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Studies of *Cantharellus cibarius* – a mycorrhizal fungus of pine and spruce

ROMAN PACHLEWSKI*, EDMUND STRZELCZYK**, JADWIGA KERMEN**

*Forest Research Institute, Sękocin, PL 05-550 Raszyn, Poland
**Department of Microbiology, Institute of Biology & Environmental Protection, Nicolaus Copernicus University, Gagarina 9, PL 87-100 Toruń, Poland

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Pure cultures of *Cantharellus cibarius* were isolated in two forms: *C. cibarius* hardwood form (isolate No. 5400) and *C. cibarius* coniferous form (isolate No.5410). Artificial mycorrhization of pine (*Pinus sylvestris*) and spruce (*Picea abies*) was applied in this work and were determinated mycorrhiza-forming properties in both isolates with differences in mycorrhiza-forming activity and in morphogenesis of ectomycorrhizas. The sporocarps of *C. cibarius* consistently contained bacteria probably belonging to the genus: *Pseudomonas*. It was possible to evaluate the culture conditions for associated bacteria using *in vitro* tests (effect of antibiotics, pH of the medium), as well as their neutral interactions with mycorrhizal fungi (*Cantharellus cibarius, Pisolithus tinctorius, Suillus bovinus*, and *Mycelium radicis atrovirens*). Results of the present work suggest that the selection of isolates of *C. cibarius* for artificial mycorrhization of seedlings of forest trees in nurseries could be very useful.

Key words: *Cantharellus cibarius*, mycorrhizal fungi.

INTRODUCTION

Mycological observations of the occurrence of sporocarps of *Cantharellus cibarius* indicate the an essential contribution of this fungus in both coniferous and in hardwood forests (O r ł o ś, 1949; H a a s, 1964; L a n g e, L a n g e, 1967). Recently a very interesting paper on this fungus was published by D a n e l l and F r i e s (1990), and D a n e l l, A l s t r o m and T e r n s t r o m (1993).

Depending on the forest habitat type and the species composition of trees, two forms of sporocarp exist: *C. cibarius* hardwood form, which has large pale yellow--coloured sporocarps with compact paronchym and is found in hardwood forests and *C. cibarius* coniferous form with smaller yellow-coloured (egg-yolk resembling colour) sporocarps with less compact parenchyma occurring in coniferous forests (L a n g e, L a n g e, 1967).

C. cibarius was recognized for the first time as a mycorrhizal fungus by Doak in 1934 (cf. T r a p p e, 1962). The authors indicated ectomycorrhiza formation of this fungus with *Pinus strobus* and *P. taeda*. In his monograph about mycohorrizal associations of trees T r a p p e (1962), pointed out that *C. cibarius* could accompany many forest trees, both coniferous (pine, fir, spruce) and hardwood (beech, oak, hornbeam) ones. In their taxonomic studies of mycorrhizal fungi O r s o n and M i l l e r (1982) indicated that *C. cibarius* probably formed ectomycorrhizas with coniferous trees. D a n e l l and F r i e s (1990) and D a n e l l, A l s t r o m and T e r n s t r o m (1993) obtained ectomycorrhizas of *C. cibarius* with *Picea abies* in axenic cultures.

In the present study two forms of *C. cibarius* were investigated. The aim of the study was to determine their mycorrhiza-forming properties under controlled conditions as well as to assess their usefulness for artificial mycorrhization of pine and spruce seedlings in forest nurseries. The studies of bacteria associated with *C. cibarius* were performed additionally in order to determine the biotic factor which can undoubtedly affect the biology of this fungus. To our knowledge no studies on this fungus were carried out in Poland.

MATERIALS AND METHODS

I s o l a t i o n o f b a c t e r i a f r o m s p o r o c a r p s. For isolation of bacteria from sporocarps of *C. cibarius* and for their culturing Pp agar medium was used (P a c h l e w s k i, P a c h l e w s k a, 1974). After the appearance of bacterial growth around the fungal inoculum bacteria were subcultured onto Pp medium, having higher pH (equal to 6.5). Bacteria were passaged several times on the same medium in order to obtain pure cultures. To optimize the isolation of *C. cibarius*, steps to eliminate bacteria were undertaken exposing them to antibiotics. Antibiotic resistance tests of the bacteria were performed at 28°C using the disc method in Pp agar medium (pH 6.5). Plates with the following 7 antibiotics were used (Biomed, Warsaw, Poland): chloramphenicol (30 µg per dish), streptomycin (30 µg), oxytetracyline (30 µg), neomycin (30 µg), erythromycin (15 µg), aureomycin (30 µg), tetracycline (30 µg).

The effect of bacterial isolates on the following species of fungi was investigated: *Cantharellus cibarius, Pisolithus trinctorius, Suillus bovinus, Mycelium radicis atrovirens*. Tests were run on Petri dishes containing Pp agar medium; dense suspension of 24 hour old bacterial culture was applied directly onto fungi colonies.

P u r e c u l t u r e s o f *C a n t h a r e l l u s c i b a r i u s*. Cultures of *C. cibarius* were obtained by isolation from selected sporocarps useing the tissue method. In order to obtain pure cultures of *C. cibarius* many subculturings and reisolations to purify the isolates from bacteria were necessary using different kinds of media and techniques of culturing. Microbial purity of cultures was determined both by visual macroscopic examination of cultures on plates and by microscopic examination of

vegetative mycelium. Cultures obtained from the sporocarp derived from the mixed forest were included into the collection as isolate No 5400 and those isolated from the sporocarp of *C. cibarius* hardwood form (L a n g e, L a n g e, 1967) growing alone underneath oak trees; isolate No. 5410 was derived from a sporocarp of *C. cibarius* coniferous form (L a n g e, L a n g e, 1967) growing in a group underneath *Pinus sylvestris*. Both isolates were isolated and cultured on Pp agar medium (P a c h l e w s k i, P a c h l e w s k a, 1974), pH 4.8-5.0, temperature 24°C.

M y c o r r h i z a l i n o c u l a t i o n s o f p i n e a n d s p r u c e s e e d l i n g s w i t h *C. c i b a r i u s* i n p e a t c o n t a i n i n g c o n t a i n e r s. In order to determine mycorrhiza-forming capabilities of *C. cibarius* isolates Nos. 5400 and 5410, an experiment with artificial mycorrhization of pine and spruce seedlings in pots containing a peat mixture was performed. Seedlings were cultured adapting the method commonly used for production of containerized forest tree seedlings for nurseries (S o b c z a k, 1992).

Nonsterile mixture of: peat – 20 parts, quartz san – 20 parts and perlite – 10 parts (parts by weight) was used as a culture medium (substrate) in containers; pH of medium was in the range: 5.7-5.8.

Inoculations of seedlings were performed 4 weeks after emergence using nonwashed solid inocula grown for 3 months in 1000 ml Erlenmayer flasks in medium of the following composition: perlite – 40 g, quartz sand – 400 g, liquid medium Pp – 230 ml. The dose of inoculum of *C. cibarius* per 60 g of substrate (one container) was equal to about 8 g.

Therefore the inoculum was spread throught the substrate surface in a container and covered with a layer of substrate about 2 cm thick. After inoculation, the substrate was watered with distilled water. The experiment was run in a greenhouse. Observations on the progress of mycorrhizal infection were performed monthly for 5 months using a stereosophic binocular microscope for analyses of root systems (magnifications: 25 x and 40 x).

RESULTS

Studies of bacterial strains isolated from sporocarps of *C. cibarius* have revealed that in most cases they probably belong to the genus *Pseudomonas*. Similarly D a n e l l, A l s t r o m and T e r n s t r o m (1993) indicated the presence of *Pseudomonas fluorescens* in sporocarps and ectomycorrhizas of *C. cibarius*.

Bacterial strains isolated from sporocarps of *C. cibarius* in antibiotics resistance tests were most strongly inhibited by oxytetracycline at the concentration of 30 μg/ml.

In tests for mycolytic activity of bacteria (*Pseudomonas* sp.) isolated from sporocarps of *C. cibarius* no lysis of mycelium of the fungi tested (*C. cibarius, S. bovinus, P. tinctorius, M. radicis atrovirens*) was noted.

Studies of the bacteria isolated from sporocarps of *C. cibarius* and tentatively classified in to the genus *Pseudomonas* enabled us to observe an interesting phenomenon. Pure cultures of these bacteria grown in 9 cm Petri dishes released (after opening a plate) a characteristic smell of fresh sporocarps of *C. cibarius*. The above mentioned smell gradually dispeared after several subculturings.

In the present study both forms of *C. cibarius* were investigated. Cultures of both isolates revealed differences in their morphology and in the development in *in vitro* cultures.

Isolate No 5400 (hardwood form) was easier to isolate and exhibited more profuse and faster growth. After 4 weeks of culturing a vegetative mycelium overgew the whote surface of the plate (diameter: 9 cm) forming well developed aerial and substrate mycelium. Aerial mycelium was white and shortly tomentose. Hyphae were 2.5-6.0 μm in diameter; they had a large number of small clamps in young cultures. This number decreased with the age of the culture. The reverse side of the colony was yellow in its central parts; its outside part (growth zone) was white.

Isolate No. 5410 (conifernous form), which was very difficult to isolate, exhibited slower growth forming a colony 5-6 cm in diameter after 4 weeks of culturing. In their central part colonies had slightly leather consistence and were slightly plicate and pale beige-white. Then were white in the growth zone, which had a velvety surface. The reverse side of the colony was dirty-yellow in its central part and white--yellow in the growth zone. Hyphae were 2.5-5.0 μm in diameter with numerous swellings in the intercalary array, usually filled with shiny vacuoles. Clamps were not detected in hyphae during microscopical analysis.

Mycorrhizal inoculations of pine and spruce seedlings with *C. cibarius*. The experiments performed enabled us to determine mycorrhiza-forming properties in the isolates of *C. cibarius* with regard to species of trees. Analyzing the time-course of mycorrhizal infection we can note high mycorrhiza-forming activity of the isolate No. 5400, as regards pine and spruce, which a manifested in the fast apperance of large number of mycorrhizas. In addition isolate No. 5400 had a high morphological specificity of ectomycorrhizas formed in pine. In most cases they are dichotomously branched frequently with multiple dichotomous branches (Fig. 1, Table 1). Isolate No. 5410, which had lower activity, formed simple mycorrhizas with a lower proportion of dichotomous ones (Fig. 3, 4). Ectomycorrhizas formed by both isolates in pine had a cream-coloured mantle with tomentose surface sometimes with veils or with mycelial threads. In spruce the mantle was brown with smooth, slightly shiny surface (Fig. 5). The mantle was 15-20 μm thick. The Hartig net was always well formed and visible as a dense net.

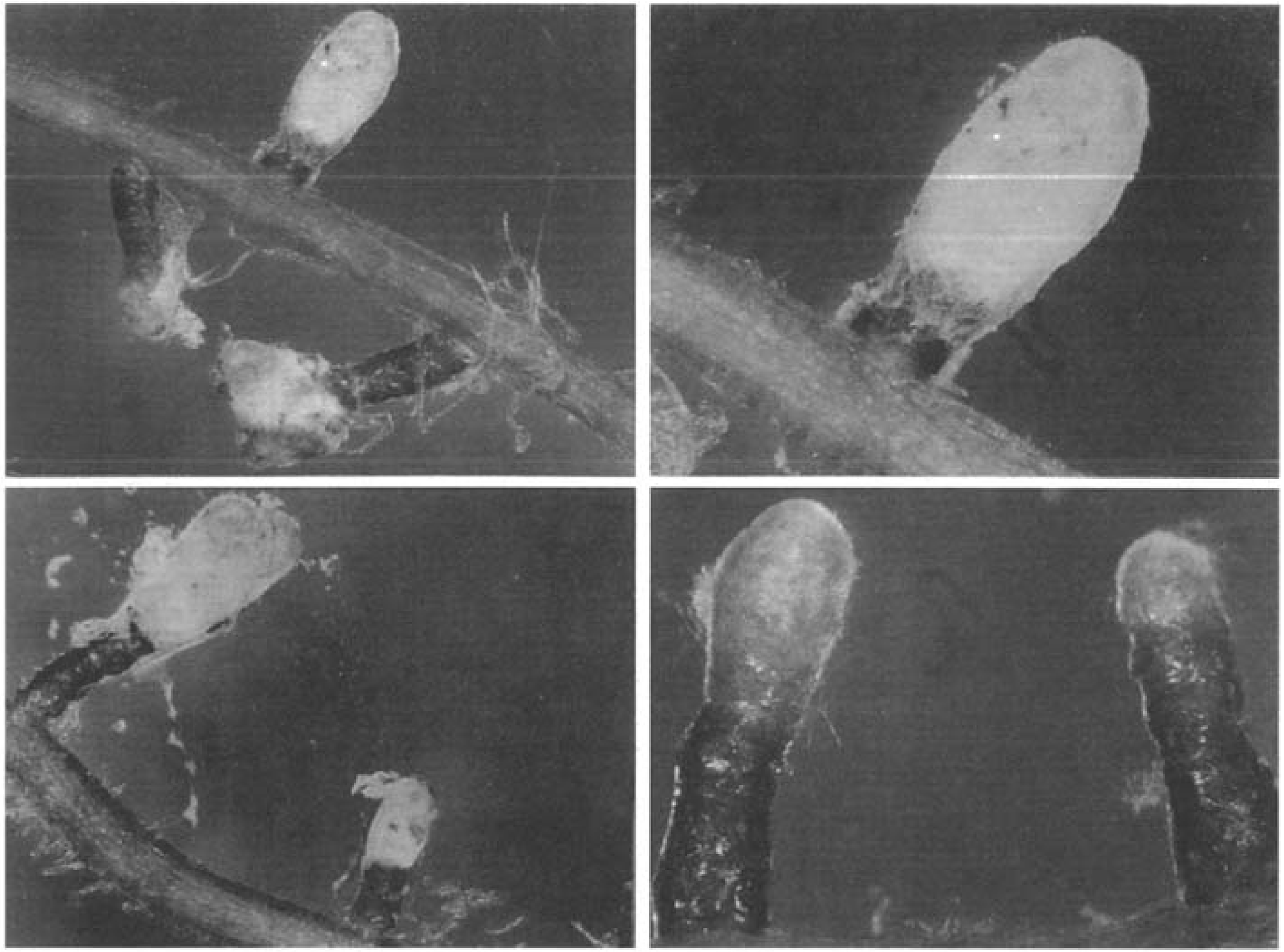

Fig. 1-4. Ectomycorrhizas of pine with *C. cibarius*

Mycorrhization in containers: 1 – isolate No. 5400 (25 x), 2 – No. 5400 (50 x), 3 – No. 5100 (25 x), 4 – No. 5410 (50 x)

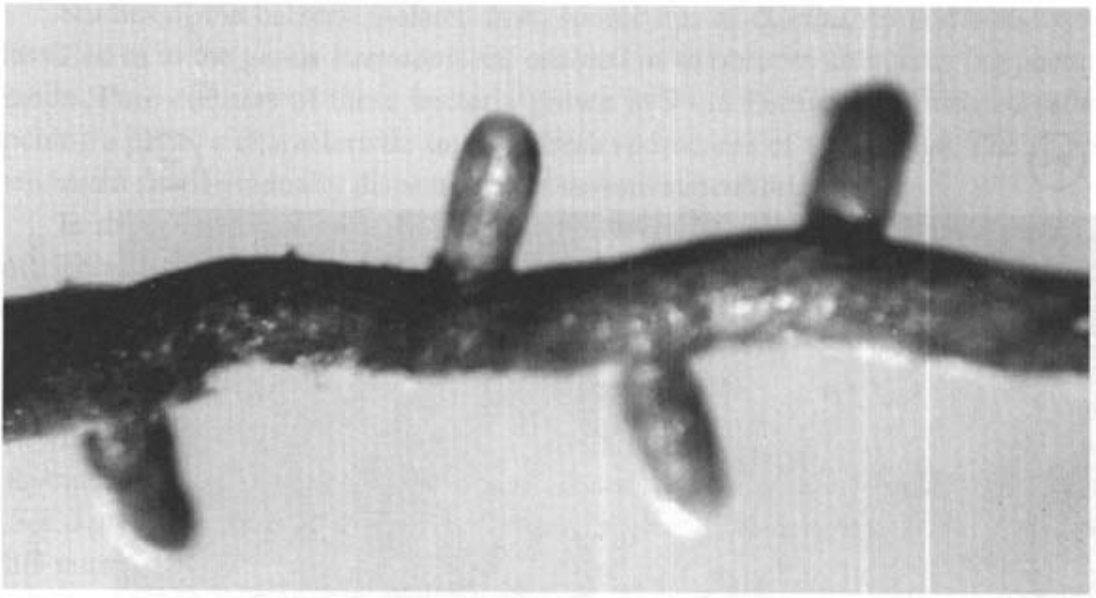

Fig. 5. Ectomycorrhizas of spruce with *C. cibarius*
Mycorrhization in containers: isolate No. 5400 (20 x)
(phot. Kieliszewska-Rokicka)

Table 1

Time-course of ectomycorrhizal infection by *Cantharellus cibarius* in pine and spruce seedlings

Fungi strain	Seedlings	Appearance of ectomycorrhizas (in months after inocultaion) 1 2 3 4	Frequency of occurrence of ectomycorrhizas*	Morphology of ectomycorrhizas	
				Simple	Dichotomous
C. cibarius 5400	Pine	x	+++	++	+++
C. cibarius 5410	Pine	x	++	+++	++
C. cibarius 5400	Spruce	x	+++	+++	–

Explanations: * four months after inoculation;
x – appearance of ectomycorrhizas;
+++ very numerous, ++ numerous, – none

DISCUSSION

Studies regarding ectomycorrhizal fungus *C. cibarius* have been not intensive. The studies performed in the present work have confirmed and extended our current knowledge on *Cantarellus cibarius* as a mycorrhizal fungus of forest trees. Our observations enabled the determination of biological properties of this fungus and their role in associations with bacteria (possibly of the genus *Pseudomonas*) which

commonly occur in sporocarps of *C. cibarius* (S c h o u t e n, W a a n d r a g e r, 1979; D a n e l l, A l s t r o m, T e r n s t r o m, 1993). This phenomenon attracts much attention nowadays, among others, due to difficulties with obtaining pure cultures of *C. cibarius* caused by bacterial infection of the fungal inoculum. Fragmentary observations of bacteria inhabiting sporocarps of *C. cibarius*, suggest that there is some association between *C. cibarius* and bacteria of the genus *Pseudomonas*. These bacteria which accompany the mycelium of *C. cibarius* in soil, infect sporocarps during their formation, and are present till to the end of their growth and development. The reproducibility of this phenomenon and its strengthening among sporocarps of *C. cibarius*, observed by us during long-term isolations and culturing of the fungus in pure cultures, suggests that a special character of these associations – advantageous to both the partners – probably due to metabolic interactions (D a n e l l, A l s t r o m, T e r n s t r o m, 1993). Further physiological studies of this species are necessary. Such studies could reveal the mechanisms of relationships between *C. cibarius* and the bacteria of the genus *Pseudomonas* as well as biological censequences of associations for both of the partners. It cannot be excluded that bacteria in the mycelium of *C. cibarius* can affect formation of sporocarps in this fungus.

The results of mycorrhization experiments of pine and spruce seedlings performed under controlled conditions (in containers) indicate possoble practical usefulness of such a treatment. The use of this fungus for inoculation can be justified by its high mycorrhiza-forming activity, advantageous influence of its ectomycorrhizas on the growth of pine and spruce seedlings. In case of the above mentioned properties it was not much inferior to the following fungi: *Hebeloma crustuliniforme*, *H. mesophaeum*, and *Laccaria laccata* – species preferentially used above all for artificial inoculation. The use of *C. cibarius* for mycorrhizal inoculations should also be considered in the aspect of protection of this fungus in the mycoflora of our forest, considering that this a species is declining in Poland. It has a great economical importance as an edible mushroom. The only drawback of the use of *C. cibarius* for inoculation is the difficulty in obtaining pure cultures of this fungus for preparation of the inoculum in a commercial/industrial scale.

The results of *in vitro* studies of isolates of *C. cibarius* Nos. 5400 and 5410 as well as results of mycorrhizal synthesis in pine and spruce seedlings with these fungi under controlled conditions indicate that two forms of *C. cibarius*: *C. cibarius* hardwood form and *C. cibarius* coniferous form (L a n g e, L a n g e, 1967) should be distinguished since they differ from each other in morphological and ecophysiological properties. Further taxonomical and physiological studies of *C. cibarius* are necessary.

When its isolates for artificial inoculation (mycorrhization) of forest trees are selected. Studies on the physiology of this fungus as well as on the associated bacteria are carried out at present.

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Mikoryza *Cantharellus cibarius*

Streszczenie

Czyste kultury *Cantharellus cibarius* izolowane z miąższu owocników tego grzyba użyto do sztucznej mikoryzacji sadzonek sosny i świerka w pojemnikach. Doświadczenie wykazało właściwości mikoryzogenne badanych szczepów ze zróżnicowaniem ich aktywności mikoryzowej i morfogenezy ektomikoryz. Stwierdzono stałe występowanie w owocnikach *C. cibarius* bakterii zaliczonych do rodzaju *Pseudomonas*. Testy *in vitro* pozwoliły określić warunki hodowlane bakterii oraz ich stosunek do grzybów mikoryzowych.

Badania wskazały także na możliwość zastosowania w leśnictwie *C. cibarius* do mikoryzacji sosny i świerka, co łączyłoby się z zagadnieniem ochrony tego gatunku grzyba w naszej mikoflorze.