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Short Communication

Survey of α-glucose 1-phosphate forming trehalose phosphorylase and trehalase in various fungi including basidiomycetous mushrooms

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The distribution of α-glucose 1-phosphate forming (α-type) trehalose phosphorylase and trehalase activities in various fungi was surveyed. α-Type phosphorylase occurred in the mycelia and fruit-bodies of Agaricales and Aphyllophorales in the Holobasidiomycetidae, and at least one species of Gasteromycetes, but not in Tremellaceae or Auriculariales of the Phragmobasidiomycetidae, Heterobasidiomycetes or Hemibasidiomycetes. The test fungi in the Ascomycotina and Deuteromycotina, and the yeasts of Basidiomycotina, showed different trehalase activities, but no trehalose phosphorylase activity. The test organisms showed different levels of trehalase activity. The fruit-bodies of most mushrooms showed higher activities of α-type trehalose phosphorylase than did the mycelia.

Key Words——basidiomycetes; α-glucose 1-phosphate; mushroom; trehalase; trehalose phosphorylase.

The mycelia of basidiomycetous mushrooms contain trehalose as the main stored soluble carbohydrate (Kitamoto and Gruen, 1976; Hammond and Nicholas, 1976; Kitamoto et al., 1978; Hammond, 1979a, b; Yoshida et al., 1986). In *Flammulina velutipes* (Curt.: Fr.) Sing., a large net loss of trehalose in the mycelia occurred as fruit-bodies developed (Kitamoto and Gruen, 1976). In conjugation with trehalose loss in the mycelia, this carbohydrate accumulated in fruit-bodies up to the mid-stage of development, then decreased remarkably thereafter. These findings suggest that a large amount of trehalose is directly translocated from mycelia to fruit-bodies, where it is metabolized as the main carbohydrate substrate for their development. The results of ^{14}C glucose labeling experiments with this fungus support this interpretation (Kitamoto and Gruen, 1976).

In many microorganisms, trehalose is hydrolyzed to produce two glucose molecules by trehalase (Elbein, 1974). However, the phospholytic cleavage of trehalose into α-glucose 1-phosphate and glucose by a novel enzyme, α-glucose 1-phosphate-forming (α-type) trehalose phosphorylase, has been demonstrated in *F. velutipes* (Kitamoto et al., 1988). In the present study, we surveyed the distribution of α-type trehalose phosphorylase in various genera of fungi and yeasts belonging to the Basidiomycotina, Ascomycotina and Deuteromycotina. We also describe the occurrence of this phosphorylase in basidiomycetous mushrooms, where it is commonly found.

Various strains of fungi and yeasts belonging to the Basidiomycotina, Ascomycotina and Deuteromycotina were used for the present experiments. *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray and *C. phlyctidosporus* Romagn. were obtained from Dr. Sagara of Kyoto University. *Hebeloma vinosophyllum* Hongo was obtained from Dr. Suzuki of Chiba University. The other strains were stock organisms of the Laboratory of Microbial Biotechonology, Tottori University.

Mushroom mycelia were cultivated in 100-ml Erlenmeyer flasks containing 20 ml of liquid medium. The compositions of media, and the culture conditions for mycelium and fruit-body cultivation of various test fungi, are summarized in Table 1. The mushrooms were cultured on DPD (Kitamoto and Gruen, 1976), MP (Kitamoto et al., 1978), or PGL (Kawamura et al., 1983) medium.

The mycelia of the different fungal stocks were grown on PDA plates for 7–10 d at 23–25°C, then agar inoculum blocks, measuring 5 × 5 × 5 mm were cut from the plates. These blocks were placed into the culture media. The cultures were allowed to grow in darkness for 1–2 wk at 23–25°C. The mycelia were harvested, washed with distilled water, and stored in a deep-freezer at −90°C as the enzyme sources.

The cultivation of mushroom fruit-bodies was carried

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Table 1. Culture conditions for basidiomycetous mushrooms and the mycelia or yeast cells of various fungi.

Organism	Medium[a]	Culture condition		
		Cultivation of mycelia or yeasts	Fruit-body production	
			Spawn running	Fruiting
Coprinus cinereus	DPD	25°C-Dark-2 wk	25°C-Dark-2 wk →	25°C-Light
Coprinus phlyctidosporus	DPD	25°C-Dark-2 wk	25°C-Dark-2 wk →	25°C-Light
Favolus arcularius	MP	25°C-Dark-1 wk	25°C-Dark-1 wk →	25°C-Light
Fistulina hepatica	DPD	25°C-Dark-2 wk	25°C-Dark-3 wk →	25°C-Light
Flammulina velutipes	DPD	23°C-Dark-2 wk	23°C-Dark-2 wk →	15°C-Light
Hebeloma vinosophyllum	DPD	25°C-Dark-2 wk	23°C-Dark-3 wk →	23°C-Light
Lentinus edodes	PGL	25°C-Dark-2 wk	23°C-Dark-3 wk →	15°C-Light
Pholiota nameko	DPD	23°C-Dark-2 wk	23°C-Dark-3 wk →	15°C-Light
Pleurotus ostreatus	DPD	25°C-Dark-2 wk	23°C-Dark-3 wk →	15°C-Light
Psilocybe panaeoliformis	DPD	25°C-Dark-2 wk	25°C-Dark-2 wk →	25°C-Light
Schizophyllum commune	DPD	25°C-Dark-2 wk	25°C-Dark-2 wk →	25°C-Light
Agaricus bisporus	(from natural or commercial source)			
Amanita virgineoides				
Amanita vaginata				
Auricularia auricula				
Grifola frondosa				
Hygrocybe conica				
Hypsizigus marmoreus				
Inocybe lacera				
Lactarius piperatus				
Lactarius volemus				
Lenzites betulina				
Lyophyllum ulmarium				
Panellus serotinus				
Pleurocybella porrigens				
Rhizopogon nigrescens				
Russula adusta				
Russula virescens				
Sparassis crispa				
Sterium gausapatum				
Tricholoma acerbum				
Tricholoma matsutake				
Boletus pulverulentus	DPD	25°C-Dark-2 wk		
Coriolus versicolor	DPD	25°C-Dark-1 wk		
Fomitopsis pinicola	DPD	25°C-Dark-1 wk		
Pycnoporus coccineus	DPD	25°C-Dark-2 wk		
Botrytis cinerea	DPD	25°C-Dark-1 wk		
Fusarium oxysporum	DPD	25°C-Dark-1 wk		
Graphiola phoenicis	DPD	25°C-Dark-2 wk		
Neurospora crassa	DPD	25°C-Dark-1 wk		
Penicillium chrysogenum	DPD	25°C-Dark-1 wk		
Phycomyces nitens	DPD	25°C-Dark-1 wk		
Tremella foliacea	DPD	25°C-Dark-1 wk		
Ustilago esculenta	DPD	25°C-Dark-2 wk		
Candida krusei	GPY	28°C-Shaking culture-24 h		
Rhodosporidium toruloides	DPD	25°C-Shaking culture-72 h		
Rhodotorula glutinis	GPY	28°C-Shaking culture-24 h		
Saccharomyces cerevisiae	GPY	28°C-Shaking culture-18 h		

a) DPD: double strength potato-dextrose medium (Kitamoto and Gruen, 1976); MP: maltose-peptone medium (Kitamoto et al., 1978); PGL: peptone-glucose-lignin medium (Kawamura et al., 1983); GPY: glucose-peptone-yeast extract medium (see the part of Materials and Methods).

out successively by incubating the cultures in the dark at 23–25°C for 1–4 wk. They were then incubated in the light at ca. 4.6 mW/cm² (about 200 lx) either at 15°C and 90% relative humidity (RH), or at 25°C and 70% RH until the formation of fruit-bodies (see Table 1). Mycelia and fruit-bodies were separately harvested, washed thoroughly with distilled water, and stored in a deep freezer. Fruit-bodies of wild mushrooms collected in the University Forest, Tottori University, were also used as the enzyme sources.

The yeasts were grown in GPY medium composed of 20 g of glucose, 2 g of peptone and 0.5 g of yeast extract in 1 L of distilled water. This was kept at 30°C for 18 h with reciprocal shaking, and the cells were harvested by centrifugation.

For extracting trehalose phosphorylase and trehalase, 50 mM potassium phosphate buffer containing 25% glycerol and 5 mM EDTA (pH 7.0) and 50 mM acetate buffer (pH 4.5) were used, respectively. The fungal materials were homogenized with a glass homogenizer in the appropriate buffer, and cell debris was removed by centrifugation. The supernatant solution was concentrated with Centriflo CF-25 (Amicon, USA) and used as the crude enzyme.

α-Type trehalose phosphorylase activity was assayed spectrophotometrically by a coupled reaction with α-phosphoglucomutase and glucose 6-phosphate dehydrogenase (Kitamoto et al., 1988). For determination of β-glucose 1-phosphate-forming (β-type) trehalose phosphorylase, β-phosphoglucomutase was substituted for α-phosphoglucomutase in the reaction mixture. The increase in absorbance at 340 nm was monitored at 30°C with a spectrophotometer. Trehalase was assayed by the method of Williams and Niederpruem (1968). The liberated glucose was measured by the method of Somogyi (1952). One unit of enzyme activity is defined as the amount of enzyme formating 1 μmol of product per min. Specific activity is expressed as units of enzyme activity per mg protein. Protein was assayed by the method of Lowry et al. (1951).

To confirm the occurrence of the trehalose phosphorylase reaction in fungi and yeasts, the products of the phospholytic reaction with trehalose were analyzed with a HPLC apparatus (Gilson) in line with a refractive index detector (Gilson, model 132) and a data processor (Hitachi, model D-2500). The reaction mixture was composed of 200 mM trehalose, 0.1 mg bovine serum albumin, 40 mM potassium phosphate buffer (pH 7.0), and an appropriate amount of the crude enzyme, in a total volume of 3 ml. The reaction was alowed to proceed at 30°C for 30 min, then stopped by boiling the reaction mixture for 5 min. The solution was then filtered with a pre-cut filter and put on an Ionpak KS-802 column (Showa Denko). The HPLC was run with water as mobile phase at 0.4 ml/min under a pressure of 49 bar at 80°C. The retention times of authentic samples of α-glucose 1-phosphate, trehalose, and glucose were 10.6, 14.7, and 18.4 min, respectively.

In many microorganisms, trehalose is hydrolyzed to produce two glucose molecules by trehalase (Elbein, 1974). Maréchal and Belocopitow (1972) reported phospholytic cleavage of trehalose into β-glucose 1-phosphate (β-G1P) and glucose by a phosphorylase from *Euglena gracilis* Klebs. In *F. velutipes*, a large amount of trehalose was accumulated in fruit-bodies up to the mid-stage of their development, and thereafter decreased by about 50% (Kitamoto and Gruen, 1976). Trehalase activity in this mushroom was higher in the mycelium than in the fruit-bodies, where the activity was at an inert level in the degradation of trehalose. However, β-glucose 1-phosphate-forming activity of trehalose phosphorylase was not detected. In this mushroom, the α-glucose 1-phosphate (α-G1P) content in fruit-bodies at an early developmental stage was higher than the content of α-glucose 6-phosphate (G6P) (Kitamoto et al., 1981; Kitamoto, 1991). However, α-phosphoglucomutase, which facilitates the conversion of α-G1P to G6P, was sufficiently active in the fruit-bodies. Therefore, we looked for a possible enzymatic phosphorylation of trehalose to produce α-G1P and glucose in mushrooms. Kitamoto et al. (1988) have found a novel trehalose phosphorylase which catalyzes the phosphorylation of trehalose to α-G1P and glucose in this fungus.

α-Type trehalose phosphorylase with an optimum pH around 7 commonly occurred in the mycelia and fruit-bodies of basidiomycetous mushrooms (Table 2). The occurrence of this enzyme was confirmed by a coupling reaction assay system (see the part of Materials and Methods) and by the HPLC products analysis in wood rotting, saprophytic and mycorrhizal mushrooms of the following genera: *Agaricus*, *Amanita*, *Boletus*, *Coriolus*, *Coprinus*, *Favolus*, *Fistulina*, *Flammulina*, *Fomitopsis*, *Grifora*, *Hebeloma*, *Hygrocybe*, *Inocybe*, *Lactarius*, *Lentinus*, *Lenzites*, *Lyophyllum*, *Panellus*, *Pholiota*, *Pleurocybella*, *Pleurotus*, *Psilocybe*, *Pycnoporus*, *Rhizopogon*, *Russula*, *Schizophyllum*, *Sterium*, and *Tricholoma*. In most of these mushrooms, the fruit-bodies showed higher specific activities of α-type trehalose phosphorylase than did the mycelia.

The distribution of α-type trehalose phosphorylase in filamentous fungi and yeasts is also shown in Table 2. The activity was detected only in the mycelia and fruit-bodies of Aphyllophorales and Agaricales in the Holobasidiomycetidae, and at least one species of Gasteromycetes. It was not found in Tremellaceae, such as *Tremella foliacea* Fr., or Auriculariales, such as *Auricularia auricula* (Hook.) Andrew. in the Phragmobasidiomycetidae. Neither α-type nor β-type phosphorylase activities was found in the Hemibasidiomycetes or Heterobasidiomycetes. The mycelia and yeast cells of fungi belonging to the Ascomycotina and Deuteromycotina, and the yeast of Basidiomycotina, produced different levels of trehalase activity but did not show trehalose phosphorylase activity.

Many researchers have reported the distribution of trehalase in filamentous fungi and yeasts (Elbein, 1974). However, if the enzyme assay had been carried out by measuring reducing sugar as the trehalase reaction product in a phosphate buffer of around pH 7 or weak alkaline range (cf. Rao and Niederpruem, 1969), the pos-

Table 2. α-Glucose 1-phosphate forming trehalose phosphorylase and trehalase activities in basidiomycetous mushrooms and the mycelia or yeast cells of various fungi.

Organism	Colony portion[a]			
	Mycelia or yeast cells		Fruit-bodies	
	TP activity	TH acitivity	TP activity	TH activity
Coprinus cinereus	39.9	21.2	49.6	8.9
Coprinus phlyctidosporus	33.0	16.3	84.4	8.5
Favolus arcularius	39.1	71.8	57.9	4.1
Fistulina hepatica	15.2	81.4	32.3	15.4
Flammulina velutipes	19.0	17.0	39.1	3.7
Hebeloma vinosophyllum	22.2	241.7	21.8	12.6
Lentinus edodes	29.5	10.0	43.1	14.2
Pholiota nameko	ND[b]	ND	31.8	14.8
Pleurotus ostreatus	66.2	58.1	142.9	41.8
Psilocybe panaeoliformis	24.7	44.0	30.1	171.3
Schizophyllum commune	72.4	18.6	19.2	1.4
Agaricus bisporus			23.0	14.6
Amanita virgineoides			95.0	ND
Amanita vaginata			35.0	15.0
Auricularia auricula			0	4.4
Grifola frondosa			48.8	84.0
Hygrocybe conica			26.0	ND
Hypsizigus marmoreus			51.0	65.0
Inocybe lacera			43.4	15.4
Lactarius piperatus			99.9	5.7
Lactarius volemus			31.4	206
Lenzites betulina			3.6	ND
Lyophyllum ulmarium			41.0	3.3
Panellus serotinus			16.5	1.0
Pleurocybella porrigens			6.1	41.2
Rhizopogon nigrescens			9.6	25.0
Russula adusta			39.0	15.2
Russula virescens			18.3	7.3
Sparassis crispa			0	ND
Sterium gausapatum			1.8	146
Tricholoma acerbum			1.3	20.6
Tricholoma matsutake			19.4	ND
Boletus pulvcerulentus	22.7	12.5		
Coriolus versicolor	39.6	654.0		
Fomitopsis pinicola	9.9	150.0		
Pycnoporus coccineus	41.5	182.0		
Botrytis cinerea	0	24.7		
Fusarium oxysporum	0	18.9		
Graphiola phoenicis	0	9.1		
Neurospora crassa	0	54.9		
Penicillium chrysogenum	0	195.0		
Phycomyces nitens	0	15.0		
Tremella foliacea	0	98.6		
Ustilago esculenta	0	3.6		
Candida krusei	0	54.6		
Rhodosporidium toruloides	0	56.0		
Rhodotorula glutinis	0	2.7		
Saccharomyces cerevisiae	0	36.0		

a) TP: trehalose phosphorylase; TH: trehalase. Activities are shown in units/mg protein.

b) ND: not determined.

sibility would remain that glucose was liberated from trehalose by the action of trehalose phosphorylase in the crude enzyme from the test organism. Accordingly, we attempted to reconfirm the presence of trehalase activity in various fungi, including basidiomycetous mushrooms.

Table 2 shows that all of the test organisms showed different levels of trehalase activity at a pH of 4.5. Maximum trehalase activity occurred at acidic pHs of 4.5 in the majority of test organisms, including the basidiomycetous mushrooms. Trehalase activities in the fruit-bodies of mushrooms were lower than those in the mycelia, except in the case of *Psilocybe panaeoliformis* Murr. and hard mushrooms such as *Coriolus versicolor* (Schwartz.: Fr.) Quel and *Pycnoporus coccineus* (Fr.) Bond. & Sing. Among the mushrooms tested, the trehalases in the fruit-bodies of *Favolus arcularius* (Fr.) Ames, *F. velutipes*, and *Schizophyllum commune* Fr.: Fr. may not actually contribute the catabolism of trehalose. The pH in the intact mycelia and fruit-body cells of mushrooms is assumed to be in the neutral range. Therefore, it is doubtful that the rapid breakdown of trehalose in the fruit-bodies was due to trehalase in basidiomycetous mushrooms.

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