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Aseptic culture of slowly growing mycorrhizal Russula and Cortinarius species

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Aseptic cultures of several *Cortinarius* and *Russula* species were obtained by transferring first a piece of the context of the sporocarp aseptically on malt extract agar, and after 2–4 weeks, if not contaminated with bacteria or molds, into liquid MMN medium. It took several months before mycelia slowly started to grow in the solution. When cultivated on MMN- agar media, most species grew only few millimetre/month, remarkably less than many other ectomycorrhizal species tested. Fungal species reacted very specifically to different sources. The slow growth rate of *Russula* and *Cortinarius* species may be related to specific requirements (nutrients and/or vitamins) not yet known. Possible role of hidden infection of bacteria is also discussed.

Key words: aseptic culture, *Cortinarius*, growth rate, *Russula*

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Introduction

Although *Russula* and *Cortinarius* species, ectomycorrhizal symbionts of many tree species, often play a dominant role in the macromycete flora of Nordic forests, relatively little is known of their exact role in the ecosystem.

Attempts to obtain aseptic cultures have been largely unsuccessful, and only a few synthesis experiments have been carried out (Agerer 1986, Taylor & Alexander 1989, Ali & Jackson 1989), compared with e.g. bolets and *Amanita* species which grow easily on cultural media. In this paper we describe the method by which aseptic cultures of several *Russula* and *Cortinarius* species were obtained.

Methods and results

A small piece of the context of the basidiocarp was taken aseptically and transferred to 1% malt extract agar slant and kept there 2–4 weeks to ensure that the piece was free from bacterial or mold infections. During this period, no clear growth was seen. From agar medium the piece was immersed into modified MelinNorkrans (MMN-) solution in 100 ml Erlenmeyer flasks. In positive cases, a sparse mycelium started to grow from the piece in 2–3 months. In all cases the liquid remained clear and evidently free from bacterial infections. After one year, there was a relatively large mycelium piece and after one and a half year the mycelium filled up the whole liquid

medium. The mycelia grew largely submerged, and had no clamp connections.

The radial growth of the mycelia was studied by cultivating them on MMN– agar in Petri dishes. The growth was compared to that of some other ectomycorrhizal species after 34 days of cultivation. During the experiment the fastest fungi grew up to 40 mm, in contrast to slower ones, which grew only few millimetres (Table 1). The results indicate that there is a continuous series from rapidly growing mycorrhizal species, such as *Paxillus involutus* and some *Suillus* species, to very slowly growing *Russula* and *Cortinarius* species.

Table 1. Radial growth (mm) of certain ectomycorrhizal fungi on MMN- agar after 34 days.

Paxillus involutus (Batsch : Fr.) Fr.	45 mm
Suillus bovinus (L. : Fr.) Roussel	40 mm
S. luteus (L. : Fr.) Roussel	37 mm
Suillus granulatus (L. : Fr.) Roussel	37 mm
Amanita rubescens (Pers. : Fr.) S.F.Gray	26 mm
Cortinarius argutus Fr.	17 mm
Boletus badius (Fr.) Fr.	13 mm
Cortinarius mucosus (Bull. : Fr.) Kickx	12 mm
C. anomalus (Fr. : Fr.) Fr.	8 mm
Leccinum versipelle (Fr.) Snell	8 mm
Tricholoma album (Fr.) Kumm.	8 mm
Lactarius mitissimus (Fr.) Fr.	7 mm
Russula vinosa Lindbl.	7 mm
Amanita citrina (Schaeff.)Pers.	6 mm
Russula delica Fr.	6 mm
R. paludosa Britz.	6 mm
Lactarius torminosus (Schaeff. : Fr.) Pers.	5 mm
Russula xerampelina (Schaeff.) Fr.	3 mm
Cortinarius cumatilis Fr.	2 mm
Russula foetens Pers. : Fr.	1 mm
R. grata Britz (R. laurocerasi)	1 mm
Cortinarius violaceus (L. : Fr.) S.F.Gray	<1 mm

The influence of different carbon sources, sugars and an amino acid L-glutamine, on the radial growth of the mycelia was studied by cultivating the mycelia on agar media containing 500 mg KH_2PO_4, 500 mg NH_4Cl, 100 mg $MgSO_4$, 10 mg $MnSO_4$, 10 mg $ZnSO_4$, 1 mg thiamine, 1 ml 1% Fe-citrate solution and 10.0 g agar in 1000 ml deionised water. After the medium had been autoclaved, it was poured into sterile erlenmeyer flasks (100 ml), and filter-sterilized (Millipore size 0.22 µm) aqueous solution of a single sugar or L-glutamine was added into 60–70° agar media. The final concentration of inositol and L-glutamine was 0.5% and that of other components 1%. Radial growth was measured after 28 days.

The species reacted specifically to different carbon sources (Table 2). Both *Russula* species preferred fructose 1,6–P, gentiobiose and also glutamine to glucose and sucrose. To *Cortinarius argutus* fungal sugars trehalose and mannitol were slightly better carbon sources than glucose and clearly better than sucrose. In *C. anomalus*, no clear difference between these sugars was observed. L-glutamine was not a suitable carbon source for *Cortinarius* species. Surprisingly, both *Russula delica* and *Cortinarius argutus* grew well without any added carbon source, although the mycelium was very thin. *Russula xerampelina* grew only on trehalose and *Russula paludosa* on maltose media, both less than 1 mm during 28 days (data not shown). *Cortinarius mucosus* produced thin aerial rhizomorphs on cellobiose medium.

Table 2. The radial growth of four ectomycorrhizal fungi on MMN- agar supplemented with different carbon sources (1%). The radial growth (mm) was measured after 28 days.

	Russula vinosa	Russula delica	Cortinarius argutus	Cortinariu anomalus
no addition	0	10	10	1
glucose	2	2	9	4
glucose 1-P	0	6	1	0
sucrose	2	+	5	3
maltose	2	4	2	2
fructose 1,6-P	5	4	3	0.5
trehalose	3	2	11	2
sellobiose	3	2	9	3
gentiobiose	4	6	5	3
malt extract	0.2	1	9	4
mannitol	0	4	12	5
inositol 0,5%	1	0	0	0
glutamine 0.5%	5	9	0	0
sorbitol	0	1	(+)	0
xylitol	2	2	6	+

Discussion

The slow growth rate of many ectomycorrhizal fungi is well known (Smith & Read 1997), especially when compared to saprophytic wood-decomposing fungi. If compared with the expansion rate of basidiocarps, the growth rate of the mycelium is surprisingly low. For example, in this study the mycelium of *Russula paludosa* grew only 6 mm in 34 days, while caps of the same species have been reported to extend up to 34 mm in a single day in suitable conditions (Hiukka 1978).

Russula and *Cortinarius* are mycorrhizal fungi, which appear mostly in the late stage of forest succession. Growth rate *in vitro* has been suggested to relate to successive changes in ectomycorrhizal colonization of pine roots (Erland & Finlay 1992). The slow growth rate of both *Russula* and *Cortinarius* species on aseptic cultures might be due to specific requirements, such as nutrients and/or vitamins, not yet known. Because both *Russula delica* and *Cortinarius argutus* grew as well or even better on the control media (no carbon source added) than on the media supplemented with several different carbon sources, the change of energy source is not the probable solution for better growth. Instead, it would be important to study possible roles of micronutrients and different forms of nitrogen in mycelial growth.

Bacteria associated with basidiocarps have been reported to inhibit the growth of mycelia of, for example, *Cantharellus cibarius* (Danell & Fries 1993) and *Suillus grevillei* (Varese et al. 1996). Also in this study, hidden infection of bacteria might reduce the growth of mycelia, although cultivation on 0.5% streptomycin agar did not result in any better growth of the mycelia. However, it should be noted that *Pseudomonas* bacteria, which occur in plant tissues and in soil, are very resistant to antibiotics (Sarvas 1976).

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