

At the control site in the spruce forest 16 terricolous saprotrophic (S) species with 263 fruit-bodies, of which 57 belong to eudominant and 206 to subdominant species, 2 lignicolous wood decaying (X) species with 13 fruit-bodies, 14 mycorrhizal (M) species with 124 fruit-bodies were found.

The sampling sites treated by *Bacillus subtilis* in the mixed forest are characterised with higher numbers of species (62) and fruit-bodies (1394) compared to the control sites (47 and 452, respectively). The increase of these numbers are caused by higher numbers of saprotrophic and wood decaying fungi. At the same time the species number is more by one, and the number of fruit-bodies is more by 14 in the treated site than those of the control site in the case of mycorrhizal fungi.

Table 2. Fungal production in treated (*Bacillus subtilis*) and control sampling sites in the spruce forest at Pilisszentkereszt between 2000–2002.

Name of species	Number of fruit-bodies		Nutrition
	Treated site	Control site	type
Agaricus silvaticus Schaeff.	22	11	S eudom.
Amanita muscaria (L.) Pers.	9	8	M
Amanita vaginata (Bull.) Vitt.	4	2	M
Auriscalpium vulgare S. F. Gray	51	16	S subdom.
Boletus piperatus Bull.	3	7	M
Boletus reticulatus Schaeff.	3	3	M
Clitocybe candicans (Pers.) Kumm.	58	32	S subdom.
Collybia butyracea (Bull.) Kumm.	61	19	S eudom
Collybia dryophila (Bull.) Kumm.	86	16	S subdom.
Cortinarius (Tel.) triformis Fr.	4	9	M
Cortinarius (Tel.) hinnuleus (Sow.) Fr.	4	8	M
Cystoderma amiantinum (Scop.) Fay.	8	–	S subdom.
Cystoderma carcharias (Pers.) Fay.	28	6	S subdom.
Gomphidius glutinosus (Schaeff.) Fr.	9	5	M
Hygrophorus agathosmus (Fr.) Fr.	11	7	M
Hygrophorus eburneus (Bull.) Fr.	2	2	M
Hypholoma fasciculare (Huds.) Kumm.	20	9	X
Laccaria laccata (Scop.) Berk. et Br.	43	28	S subdom.
Lactarius mitissimus Fr.	43	32	M
Lepista flaccida (Sow.) Pat.	22	6	S eudom.
Lepista nuda (Bull.) Cke.	10	–	S eudom.
Lycoperdon perlatum Pers.	28	8	S eudom.
Macrolepiota procera (Scop.) Sing.	23	13	S eudom.
Macrolepiota rhacodes (Vitt.) Sing	36	–	S eudom.
Marasmius androsaceus (L.) Fr.	71	31	S subdom.
Mycena aurantiomarginata (Fr.) Quél.	16	14	S subdom.
Mycena leptocephala (Pers.) Sacc.	33	21	S subdom.
Mycena pura (Pers.) Kumm.	77	16	S subdom.
Mycena rosea (Bull.) Gramberg	51	16	S subdom.
Mycena stylobates (Pers.) Kumm.	8	–	S subdom.
Pholiota lenta (Pers.) Sing.	3	–	S eudom.
Ripartites tricholoma (Alb. et Schw.) P. Karst.	10	–	S subdom.
Russula nauseosa (Pers.) Fr.	4	6	M
Russula queletii Fr. in Quél.	8	8	M
Strobilurus esculentus (Wulf.) Sing.	35	10	S subdom.
Stropharia aeruginosa (Curtis) Quél.	4	–	S eudom.
Suillus granulatus (L.) O. Kuntze	9	12	M
Tricholoma vaccinum (Pers.) Kumm.	22	15	M
Tricholomopsis rutilans (Schaeff.) Sing.	11	4	X
Total number of species	950	400	39

Table 3. Fungal production in treated and control sites of the mixed deciduous and spruce forests.

	Saprotrophic		Wood decaying		Mycorrhizal		Total	
	species	fruit-body	species	fruit-body	species	fruit-body	species	fruit-body
Treated (mixed forest)	31	952	15	279	16	163	62	1394
Control (mixed forest)	23	201	7	74	17	177	47	452
Total	–	1153	–	353	–	340	70	1846
Treated (spruce forest)	23	784	2	31	14	135	39	900
Control (spruce forest)	16	263	2	13	14	124	32	400
Total	–	1047	–	44	–	259	39	1300

The number of fruit-bodies is much higher in the genus *Agaricus* on the treated area (262) compared to the control (83). The number of species is almost the same on both the treated (6) and the control (5) sites. The number of fruit-bodies in genus *Collybia* is increased strikingly. If the wood decaying *Collybia fusipes* is omitted from the calculations, 4 species are represented by 273 fruit-bodies, while on the control site 3 species are represented by 51 fruit-bodies. A similar tendency is seen in *Clitocybe* and *Lepista*. In the case of *Clitocybe*, 112 fruit-bodies of 5 species were found on the treated sites, 11 fruit-bodies of 3 species on the control sites. The genus *Lepista* is characterised by 3 species with 78 individuals on the treated, and 3 species with 15 individuals on the control sites. There is a conspicuous increase in the numbers of species and fruit-bodies of the wood decaying species compared to those of the control quadrates: these became twice as much in the last year of the 3 year period. A total of 15 species with 279 fruit-bodies were found on the treated, and 7 species with 74 fruit-bodies on the control sites.

The sampling sites in the spruce forest at the edge of Pilisszentkereszt have already been studied before, when they were sampled monthly from April to November in 1983–1984, and also mycocoenological investigations were carried out (VASAS 1985). As a result of this former research it was found that saprotrophic fungi predominate over mycorrhizal ones in spruce forests. Furthermore, the small-size subdominant species (with mean weight is lower than 1 g) are represented with higher numbers than those of the eudominant species (with mean weight usually higher than 10 g). Among the subdominant species the tiny saprotrophic species predominated over the mycorrhizal ones. This could be explained by the higher amount of resin-containing litter, which was decomposed by the subdominant saprotrophic fungi and other, litter decomposer organisms.

During our investigations carried out in the spruce forest in 2001–2002 it was established that numbers of saprotrophic species and their fruit-bodies are still

higher than those of mycorrhizal ones at both the *Bacillus subtilis* treated and the control sites. The number of fruit-bodies (206) of the subdominant saprotrophic species is higher than that of the eudominant saprotrophic species (57) similarly to the investigations carried out 16 years ago.

Due to a monthly treatment by *Bacillus subtilis* the number of fruit-bodies of saprotrophic fungi became 3 times as much (784) compared to that of the control site (263). It is interesting that while the number of subdominant species (575) became almost 3 times as much in the control site, the number of fruit-bodies of eudominant species became 4 times as much (209).

The numbers of species of wood decaying fungi are equal in the two sampling sites in the spruce forest, however, the number of fruit-bodies in the treated area is twice as much as in the control.

The results received in the spruce forest are similar to those obtained in the deciduous forest: the production of the secondary decomposer saprotrophic fungi species became multiple due to the *Bacillus subtilis* treatment. In the case of wood decaying species the number of species and fruit-bodies became twice as much after the 3-year *Bacillus subtilis* treatment. Supposedly as a result of the treatment, new species entered in the succession in the last year while in the spruce forest, where the investigation lasted for 2 years only, the number of species remained unchanged but the number of fruit-bodies was already effected by the bacterium.

CONCLUSIONS

In the two sampling sites (deciduous forest, spruce forest) treated by *Bacillus subtilis*, the primary decomposer and mycorrhizal fungi did not show obvious difference between the treated and control sites in the number of fruit-bodies, but in the case of the secondary decomposer saprotrophic species the number of fruit-bodies increased strikingly.

One of the reasons might be the assisted nutrient uptake, since in case of the secondary decomposer species *Bacillus subtilis* starts the decomposition, thus the nutrient uptake is easier for the species of macrofungi.

The other possible reason is the protective function. *Bacillus subtilis* produces fungistatic substances (polypeptide antibiotics: subtilin) which inhibit or slow down the growth of other competitor organisms. It has some, (though limited) inhibiting effect also on macrofungi, so they have an advantage compared to those of other soil-inhabiting competitor micro-organisms. Consequently, fungi can develop faster, which is clearly seen also in the production of fruit-bodies according to our results. Fungistasis is presumably important also in the case of mycorrhizal fungi,

but only in the early stage of their development. A protective effect is necessary until the hypha developing from the spore reaches the rhizosphere, where the stimulating effect of the root acids and the protective effect of the rhizospheric bacteria are manifested. Since the mycorrhizal connection appears at the early stage of the development of the host tree and the fruit-bodies are formed only at a certain development stage, therefore longer time is necessary for the manifestation of the protective or stimulating effect of *Bacillus subtilis*.

In the case of wood decaying fungi the results of the treatment require also a longer period of time, since the bacteria help the process of decomposition only at its initial phase. These fungi are primary decomposers and so the selection of substrates is not so important than in the case of the secondary decomposer species. They are capable to decompose wood thanks to their high cellulase and ligninase activity. *Bacillus subtilis*, on the other hand, cannot decompose wood, although it provides protection against competitor organisms during the germination of spores.

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REFERENCES

DIX, N. J. and WEBSTER, J. (1994): *Fungal Ecology*. – Chapman and Hall, London.

HUBBARD, J. P., HARMAN, G. E. and HADAR, Y. (1983): Effect of soil borne Pseudomonas spp. on the biological control agent Trichoderma hamatum on pea seeds. – *Phytopathology* **73**: 655–659.

LOCSMÁNDI, CS. and VASAS, G. (2001): A fungisztázis jelensége a gombák körében. Irodalmi összefoglaló. (Fungistasis in the fungal world). – *Mikol. Közlem., Clusiana* **40**(3): 75–84.

PARK, J. Y. and AGNIHOTRI, V. P. (1969): Bacterial metabolites trigger sporophore formation in Agaricus bisporus. – *Nature* **222**: 984.

VASAS, G. (1985): *Telepített fenyvesek és természetes lomberdei társulások nagygombáinak vizsgálata a Bükk- és a Pilis-hegységben*. – Egyetemi doktori értekezés, 119 pp.

VASAS, G., BOHUS, G. and LOCSMÁNDI, CS. (1997): Gombagénbank a Magyar Természettudományi Múzeum Növénytárában. (Mushroom culture collection in the Botanical Department of the Hungarian Natural History Museum). – *Mikol. Közlem., Clusiana* **37**(1–3): 41–54.

VASAS, G., BOHUS, G. and LOCSMÁNDI, CS. (1998): Genetic resource collection of macrofungi in Hungary. – *Studia bot. hung.* **29**: 17–34.

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