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DIVERSITY OF CULTURABLE BACTERIA ASSOCIATED WITH FRUITING BODIES OF ECTOMYCORRHIZAL FUNGI[1]

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Abstract

The diversity of the culturable bacteria associated with fruiting bodies of ectomycorrhizal fungi was studied. Gram-negative rods were the predominant type of bacteria isolated from inside the sporocarps, with the exception of *Laccaria amethistina*. The majority of bacteria derived from the fruiting bodies of this fungus were Gram-positive cocci. Bacteria highly similar (p = 0.8–0.98) to *Sphingomonas paucimobilis, Pseudomonas aeruginosa, P. pickettii, Chromobacterium violaceum* were the predominant taxa among the Gram-negative rods.

In the soil surrounding sporocarps Gram-variable coryneform bacteria were more numerous than Gram-negative rods which predominated within sporocarps. Streptomycetes, not detected among endobiotic bacteria, were present in soil.

Key words: bacterial endobionts, sporocarps, ectomycorrhizal fungi

Introduction

Bacteria of several genera have been isolated from fruiting bodies of ectomycorrhizal fungi (Li and Castellano 1987, Garbaye et al. 1990, Danell et al. 1993, Varese et al. 1996).

Different taxa of microbes in mycorrhizosphere have been reported to be associated either with the hyphae or with the sporocarps of different mycorrhizal fungi (Garbaye et al. 1990, Varese et al. 1996). Aerobic bacteria were isolated from fruiting bodies of *Cantharellus cibarius* (L.) Fries. The dominating bacterium was *Pseudomonas fluorescens* (Danell et al. 1993). Li and Castellano (1987) isolated and identified as *Azospirillum* acetylene-reducing bacteria from within sporocarps of

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ectomycorrhizal fungi *Suillus ponderosa* Smith et Thiers, *Hymenogaster parksii* Zeller et Dodge, *Tuber melanosporum* Vitt., *Hebeloma crustuliniforme* (Bull.) Quél., *Laccaria laccata* (Scop.: Fr.) Berk. et Br. and *Rhizopogon vinicolor* Smith.

Varese et al. (1996) isolated 27 bacterial species from both the sporocarps of *Suillus grevillei* (Klotzsch) Sing. and the ectomycorrhizae of *S. grevillei – Larix decidua* Mill. The genera *Pseudomonas, Bacillus* and *Streptomyces* were predominant. Several species: *Bacillus cereus, B. mycoides, Enterobacter agglomerans, Micrococcus luteus, Pseudomonas cepacia, P. chlororaphis, P. fluorescens, P. putida* were common to both the sporocarps and the ectomycorrhizae.

The results obtained by Varese et al. (1996) suggest for some bacterial isolates a very high fungus specificity at the intraspecific level.

Gazzanelli et al. (1999) isolated a microbial population from young sporocarps of *Tuber borchii* Vittad. The mean bacterial count in the sporocarps was equal to 10^6 cfu/g. In general, these counts were higher that those in the bulk soil (10^3 cfu/g). In the sporocarps examined the predominant bacteria were *P. fluorescens* (30% of the total populations) and spore forming Gram-positive bacteria (15% of total).

The purpose of our work was to evaluate the diversity of culturable bacteria inside the fruiting bodies of different ectomycorrhizal fungi growing in coniferous forest, mixed hardwood-coniferous forest and non-forest habitat. The number of bacteria and their morphological types in the upper part of the podzolic soil surrounding the sporocarps were also studied.

Materials and methods

Ectomycorrhizal fungi, media and culture conditions

Bacteria were isolated from inside unripe fruiting bodies of following ectomycorrhizal fungi: *Suillus luteus* (Fr.) S.F. Gray, *S. grevillei, Lycoperdon* sp., *Xerocomus* sp., *Hebeloma crustuliniforme, Thelephora terrestris* Pers.: Fr., *Scleroderma* sp., *Laccaria laccata, L. amethistina* (Bull.) Murr., *L. proxima* (Boud.) Pat. and *Amanita muscaria* (L.) Hooker. The fungi derived from different forest habitats of north-western Poland: coniferous forest, mixed hardwood-coniferous forest and non-forest habitat which was over 20 m away from the nearest forest.

Fungi for the study were collected on 3rd October 1998 and the same day studied in the laboratory. Five sporocarps of each fungal species (of each habitat) were washed with sterile distilled water and then surface disinfected (Petrini and Müller 1986) with: 96% ethyl alcohol (1 min), 5% sodium hypochloride (3 min) and again: 96% ethyl alcohol (0.5 min). Then the fruiting bodies were washed in sterile distilled water (excess water was removed by squeezing between two sterile sheets of filter paper) and ground in a sterile handheld tissue grinder with a pestle. Cultural media containing Bacto R2A agar (Dahm et al. 2001, Pokojska-Burdziej et al. 2001) were inoculated with tenfold dilutions of homogenized sporocarps. Bacteria

were grown at 26°C for 14 days, subsequently counted and isolated randomly from Petri dishes in the number of 30 for each source of isolation.

Identification of bacteria

The preliminary grouping of bacteria involved Gram staining, testing ability to grow on Bacto EMB agar (Difco, Gdańsk, Poland) and formation of soluble pigments that fluorescence under a UV lamp with the Wood's filter.

Identification of bacteria was carried out by the probabilistic method using the PIB computer program (Bryant 2001), using the probability matrix for non-enteric Gram-negative rods for API 20 NE system (bioMerieux, Marcy l'Etoile, France). In the API 20 NE system following tests were performed: reduction of nitrate, production of indole, acidification of glucose, production of dihydrolase of arginine, production of urease, hydrolysis of aesculin, hydrolysis of gelatine, production of β-galactosidase, utilization of glucose, arabinose, mannose, maltose, mannitol, N-acetylglucosamine, gluconate, capronate, adipinate, malonate, citrate, phenylacetate, production of cytochrome oxidase. 98% similarity to a known species was accepted as the threshold value of the Wilcox coefficient for satisfactory identification (Bryant 2001).

Transmission electron microscopy (TEM)

The sporocarps for TEM studies were washed twice in 0.05 M phosphate buffer, pH 6.8 and fixed in 3% glutaraldehyde in the same buffer, for 4 h at 4°C. They were rinsed several times in the same buffer and postfixed in 1% osmium tetroxide for 12 h at room temperature (24°C). The material was dehydrated in a series of 96% ethyl alcohol and embedded in LR Gold resin (Sigma, Poznań, Poland). Polymerization with 1% benzoyl peroxide as the accelerator occurred for seven days at room temperature. Ultrathin sections were cut with a glass knife on a Leica Ultracut UCT ultramicrotome and collected on nickel grids coated with 3% Formvar (Sigma). The sections were stained after Reynolds (1963) and observed in a JOEL 1010 electron microscope. Micrographs were taken on FOTON TN-12 films.

Results

Almost a half of sporocarps tested contained $1.8–5.8 \times 10^7$ cfu of bacteria per 1 g of fresh weight (Table 1). The lowest number of culturable bacteria was detected in the sporocarps of *A. muscaria* (6.8×10^2 cfu per 1 g of fresh weight) (Table 1). Sporocarps of the same fungal species, but growing in different habitats, contained different number of culturable bacteria. The sporocarps of *H. crustuliniforme* growing in the non-forest habitat harboured a greater number of bacteria than the sporocarps of the same species growing in the forest. Number of bacteria isolated

Table 1

Number and morphological types of bacteria isolated from inside of sporocarps of ectomycorrhizal fungi

Fungi	Cfu per 1 g of fresh weight	Number of isolates studied	Number of Gram--negative rods	Number of Gram--positive spore-forming bacilli	Number of Gram--positive cocci	Number of Gram--variable pleomorphic bacteria
Amanita muscaria[2]	6.8×10^2 a	3	3	–	–	–
Hebeloma crustuliniforme[1]	2.4×10^4 bc	30	21	6	2	1
Hebeloma crustuliniforme[2]	1.2×10^4 bc	30	18	–	12	–
Hebeloma crustuliniforme[3]	2.0×10^6 c	30	28	–	–	2
Laccaria amethistina[2]	5.1×10^3 bc	29	5	–	24	–
Laccaria laccata[1]	1.1×10^4 bc	30	30	–	–	–
Laccaria laccata[2]	1.8×10^7 c	29	29	–	–	–
Laccaria laccata[3]	3.3×10^7 c	30	27	1	–	–
Laccaria proxima[1]	1.1×10^5 bc	30	27	–	–	3
Lycoperdon sp.[2]	5.8×10^7 c	30	30	–	–	–
Scleroderma sp.[3]	2.0×10^7 c	30	30	–	–	–
Suillus grevillei[1]	7.8×10^3 b	30	20	1	9	–
Suillus luteus[2]	3.4×10^7 c	30	28	2	–	–
Thelephora terrestris[3]	4.0×10^7 c	30	30	–	–	–
Xerocomus sp.[3]	3.0×10^7 c	29	27	2	–	–

[1]Coniferous forest.
[2]Mixed hardwood-coniferous forest.
[3]Non-forest habitat (considerably distant – over 20 m – from the closest forest).
– – absent.
Mean values marked with the same letter do not differ significantly ($p \leq 0.05$).

from *L. laccata* sporocarps growing in non-forest habitat and mixed forest was similar and higher than in those from coniferous forest.

A greater number of bacteria was isolated from the soil with pH 6.3 than from the soil with pH 4.58 (Table 2).

Gram-negative rods were the predominant type of bacteria isolated from the sporocarps of ectomycorrhizal fungi, with the exception of *L. amethistina* (Table 1). The majority of bacteria obtained from fruiting bodies of *L. amethistina* were Gram-positive cocci. Coccoid forms of bacteria were also isolated in high number from the sporocarps of *H. crustuliniforme* and *S. grevillei*. Gram-positive spore-forming aerobic bacilli and Gram-variable pleomorphic bacteria were also detected in the sporocarps. The sporeforming bacteria were most frequently isolated from fruiting bodies of *H. crustuliniforme* (from coniferous forest). The pleomorphic forms were detected in sporocarps of *L. proxima* and *H. crustuliniforme* growing in coniferous forest and non-forest habitat.

Table 2

Number and morphological types of bacteria isolated from soil surrounding sporocarps

Source of isolation	pH	Cfu per 1 g of fresh weight	Number of isolates studied	Number of Gram-negative rods	Number of Gram-positive spore-forming bacilli	Number of Gram-positive cocci	Number of Gram-variable pleomorphic bacteria	Number of streptomycetes
Soil from mixed hardwood-coniferous forest	4.58	7.5×10^5 a	30	2	2	–	19	7
Soil from non-forest habitat	5.09	1.1×10^6 b	30	12	4	1	8	5
Soil from coniferous forest	6.30	3.2×10^6 c	30	10	3	1	10	6

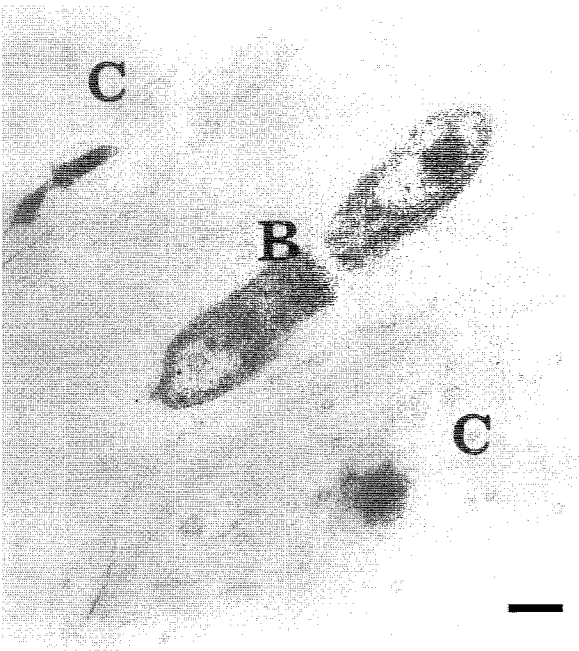
Mean values marked with the same letter do not differ significantly ($p \leq 0.05$).

Table 3

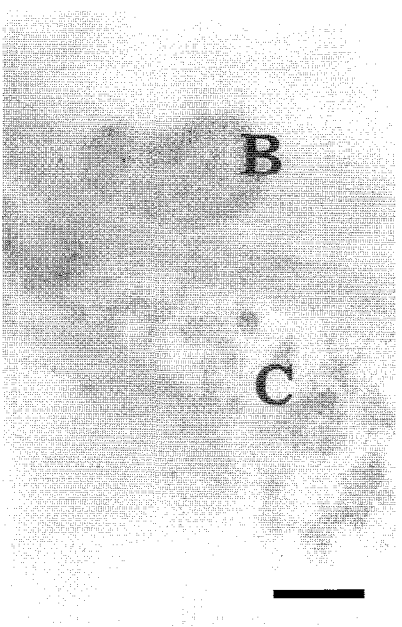
Predominant species of Gram-negative rods isolated from sporocarps of ectomycorrhizal fungi

Fungi	Predominant species (probability of taxon membership: 0.80–0.98)
Hebeloma crustuliniforme[1]	*Sphingomonas paucimobilis*
Hebeloma crustuliniforme[2]	*Sphingomonas paucimobilis*
Hebeloma crustuliniforme[3]	*Chromobacterium violaceum*
Laccaria amethistina[2]	*Sphingomonas paucimobilis*
Laccaria laccata[1]	*Sphingomonas paucimobilis*
Laccaria laccata[2]	*Chromobacterium violaceum*
Laccaria laccata[3]	*Chromobacterium violaceum*
Laccaria proxima[1]	*Pseudomonas aeruginosa*
Lycoperdon sp.[2]	*Pseudomonas aeruginosa*
Scleroderma sp.[3]	*Chromobacterium violaceum*
Suillus grevillei[1]	*Sphingomonas paucimobilis*
Suillus luteus[2]	*Pseudomonas aeruginosa*
Thelephora terrestris[3]	*Pseudomonas pickettii*
Xerocomus sp.[3]	*Pseudomonas aeruginosa*

[1]Coniferous forest.
[2]Mixed hardwood-coniferous forest.
[3]Non-forest habitat (considerably distant – over 20 m – from the closest forest).



Phot. 1. Dividing bacteria in the intercellular space of the hypha of *Xerocomus* sp. (transmission electron micrograph); B – bacteria, C – cytoplasm; bar – 0.5 μm (photo by E. Bednarska)

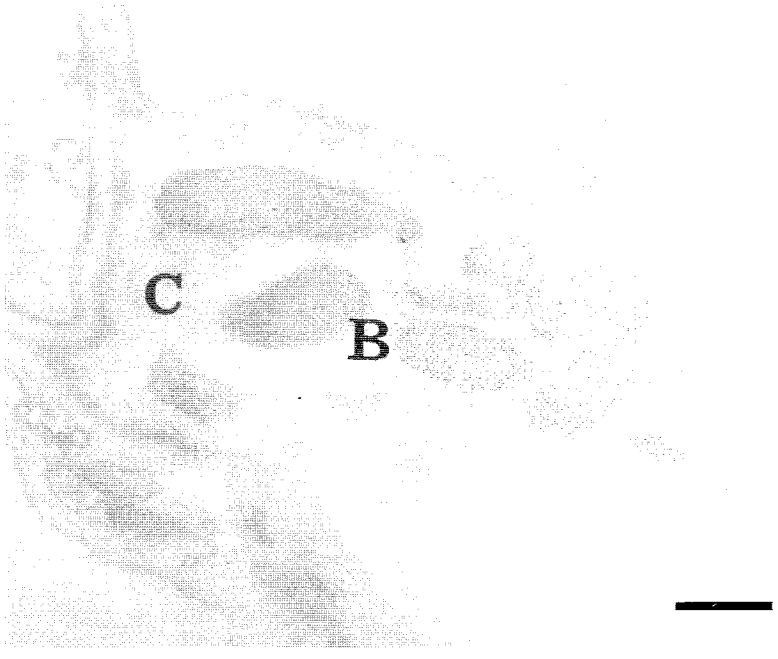


Phot. 2. Bacteria adhering to the wall of *Xerocomus* sp. hypha cell (transmission electron micrograph); B – bacteria, C – cytoplasm; bar – 0.5 μm (photo by E. Bednarska)

Sporocarps of *H. crustuliniforme* were harboured by greatest range of different morphological bacteria types.

Among the soil bacteria, Gram-variable coryneform bacteria were more numerous than Gram-negative rods, which predominated within the sporocarps. Streptomycetes were also numerous in soil (Table 2).

The isolates of *Sphingomonas paucimobilis, Pseudomonas aeruginosa, P. pickettii* and *Chromobacterium violaceum* were predominant among the Gram-negative rods of endobionts (Table 3) (probability of the reference taxon membership: $p = 0.80–0.98$).



Phot. 3. Bacteria inside the cell of *Xerocomus* sp. sporocarp (transmission electron micrograph); B – bacteria, C – cytoplasm; bar – 0.5 μm (photo by E. Bednarska)

In general, among the endobiotic bacteria from sporocarps growing in non-forest habitat *C. violaceum* predominated, while *S. paucimobilis* was predominating in the sporocarps growing in coniferous forest.

The transmission electron microscopy study revealed bacterial cells inside fruiting body of *Xerocomus* sp. (Phots 1–3). Bacteria were observed in intercellular space of hypha (Phot. 1) and adhering to the wall of hypha cell (Phot. 2). Many bacteria were found inside the cells of sporocarp (Phot. 3).

Discussion

Bacterial endosymbiosis is characterized by intracellular localization of the bacteria, regardless of their parasitic or mutualistic behaviour (Bertaux et al. 2003).

Only a few cases of bacterial endosymbiosis with fungi have been reported, as yet, mostly for *Glomeromycota* species (Schüßler et al. 2001). According to the authors, most intrafungal bacteria are non-culturable. Electron microscopy and molecular methods had allowed the detection of fungus-associated bacteria, both intra- and extracellular in pure culture of mycorrhizal fungi.

Bianciotto et al. (1996) identified endobacteria connected with several species of endomycorrhizal fungi (*Gigaspora* spp. and *Scutellospora* spp.) as *Burkholderia* spp. Barbieri et al. (2000) discovered by fluorescence *in situ* hybridization bacteria be-

longing to a *Cytophaga – Flexibacter – Bacteroides* phylogroup embedded in the cell wall of hyphae of an ectomycorrhizal fungus *Tuber borchii* Vitt. Bertaux et al. (2003), using the same methods as previously mentioned authors, as well as confocal laser scanning microscopy, detected intracellular bacteria (0.5 μm in diameter), rarely and heterogeneously distributed in the mycelium of *L. bicolor* (Maire) Orton. These bacteria were identified as *Paenibacillus* spp. (16S rRNA directed oligonucleotide probe).

Darbyshire and Greaves (1973) classified endophytic bacteria as a part of the rhizosphere bacterial community. These bacteria would colonize sporocarps randomly when carried along the hyphae. Also numerous other authors stated that Gram-positive and Gram-negative bacterial endophytes living in roots, shoots, leaves, seeds and ovules are in general similar to those found in the adjacent root zone soils (Shishido et al. 1995, 1999, McInroy and Kloepper 1995, Lamb et al. 1996, Strzelczyk and Li 2000). In contrast, van Peer et al. (1990), using an analysis of lipopolysaccharide patterns, reported that endophytic bacteria and rhizobacteria belonging to the same genera formed subpopulations, each specifically suited to colonize their respective niches.

Our present study showed that the sporocarps and their surrounding soil had different bacterial populations. In the podzolic soil, Gram-variable coryneform bacteria were more numerous than Gram-negative rods, which were predominant in the sporocarps. Moreover, in the soil 20% of isolates were actinomycetes, which were not detected in the sporocarps in this study. In our study Gram-negative rods predominated among bacteria occurring inside the sporocarps of all fungi studied. Gram-positive cocci were predominant bacteria among isolates from sporocarps of *L. amethistina*. The sporocarps of *A. muscaria* harboured the lowest number of bacteria (slowly growing ones).

According to Danell (1994) the concentration of aerobic bacteria inside fruiting body of *C. cibarius* was about the same as that in the upper part of the podzolic soil.

Our study demonstrated that the numbers of bacteria in the surrounding soil and in the fungal sporocarps were similar or higher in the soil, depending on the mycorrhizal species and the soil from which the sporocarp was collected. The number of culturable bacteria in *C. cibarius* sporocarps varied from 3×10^5 to 7×10^6 cfu per 1 g of fresh weight (Danell 1994). This value was about 100–1000 times greater than the bacterial concentration in various species of *Agaricales*. *Boletus edulis* (Bull.) Fries and *A. muscaria* appeared to be bacteria free.

Pseudomonas fluorescens was the predominant bacterium isolated from the sporocarps of *C. cibarius* (Danell et al. 1993). It represented 12% of the total bacterial isolates in soil and 78% in fruiting bodies of *C. cibarius*. Endobionts isolated in our studies belonged to different bacterial species; Gram-negative rods mainly to *S. paucimobilis*, *C. violaceum* and *P. aeruginosa*.

Bacterial populations in the host appear to be under the influence of both the host and the environment. Mycorrhizal fungi have been shown to provide a physical and nutritional substrate for many soil microbes (Tisdall 1991). The significance of the endobiotic bacteria for a fungus (as well as for a plant) is still not completely recognized. Bacteria associated with mycorrhizal fungi could play an

important role in sporocarp formation (Sbrana et al. 2000) and in promotion of mycorrhizal symbiosis (Garbaye 1994, Frey-Klett et al. 1997). Ectomycorrhizal symbiosis modifies qualitatively and quantitatively root exudation. In ectomycorrhizosphere bacterial equilibrium differs from that in the bulk soil. Frey-Klett et al. (1997) characterized functional diversity of *P. fluorescens* populations associated with Douglas fir-*L. bicolor* symbiosis, in comparison to the bulk soil. They found that the ectomycorrhizosphere structures contained *P. fluorescens* populations. It favoured and selected isolates which used sugars and carboxylic acids rather than amines and aminoacids, did not produce HCN (a metabolite which completely inhibited the growth of *L. bicolor* mycelium), efficiently mobilized soil iron and phosphorus and did not affect *in vitro* growth of *L. bicolor* or formation of its mycorrhizas with Douglas fir seedlings. The study quoted demonstrated that mycorrhizal roots selected *P. fluorescens* strains which were beneficial to the symbiosis.

Bacteria intimately associated with spore walls of endomycorrhizal fungus *Glomus mosseae* Mosse, have been reported to occur also in ecto- and arbutoid mycorrhizae (Buscot 1994, Filippi et al. 1995, Varese et al. 1996). The occurrence of bacteria in the wall of *G. mosseae* spores and hyphae probably was responsible for the lytic activity observed in the wall. Bacteria occurring within the sporocarps of *G. mosseae* were able to make tunnels while embedded in the wall (Filippi et al. 1998). Dahm and Wrótniak (2003) detected proteolytic and chitinolytic activity of the pseudomonads isolated from inside the sporocarps of *C. cibarius*. Higher chitinolytic activity of bacteria was found in the medium with mycelium of *C. cibarius* than with mycelium of *Fusarium oxysporum* Schlecht. emend. Snyd. et Hans. or colloidal chitin. We previously stated that chitinolytic activity was a general feature of the bacteria isolated from sporocarps discussed in this paper. However, isolates derived from sporocarps of *Xerocomus* sp. and *A. muscaria* had no such ability (unpublished data). The bacterial chitinolytic activities could facilitate release of spores from the sporocarp for germination (Gazzanelli et al. 1999).

Many other mechanisms are supposed to be involved in the process, that is why further studies are required to unravel the specific role of these organisms.

Streszczenie

RÓŻNORODNOŚĆ DAJĄCYCH SIĘ HODOWAĆ BAKTERII ZWIĄZANYCH Z OWOCNIKAMI GRZYBÓW EKTOMIKORYZOWYCH

Przeprowadzono badania nad różnorodnością dających się hodować bakterii zasiedlających owocniki grzybów ektomikoryzowych. Bakterie w większości przypadków były liczne we wnętrzu owocników (ponad 10^7 jtk na 1 g świeżej masy owocników), z wyjątkiem owocników *Amanita muscaria* ($6{,}8 \times 10^2$ jtk na 1 g świeżej masy). Pałeczki Gram-ujemne były dominującym typem bakterii wyizolowanych z owocników grzybów ektomikoryzowych, z wyjątkiem *Laccaria amethistina* (większość bakterii zasiedlających owocniki tego grzyba była Gram-dodatnimi

ziarniakami). Owocniki *Hebeloma crustuliniforme* były zasiedlone przez najbardziej zróżnicowaną pod względem morfologicznym grupę bakterii. Bakterie bardzo podobne (p = 0,80–0,98) do *Sphingomonas paucimobilis, Pseudomonas aeruginosa, P. pickettii* i *Chromobacterium violaceum* były dominującymi taksonami wśród pałeczek Gram-ujemnych.

W glebie otaczającej owocniki bakterie pleomorficzne Gram-zmienne były liczniejsze od pałeczek Gram-ujemnych, dominujących wewnątrz owocników. Stosunkowo liczną grupą bakterii wyizolowanych z gleby były promieniowce, których nie wykrywano wśród bakterii endobiotycznych.

Badania z zastosowaniem transmisyjnej mikroskopii elektronowej wykazały obecność komórek bakteryjnych wewnątrz owocników.

Literature

Barbieri E., Potenza L., Rossi I., Sisti D., Giamoro G., Rossetti S., Beinfohr C., Stocchi V., 2000: Phylogenetic characterisation and *in situ* detection of a *Cytophaga – Flexibacter – Bacteroides* phylogroup bacterium in *Tuber borchii* Vittad. ectomycorrhizal mycelium. Appl. Environ. Microbiol. 66: 5035–5042.

Bertaux J., Schmid M., Chemidlin Prevost-Boure N., Churin J.L., Hartmann A., Garbaye J., Frey-Klett P., 2003: *In situ* identification of intracellular bacteria related to *Paenibacillus* spp. in the mycelium of the ectomycorrhizal fungus *Laccaria bicolor* S 238 N. Appl. Environ. Microbiol. 69: 4243–4248.

Bianciotto V., Bandi C., Minerdi M., Sironi M., Tichy H.V., Bonfante P., 1996: An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Appl. Environ. Microbiol. 62: 3005–3010.

Bryant T.N., 2001: Probabilistic identification of bacteria (MS-DOS computer program, Version 1,20 – freeware). http://www.som.soton.ac.uk/staff/tnb/microsoftware/PIB120.EXE.

Buscot F., 1994: Ectomycorrhizal types and endobacteria associated with ectomycorrhizas of *Morchella elata* (Fr.) Boudier with *Picea abies* (L.) Karst. Mycorrhiza 4: 223–232.

Dahm H., Wrótniak W., 2003: Proteolytic and chitinolytic activity of the pseudomonads isolated from within sporocarps of *Cantharellus cibarius*. Bull. Pol. Acad. Sci. 51, Biol. Sci. 3: 167–175.

Dahm H., Wrótniak W., Ciesielska A., 2001: Effect of ectomycorrhizal fungus *Laccaria laccata*, mycorrhization helper bacteria and soilborne pathogenic fungi on the development of pine seedlings (*Pinus sylvestris* L.). Bull. Pol. Acad. Sci. 49, Biol. Sci. 3: 137–148.

Danell E., 1994: *Cantharellus cibarius*: mycorrhiza formation and ecology. Acta Univ. Ups. Compr. Summ. Upps. Diss. Fac. Sci. Technol. 35.

Danell E., Alström S., Ternström A., 1993: *Pseudomonas fluorescens* in association with fruit bodies of the ectomycorrhizal mushroom *Cantharellus cibarius*. Mycol. Res. 97: 1148–1152.

Darbyshire J.F., Greaves M.P., 1973: Bacteria and protozoa in the rhizosphere. Pestic. Sci. 4: 349–360.

Filippi C., Bagnoli G., Citernesi A.S., Giovannetti M., 1998: Ultrastructural spatial distribution of bacteria associated with sporocarps of *Glomus mosseae*. Symbiosis 24: 1–12.

Filippi C., Bagnoli G., Giovannetti M., 1995: Bacteria associated to arbutoid mycorrhizae in *Arbutus unedo* L. Symbiosis 8: 57–68.

Frey-Klett P., Pierratt J.C., Garbaye J., 1997: Location and survival of mycorrhiza helper *Pseudomonas aeruginosa* during establishment of ectomycorrhizal symbiosis between *Laccaria bicolor* and Douglas fir. Appl. Environ. Microbiol. 63: 139–144.

Garbaye J., 1994: Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. 128: 197–210.

Garbaye J., Duponnois R., Wahl J.L., 1990: The bacteria associated with *Laccaria laccata* ectomycorrhizas or sporocarps: effect on symbiosis establishment on Douglas fir. Symbiosis 9: 267–273.

Gazzanelli G., Malatesta M., Pianetti A., Baffone W., Stocchi V., Citterio B., 1999: Bacteria associated to fruit bodies of ectomycorrhizal fungus *Tuber borchii* Vittad. Symbiosis 26: 211–222.

Lamb T.G., Tonkyn D.W., Kluepeel D.A., 1996: Movement of *Pseudomonas aureofaciens* from the rhizosphere. Can. J. Microbiol. 42: 1112–1120.

Li C.-Y., Castellano M.A., 1987: *Azospirillum* isolated from within sporocarps of the mycorrhizal fungi *Hebeloma crustuliniforme, Laccaria laccata* and *Rhizopogon vinicolor*. Trans. Br. Mycol. Soc. 88: 563–565.

McInroy J.A., Kloepper J.W., 1995: Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil 173: 337–342.

Peer R. van, Punte H.L.M., Vegerl A. de, Schippers B., 1990: Characterization of root surface and endorhizosphere pseudomonads in relation to their colonization of roots. Appl. Environ. Microbiol. 56: 2462–2470.

Petrini O., Müller E., 1986: Die praktische Bedeutung der Endophyteforschung. Swiss Biotechnol. 4: 11–14.

Pokojska-Burdziej A., Różycki H., Strzelczyk E., 2001: Utilization of different carbon sources by bacteria isolated from the roots of pine seedlings (*Pinus sylvestris* L.) inoculated with root-free, rhizosphere and mycorrhizosphere soil. Acta Microbiol. Pol. 50: 65–73.

Reynolds E.S., 1963: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208–212.

Sbrana C., Bagnoli G., Bedini S., Filippi C., Giovannetti M., Nuti M.P., 2000: Adhesion to hyphal matrix and antifungal activity of Pseudomonas strains isolated from *Tuber borchii* ascoscarps. Can. J. Microbiol. 46: 259–268.

Schüßler A., Schwarzott D., Walker C., 2001: A new fungi phylum, the *Glomeromycota*: physiology and evolution. Mycol. Res. 105: 1413–1421.

Shishido M., Breuil C., Chanway C.P., 1999: Endophytic colonization of spruce by plant growth-promoting rhizobacteria. FEMS Microbiol. Ecol. 29: 191–196.

Shishido M., Loeb B.M., Chanway C.P., 1995: External and internal root colonization of lodgepole pine seedlings by two growth-promoting *Bacillus* strains originated from different root microsites. Can. J. Microbiol. 41: 707–713.

Strzelczyk E., Li C.-Y., 2000: Bacterial endobionts in the big non-mycorrhizal roots of Scots pine (*Pinus sylvestris* L.). Microbiol. Res. 155: 1–4.

Tisdall J.M., 1991: Fungal hyphae and structural stability of soil. Austr. J. Soil Res. 29: 729–743.

Varese G.C., Portinaro S., Trotta A., Scannerini S., Luppi-Mosca A.M., Martinotti G., 1996: Bacteria associated with *Suillus grevillei* sporocarps and ectomycorrhizae and their effects on *in vitro* growth of mycobiont. Symbiosis 21: 129–147.

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