Food Sci. Biotechnol. Vol. 15, No. 2, pp. 308 ~ 311 (2006)

Food Science and Biotechnology

Calcium Absorption by the Fruitbody of *Saesongi* (*Pleurotus eryngii*) Mushroom

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Abstract *Saesongi (Pleurotus eryngii)* was cultivated in both potato dextrose agar (PDA) and sawdust media supplemented with Ca salts. The addition of Ca phosphate and Ca carbonate to sawdust media did not affect the growth, whereas Ca sulfate addition suppressed the mycelial growth appreciably. The efficiencies of Ca accumulation in the fruiting were studied based on mycelial growth experiments on Ca-supplemented sawdust media. Supplementation with 0.1 to 5% Ca phosphate increased the Ca content in the fruiting body by 4.5-6.5 fold, to a level of 314.6 ± 22.7 to 449.7 ± 29.3.

Keywords: *Saesongi*, fruit body, calcium, sawdust culture media

Introduction

Saesongi (*Pleurotus eryngii*) mushroom is the saprophyte belonging taxologically to *Basidiomycotina, Agaricales, Pleurotaceae,* and *Pleurotus*. While most agaric naturally live by adhering to the stump or trunk of decayed trees as wood-destroying fungi, it is reported that *Saesongi* grows gregariously as a single organism or bundle in the grassland soil of the subtropical regions (1, 2).

Saesongi mushroom termed King oyster mushroom in Europe, has not been collected in Korea (3). In Korea an artificial cultivation technique using saw dust has been developed (4), and currently this mushroom is cultivated in bottle mushroom cultivating farmhouses equipped with automatic facilities.

In Korea, consumption of *Saesongi* is increasing due to its good taste and aroma. In addition, as its moisture content is lower than that of other mushrooms, its value as an exportable item is very high and it is expected to become a greater source of agricultural income (5, 6). Also, the mushroom's antitumor effect (7), antioxidant activity (8-11), angiotensin converting enzyme inhibition activity (12), and immune cell activation activity (13) have all attracted research attention.

The total amount of Ca contained in the mushrooms is less than that of vegetables (14, 15). Several studies have investigated the absorption of heavy metals from sawdust culture media by such mushrooms as *Saesongi, Hypsizygus marmoreus, Pholiota nameko, Flammulina velutipes,* and *Ganoderma lucidum* (16-20). Tabata and Ogura (21) studied mycelial growth of *H. marmoreus* in potato sucrose agar (PSA) and in sawdust media supplemented with Ca salts. Tabata and Shinohara (22) reported the Ca absorption of *P. ostreatus* and *P. nameko* in both PSA and sawdust medium supplemented with Ca salts. However, the absorption of Ca by *Saesongi* from sawdust culture medium supplemented with these salts has not been established yet.

In this paper, the mycelial growth of *Saesongi* was studied in potato dextrose agar (PDA) and in sawdust media supplemented with Ca salts. Furthermore, the absorption of this element from the sawdust into the fruiting body is discussed.

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Received January 17, 2006; accepted February 23, 2006

Materials and Methods

Strain and Ca sources The strain of *Saesongi*, originally cultured at a mushroom farm (Mushheart Co., Anseong, Korea), was purchased from the National Institute of Agricultural Science and Technology. Calcium chloride ($CaCl_2 \cdot 2H_2O$), calcium sulfate ($CaSO_4 \cdot 2H_2O$), calcium carbonate ($CaCO_3$) and calcium phosphate ($CaHPO_4 \cdot 2H_2O$) were of analytical grade.

Mycelial growth on PDA supplemented with Ca The PDA medium was supplemented with a calcium salt to a concentration of 1, 5, or 10%. The calcium salts tested were calcium chloride, calcium sulfate, calcium carbonate, and calcium phosphate. The medium was then autoclaved at 121°C for 15 min before being poured into 5 petri dishes.

Saesongi was previously grown on the PDA medium without supplementation in a petri dish. Agar discs covered with well-grown mycelia from a 7-day old colony that had been made using a sterile cork borer. An agar disc was placed at the center of each plate supplemented with calcium salt. The plate was then incubated for 5 days at 25°C and the diameter of the mycelial growth was determined. All experiments were conducted in 5 repetitions.

Mycelial growth on the sawdust medium supplemented with calcium salts A mixture of sawdust and rice bran (4:1 w/w) was supplemented with 0.5, 1.0, or 2.0% of calcium salts. Then tap water with a calcium content of 10.9 ppm was added to the mixture to give a moisture

content of 65%, and it was mixed well to ensure homogeneity. Thirty grams of the medium were tightly packed into a petri dish which was autoclaved at 121°C for 50 min and allowed to cool for 6 hr. A mycelium disc was placed on the sawdust medium via the same method as the agar medium described above.

Harvesting the fruit bodies of *Seasongi* from sawdust media supplemented with calcium salts Sawdust media supplemented with 0.5, 1, or 2% of Calcium phosphate were prepared. A heat-resistant polypropylene bottle (approximate capacity, 850 mL) was filled with the sawdust medium. A cavity (1.5 cm diameter × 10 cm deep) was made in the center of the medium for the inoculation. The sterilized medium was inoculated with 10 mL of liquid spawn and incubated at 25±1°C in the dark. When the spawn had grown well throughout the medium, the culture was subjected to the kinkaki treatment, which scratches away and removes the surface of mycelia to induce budding (23). The culture was then transferred to a culture room maintained at 15±2°C in 80±5% relative humidity. The fruiting bodies of *Saesongi* were harvested and dried in a forced-air oven at 60-70°C for 72 hr. A homogeneous sample was prepared by grinding the dried fruiting bodies in a stainless-steel mill.

Analysis of Calcium contents in fruit bodies The Calcium content in the fruit body of *Saesongi* was analyzed with analytical methods for atomic absorption spectroscopy (24).

Results and Discussion

Effect of calcium salts on mycelial growth on PDA The effect of Ca supplementation was expressed as a growth index dividing the diameter of the mycelia on the calcium-supplemented PDA by that on the plate without supple-mentation. Fig. 1 shows the indices for calcium phosphate, calcium sulfate, calcium chloride, and calcium carbonate. The small concentration dependency of mycelial growth on calcium phosphate and calcium sulfate might be attributable to their low solubility. As Fig. 1 shows, the supplementation of 0.5, 1.0, and 2.0% calcium chloride suppressed the growth by 15, 31, and 90%, respectively. The fact that no mycelial growth was shown by the addition of more than 1% of calcium carbonate might be attributable to the high pH values (>8.4) of the media, as reported by Tabata and Shinohara (22).

Effect of Calcium salts on the mycelial growth on the sawdust medium Figure 2 shows the effect of supplementtion with 0.5, 1.0, or 2.0% of calcium phosphate, calcium sulfate, calcium chloride, and calcium carbonate on growth of *saesongi* on the sawdust medium. The addition of calcium phosphate and calcium carbonate did not affect the growth, whereas calcium sulfate suppressed the mycelial growth appreciably.

As reported by Tabata and Ogura (21), the sawdust medium containing calcium carbonate showed notable lower initial pH value than the corresponding potato sucrose agar medium, while the mycelial growth of *H. marmoreus* was slightly increased by augmentation with Calcium carbonate compared to the control and complete suppression was achieved in potato sucrose agar.

Absorption of Calcium by the fruiting body Based on mycelial growth experiments on ca-supplemented sawdust media, the efficiencies of calcium accumulation in the fruiting were studied. As Fig. 3 shows, only 69.3±4.1 ppm of calcium was found in dried mushroom cultivated on a nonsupple-mented sawdust medium. Supplementation with 0.1 to 5% of calcium phosphate increased the calcium content in the fruiting body by 4.5-6.5 fold, to a level of 314.6 ± 22.7 to 449.7 ± 29.3. The calcium content in the fruit body of *Saesongi* exhibited only weak differences with the addition of calcium source until 10 days after the growth commencement, but after 15 days the differences

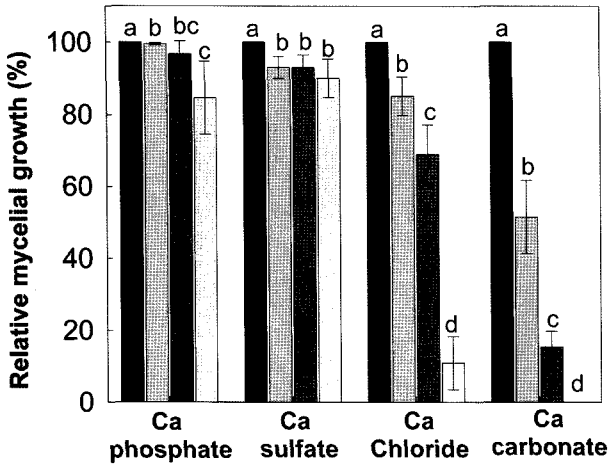

Fig. 1. Mycelial growth of *Saesongi* on the PDA medium. ▬ no addition. ▭ 0.5% addition, ▬ 1% addition, ▭ 2% addition, Values with different letters are significantly different among the experimental groups at $p<0.05$ by Duncan's multiple range test.

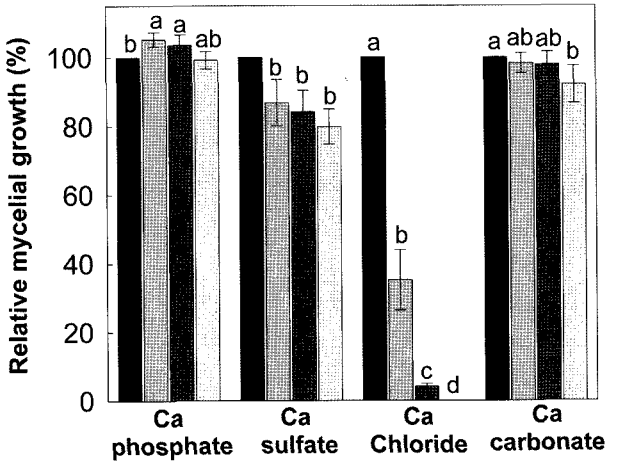

Fig. 2. Mycelial growth of *Saesongi* on the Sawdust medium. ▬ no addition, ▭ 0.5% addition, ▬ 1% addition, ▭ 2% addition, Values with different letters are significantly different among the experimental groups at $p<0.05$ by Duncan's multiple range test.

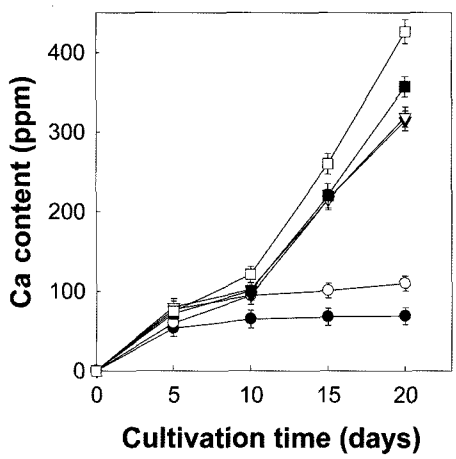

Fig. 3. Calcium contents of *Saesongi* harvested from the sawdust medium supplemented with various Calcium phosphate contents. (Non addition ● ; 0.1% addition ○ ; 0.5% addition ▼ ; 1.0 % addition ▽ ; 2.0% addition ■ ; 5.0% addition □)

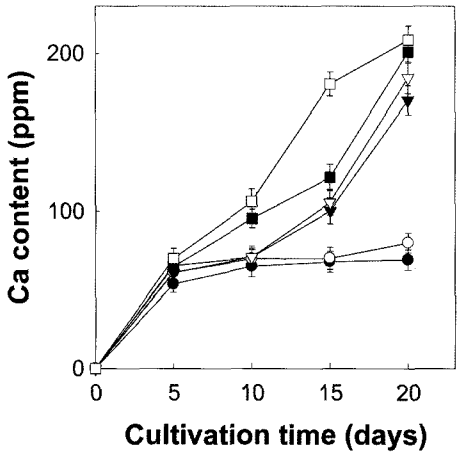

Fig. 4. Calcium contents of *Saesongi* harvested from the sawdust medium supplemented with various calcium carbonate contents. (Non addition ● ; 0.1% addition ○ ; 0.5% addition ▼ ; 1.0 % addition ▽ ; 2.0% addition ■ ; 5.0% addition □).

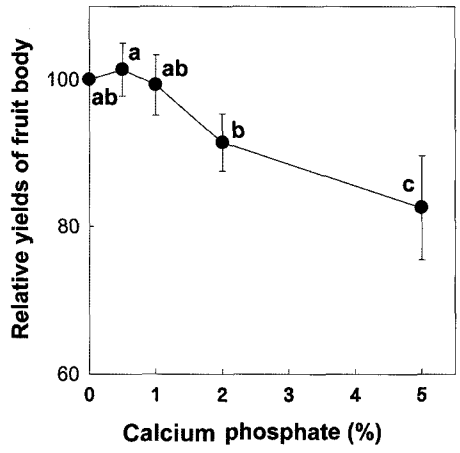

Fig. 5. Relative fruit body yields of *Saesongi* harvested from the sawdust medium supplemented with calcium phosphate. *Values with different letters are significantly different among the experimental groups at $p<0.05$ by Duncan's multiple range test.

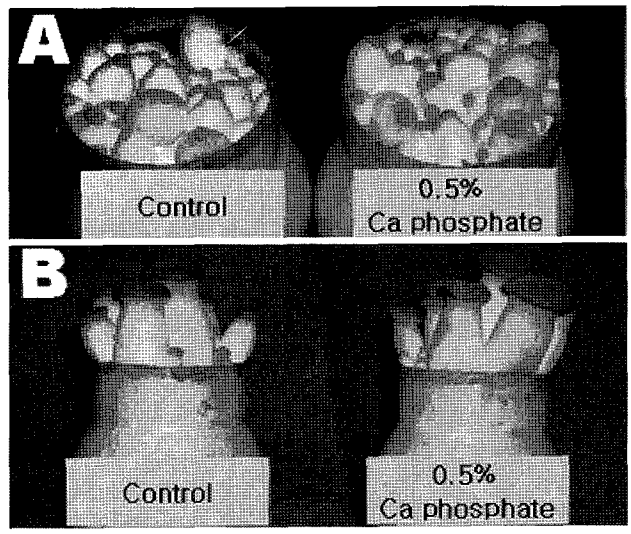

Fig. 6. Effect of 0.5% calcium phosphate on the budding and growth of *Saesongi*. A: The number of buds did not differ according to the addition of 5% Calcium phosphate; B: The growth of fruit was not effected by the addition of 5% Calcium phosphate.

of calcium content became significant. The Ca content in the fruit body of *Saesongi* tended to increase with increasing content of calcium phosphate that was added to the culture media. However, adding more than 2% of calcium phosphate reduced the yield of fruit body significantly, suggesting a low level of industrial usefulness (Fig. 5, 6).

Both the calcium content and yield of fruit body did not significantly differ between 1 and 0.5% addition of Ca phosphate to the sawdust medium. Therefore, it is expected that a mere 0.5% addition of calcium phosphate to the sawdust medium is the desirable level to produce good quality *Saesongi* containing a higher Calcium concentration. As Fig. 4 shows, supplementation with 0.1 to 5% Ca carbonate increased the Ca content in the fruiting body by 1.2-3.1 fold, to a level of 80.2 to 208.6 ppm. The variation of Ca content in the fruit body of *Saesongi* according to cultivation time was similar to that of calcium phosphate addition. It appeared that the calcium content in *Saesongi* tended to increase with increasing calcium carbonate content in the culture media. However, the efficiency was confirmed to decline compared to the addition of calcium

Table 1. Spawn run periods and days to primordia of *Saesongi* according to calcium phosphate and calcium carbonate supple-mentation

		Supplementation (%)					
		0	0.1	0.5	1.0	2.0	5.0
Ca phosphate	Spawn run periods (days)	20	20	20	20	22	25
	Days to primordia (days)	6	6	6	6	7	8
Ca carbonate	Spawn run periods (days)	20	20	21	21	23	25
	Days to primordia (days)	6	6	6	7	7	8

phosphate and the yield of fruit body according to the addition of calcium carbonate was lower than that for calcium phosphate addition (data not shown). Spawn run periods and days to primordia of *Saesongi*, according to addition of calcium sources, are shown in Table 1.

In the case of calcium phosphate addition, both spawn run periods and days to primordia did not show any difference until 1.0% addition. calcium carbonate addition above 2.0% suppressed the growth of *Saesongi*. Mushroom, including *Saesongi*, was found to be an excellent agricultural product having various functional materials and preferences in spite of its disadvantage of having a lower calcium content than vegetables. Several studies have investigated the absorption of metal ions from the culture media that occurs during the inorganic absorption of mushrooms and *P. ostreatus*, *H. marmoreus*, *P. nameko*, *F. velutipes* and *G. lucidum*. Therefore, these study results suggest that the usefulness of mushrooms as foods will be greatly improved by increasing the presently sub-optimal level of calcium content in the mushroom. Hereafter, research will focus on Ca-enriched *Saesongi* using various natural calcium sources such as crab-shell, shrimp-shell, clam-shell, oyster shell and starfish. If these waste resources are developed as a practical medium additive for mushroom, then the functional enlargement of mushroom consumption and the development of allied industries can be expected.

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