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Ability of ectomycorrhizal fungi to utilize starch and related substrates

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Basidiomycetous fungi of 55 strains of 33 species in 15 genera which are thought to be ectomycorrhizal were grown on starch and related substrates as a sole carbon source, and their ability to utilize these substrates was determined. Mycelial weights of the fungi grown on agar media containing starch and amylose varied between the strains from 1.1 to 94.9 mg/flask and from 0.4 to 93.3 mg/flask, respectively. Mycelial growth rates ranged from 0 to 1.17 mm/d on barley grain medium and from 0 to 2.03 mm/d on rice grain medium; the highest rate corresponded to about half of the average of reference wood-rotting fungi. Most of the mycorrhizal fungi that grew well on amylose gave higher growth rates on barley. Several strains in *Lyophyllum*, *Hebeloma*, *Sarcodon*, and *Tricholoma* grew well on both glucose and starch media.

Key Words——amylose; barley; ectomycorrhizal fungi; mycelial growth; starch.

Microorganisms require carbohydrates in greater quantity than other essential nutrients. Most saprophytic fungi can decompose polysaccharides such as cellulose and starch and utilize them as a carbon source. On the other hand, many ectomycorrhizal fungi have been thought to have no or only slight ability to decompose polymer substrates. Therefore, glucose and a few other monosaccharides have been used as carbon sources for their cultivation in pure culture. These aspects were briefly reviewed by Hacskaylo (1973).

When fungi form fruit-bodies, large amounts of mycelia may be needed either to store nutrients or to transport nutrients to the fruit-bodies, or both. However, it is very difficult in practice to cultivate large amounts of mycelia using monosaccharides in pure culture. Addition of low molecular substrates at high concentration increases the osmotic pressure in the medium, which suppresses the growth of the mycelium; and the continuous addition of a low concentration of monosaccharide entails a high risk of contamination.

A few ectomycorrhizal species in *Tricholoma* can grow on starch and inulin when a small amount of glucose is added as a starter (Norkrans, 1950). I have reported that an ectomycorrhizal fungus, *Lyophyllum shimeji* (Kawamura) Hongo, forms mature fruit-bodies on a medium mainly consisting of barley grains (Ohta, 1994b). These facts indicate that some mycorrhizal fungi have the ability to utilize polysaccharides. I also showed that an ectomycorrhizal fungi can form fruit-bodies without a host plant when sufficient mycelia were grown. The present paper reports the ability of ectomycorrhizal fungi to utilize starch and related substrates, as assessed by two methods in pure culture.

Materials and Methods

Fungal strains Basidiomycetous fungi that are known or thought ectomycorrhizal (Trappe, 1962; Ogawa, 1988; Agerer, 1995) were used. Three saprophytic species were also used as a reference, being chosen from among wood-rotting fungi that are commonly cultivated commercially in Japan (Table 1). Fungal strains were maintained at 5°C in test tubes containing stock culture medium until use. Composition of the stock culture medium was: glucose, 4 g; yeast extract, 0.4 g; agar, 15 g; and distilled water, 1,000 ml (adjusted to pH 5.1 with 0.1 M HCl).

Determination of growth in weight on starch and amylose Starch utilization was estimated from the mycelial weight of the fungi grown on an agar medium containing starch. Starch (from wheat, 0.2 g), glucose as a starter (5 mg), agar (0.3 g), and water (10 ml) were added to a 100 ml flask, and the synthetic nutrient solution described below (10 ml) was added to another flask. To avoid decomposition of the elements, the solutions were autoclaved separately for 10 min at 120°C, then combined after cooling to 48–50°C. The resulting medium is here referred to as starch medium. Amylose (from corn) and glucose were also used instead of starch (amylose medium and glucose medium), and a similar medium containing only starter glucose as a carbon source was prepared as a reference (control medium). Three flasks of each medium were used for each strain.

Three agar blocks about 5 × 5 × 5 mm cut from the stock culture of the fungus were inoculated into each flask and incubated at 22°C for 21 d for mycorrhizal fungi and 14 d for wood-rotting fungi. Grown mycelia were removed from the flask together with agar, washed

Table 1. Derivation of the fungi examined.

Strain no.[a]	Strain	Species	Japanese name	Locality collected	Date isolated	Accession no. etc.
	(Mycorrhizal fungi)					
1	NR2A	*Amanita hemibapha* (Berk. et Bref.) Sacc. subsp. *hemibapha*	Tamagotake	Kamikitayama, Nara	July 14, 1993	
2	NR3A			Azumi, Nagano	Aug. 21, 1993	
3	NR1A	*A. hemibapha* subsp. *similis* (Boedijn) Corner et Bas	Chatamagotake	Takatori, Nara	Aug. 20, 1993	
4	Ap2	*A. pantherina* (DC.: Fr.) Secretan	Tengutake	Kouka, Shiga	Oct. 10, 1992	IFO32788
5	WK1A			Kukizaki, Ibaraki	Sep. 29, 1986	W25
6	KY-A1	*A. pseudoporphyria* Hongo	Kotengutakemodoki	Tanba, Kyoto	Nov. 4, 1994	IFO32772
7	Bl1	*Boletopsis leucomelas* (Pers.) Fayod	Kurokawa	Kounan, Shiga	Oct. 25, 1988	IFO32789
8	NR4B	*Boletus fraternus* Peck	Koujitake	Takatori, Nara	Sep. —, 1994	
9	NBR-1	*B. reticulatus* Schaeffer	Yamadoritakemodoki	Kashihara, Nara	June 26, 1994	IFO32773
10	NBR-3			Kashihara, Nara	Oct. 3, 1994	
11	Cp1	*Cortinarius purpurascens* (Fr.) Fr.	Kawamurahusentake	Yasu, Shiga	Sep. 21, 1993	
12	HY1Hn	*Hebeloma radicosum* (Bull.: Fr.) Ricken	Nagaenosugitake	Haga, Hyogo	Oct. 7, 1989	
13	Hr2	*Hygrophorus russula* (Schaeffer: Fr.) Quélet	Sakurashimeji	Minakuchi, Shiga	Oct. 13, 1986	ATCC76005
14	Hr4			Shichigashuku, Miyagi	— —, 1991	Hr-3
15	Lb1	*Laccaria bicolor* (Maire) P.D. Orton	Kitsunetake	Yasu, Shiga	Oct. 20, 1992	
16	KY-Lc1	*Lactarius chrysorrheus* Fr.	Kichichitake	Wachi, Kyoto	Oct. 25, 1994	IFO32775
17	Lc1			Minakuchi, Shiga	Oct. 20, 1992	
18	KY-H1	*L. hatsudake* Tanaka	Hatsutake	Tanba, Kyoto	Nov. 4, 1994	IFO32778
19	Lh2			Yasu, Shiga	Nov. 9, 1986	
20	Lf1	*Lyophyllum fumosum* (Pers.: Fr.) P.D. Orton	Shakashimeji	Minakuchi, Shiga	Oct. 13, 1986	ATCC66997
21	LF-Ky8			Kyoto	Oct. 13, 1987	
22	Ls3	*L. shimeji* (Kawamura) Hongo	Honshimeji	Shigaraki, Shiga	Oct. 22, 1987	ATCC76004
23	Ls6			Yamada, Hyogo	Nov. 18, 1987	
24	Pt1	*Pisolithus tinctorius* (Pers.) Coker et Couch	Kotsubutake	Yasu, Shiga	Oct. 5, 1994	IFO32811
25	SM1R	*Rhizopogon rubescens* (Tul.) Tul.	Shouro	Shinji, Shimane	Sep. 20, 1991	
26	Rr4			Amino, Kyoto	Apr. 8, 1986	IFO32812
27	MG-RS1	*Russula subnigricans* Hongo	Nisekurohatsu	Yamato, Miyagi	July 3, 1992	IFO32784
28	Sa1	*Sarcodon aspratus* (Berk.) S. Ito	Koutake	Shigaraki, Shiga	Oct. 30, 1987	ATCC76006
29	Sa3			Minakuchi, Shiga	Nov. 2, 1991	IFO32815
30	SB-ME1	*Suillus bovinus* (L.: Fr.) O. Kuntze	Amitake	Hakusan, Mie	Oct. 4, 1993	
31	Sb4			Mizuho, Kyoto	Oct. —, 1986	
32	OK-S2	*S. grevillei* (Klotzsch) Singer	Hanaiguchi	Okutsu, Okayama	Oct. 26, 1994	IFO32786
33	Sg1			Nagano	—	
34	Sl1	*S. luteus* (L.: Fr.) S.F. Gray	Numeriiguchi	Mizuho, Kyoto	Oct. 24, 1986	
35	Sl6			Yasu, Shiga	Oct. 6, 1993	
36	IS-K1	*Tricholoma auratum* (Fr.) Gillet	Shimokoshi	Kaga, Ishikawa	Dec. 1, 1986	IFO32788
37	Tk1			Mikuni, Fukui	Nov. 1, 1986	IFO32821
38	Tb1	*T. bakamatsutake* Hongo	Bakamatsutake	Kyoto	Oct. 6, 1953	
39	WK2B			Nakaheji, Wakayama	Sep. 24, 1991	W152
40	Tc1	*T. caligatum* (Viviani) Ricken		Algeria	—, —, 1964	Bg4-15
41	Tc2			Morocco	—, —, 1991	

42	WK1N	*T. fulvocastaneum* Hongo	Nisematsutake	Nakaheji, Wakayama	Oct. 15, 1988	W112
43	0Tj2	*T. japonicum* Kawamura	Shiroshimeji	Kounan, Shiga	Nov. 10, 1986	ATCC76001
44	Tj3			Shigaraki, Shiga	Oct. 22, 1987	IFO32820
45	KY-K1	*T. flavovirens* (Pers.: Fr.) Lund.	Kishimeji	Wachi, Kyoto	Oct. 27, 1994	IFO32805
46	Tk3			Kounan, Shiga	Nov. 10, 1986	ATCC76003
47	Mg1T	*T. matsutake* (S. Ito et Imai) Singer	Matsutake	Karakuwa, Miyagi	Oct. 26, 1986	11-1
48	Tm67			Kounan, Shiga	Nov. 2, 1992	
49	Tp1	*T. ponderosum* (Sacc.) Singer		British Columbia, Canada	Oct. 16, 1973	Bg5-1
50	Tp3			Canada	Sep. 26, 1993	
51	Tr3	*T. robustum* (Alb. et Schwein.: Fr.) Ricken	Matsutakemodoki	Kounan, Shiga	Nov. 11, 1986	ATCC76000
52	Tr4			Kounan, Shiga	Oct. 31, 1990	
53	Ts2	*T. saponaceum* (Fr.) Kummer	Mineshimeji	Kutsuki, Shiga	Nov. 9, 1994	
54	KY-Tu1	*T. ustale* (Fr.: Fr.) Kummer	Kakishimeji	Wachi, Kyoto	Oct. 25, 1994	IFO32808
55	Tu1			Hino, Shiga	Nov. 10, 1994	IFO32825
	(Wood-rotting fungi)					
56	Fv1	*Flammulina velutipes* (Curtis: Fr.) Singer	Enokitake	Commercial strain	—	Nichinou A
57	FLV			Stock culture, Kyoto Univ.	—	FLV0904
58	Le1	*Lentinus edodes* (Berk.) Singer	Shiitake	Commercial strain	—	Mori 465
59	Le5			Commercial strain	—	Nichinou 270
60	Po1	*Pleurotus ostreatus* (Jacq.: Fr.) Kummer	Hiratake	Commercial strain	—	Mori 39
61	Po3			Yasu, Shiga	Sep. 24, 1987	

a) Tentatively used in this paper.

with boiling water three times, and dried for 40 h at 65°C in a forced air oven, then weighed.

Composition of the nutrient solution (1,000 ml) was: citric acid, 1 g; ammonium tartrate, 1 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 1 g; $CaCl_2$, 50 mg; HEPES, 7 g; mineral solution, 10 ml; and vitamin solution, 10 ml (adjusted to pH 5.1 with 1 M KOH). Composition of the mineral solution (1,000 ml) was: $FeCl_3$, 5 g; $ZnSO_4 \cdot 7H_2O$, 300 mg; $NiSO_4 \cdot 6H_2O$, 200 mg; $CuSO_4 \cdot 5H_2O$, 100 mg; $MnSO_4 \cdot 4$-$6H_2O$, 50 mg; $CoSO_4 \cdot 7H_2O$, 50 mg; and acetylacetone, 1 ml. That of the vitamin solution (1,000 ml) was: thiamine·HCl, 300 mg; nicotinic acid, 5 mg; folic acid, 3 mg; biotin, 5 mg; pyridoxine·HCl, 0.5 mg; calnitine chloride, 1 mg; adenine·$H_2SO_4 \cdot 2H_2O$, 3 mg; choline chloride, 3 mg.

Determination of growth in length on barley and rice

Crop grains consist chiefly of starch and have a large surface area, which facilitates the growth of mycelia. Mycelial growth of the fungi was determined using barley and rice grains, which were chosen because they would be easily available in large quantity and constant quality for commercial cultivation of mycorrhizal fungi. Barley medium was prepared as follows. Polished barley grains (7 g) and the synthetic nutrient solution diluted to 1/10 concentration (8 ml) were added to an 18 mm diam × 180 mm test tube, left to stand for 4 h, mixed, then autoclaved for 20 min at 120°C. Polished rice grains (7 g) were also used instead of barley grain (rice medium). A mycelial block cut from the stock culture was put on the medium, and the culture was incubated at 22°C. Linear growth of the mycelia was measured every third day. The growth rate was estimated from the linear part of the plot of the length against incubation time, as previously described (Ohta, 1994a). Six test tubes of each medium were used for each strain.

Results

Mycelial growth of 33 species of ectomycorrhizal fungi and 3 species of wood-rotting fungi grown on the agar media containing starch, amylose, and glucose are shown in Table 2. Mycelial weights of the mycorrhizal strains ranged from 1.1 to 94.9 mg/flask on the starch medium and from 0.4 to 93.3 mg/flask on the amylose medium at 21 d incubation.

Growth of the strains on starch and amylose relative to glucose were estimated as (Ws-Wo)/(Wg-Wo) and (Wa-Wo)/(Wg-Wo), respectively, where Ws, Wg, Wa, and Wo are mycelial weights on starch, glucose, amylose, and control media. The ratios for the mycorrhizal strains ranged from 0 to 2.2 on starch and from 0 to 3.1 on amylose, except for one strain of *Boletus reticulatus* that grew only slightly on the glucose medium. The ratios for the wood-rotting strains were higher than 0.78 on both substrates.

Rates of mycelial growth in length on the barley and rice media are also shown in Table 2. Mycorrhizal strains gave growth rates ranging from 0 to 1.17 mm/d

Table 2. Mycelial growth in weight and length.

Strain no.[a]	Species	Strain	Mycelial weight (mg/flask)[b]				Growth rate (mm/d)[c]	
			S	A	G	C	B	R
1	*Amanita hemibapha* subsp. *hemibapha*	NR2A	5.6	6.8	27.6	4.0	0	0
2		NR3A	4.2	3.8	8.6	2.3	0.02	0
3	*A. hemibapha* subsp. *similis*	NR1A	1.1	2.3	2.0	1.1	0	0
4	*A. pantherina*	Ap2	28.6	21.0	45.4	7.8	0.77	0.75
5		WK1A	14.1	10.1	38.6	3.2	0.06	0.18
6	*A. pseudoporphyria*	KY-A1	5.6	6.0	15.5	3.2	0	0
7	*Boletopsis leucomelas*	Bl1	4.2	4.3	2.4	0.8	0.31	0.14
8	*Boletus fraternus*	NR4B	6.2	2.3	15.2	2.3	0	0.14
9	*B. reticulatus*	NBR-1	3.4	8.0	3.8	4.7	0.12	0.04
10		NBR-3	5.0	5.3	24.8	2.5	0.05	0.08
11	*Cortinarius purpurascens*	Cp1	39.2	26.2	58.2	11.3	0.29	0.30
12	*Hebeloma radicosum*	HY1Hn	38.6	45.3	83.5	9.7	0.60	0.33
13	*Hygrophorus russula*	Hr2	9.0	12.7	21.5	4.7	0.50	0.09
14		Hr4	18.0	14.2	22.0	0.8	0.35	0.09
15	*Laccaria bicolor*	Lb1	7.3	11.8	47.6	5.4	1.14	2.03
16	*Lactarius chrysorrheus*	KY-Lc1	9.5	10.8	18.4	4.0	0	0
17		Lc1	17.9	15.5	26.4	6.4	0.20	0
18	*L. hatsudake*	KY-H1	23.8	27.1	30.0	9.5	0.30	0.41
19		Lh2	18.5	27.3	15.1	6.5	0.23	0.37
20	*Lyophyllum fumosum*	Lf1	40.9	36.0	46.0	4.0	0.54	0.64
21		LF-Ky8	43.4	25.3	48.1	1.7	0.70	1.09
22	*L. shimeji*	Ls3	69.0	93.3	57.3	6.8	1.17	1.27
23		Ls6	94.9	65.9	73.5	9.5	1.00	0.26
24	*Pisolithus tinctorius*	Pt1	6.1	13.6	64.4	5.2	0.24	0.35
25	*Rhizopogon rubescens*	SM1R	22.0	21.3	77.5	10.4	0.49	0.75
26		Rr4	15.4	10.5	79.8	8.4	0.53	0.67
27	*Russula subnigricans*	MG-RS1	21.4	47.1	18.1	4.0	0.85	1.03
28	*Sarcodon aspratus*	Sa1	62.9	43.6	69.5	13.1	0.19	0.26
29		Sa3	59.9	34.5	66.0	9.9	0.16	0.23
30	*Suillus bovinus*	SB-ME1	18.7	41.9	76.6	8.6	0.40	0.31
31		Sb4	7.3	7.5	56.4	5.7	0.13	0.17
32	*S. grevillei*	OK-S2	15.1	23.7	40.1	6.8	0.35	0.41
33		Sg1	15.9	15.4	26.3	1.6	0.28	0.26
34	*S. luteus*	Sl1	8.5	8.2	71.3	5.6	0.19	0.40
35		Sl6	12.4	23.5	85.5	9.0	0.25	0.42
36	*Tricholoma auratum*	IS-K1	18.1	13.8	55.0	5.8	0	0
37		Tk1	4.7	3.9	12.9	1.8	0	0
38	*T. bakamatsutake*	Tb1	15.5	16.1	34.2	8.5	0.15	0
39		WK2B	8.1	6.3	14.9	5.8	0.03	0.02
40	*T. caligatum*	Tc1	40.3	31.8	76.3	9.3	0.06	0.16
41		Tc2	5.0	6.5	20.5	4.4	0	0.05
42	*T. fulvocastaneum*	WK1N	28.7	13.2	44.5	7.3	0.24	0.06
43	*T. japonicum*	Tj2	29.1	27.7	38.8	7.1	0.39	0.05
44		Tj3	23.0	16.4	38.1	4.6	0.34	0.04
45	*T. flavovirens*	KY-K1	12.9	12.8	21.0	7.6	0	0
46		Tk3	15.3	13.0	23.4	3.1	0	0.14
47	*T. matsutake*	Mg1T	47.9	39.0	97.5	10.9	0.45	0.46
48		Tm67	27.8	16.4	49.6	8.9	0.14	0.16
49	*T. ponderosum*	Tp1	17.5	17.3	46.9	5.5	0.30	0.04
50		Tp3	12.5	11.2	39.3	5.9	0.19	0
51	*T. robustum*	Tr3	7.0	4.8	9.5	2.7	0	0
52		Tr4	2.6	0.4	11.1	0	0	0
53	*T. saponaceum*	Ts2	13.5	37.5	27.1	5.1	0	0
54	*T. ustale*	KY-Tu1	14.7	17.5	40.2	6.5	0.22	0
55		Tu1	61.4	35.1	51.9	10.0	0.26	0
	(Average of mycorrhizal strains)		21.4	20.2	40.3	5.8	0.28	0.27
56	*Flammulina velutipes*	Fv1	64.1	58.6	59.3	6.7	4.45	4.80
57		FLV	39.2	44.3	34.0	6.8	3.04	4.14
58	*Lentinus edodes*	Le1	14.9	25.5	9.6	5.7	3.81	3.72
59		Le5	9.0	14.7	10.7	3.1	4.91	1.61
60	*Pleurotus ostreatus*	Po1	66.2	61.6	53.1	23.1	5.44	6.31
61		Po3	70.4	84.2	30.4	7.2	5.65	5.15
	(Average of wood-rotting strains)		44.0	48.1	32.9	8.8	4.55	4.29

a) Numbers are identical to Table 1.
b) Average of 3 flasks each containing 3 colonies; A, amylose medium; S, starch medium; G, glucose medium; C, control medium. Incubation time was 21 d for mycorrhizal fungi and 14 d for wood-rotting fungi.
c) Average of 6 test tubes; B, barley medium; R, rice medium.

on the barley medium and from 0 to 2.03 mm/d on the rice medium. The highest value of 2.03 mm/d was 0.47 times the average of the wood-rotting fungi. Of the 45 mycorrhizal strains which grew on either the barley or the rice medium, 27 strains grew faster on rice than on barley.

Figure 1 shows the relationship between weight growth on the amylose medium and growth rate on the barley medium for the mycorrhizal strains. This gave the highest correlation coefficient in simple regression analysis ($r=0.65$) among all combinations between weight growth on starch or amylose medium and growth rate on barley or rice medium, followed by the relationship between weight growth on starch medium and growth rate on barley medium ($r=0.52$).

Discussion

Norkrans (1950) reported that ectomycorrhizal species in *Tricholoma* have a wide range of starch-utilization abilities. Kawai and Abe (1976) showed that *T. matsutake* and *T. ponderosum* (=*T. magnivelare*) grow slowly on starch. The present study revealed that many other mycorrhizal fungi show a wide range of starch-utilization abilities, as estimated by the mycelial weight grown on the starch medium.

A similar result was obtained with amylose, one of the major components of starch, which has no branches in its chemical structure. The mycorrhizal strains also showed a wide range of growth rates on barley and rice media, with the highest value being about a half of the rate of reference wood-rotting fungi. A high correlation coefficient was observed between weight growth on the amylose medium and growth rate on the barley medium (Fig. 1), suggesting that amylose in barley grains may preferentially be decomposed by the ectomycorrhizal fungi.

The relationship between weight growth on the glucose medium and on the starch medium shown in Fig. 2 suggests several features of the cultural characteristics of the ectomycorrhizal fungi. In the strains that grew slowly on both glucose and amylose, growth may have been suppressed either by the use of inadequate culture conditions in this study or by a combination of low starch utilization ability and inhibition by glucose, at least at the initial concentration. These strains need further examination to determine their starch-utilization ability after eliminating the cause of inhibition.

In the strains that grew rapidly on glucose and slowly on starch, it is likely that sufficient glucose to support fungal growth was not produced by degradation of starch during autoclaving. In this connection, Kawai and Abe (1976) have pointed out that sterilization method affects utilization of sucrose of *Tricholoma* species. The good growth of these strains on glucose shows that culture conditions other than carbon source were adequate for growth, thus indicating that these strains have no or slight enzyme activity to utilize starch or partly degraded starch. Strains in *Laccaria bicolor*, *Pisolithus tinctorius*, *Rhizopogon rubescens*, *Suillus bovinus*, and *S. luteus* were included in this group.

There was no typical strains that grew slowly on glucose and rapidly on starch, as assessed by the averages of growth on both substrates.

The group of strains that grew rapidly on both glucose and starch included several strains in *Cortinarius*

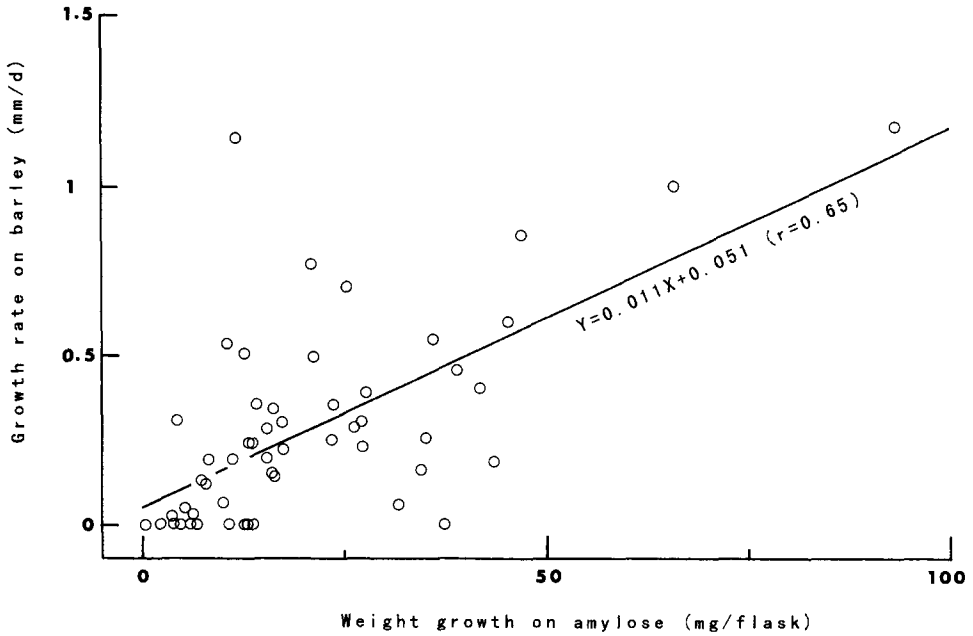

Fig. 1. Relationship between mycelial weight growth of ectomycorrhizal strains on amylose medium and mycelial growth rate on barley medium.
The regression is significant at 99% level.

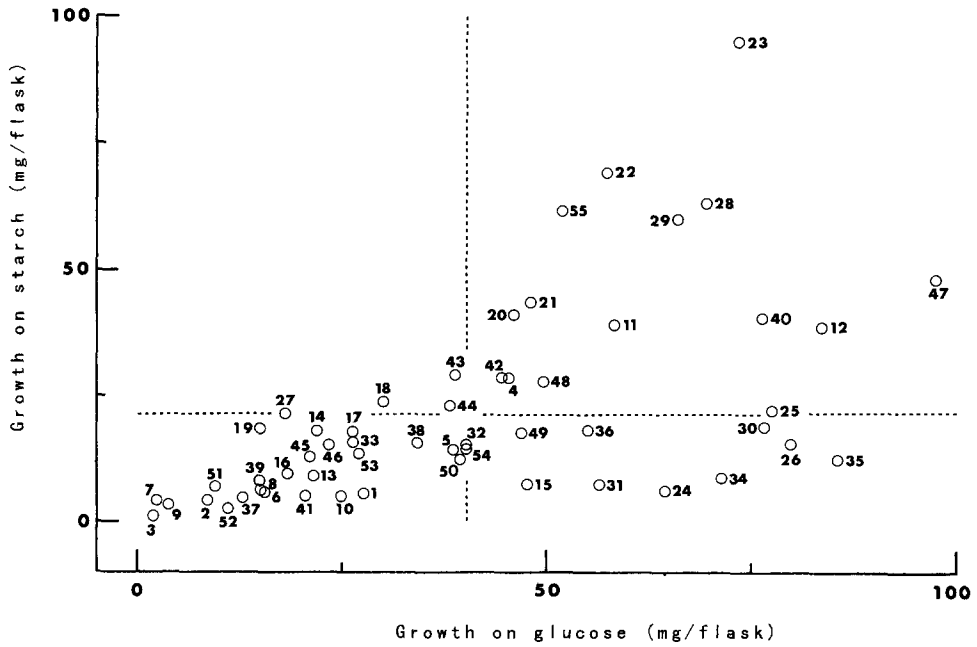

Fig. 2. Relationship between mycelial growth (weight) of ectomycorrhizal strains on glucose medium and on starch medium. Broken lines indicate averages of the data on each axis. Numbers beside the circles indicate strain numbers shown in Tables 1 and 2.

purpurascens, *Hebeloma radicosum*, *Lyophyllum fumosum*, *L. shimeji*, *Sarcodon aspratus*, and *Tricholoma matsutake*. These strains can be easily cultivated on the ordinary media containing glucose. Large amounts of mycelia could also easily be cultivated on starch in pure culture, avoiding the problem of osmotic pressure in the medium. This feature will contribute to making inocula for cultivation of the ectomycorrhizal fungi in forests and producing fruit-bodies in pure culture.

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