Absorption of Calcium and Magnesium to the Fruit Body of Aragekikurage (*Auricularia polytricha* (Mont.) Sacc.) from Sawdust Culture Media Supplemented with Calcium and Magnesium Salts

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Auricularia polytricha was incubated in potato-sucrose-agar (PSA) and in a sawdust medium supplemented with Ca or Mg salts. The mycelia grew well on the PSA supplemented with Ca sulfate, Ca phosphate, Mg sulfate and Mg chloride. On sawdust medium, the supplementation with Ca phosphate, Ca carbonate, Mg sulfate or Mg carbonate resulted in good mycelial growth. The Ca content of fruit body grown on the sawdust medium was increased 1.1–1.5 times by supplementation of 1–5% of Ca phosphate or Ca carbonate. The Mg content was increased 1.7–2.2 times by 0.5% of Mg carbonate, Mg hydroxide, Mg sulfate and Mg chloride.

Keywords: Aragekikurage (*Auricularia polytricha*), mycelial growth, Mg-Ca-supplementation, Mg-Ca-absorption

Auricularia polytricha (Aragekikurage in Japanese) is one of the wood-rot fungi of the broadleaf tree. Though *A. polytricha* is a mushroom which is as important for Chinese-cooking as *Auricularia auricula* (Kikurage in Japanese), in many cases, the mushroom is sold in the market without distinguishing it from *A. auricula*. It generally resembles *A. auricula*, however, it is covered with small grayish brown hair, and the underside is darkish brown or light brown (Imazeki *et al.*, 1970).

In research of *A. polytricha*, the culture media (Kinjo & Kon-do, 1979; Lin, 1991), the components (Yoshida *et al.*, 1986, 1996; Lee *et al.*, 1995; Cheung, 1997; Mau *et al.*, 1998), antitumor activity (Misaki *et al.*, 1979), antioxidative activity (Zhou & Yu, 2000) and effects of cholesterol (Arakawa *et al.*, 1977; Tamaki *et al.*, 1997) have been reported.

The Ca and Mg content of mushrooms are generally low in comparison with vegetables and seaweed (Kagaku Gijitsu-cho, 1995). However, it is known that in the Jew's-ear mushrooms the content of Ca and Mg in mushrooms is very high (Kawai *et al.*, 1986; 1990). Apparently, Ca and Mg are especially necessary for the maintenance of the human physiological function.

Several studies have been directed on the absorption of heavy metals from sawdust culture media by the mushrooms *Pleurotus ostreatus*, *Hypsizigus marmoreus*, *Pholiota nameko*, *Flammulina velutipes* and *Ganoderma lucidum* (Yasui *et al.*, 1988; Sasaki *et al.*, 1995; Tham *et al.*, 1999). However, the absorption of Ca and Mg by *A. polytricha* from sawdust culture medium supplemented with these salts has not yet been established. Previously, we studied *P. ostreatus* and *P. nameko* in PSA and in sawdust medium supplemented with Ca or Mg salts (Tabata & Shinohara, 1995; Tabata & Ogura, 2003).

In this paper, the mycelial growth of *A. polytricha* was studied in PSA and in sawdust medium supplemented with diverse Ca or Mg salts; the absorption of these elements from the sawdust into

the fruit body is discussed. In addition, the pH change of medium supplemented with these salts during mycelial growth was studied.

Materials and Methods

The strain of *A. polytricha* was purchased from the Onuki Kinjin Co., Utsunomiya-shi, Japan. The mushrooms were cultivated in the farm of Kobe Women's University Biotechnology Institute. Ca chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), Ca sulfate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), Ca carbonate (CaCO_3), Ca phosphate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), Mg sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), Mg chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), Mg carbonate (approximate composition $4\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 5\text{H}_2\text{O}$), and Mg hydroxide ($\text{Mg}(\text{OH})_2$) were purchased from Wako Pure Chemical Industries Co. and used without further purification.

Mycelial growth on the PSA supplemented with Ca or Mg salts The PSA medium was supplemented with a Ca salt to 0.5, 1 or 10%, or with an Mg salt to 0.5, 1 or 5%. The Ca and Mg salts tested were Ca chloride, Ca sulfate, Ca carbonate, Ca phosphate, Mg sulfate, Mg chloride, Mg carbonate and Mg hydroxide. Then, the medium was autoclaved at 120°C (1.0 kg/cm²) for 15 min and poured into petri dishes. *A. polytricha* was previously grown on the PSA medium without supplementation in a petri dish. Agar discs (7 mm in diameter) covered with well-grown mycelia from a 7-day-old colony were placed at the center of each plate supplemented with Ca or Mg salt. The plate was then incubated for 7 days at 25°C in the dark and the diameter of the mycelial growth was determined. All experiments were conducted with three replicates per treatment.

Mycelial growth on the sawdust media supplemented with Ca or Mg salts A mixture of beech sawdust and rice bran (4 : 1 w/w) was supplemented with 1, 5, or 10% of a Ca salt, or with 0.5, 1 or 5% of a Mg salt. Then, tap water was added to the mixture to give a moisture content of 70%, and it was mixed well for homogeneity. Approximately 30 g of the medium was tightly packed into a petri dish. The dish was autoclaved at 120°C for 60 min, and allowed to cool for about 5 h. A mycelium disc was put

on the center of the sawdust medium in the same method as the agar medium described above and allowed to incubate for 10 days at 25°C in the dark. The diameter of the mycelial growth was measured.

Harvesting of the fruit body of *A. polytricha* from sawdust media supplemented with Ca or Mg salts Sawdust medium supplemented with 1, 5 or 10% of Ca carbonate or Ca phosphate, or 0.5% of Mg salts was prepared as described above. A heat-resistant polypropylene culture bag containing approximately 800 g of the medium was autoclaved at 120°C (1.0 kg/cm²) for 120 min. After cooling for overnight, it was inoculated with 10 to 14 g of *A. polytricha* mushroom seed and incubated at 25°C.

Approximately 80 days after the inoculation, the mycelia spread thoroughly over the medium. Then, the bag was opened, the culture was sprinkled with water once a day to keep it moist and to maintain the relative humidity at about 90%. The temperature during the active fruiting period was kept between 25 and 28°C. The fruit body of *A. polytricha* were harvested and dried in a forced air-oven at 60–70°C for 70 h. A homogeneous sample was prepared by grinding the dried fruit body using a stainless steel mill.

Analysis of Ca and Mg contents in fruit body Ca and Mg were analyzed as described previously (Tabata & Shinohara 1995). Silicic acid was removed from the ashed samples by HCl digestion. Atomic absorption spectrophotometry (Shimadzu AA6400F) was used to determine the elements, adding SrCl₂ and employing a nitrous oxide-acetylene flame.

Analysis of Ca and Mg contents in sawdust medium One gram of a sawdust medium placed in a quartz crucible was calcinated carefully using a Bunsen burner and kept at 550°C in a muffle furnace for 5 h. Ca and Mg were determined as described above. It was found that 100 g of the sawdust medium described above (wet weight) contained 64.4 mg of Ca and 82.1 mg of Mg. Ca and Mg contents in sawdust used in this experiment were found to be 279 and 35 mg/100 g (dry weight), respectively.

Results and Discussion

The mycelial growth of *A. polytricha* was studied supplementing a Ca or Mg salt to PSA and sawdust medium. The absorption of these elements to the fruit bodies was studied growing the mushroom on sawdust medium supplemented with the Ca or Mg salts.

Fig. 1. Mycelial growth of *A. polytricha* on PSA supplemented with Ca salts. Marks in the Figure are as follows; ■, no addition; □, addition of 1%; ▨, addition of 5%; ▩, addition of 10%. Standard deviations (mm): none 1.1; Ca phosphate 1% 0.5, 5% 1.0, 10% 0.6; Ca sulfate 1% 2.3, 5% 1.3, 10% 2.0; Ca carbonate 1% 0.6, 5% 0.3, 10% 0.3; Ca chloride 1% 0.4.

Fig. 2. Mycelial growth of *A. polytricha* on PSA supplemented with Mg salts. * Marks in the Figure are as follows: ■, no addition; □, addition of 0.5%; ▨, addition of 1.0%; ▩, addition of 5%. Standard deviations (mm): none 0.9. Mg sulfate 0.5% 0.6, 1% 0.3, 5% 0.4; Mg chloride 0.5% 0.5, 1% 0.4; Mg carbonate 0.5% 0.7, 1% 0.5, 5% 0.4; Mg hydroxide 0.5% 0.2.

Effect of Ca salts on the mycelial growth in the PSA Figure 1 shows the effect of diverse Ca salts on the mycelial growth on the PSA. The mycelia grew very well on PSA medium supplemented by Ca phosphate and Ca sulfate. However, the growth was suppressed remarkably when supplemented with Ca carbonate at all of the three concentrations and completely by Ca chloride at 5 and 10%.

The supplementation with 1% a chloride slightly suppressed the mycelial growth, however, 5 and 10% additions did not allow any growth. Therefore, chloride ion exerts considerably high toxicity on mycelial growth of *A. polytricha*.

Effect of Mg salts on the mycelial growth on the PSA Figure 2 shows the effect of the mycelial growth on the supplementation with 0.5, 1, and 5% of Mg salts to agar medium. The supplementation of 0.5% Mg sulfate and Mg chloride significantly increased the growth compared with non supplement. However, the growth was decreased critically by the supplementation with 5% Mg chloride. It is assumed that Mg chloride is a considerably toxic substance to *A. polytricha* at 1% or more.

The growth was depressed remarkably by Mg carbonate and Mg hydroxide. Moreover, supplementation of 1% and 5% Mg hydroxide did not allow any growth.

Effect of Ca salts on the mycelial growth on the sawdust media Figure 3 shows the effects of supplementation with 1, 5, and 10% of Ca salts on the mycelial growth in mm. The addition of Ca phosphate did not affect the growth while Ca sulfate and Ca chloride remarkably suppressed it.

On the other hand, the mycelial growth was considerably increased with the addition of Ca carbonate. Previously it was found that the sawdust medium containing Ca carbonate showed notably lower initial pH values than that of the corresponding PSA (Tabata & Shinohara, 1995). The observed promotion suggests that the acidic wood extracts having toxicity on fungi in a sawdust medium, such as fatty acids, aromatic compounds and terpenoids (Migita *et al.*, 1968) were neutralized and converted to nontoxic compounds by supplementation with Ca carbonate. Therefore, Ca carbonate supplementation might be recommendable when softwood sawdust is used for mushroom culture. In fact, the advantage of the supplementation of a sawdust medium with Ca carbonate has been widely recognized in the culture of mushrooms (Balasubramanyam, 1988; Tripathi *et al.*, 1993; Sanchez & Royse, 2001).

Effect of Mg salts on mycelial growth in the sawdust medium Figure 4 shows the effect of the supplementation of the sawdust medium with 0.5, 1, and 5% of Mg salts on the growth of mycelia. In contrast to the control, the supplementation with 0.5% Mg carbonate and Mg hydroxide increased the mycelial growth. However, supplementation of 5% Mg carbonate and Mg hydroxide did not allow any growth.

Effect of pH in the medium on mycelial growth Table 1 shows the pH values before and after incubation of *A. polytricha* on sawdust medium supplemented with Ca salts. On the 14th day, the media were completely covered by mycelia, when the pH decreased by 0.3–1.5 units from the initial. These decreases might be responsible for organic acids produced in the vegetative mycelia. Yoshida *et al.* (1996) showed that malic acid was the predominant organic acid detected in the vegetative mycelia.

The pH of the medium supplemented with Mg salts before and after the incubation is shown in Table 2. Most of the initial pH values are from 5.3–6.8 except for the higher values for 5% supplementations of Mg carbonate and hydroxide, which critically inhibited the mycelial growth. The pH of media lowered by 0.4–1.2 units on the 14th day of the cultivation except for the 5% supplementations of Mg carbonate and hydroxide.

Kinjo and Kondo (1979) found that the optimum pH of *A. polytricha* growth is 7–8 and that the mycelia grew over 50%

even in lower medium pH 4. On the other hand, the two highest pH were found in the medium supplemented by 5% Mg hydroxide and carbonate, 8.83 and 7.43, respectively. The media of these pH, at least that of the latter, must be the optimum condition; however, the growth was completely inhibited in them.

Consequently, it is concluded that the relatively high pH of media supplemented with these salts is not responsible for the growth inhibition. However, further study is awaited to understand the toxicity of the salts.

Absorption of Ca by the fruit body Based on the mycelial growth experiments on the Ca-supplemented sawdust medium, the efficiencies of Ca accumulation in the fruit body were studied. As Table 3 shows, only 114 mg of Ca in 100 g of dried mushroom was found in the mushroom cultivated on a nonsupplemented sawdust medium. Supplementation with 1–10% Ca carbonate or Ca phosphate increased the Ca content in the fruit-body 1.1–1.5 times, attaining 130–173 mg/100 g. The supplementation with 5% Ca carbonate or 1% Ca phosphate resulted in the highest Ca content.

Absorption of Mg by the fruit body The Mg contents in the fruit body are shown in Table 3. The supplementation with

Fig. 3. Mycelial growth of *A. polytricha* on sawdust media supplemented with Ca salts. Marks in figure were the same as those in Fig. 1. Standard deviations (mm): none 0.5; Ca phosphate 1% 1.2, 5% 2.5, 10% 2.1; Ca sulfate 1% 2.3, 5% 2.1, 10% 1.6; Ca carbonate 1% 0.9, 5% 0.4, 10% 0.7; Ca chloride 1% 3.7.

Fig. 4. Mycelial growth of *A. polytricha* on sawdust media supplemented with Mg salts. Marks in Figure were the same as those in Fig. 2. Standard deviations (mm): none 1.0; Mg sulfate 0.5% 1.7, 1% 1.7, 5% 2.3; Mg chloride 0.5% 1.0, 1% 1.7, 5% 1.4; Mg carbonate 0.5% 1.4, 1% 2.4%; Mg hydroxide 0.5% 1.6, 1% 1.3.

Table 1. pH Value before and after incubation of *A. polytricha* on sawdust media supplemented with Ca salts.

Salts	Supplementation			
	0	1%	5%	10%
	5.50 (4.73)			
Ca phosphate		5.56 (4.87)	5.55 (4.93)	5.51 (4.97)
Ca sulfate		5.39 (4.81)	5.05 (4.63)	4.94 (4.59)
Ca carbonate		6.17 (5.01)	6.64 (5.15)	6.78 (5.42)
Ca chloride		5.23 (4.89)	4.69 (4.70) ^{a)}	4.52 (4.52) ^{a)}

The initial and final pH are shown without and in parenthesis, respectively. The sawdust media were incubated at 25°C under 85% humidity in the dark for 14 days to allow full colonization of mycelia.

^{a)} Mycelial growth was critically inhibited.

Table 2. pH Value before and after incubation of *A. polytricha* on sawdust media supplemented with Mg salts.

Salts	Supplementation			
	0	0.5%	1%	5%
	5.54 (4.78)			
Mg sulfate		5.59 (4.82)	5.56 (4.84)	5.35 (4.79)
Mg chloride		5.63 (4.82)	5.53 (4.78)	5.27 (4.86)
Mg carbonate		6.25 (5.19)	6.39 (5.37)	7.43 (7.40) ^{a)}
Mg hydroxide		6.40 (5.33)	6.83 (5.67)	8.83 (8.70) ^{a)}

Number and mark in Table are the same as those in Table 1.

Table 3. Ca contents in fruit body of *A. polytricha* harvested from the sawdust medium supplemented with Ca carbonate and Ca phosphate.

Compound	Supplementation (%)	Ca content (mg/100 g, dry weight)	Absorption ratio ^{b)}
Ca carbonate	1	142.3 ± 2.7 ^{a)}	1.2
	5	172.1 ± 3.8 ^{a)}	1.5
	10	114.4 ± 3.1 ^{a)}	1
Ca phosphate	1	173.3 ± 5.4 ^{a)}	1.5
	5	130.2 ± 1.2 ^{a)}	1.1
Control	0	114.3 ± 5.3 ^{a)}	—

^{a)} Standard deviation.

^{b)} The absorption ratio was obtained by dividing the calcium content of fruit body from supplemented medium with that harvested from control.

Table 4. Mg contents in fruit body of *A. polytricha* harvested from the sawdust medium supplemented with 0.5% of Mg salts.

Compound	Ca content (mg/100 g, dry weight)	Absorption ratio
Mg sulfate	222.2±5.4 ^a	1.8
Mg chloride	280.4±6.2 ^a	2.2
Mg carbonate	210.1±4.1 ^a	1.7
Mg hydroxide	238.3±4.8 ^a	1.9
Control	125.1±5.0	—

^aStandard deviation.

Mg sulfate, Mg chloride, Mg carbonate and Mg hydroxide increased the Mg content in the fruit-body 1.7–2.2 times, attaining 210–280 mg/100 g. In particular, the supplementation with Mg chloride resulted in the highest Mg content.

It is well documented that rice bran has a high Mg content (Idouraine *et al.*, 1995; Claye *et al.*, 1998). Idouraine *et al.* (1996) reported that the defatted rice bran they studied contained 580 and 120 mg/100 g of Mg and Ca, respectively. The Mg and Ca contents in Japanese rice bran are reported to be 1000 and 46 mg/100 g, respectively (Kagaku Gijutsu-cho, 1995). We found that the sawdust medium employed in this study had 75.1 mg of Ca and 95.8 mg of Mg in 100 g of the medium (wet weight). Therefore, a considerable amount of Ca and Mg was already in the culture medium before the supplementations. However, it is known that Ca and Mg in rice bran are a form of hardly soluble phytin (Hayakawa & Igaue, 1992; Sugahara *et al.*, 1994) and are difficult to be absorbed. From this experimental result, it is considered that the absorption of Ca or Mg from the medium is closely related to the type and concentration of supplemented diverse Ca and Mg salts.

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