

Effects of Fruiting Bodies of *Basidiomycetes* on Yeast Growth Rates

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The effects of edible fruiting of Basidiomycetes on growth of Saccharomyces cerevisiae and other yeast strains were examined. The growth rates were significantly increased in the presence of fruiting bodies but there was no significant difference growth yield between cultures with and without fruiting bodies. Growth rates of yeast cells were promoted in both synthetic and natural media.

Key Words: Fruiting body, Basidiomycetes, yeast, growth promotion, specific growth rate.

INTRODUCTION

Nearly 2000 mushroom species belonging to the class *Basidiomycetes* are edible, but only a few are consumed and cultivated on a large scale¹⁻⁶. We have investigated new ways of utilizing edible fruiting bodies of *Basidiomycetes*, including brewing alcoholic drinks with a fruiting body flavour have been investigated. Alcohol production by the yeast *Saccharomyces cerevisiae* is markedly increased in medium supplemented with fruiting bodies. Tokumitsu *et al.* reported that addition of edible mushroom *Grifola frondosa* to white bread dough affected gas production by baker's yeast⁹. A factor which promotes growth of yeast cells was isolated from *Chlorella* by Kanno *et al.*⁸. However, there have been no reports of isolation of a growth-promoting factor from fruiting bodies of *Basidiomycetes*. The present report describes the positive impact of fruiting bodies of *Basidiomycetes* on growth of yeast cells.

MATERIALS AND METHODS

Yeast strains

The following yeast strains were used in this study: *Kluyveromyces marxianus* IFO 0288, *Saccharomyces bayanus* IFO 0215, *Saccharomyces cerevisiae* IFO 0224, *Saccharomyces cerevisiae* IFO 2377, and *Schizosaccharomyces pombe* IFO 0342. All strains were purchased from IFO (Institute for Fermentation Osaka, Japan).

Edible mushrooms

The following mushrooms were used in this study: *Agaricus arvensis*, *Elammulina velutipes*, *Grifola frondosa*,

Lentinus edodes, *Lyophyllum ulmarinum* and *Poliota nameko*. All mushrooms were purchased from a local market. Mushrooms were freeze-dried after being cut into pieces about 1 cm in length.

Media for yeast

Malt extract-yeast extract medium (MY medium; 2% glucose, 0.3% malt extract, 0.3% yeast extract, 0.5% polypepton) was used for preculture. The synthetic medium⁷ (see Table I), malt extract-yeast extract-sucrose medium (MYS medium; 2% sucrose, 0.3% malt extract, 0.5% polypeptone, 0.3% yeast extract) and yeast extract-polypeptone-sucrose medium (YPS medium; 2% sucrose, 1% yeast extract, 2% polypeptone) were used for growth tests. All chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Analysis

Viable cell number was determined using a hemocytometer, after staining with methylene blue. Growth was monitored by measuring absorbance at 660 nm.

Culturing procedure for growth test

To investigate the effect of fruiting bodies on growth, a growth test was performed as follows. One loopful of yeast cells was inoculated into 100 ml of MY medium (in a 300-ml Erlenmeyer flask), and incubated on a rotary shaker at 180 rpm at 30°C. Twenty-four hours later, 20 ml of the culture was transferred into 400 ml of synthetic medium (in a 500-ml Erlenmeyer flask) and *statically* incubated at 30°C for 18h. Viable cells were then inoculated into 300 ml of medium (in a 500-ml narrow-mouth reagent bottle with an air duct), at a density of 50 cells/ml, and *statically* cultured.

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TABLE I. Composition of synthetic medium used for growth tests.

Components	Amount per litre
Carbon source	
Sucrose	20 g
Nitrogen source	
Ammonium citrate, Tribasic	10 g
Vitamins	
Thiamine hydrochloride	10 mg
Riboflavin	2 mg
Pyridoxine hydrochloride	1 mg
Nicotinamide	6 mg
Calcium pantothenate	10 mg
Biotin	40 µg
Folic acid	40 µg
p- Aminobenzoic acid	1 mg
Inositol	100 mg
Organic bases	
Adenine sulphate	10 mg
Guanine hydrochloride	10 mg
Uracil	10 mg
Xanthine	10 mg
Salts	
Potassium phosphate, Monobasic	2.2 g
Potassium chloride	1.7 g
Calcium chloride	250 mg
Magnesium sulphate	500 mg
Ferric chloride	10 mg
Manganese sulphate	10 mg
Zinc sulphate	10 mg
Copper sulphate	1 mg
Boric acid	100 µg
Sodium molybdate	100 µg
Potassium citrate, Monohydrate	4 g
Citric acid	0.8 g

RESULTS AND DISCUSSION

Effect of fruiting bodies on growth of *Saccharomyces cerevisiae* IFO 2377

The first experiment was designed to compare the effects of fruiting bodies of several edible species of *Basidiomycetes* on growth of yeast cells. The sake yeast *Saccharomyces cerevisiae* IFO 2377 was inoculated into 300 ml of synthetic medium, with or without 600 mg of freeze-dried fruiting bodies of *Basidiomycetes* (in a 500-ml narrow-mouth reagent bottle with an air duct). The media were sterilized by autoclaving at 110°C for 20 min. Figure 1 shows that the growth rate of strain 2377 was increased in the presence of 6 species of fruiting bodies. However, growth yield did not differ significantly. In order to examine whether this promotive effect on growth is unique to mushrooms, the effects of the following vegetables on yeast growth: carrot, broccoli, cabbage, pumpkin, potato, onion, green bean sprout and radish were examined but no vegetable elicited similar effects.

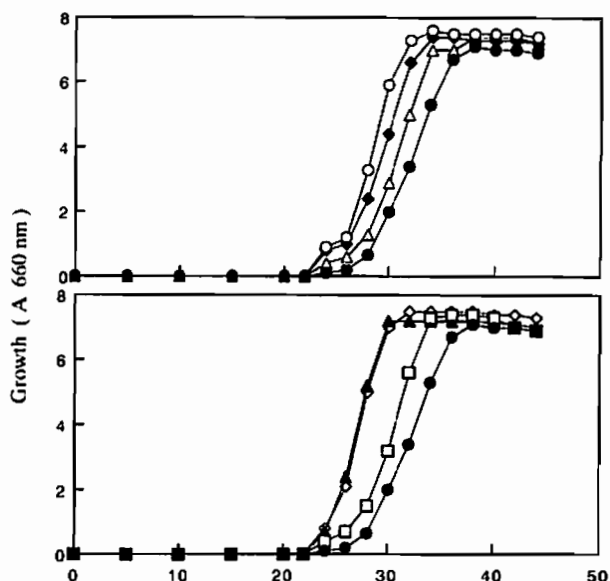


FIG. 1. Effect of fruiting body on the growth of *Saccharomyces cerevisiae* IFO 2377. Symbols: ●, control; ▲, *Agaricus arvensis*; ◊, *Elammulina velutipes*; ◆, *Grifola frondosa*; ◆, *Lentinus edodes*; ◊, *Lyophyllum ulmarinum*; △, *Pholiota nameko*.

Effect of concentration of fruiting bodies on growth of *S. cerevisiae* IFO 2377

To examine the relationship between the concentration of fruiting bodies of *Basidiomycetes* and cell growth, *S. cerevisiae* IFO 2377 was statically cultivated in 300 ml of synthetic medium with freeze-dried fruiting bodies (0-2 mg/ml), at 30°C for 30h. The mushroom species used for this growth test was *Elammulina velutipes*, which is available year-round at a local market. Figure 2 shows that growth increased in a manner indicating dependence on the concentration of fruiting bodies of *Basidiomycetes*, but the dependence was not linear. The promotive effect was maximal at concentrations of about 1 mg/ml. At concentrations of fruiting bodies above 2 mg/ml, increased concentration did not result in greater growth.

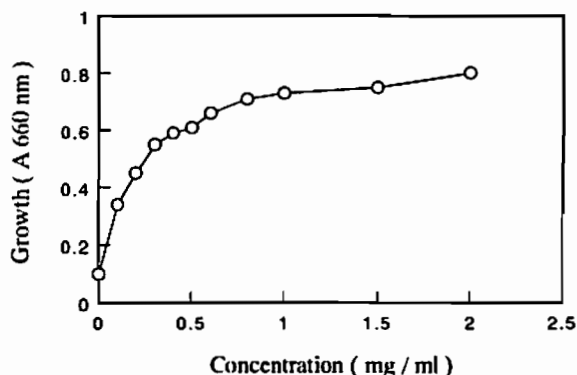


FIG. 2. Effect of concentration of fruiting body of *Elammulina velutipes* on growth of *Saccharomyces cerevisiae* IFO 2377. Strain IFO 2377 was statically cultivated in 300 ml of synthetic medium in a 500 ml-narrow mouth reagent bottle at 30°C.

Effects of fruiting bodies on growth of four yeast strains

The effects of fruiting bodies of *Elammulina velutipes* on the following yeast strains: *Kluyveromyces marxianus* IFO

0288, *Saccharomyces bayanus* IFO 0215, *Saccharomyces cerevisiae* IFO 0224, and *Schizosaccharomyces pombe* IFO 0342 were examined. Growth was monitored by measuring absorbance at 660 nm, and specific growth rates were estimated from these values. These results, along with results for *S. cerevisiae* IFO 2377, are shown in Figure 3 and Table II. In all strains, lag phases were markedly reduced by addition of fruiting bodies, although there were only slight differences in specific growth rates between cultures with and without fruiting bodies. For *S. bayanus* IFO 0215, there was a slight difference in growth yield between cultures with and without fruiting bodies. Among the yeast strains, the growth rate of IFO 0224 (which has a high alcohol-producing capacity) was greatest. For this reason, *S. cerevisiae* IFO 0224 was used in further experiments on the promotive effect of fruiting bodies in different media. Although growth was promoted in synthetic medium containing fruiting bodies of *Elammulina velutipes*, the effect may have been caused by fruiting bodies supplying nutrients that were lacking in the synthetic medium. This growth test was therefore repeated with 2 kinds of natural media.

TABLE II. Specific growth rates of yeast strains cultivated with and without *Elammulina velutipes*.

Strain	Specific growth rate (h ⁻¹)	
	<i>Elammulina velutipes</i>	
	without	with
<i>Kluyveromyces marxianus</i> IFO 0288	0.30	0.31
<i>Saccharomyces bayanus</i> IFO 0215	0.27	0.29
<i>Saccharomyces cerevisiae</i> IFO 0224	0.37	0.38
<i>Saccharomyces cerevisiae</i> IFO 2377	0.33	0.36
<i>Schizosaccharomyces pombe</i> IFO 0342	0.19	0.20

Yeast cells were inoculated into 300 ml of synthetic medium without and with *Elammulina velutipes* (300 mg) in a 500 ml narrow mouth reagent bottle with an air duct and statically cultivated at 30°C.

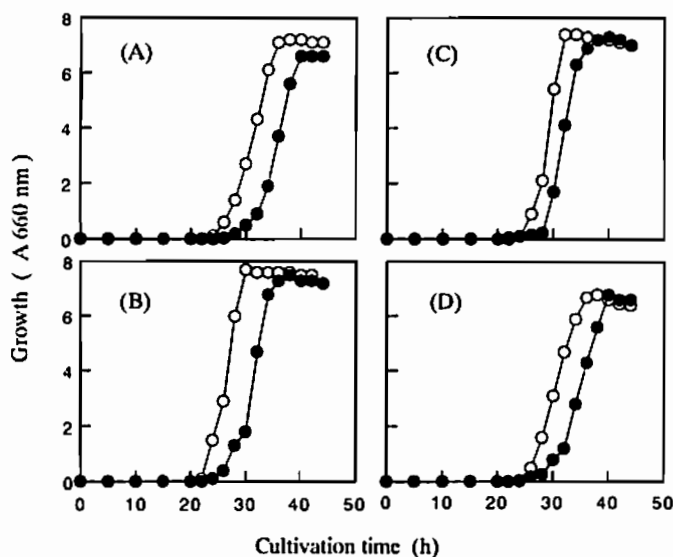


FIG. 3. Effect of fruiting body of *Elammulina velutipes* on growth of yeast strains. Yeast cells were statically cultivated in 300 ml of synthetic medium with and without fruiting body of *Elammulina velutipes*. (300 mg) (A), *Saccharomyces bayanus* IFO 0215; (B), *Saccharomyces cerevisiae* IFO 0224; (C), *Kluyveromyces marxianus* IFO 0288; (D), *Schizosaccharomyces pombe* IFO 0342. Symbols: ●, without fruiting body; ○, with fruiting body.

Effects of fruiting bodies on growth in different media

In this investigation, MYS and YPS medium were used. Fruiting bodies (300 mg) were added to 300 ml of medium, which was then sterilized by autoclaving at 110°C for 20 min. The results are shown in Figure 4 and Table III. In MYS and YPS medium, fruiting bodies induced 53 and 80% increases in maximum specific growth rates, respectively. The time taken to reach maximal cell growth was reduced from 48 h to 38 h in both media. Therefore, fruiting bodies of *Elammulina*

velutipes promoted the growth of *S. cerevisiae* IFO 0224 in both types of medium. On the other hand, fruiting bodies did not induce any significant difference in specific growth rate in synthetic medium (see Table II). It is unclear what role medium type played in these differences in specific growth rate. Tokumitsu *et al.*⁹ reported that adding *Grifola frondosa* to white bread dough affects gas production by baker's yeast. They concluded that this was due to the fermentable sugar contained in fruiting bodies, and the release of fermentable sugar from wheat flour by amylase contained in *Grifola frondosa*⁹. In our experiments, media were immediately autoclaved after addition of fruiting

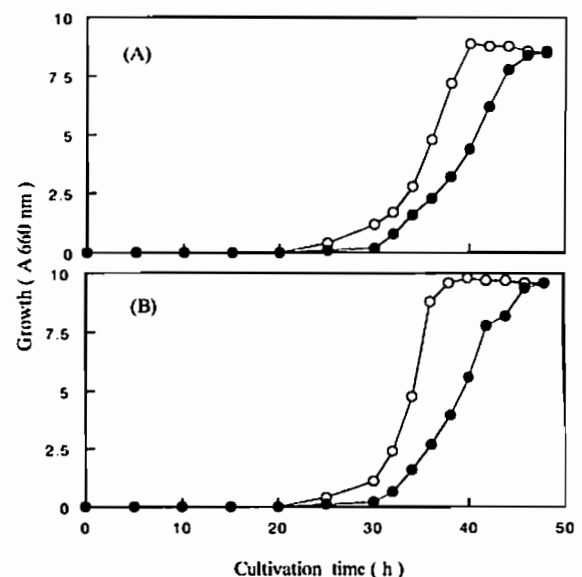


FIG. 4. Effect of fruiting bodies on growth of *Saccharomyces cerevisiae* IFO 0224 in (A) MYS medium and (B) YPS medium. Symbols: ●, without fruiting body; ○, with fruiting body.

TABLE III. Maximum specific growth rates of *Saccharomyces cerevisiae* IFO 0224 cultivated in natural media with and without fruiting body of *Elammulina velutipes*.

Medium	Maximum Specific growth rate (h ⁻¹)	
	without	with
MYS	0.17	0.26
YPS	0.16	0.29

bodies. It was thought that amylase was denatured during autoclaving, and that, as a result, the amount of fermentable sugar was not increased by amylase activity. The present study found no significant differences in growth yield between cultures with and without fruiting bodies of *Grifola frondosa* (see Fig. 1). The promotive effect of fruiting bodies on yeast cell growth did not appear to have been caused by an increase in the amount of fermentable sugar. Further investigations are currently in progress, in an effort to examine the effects of mushroom fruiting bodies on alcohol fermentation and identify the substance responsible for growth promotion.

CONCLUSION

Cell growth of 5 yeast strains (in synthetic medium) was promoted by supplementation with edible fruiting

bodies of several species of *Basidiomycetes*. In all strains, lag phases were reduced by the presence of fruiting bodies of *Elammulina velutipes*. Specific growth rates in natural media were markedly increased by supplementation, but in synthetic medium there were only slight differences between cultures with and without fruiting bodies.

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