FULL PAPER

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Mycelial growth of strains of the genera *Suillus* and *Boletinus* in media with a wide range of concentrations of carbon and nitrogen sources

Abstract *Suillus* and *Boletinus* were studied using Ohta medium. In media with glucose or trehalose, all tested strains grew well. With mannose and cellobiose, strains generally grew well, except for one strain of *Suillus*. Utilization of dextrin and soluble starch differed with each strain, and that of sucrose and glycerol was low for all strains. Utilization of four amino acids, arginine, glutamic acid, aspartic acid, and alanine, was similar to that of ammonium tartrate for *Suillus* strains, but mycelial growth with amino acids was clearly lower than with ammonium tartrate for the *Boletinus* strain. The effect of glucose and ammonium tartrate concentrations for nine strains of the genera *Suillus* and *Boletinus* was studied with ranges for glucose of 1–100 and 200 g/l, respectively, and for ammonium tartrate of 0.2–5 and 20 g/l, respectively. Six strains showed maximal growth at a glucose concentration greater than 25 g/l, and one strain showed maximal growth at 70 g/l. The results indicate that these fungi are adapted to relatively high concentrations of carbon sources. In general, glucose concentration at mycelial growth maximum decreased as ammonium tartrate concentration increased, and at higher concentrations of glucose, mycelial growth decreased more rapidly in higher concentrations of ammonium tartrate.

Key words *Boletinus* species · Carbon and nitrogen sources · High concentration of carbon source · Mycelial growth · *Suillus* species

Introduction

Because *Suillus* and *Boletinus* are popular in the mountainous region of the central districts of Japan and some species

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have been reported to stimulate growth of seedlings of *Larix kaempferi* (Murata 1991), the development of techniques to culture these fungi is important for their use. Many studies of the cultivation of *Suillus* species have been carried out (Melin 1954, 1959; Ferry and Das 1968; Palmer and Hacskaylo 1970; Lamb 1974; Lindeberg and Lindeberg 1977; Giltrap and Lewis 1981; Hung and Trappe 1983; Ohta 1990; Murata 1991, 1993; Liangqing and Zhida 1998). For *Boletinus* species, the utilization of chitin and the enzymatic degradation of some carbon and nitrogen sources were reported (Hutchison 1990a,b; Hodge et al. 1995; Dickinson and Hutchison 1997).

Numerous studies have been performed on the cultivation of mycorrhizal fungi (Melin and Norkrans 1948; Mikola 1948; Norkrans 1950; Melin 1954, 1959; Jayko et al. 1962; Lundeberg 1970; Hacskaylo 1973; Molina and Palmer 1982; Fries 1983; Hung and Trappe 1983; Harley and Smith 1983; Ohta 1990; Finlay et al. 1992; Mischiati and Fontana 1993; Brundrett et al. 1996; Keller 1996; Vaario et al. 2002), including studies on the utilization of carbon sources (Ferry and Das 1968; Palmer and Hacskaylo 1970), nitrogen sources (Melin and Norkrans 1948; Norkrans 1953; Finlay et al. 1992; Keller 1996), the effect of phosphate on the availability of glucose (Giltrap and Lewis 1981), the effect of chitin (Hodge et al. 1995), and the effect of small amounts of glucose on the utilization of other carbon sources (Lamb 1974).

Because ectomycorrhizal fungi are believed to obtain much of the carbon necessary for growth from the host through ectomycorrhizae, they must be adapted to nutrient conditions of the inside of the plant root tissue, especially that of the apoplast. In plants, the phloem sap flows down to the apoplast of the root and the nutrient conditions of the apoplast may reflect the components of the phloem sap, which may contain rather high concentrations of sucrose and amino acids. Hayashi and Chino (1986) reported that 251 mM sucrose, 261.7 mM total amino acids, and 299 mM K^+ were present in the phloem sap of wheat, and Fisher (1983) reported a high osmolality of the sieve-tube sap in field specimens of willow. These data suggest that the sugar concentration in the apoplast of tree roots may increase to

relatively high levels during seasonal changes or in response to a change in environmental conditions, and that ectomycorrhizal fungi may be able to grow in relatively high concentrations of sugars.

In reports on the nutrition of mycorrhizal fungi, the range of carbohydrate concentrations analyzed was rather limited, and tests of carbon source concentrations of more than 20 g/l were rare (Mikola 1948; Jayko et al. 1962). Because the utilization of carbohydrates by fungi has been reported to be dependent on the amount of nitrogen source present (Chang and Miles 1989), to elucidate the nutritional characteristics of ectomycorrhizal fungi, growth tests in high concentrations of carbon sources in the presence of varying amounts of nitrogen are necessary. As far as we can determine, however, no such tests have been performed for *Suillus* and *Boletinus* species. In this research, the mycelial growth of several strains of *Suillus* and *Boletinus* species was studied with a wide range of carbon sources, from 1 to 100 g/l (in one case to 200 g/l), and nitrogen sources from 0.2 to 5 g/l (in one case to 20 g/l), to characterize the nutritional requirements of these fungi.

Materials and methods

Fungal strains

In this study, we used two strains of *Suillus luteus* (L.: Fr.) S.F. Gray, two strains of *S. grevillei* (Klotz.) Sing., a strain of *S. laricinus* (Berk. in Hook.) O. Kuntze, three strains of *Boletinus cavipes* (Opat.) Kalchbr., and a strain of *B. paluster* (Peck) Peck (Table 1). All these strains were stock cultures of the Laboratory of Applied Mushroom Science of the Faculty of Agriculture, Shinshu University.

Media used for cultivation of mycelia

The synthetic culture medium developed by Ohta (1990) was used as the basic culture medium. The liquid medium used for evaluation of the mycelial growth was modified by the addition of different concentrations of various carbon and nitrogen sources.

Preparation of the inoculum

Each strain was inoculated onto basic agar [2% (w/v)] medium in a Petri dish and was cultured for 30 days at 25°C in the dark. Mycelial disks with a diameter of 5 mm were punched out from the culture with a cork borer. Each mycelial disk was inoculated into 30 ml basic medium in a 100-ml Erlenmeyer flask and grown in a static culture at 25°C in the dark for 30 days. After the mycelium was washed in sterilized distilled water in a beaker, washed mycelia from three flasks were combined and homogenized for 30 s at 6000 rpm using a homogenizer (Ace Homogenizer, AM-8; Nihon Seiki Kaisha, Tokyo, Japan) in 100 ml sterile distilled water. The suspension of homogenized mycelium was filtered using a nylon mesh with a pore size of 37 μm to remove large fragments of mycelium.

Determination of the mycelial growth in culture media

To evaluate the mycelial growth in various media, 2 ml mycelial suspension was inoculated into 18 ml liquid medium in a 100-ml Erlenmeyer flask and grown in a static culture at 25°C for 18 days in the dark. Each condition was tested in seven replications. After cultivation, the mycelium was collected on filter paper (Advantec, No. 2) and washed with distilled water. Each mycelium was placed on a preweighed piece of aluminum foil and dried in a drying oven for 2 days at 60°C, then with a dry heat sterilizer for 2 h at 105°C; its dry weight was then measured.

The average mycelial growth was calculated from the value of seven replicate flasks for each medium and analyzed statistically using ANOVA with $P < 0.05$.

Utilization of the carbon and nitrogen sources

To study carbon source utilization, glucose in the basic culture medium was replaced with sucrose, cellobiose, mannose, glycerol, dextrin, trehalose dihydrate, or soluble starch. The concentration of each carbon source in the media was set at 10 g/l.

To study nitrogen source utilization, ammonium tartrate in the basic culture medium was replaced with arginine, glutamic acid, aspartic acid, or alanine. The concentration of each nitrogen source was adjusted so that the amount of nitrogen in each medium was equal to the amount of

Table 1. Fungal strains used in this study

Species	Strain	Date of collection	Location
Boletinus cavipes (Opat.) Kalchbr.	SA286	Oct. 1995	Japan, Nagano
	SA473	Sept. 2000	Japan, Nagano
	SA474	Sept. 2000	Japan, Nagano
Boletinus paluster (Peck) Peck	SA348	Sept. 1997	Japan, Nagano
Suillus grevillei (Klotz.) Sing.	SA52	Nov. 1992	Japan, Nagano
	SA56	June 1985	Japan, Hokkaido
Suillus laricinus (Berk. in Hook.) O. Kuntze	SA525	June 2001	Japan, Nagano
Suillus luteus (L.: Fr.) S.F. Gray	SA50	Oct. 1991	Japan, Hiroshima
	SA51	Mar. 1992	New Zealand

nitrogen in the basic medium. Moreover, considering the influence of the tartaric acid in the basic medium, 1.534 g/l potassium tartrate tetrahydrate was added as well as the added source of nitrogen.

Effect of glucose and ammonium tartrate concentrations in the medium on the mycelial growth of fungi

Each strain was cultured in media of varying glucose concentrations [1 g/l, 3.33 g/l, 10 g/l (basic medium), 33.3 g/l, and 100 g/l] and varying ammonium tatrate concentrations [0.2 g/l, 1 g/l (basic medium), and 5 g/l] in a total of 15 blocks. For *S. luteus*, SA50, in addition to the aforementioned experimental design, another design was used in which the glucose concentration was 10, 33.3, 100, or 200 g/l, and the ammonium tatrate was 1, 5, 10, or 20 g/l, in a total of 16 blocks.

Results

Utilization of carbon sources

Four strains of the genus *Suillus* (SA50, -51, -52, and -56) and one strain of the genus *Boletinus* (SA286) were tested to investigate the utilization of the various carbon sources.

For all five strains, the mycelial growth in media with glucose or trehalose dihydrate was good, the mycelial growth in media with sucrose was poor, and growth with glycerol was very poor.

The *S. luteus* strain SA50 showed the best mycelial growth in medium that contained mannose or cellobiose (Fig. 1A), and SA51 showed the best in medium with trehalose dihydrate (Fig. 1B). They showed slightly poorer growth in medium with glucose compared to the saccharides, in which they showed the best mycelial growth. In media that contained mannose, sucrose, or dextrin, the mycelial growth of the two strains was different. In media that contained soluble starch, mycelial growth was very poor. *S. grevillei* SA52 and SA56 showed the best mycelial growth in media that contained glucose, mannose, or trehalose dihydrate (Fig. 1C,D), but grew very differently in media containing cellobiose, dextrin, or soluble starch. In the medium with the latter three saccharides, the growth of SA52 was better than that of SA56.

B. cavipes SA286 showed the best mycelial growth in a medium that contained glucose or trehalose dihydrate (Fig. 1E). In the culture medium that contained cellobiose, the growth was about 70% of that with glucose, and in medium with mannose, 60%. The mycelial growth of SA286 in media containing soluble starch, sucrose, dextrin, or glycerol was very poor, and there was no significant difference among the four types of culture media.

Utilization of nitrogen sources

The utilization of various sources of nitrogen was tested for four strains of the genus *Suillus*, SA50, -51, -52, and one strain of the genus *Boletinus,* SA286.

Four strains of the genus *Suillus* showed no clear difference in mycelial growth with the five nitrogen sources tested. For *S. luteus*, the mycelial growth of SA50 in medium with alanine was statistically significantly better than that in media with other nitrogen sources (Fig. 2A), but the difference was not very large. For SA51, growth in the medium with glutamic acid appeared to be best, but there was no statistically significant difference among the five nitrogen sources tested (Fig. 2B). Similar patterns of

Fig. 1. Utilization of carbon sources by *Suillus luteus, S. grevillei*, and *Boletinus cavipes*. *Vertical axis*, mycelial growth; *horizontal axis*, carbon source; each *column* shows average amount of mycelial growth for seven replicates with each carbon source; *bars* show standard deviations; and columns labeled with the same letter (*a–d*) are not significantly different (*P* < 0.05). These symbols have similar meanings in Figs. 2, 5, 6. **A** *S. luteus* (SA50); **B** *S. luteus* (SA51); **C** *S. grevillei* (SA52); **D** *S. grevillei* (SA56); **E** *B. cavipes* (SA286). *DW*, dry weight
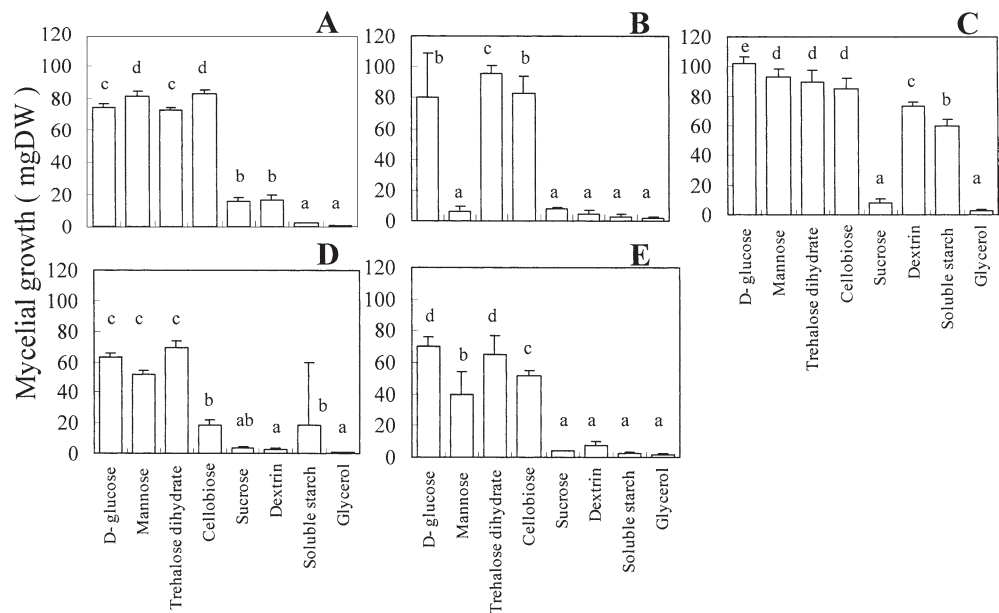
Carbon sources used in the medium

Fig. 2. Utilization of nitrogen sources by *S. luteus*, *S. grevillei*, and *B. cavipes*. *Vertical axis*, mycelial growth; *horizontal axis*, nitrogen source. Symbols as in Fig. 1. **A** *S. luteus* (SA50); **B** *S. luteus* (SA51); **C** *S. grevillei* (SA52); **D** *S. grevillei* (SA56); **E** *B. cavipes* (SA286)

Nitrogen sources in the medium
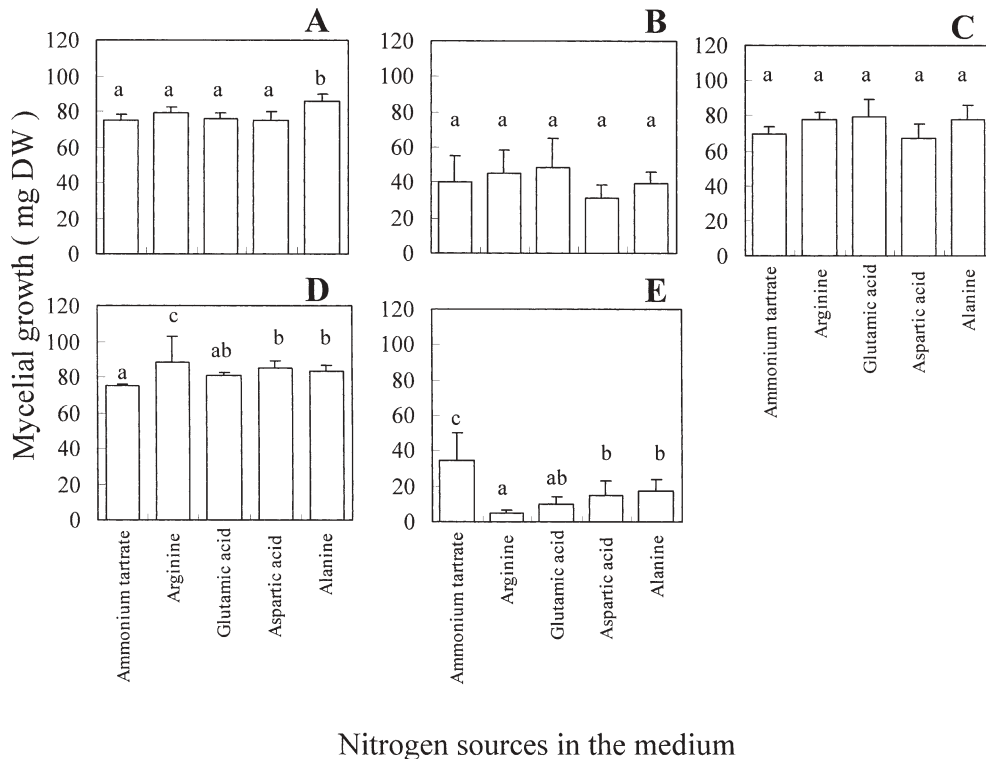
nitrogen source usage were observed for *S. grevillei* SA52 and SA56 (Fig. 2C, D).

For *B. cavipes* SA286, the pattern of nitrogen source usage was clearly different from that for species of *Suillus*. SA286 showed the best mycelial growth in the medium that contained ammonium tartrate (Fig. 2E). Among the four amino acids used in this test, the mycelial growth of the strain was maximum with alanine and minimum with arginine.

Change in the mycelial growth with glucose concentration

In media containing glucose concentrations of 3.33 or 1 g/l, mycelial growth for all strains was minimal and the difference in the mycelial growth in media containing different levels of nitrogen sources was small. In media containing 100 g/l glucose, the growth of mycelium of many strains was relatively low, except for one strain, *Suillus* SA50.

Five strains of the genus *Suillus* showed hyphal growth with glucose concentrations of 100 g/l or less (Fig. 3). The typical pattern of mycelial growth dependence on the glucose concentration in the media is that of *S. luteus* SA51. In this strain, for all ammonium tartrate concentrations, the mycelial growth increased with an increase in glucose concentration and showed the highest value at 33.3 g/l, which is higher than the glucose concentration of the basic medium, 10 g/l (Fig. 3B). The growth of mycelia with 33.3 g/l glucose, was more than twice of that in media with 10 g/l glucose, with either 1 or 5 g/l ammonium tartrate. *S. luteus* SA50 showed the highest mycelial growth with a glucose concentration of 100 g/l (Fig. 3A,A′). Mycelial growth curves of *S. grevillei* SA52 and SA56 were similar to that of *S. luteus*

SA51 and increased with increasing glucose concentrations, showing the highest value at 33.3 g/l (Fig. 3C,D). For *S. luteus* SA50 and *S. laricinus* SA525, glucose concentration at the peak of the mycelial growth was clearly dependent on ammonium tartrate concentration (Fig. 3A′,E). Glucose concentration at the peak of the mycelial growth of all strains at each ammonium tartrate concentration (more than 0.2 g/l) as well as values of C/N ratio at the peak point is shown in Table 2.

The results for strains of the genus *Boletinus* are shown in Fig. 4. Four strains of the genus *Boletinus* were tested, and three strains of *B. cavipes* showed a rather broad peak of dependence of mycelial growth on glucose concentration. For all strains, as the glucose concentration increased, the mycelial growth increased from the glucose concentration 1 g/l, reached a maximam at 10–33.3 g/l, and decreased thereafter. The concentrations of glucose at which the best mycelial growth was observed are shown in Table 2. Two strains, *B. cavipes* SA473 and SA474, showed relatively good mycelial growth at a glucose concentration of 100 g/l with all concentrations of ammonium tartrate. *B. paluster* SA348 showed the best mycelial growth at a glucose concentration of about 30 g/l with all concentrations of ammonium tartrate (Fig. 4D); it showed only a small difference in mycelial growth with ammonium tartrate between 1 and 5 g/l at all glucose concentrations.

Effect of the nitrogen source concentration on mycelial growth

The concentration of ammonium tartrate also affected mycelial growth greatly. The mycelial growth was good at

Fig. 3. Change in mycelial growth of strains of the genus *Suillus* according to glucose concentration in the medium. *Vertical axis*, amount of mycelial growth; *horizontal axis*, glucose concentration in the medium. The symbols (■, 20 g/l; ◆, 10 g/l; □, 5 g/l; ◇, 1 g/l; △, 0.2 g/l) indicate the concentration of ammonium tartrate in the medium. *Bar* shows ± SD. **A,A′** *S. luteus* (SA50); **B** *S. luteus* (SA51); **C** *S. grevillei* (SA52); **D** *S. grevillei* (SA56); **E** *S. laricinus* (SA525)
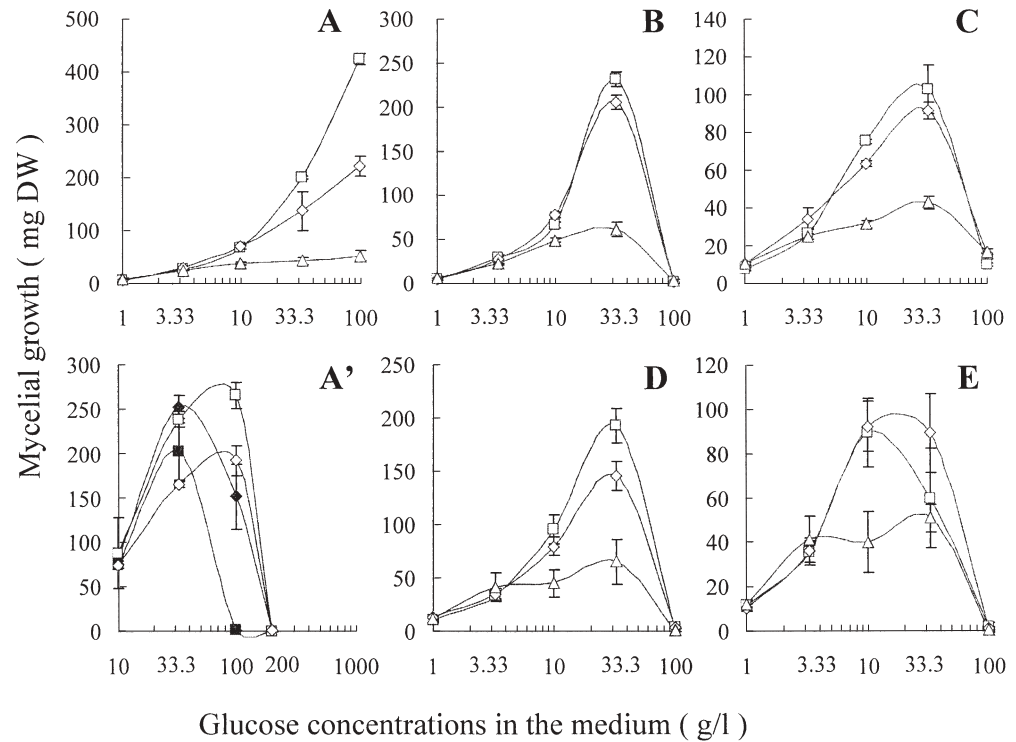

Glucose concentrations in the medium (g/l)

Table 2. Glucose concentration at the peak of mycelial growth of the genera *Suillus* and *Boletinus*

Species	Strain	AT (g/l)[a]	Glucose (g/l)[b]	C/N ratio
S. luteus	SA50	1	80	210.00
		5[c]	70	36.80
		10	35	9.20
		20	30	3.94
	SA51	1	33.3	87.62
		5[c]	33.3	17.52
S. grevillei	SA52	1	30	78.90
		5[c]	28	15.80
	SA56	1	33.3	87.62
		5[c]	33.3	17.52
S. laricinus	SA525	1[c]	20	52.60
		5	10	5.26
B. cavipes	SA286	1	20	52.60
		5[c]	10	5.26
	SA473	1	20	52.60
		5[c]	15	5.26
	SA474	1[c]	25	65.70
		5	15	5.26
B. paluster	SA348	1	30	78.90
		5[c]	28	14.72

[a] Ammonium tartrate concentration in the medium
[b] Glucose concentration at the peak of mycelial growth
[c] Ammonium tartrate concentration that showed the highest mycelial growth in each strain

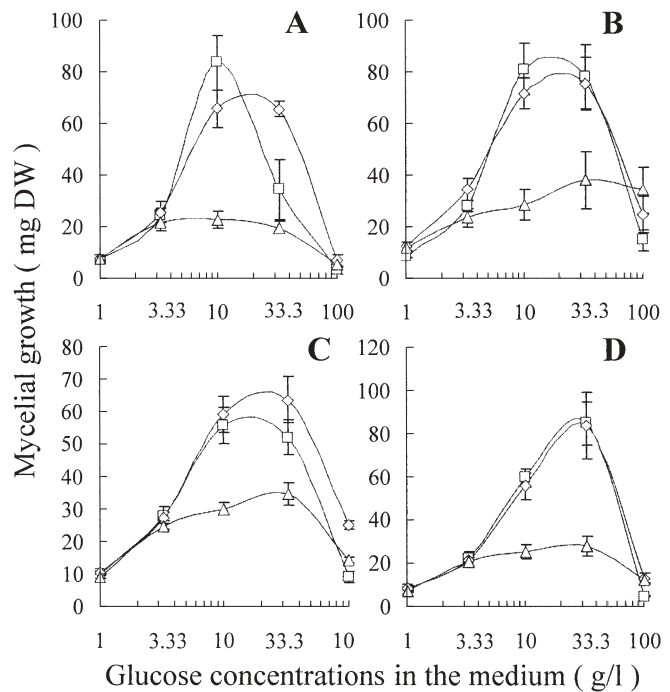Glucose concentrations in the medium (g/l)

Fig. 4. Change in mycelial growth of strains of the genus *Boletinus* according to the glucose concentration in the medium. Symbols as in Fig. 3. **A** *B. cavipes* (SA286); **B** *B. cavipes* (SA473); **C** *B. cavipes* (SA474); **D** *B. paluster* (SA348)

Fig. 5. Change in relative mycelial growth of *Suillus* and *Boletinus* strains according to ammonium tartrate concentration in the medium that showed mycelial growth greater than 10 mg at the glucose concentration 100 g/l. *Vertical axis*, percentage of relative mycelial growth; *horizontal axis*, ammonium tartrate concentration in the medium. The symbols (○, 10 g/l; ▲, 33.3 g/l; ×, 100 g/l) indicate the concentration of glucose in the medium. **A** *S. luteus* (SA50); **B** *S. grevillei* (SA52); **C** *B. cavipes* (SA473); **D** *B. cavipes* (SA474); **E** *B. paluster* (SA348)

Fig. 6. Change in relative mycelial growth of *S. laricinus* and *B. cavipes* according to ammonium tartrate concentration in the medium. *Vertical axis*, percentage of relative mycelial growth; *horizontal axis*, ammonium tartrate concentration in the medium. The symbols (○, 10 g/l; ▲, 33.3 g/l) indicate the concentration of glucose in the medium. **A** *S. laricinus* (SA286); **B** *B. cavipes* (SA525)
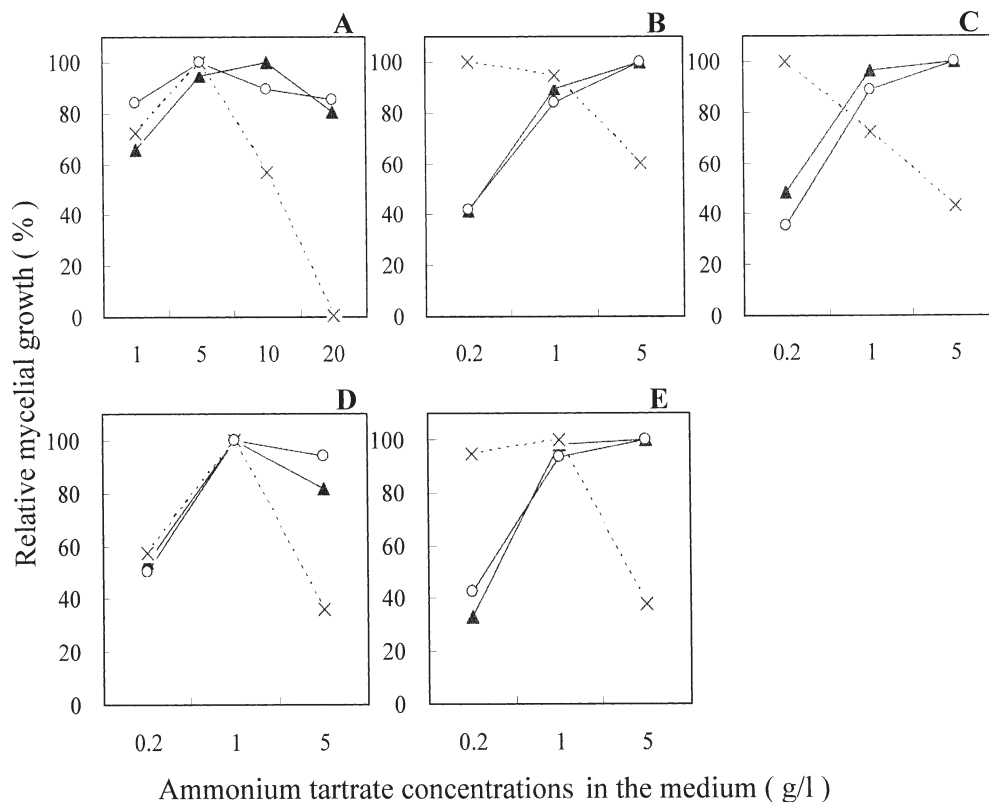
concentrations of ammonium tartrate greater than 0.2 g/l. At an ammonium tartrate concentration of 0.2 g/l, all strains showed low levels of mycelial growth. Generally, a large difference in mycelial growth in media with different concentrations of ammonium tartrate was observed with glucose concentrations of 10 and 33.3 g/l (see Figs. 3,4). At the glucose concentration 100 g/l, a large difference was observed for *S. luteus* SA50, and a relatively large difference was observed for *B. cavipes* SA473 and SA474.

Glucose utilization was considerably changed with the concentration of ammonium tartrate in the medium. For example, *B. cavipes* SA286 showed the best mycelial growth at a glucose concentration of 10 g/l and an ammonium tartrate concentration of 5 g/l, with a peak of mycelial growth at 20 g/l glucose in the presence of 1 g/l ammonium tartrate. As seen in Table 2, generally both the glucose concentration at the peak point and the value of C/N ratio at the point became lower as the ammonium tartrate concentration in the medium became higher. Figure 5 shows a comparison of patterns of mycelial growth dependence on ammonium tartrate concentrations for glucose concentrations 10, 33.3, and 100 g/l in the medium for strains that showed mycelial growth greater than 10 mg at the glucose concentration 100 g/l. At the glucose concentration 100 g/l, mycelial growth decreased rapidly at higher concentrations of ammonium tartrate. Figure 6 shows a comparison of patterns of mycelial growth dependence on ammonium tartrate concentrations between glucose concentrations 10 and 33.3 g/l in the medium for strains *S. laricinus* SA525 and *B. cavipes* SA286. In these strains, at 33.3 g/l glucose, mycelial growth decreased rapidly at 5 g/l ammonium tartrate.
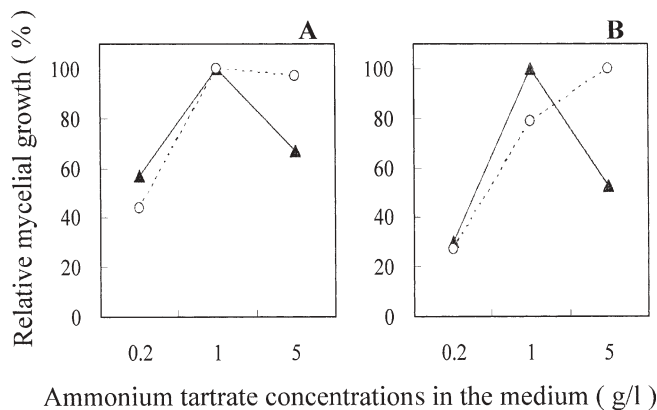
Discussion

In a preliminary set of experiments, we examined the effects of 21 carbohydrate sources and 17 nitrogen sources on the growth of *S. grevillei* SA52 (data not shown). In this study, we examined 8 carbohydrate sources and 5 nitrogen sources, all of which allowed good growth in the preliminary experiments, except for sucrose and glycerol.

The utilization of mannose and cellobiose differed considerably among different strains. None of the strains in this

study was unable to utilize both mannose and cellobiose. Ferry and Das (1968), Lamb (1974), and Murata (1993) also reported a considerable difference in utilization of these carbohydrates among various strains of *Boletus luteus* and *Suillus* strains. In this study, only *S. grevillei* SA52 used dextrin and soluble starch to any extent in the absence of glucose. Lamb (1974) also reported that one strain of *S. luteus* was able to use dextrin and starch moderately without the addition of glucose. The utilization of sucrose by four *Suillus* strains and a *Boletinus* strain that we tested was low. Murata (1993), Palmer and Hacskaylo (1970), Ferry and Das (1968), and Lamb (1974) obtained different results in the utilization of sucrose for their strains of *Suillus* and *Boletus*. These characters seemed dependent on the genetic background of strains. The utilization of glycerol was low for all our strains, and Ferry and Das (1968) reported similar results.

The growth of the four strains of *Suillus* tested in this study was good in medium with each of the five nitrogen sources. Murata (1993) reported some differences in the spectrum of usage of glutamic acid, arginine, and aspartic acid among three strains of *S. grevillei*. Our strain seemed to show better usage of arginine. Growth of *B. cavipes* SA286 was good with ammonium tartrate, but poor with each of the four amino acids. These facts may be related to the report of Hutchison (1990a), which indicated that some strains of *S. grevillei* and *S. luteus* are able to degrade urea, but *B. cavipes* cannot degrade the substance.

All strains used in this study grew well at a glucose concentration of 33.3 g/l. *S. luteus* SA50 showed best growth at 100 g/l. The result shows that these strains can grow well at relatively high glucose concentrations. This fact may indicate that these fungi frequently experience sugar concentration conditions such as 33.3 g/l in the root system of plants. If we assume that the concentration of nutrients in the apoplast is directly derived from the phloem sap, and that sucrose in the sap is efficiently transformed to glucose and fructose by the plant invertase in the apoplast (Salzer and Hager 1991; Hampp et al. 1995), the concentration of saccharides available to mycorrhizal fungi may be within the same order of magnitude as the concentration of saccharides in the phloem sap solution. Concerning the willow, Fisher (1983) reported that the seasonal variation of sieve-tube sap osmolality in field specimens was 400–800 mosmole and that sucrose accounted for virtually all of the sugars in exudates collected during the winter. If we assume that one third of the solute is sucrose, the sucrose concentration would be approximately 130–270 mM, that is, 44.5–92.4 g/l. This value could explain the relatively high glucose concentration optima for *Suillus* and *Boletinus* that were obtained in this study. The data reported by Dixon et al. (1981) for black oak and by Gall et al. (2002) for Norway spruce are also consistent with our results.

As described in Results, the difference in mycelial growth in media containing different levels of glucose was relatively small at an ammonium tartrate concentration of 0.2 g/l, which indicates that nitrogen concentration was the limiting factor for the mycelial growth at this concentration. Similarly, glucose concentration at or below 3.33 g/l seems to be the limiting factor for mycelial growth under these conditions.

The C/N ratio of a medium is considered to be an important factor for the growth of fungi (Chang and Miles 1989) and for regulation of fungal metabolites (Park et al. 1991; Gharieb 2000). Murata (1993) reported C/N ratios for *S. grevillei* as 40 for one strain and below 80 for two other strains. Liangqing and Zhida (1998) reported a value of 20 for a strain of *S. luteus*. Table 2 in this study shows for the *Suillus* and *Boletinus* species studied in the present work that the C/N ratio at the peak of the growth curve is dependent on the ammonium tartrate concentration in the medium and decreases as the concentration increases.

As shown in Fig. 3 for *S. luteus* SA50, mycelial growth in the media with a glucose concentration of 100 g/l and an ammonium tartrate concentration of 20 g/l was very poor and very much worse than with a glucose concentration of 100 g/l and an ammonium tartrate concentration of 10 g/l, or a glucose concentration of 33.3 g/l and an ammonium tartrate concentration of 20 g/l. These facts indicate that at the higher glucose concentration the mycelial growth decreases more rapidly at a higher concentration of ammonium tartrate. Figures 5 and 6 show that this tendency is observed in most strains used in this study. The fact that the tendency is seen at the glucose concentration 33.3 g/l (Fig. 6) indicates that it is not related merely to the osmotic effect of the glucose.

References

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Pure culture isolation of fungi and the production of inoculum. In: Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research, Canberra, pp 217–253

Chang S-T, Miles PG (1989) Edible mushrooms and their cultivation. CRC Press, Boca Raton

Dickinson TA, Hutchison LJ (1997) Numerical taxonomic methods, cultural characters, and the systematics of ectomycorrhizal agarics, boletes and gasteromycetes. Mycol Res 101:477–492

Dixon RK, Garrett HE, Bixby JA, Cox GS, Tompson JG (1981) Growth, ectomycorrhizal development and root soluble carbohydrates of black oak seedlings fertilized by two methods. For Sci 27:617–624

Ferry BW, Das N (1968) Carbon nutrition of some mycorrhizal *Boletus* species. Trans Br Mycol Soc 51:795–798

Finlay RD, Frostegard A, Sonnerfeldt A-M (1992) Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. ex Loud. New Phytol 120:105–115

Fisher DB (1983) Year-round collection of willow sieve-tube exudates. Planta (Berl) 159:529–533

Fries N (1983) Basidiospore germination in species of Boletaceae. Mycotaxon 18:345–354

Gall R, Landolt W, Schleppi P, Michellod V, Bucher JB (2002) Water content and bark thickness of Norway spruce (*Picea abies*) stems: phloem water capacitance and xylem sap flow. Tree Physiol 22:613–622

Gharieb MM (2000) Nutritional effects on oxalic acid production and solubilization of gypsum by *Aspergillus niger*. Mycol Res 104:550–556

Giltrap NJ, Lewis DH (1981) Inhibition of growth of ectomycorrhizal fungi in culture by phosphate. New Phytol 87:669–675

Hacskaylo E (1973) Carbohydrate physiology of ectomycorrhizae. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae. Academic Press, New York, pp 207–230

Hampp R, Schaeffer C, Wallenda T, Stulten C, Johann P, Einig W (1995) Changes in carbon partitioning or allocation due to ectomycorrhiza formation: biochemical evidence. Can J Bot 73:548–556

Harley JL, Smith SE (1983) Growth physiology of ectomycorrhizal fungi. In: Mycorrhizal symbiosis, 1st edn. Academic Press, London, pp 145–169

Hayashi H, Chino M (1986) Collection of pure sap from wheat and its chemical composition. Plant Cell Physiol 27:1387–1393

Hodge A, Alexander IA, Gooday GW (1995) Chitinolytic enzymes of pathogenic and ectomycorrhizal fungi. Mycol Res 99:935–941

Hung L-L, Trappe JM (1983) Growth variation between and within species of ectomycorrhizal fungi in response to pH in vitro. Mycologia 75:234–241

Hutchison LJ (1990a) Studies on the systematics of ectomycorrhizal fungi in axenic culture. II. The enzymatic degradation of selected carbon and nitrogen compounds. Can J Bot 68:1522–1530

Hutchison LJ (1990b) Studies on the systematics of ectomycorrhizal fungi in axenic culture. IV. The effect of some selected fungitoxic compounds upon linear growth. Can J Bot 68:2172–2178

Jayko LG, Baker TI, Stubblefield RD, Anderson RF (1962) Nutrition and metabolic products of *Lactarius* species. Can J Microbiol 8:361–371

Keller G (1996) Utilization of inorganic and organic nitrogen sources by high-subalpine ectomycorrhizal fungi of *Pinus cembra* in pure culture. Mycol Res 100:989–998

Lamb RJ (1974) Effect of glucose on utilization of single carbon sources by ectomycorrhizal fungi. Trans Br Mycol Soc 63:295–306

Liangqing C, Zhida P (1998) A study on the liquid cultural conditions for a mycorrhiza fungi (*S. l.*) with *Pinus massoniana*. For Res 11:443–446

Lindeberg G, Lindeberg M (1977) Pectinolytic ability of some mycorrhizal and saprophytic Hymenomycetes. Arch Microbiol 115:9–12

Lundeberg G (1970) Utilisation of various nitrogen sources, in particular bound soil nitrogen, by mycorrhizal fungi. Stud For Suec 79:1–95

Melin E (1954) Growth factor requirements of mycorrhizal fungi of forest trees. Sven Bot Tidskr 48:86–94

Melin E (1959) Studies on the physiology of tree mycorrhizal Basidiomycetes. I. Growth response to nucleic acid constituents. Sven Bot Tidskr 53:135–154

Melin E, Norkrans B (1948) Amino acids and the growth of *Lactarius deliciosus* (L.) Fr. Physiol Plant 1:176–184

Mikola P (1948) On the physiology and ecology of *Cenococcum graniforme*. Especially as a mycorrhizal fungi of birch. Commun Inst For Fenn 36:1–104

Mischiati P, Fontana A (1993) In vitro culture of *Tuber magnatum* mycelium isolated from mycorrhizas. Mycol Res 97:40–44

Molina R, Palmer JC (1982) Isolation, maintenance, and pure culture manipulation of ectomycorrhizal fungi. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, MN, pp 115–129

Murata Y (1991) In vitro mycorrhizal synthesis on Japanese larch (*Larix leptolepis* Gordon) seedlings and their growth. Bull Hokkaido For Res Inst 29:1–13

Murata Y (1993) Cultural characteristics of *Suillus grevillei* and *S. laricinus*. Bull Hokkaido For Res Inst 30:74–87

Norkrans B (1950) Studies in growth and cellulolytic enzymes of *Tricholoma*. Symb Bot Ups 11:1–126

Norkrans B (1953) The effect of glutamic acid, aspartic acid, and related compounds on the growth of certain *Tricholoma* species. Physiol Plant 6:584–593

Ohta A (1990) A new medium for mycelial growth of mycorrhizal fungi. Trans Mycol Soc Jpn 31:323–334

Palmer JG, Hacskaylo E (1970) Ectomycorrhizal fungi in pure culture. I. Growth on single carbon sources. Physiol Plant 23:1187–1197

Park Y-H, Stack JP, Kenerley CM (1991) Production of gliotoxin by *Gliocladium virens* as a function of source and concentration of carbon and nitrogen. Mycol Res 95:1242–1248

Salzer P, Hager A (1991) Sucrose utilization of the ectomycorrhizal fungi *Amanita muscaria* and *Hebeloma crustuliniforme* depends on the cell wall-bound invertase activity of their host *Picea abies*. Bot Acta 104:439–445

Vaario L-M, Guerin-Laguette A, Matsushita N, Suzuki K, Lapeyrie F (2002) Saprobic potential of *Tricholoma matsutake*: growth over pine bark treated with surfactants. Mycorrhiza 12:1–5