African Journal of Biotechnology Vol. 9(43), pp. 7379-7382, 25 October, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1085
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Breaking the spores of *Ganoderma lucidum* by fermentation with *Lactobacillus plantarum*

Chaiyavat Chaiyasut*, Chakkrapong Kruatama and Sasithorn Sirilun

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiangmai University, Thailand.

Accepted 30 September, 2010

***Ganoderma lucidum* has been utilized in medical field from ancient time. In recent studies, it is found that spores of *G. lucidum* consists of several bioactive substances which are much abundant than in the fruiting body. A hard sporoderm limits the absorption of these active compounds. Therefore, it is necessary to break down the sporoderm to allow complete absorption. The spore breaking methods which have been developed are physical smashing, ultrasonic, high pressure and enzymes application. A few limitations have come forward; they are low yield, high cost, high technology tools requirement and loss valuable substances during production. In this paper, fermentation of *G. lucidum* with *Lactobacillus plantarum* was applied to break down the sporoderm. Scanning electron microscope (SEM) was used to characterize the spores. The broken spores were found on the 3rd day and complete breaking on the 5th day of fermentation. Lactic acid, acetic acid and combination of these acids did not cause the spore to break down. This technique is simple, less cost. It can be used to produce fermented juices with the benefit from *G. lucidum* spores and lactic acid bacteria. Mechanism of spore break down by lactic acid bacteria is further investigated.**

Key words: *Ganoderma lucidum*, break spore, *Lactobacillus plantarum*, lactic acid bacteria, fermentation.

INTRODUCTION

Ganoderma lucidum, a fungus in a group of Basidiomycetes, has been used as a Chinese medicine for over 2000 years (Ping et al., 2009) for promoting vitality and longevity. It has been used for prevention and treatment of various kinds of diseases such as diabetes, hypertension, asthma, tumorigenic disease, hypercholesterolemia, immunological disorders, AIDS and cancer (Wasser and Weis, 1999; Mohammed et al., 2007; Fu et al., 2009). It is also safe and does not reveal any toxic effects toward the body (Eo et al., 1999a, b).

The spores of *G. lucidum* were realized in medical fields during the 20th century due to the amount of bioactive substances of the spores that are much higher than in the fruiting body. Bioactive compounds such as polysaccharides, triterpenoids, sterols, proteins, nucleosides and fatty acid were found (Russell and Paterson, 2006). Recent studies on *G. lucidum* spores show significant anti-tumor activity (Zhu et al., 2000; Liu et al., 2002; Xie et al., 2006), anti-aging (Gan et al., 1998) and free radical-scavenging (Yen and Wu, 1999; Ping et al., 2009). These bioactive substances are protected by hard sporoderm (Jungjing et al., 2007). In order to completely absorb the bioactive substances within the spores, the sporoderm have to first break down (Jungjing et al., 2007). Therefore, processes in making spores break down have been developed such as physical smashing, ultrasonic, high pressure and enzyme utilization. However, high temperature operation in physical smashing causes the decomposition of bioactive substances. The methods of ultrasonic and high pressure have low broken spore yield with high expense and requirement of specific equipment (Fu et al., 2009). As for method of using different enzymes to decompose spores' outer wall, the process is expensive and will also have effects on decomposition of important bioactive substance (Fu et al., 2009).

From the aforementioned limitations, the investigators had developed new spore breaking method which is simple, low cost and no need for expensive tools.

*Corresponding author. E-mail: chaiyavat@gmail.com. Tel: +6653944340. Fax: +6653894163.

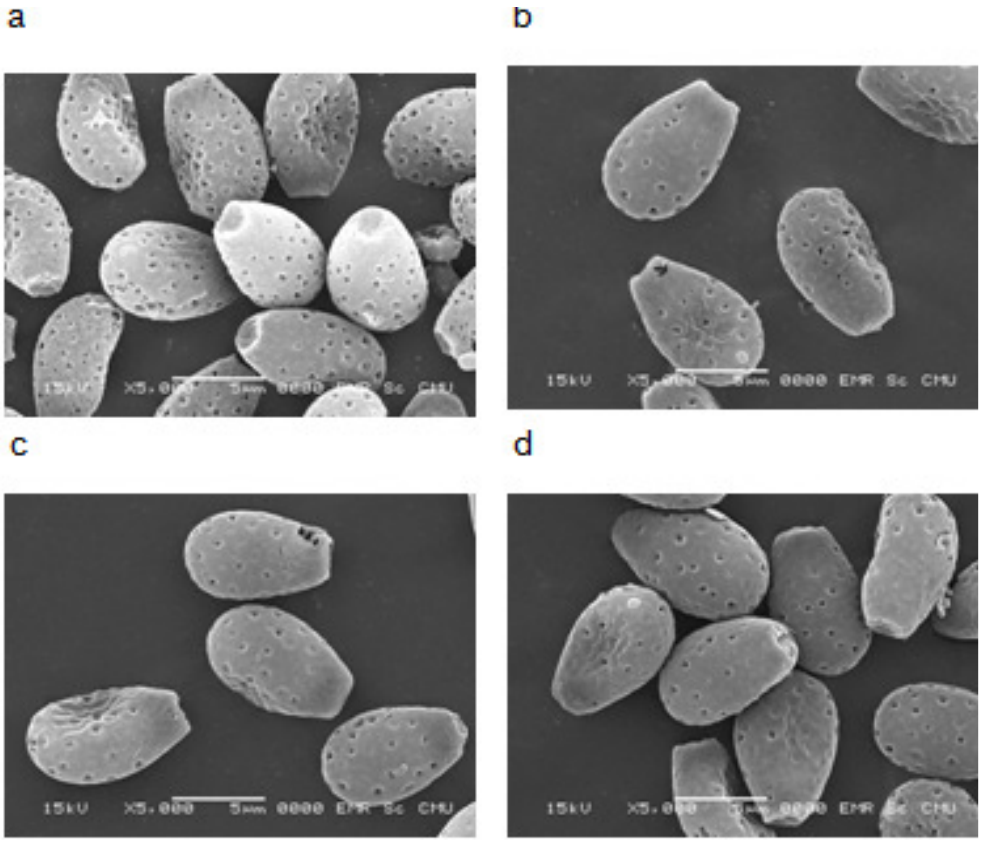

Figure 1. SEM images of *G. lucidum* spores at day 7. (a) Incubated with MRS broth. (b) Incubated with lactic acid (3.5%). (c) Incubated with acetic acid (1.5%) (d) Incubated with lactic acid (3.5%) and acetic acid (1.5%).

Fermentation of *G. lucidum* spores with lactic acid bacteria were investigated in order to break down the spores of *G. lucidum.*

MATERIALS AND METHODS

The spores of *G. lucidum* L6 were received from Huai Hong Khrai Royal Development Study Center, Chiang Mai city, Thailand.

Fermentation of *G. lucidum* with lactic acid bacteria

1.0 g spores of *G. lucidum* and 2% pasteurized DE MAN, ROGOSA and SHARPE (MRS) broth media were fermented with *Lactobacillus plantarum* 10% (about $1x10^8$ CFU/ml) in incubator at 37°C. The samples were collected at day 0, 3, 5 and 7, respectively. Whereas, control is spores in MRS broth without *L. plantarum* at 37°C. The samples were then filtered using Whatman paper No.1 and dried at 60°C for 3 h.

Effect of organic acid on *G. lucidum* spores

1.0 g spores of *G. lucidum* was fermented with lactic acid at concentration 0.5, 1.0, 2.0 and 3.5% in 2% pasteurized MRS broth 50 ml in incubator at 37°C. Acetic acid at concentration 0.5, 1.0, 1.5 and 2.0% and combination of lactic acid and acetic acid at the mentioned concentration were also studied. The samples were collected at day 0, 3, 5 and 7, respectively.

Characterization of broken Ganoderma spores

The morphological change of the spores of *G. lucidum* during fermentation period was observed by a scanning electron microscope (SEM). The samples were prepared by filtering through Whatman paper no 1. The spores, which remained on filter paper, were dried at 60°C for 3 h. The observation was performed by mounting spores on aluminium stub, coated with gold-palladium in SPI module™ carbon coater for 1 min and viewed at 1000, 5000 and 10000×, respectively.

RESULTS AND DISCUSSION

SEM images of *G. lucidum* spores without *L. plantarum* (control) and fermented spores with *L. plantarum* are shown in Figures 1 and 2, respectively. On 3rd day as in Figure 2b, partial broken spores of *G. lucidum* were observed and complete broken spores were achieved on 5th day (Figure 2c) and 7th day (Figure 2d). The result indicated that the fermentation of *G. lucidum* spores with *L. plantarum* had contributed to break the spore with higher decomposition along the fermentation time.

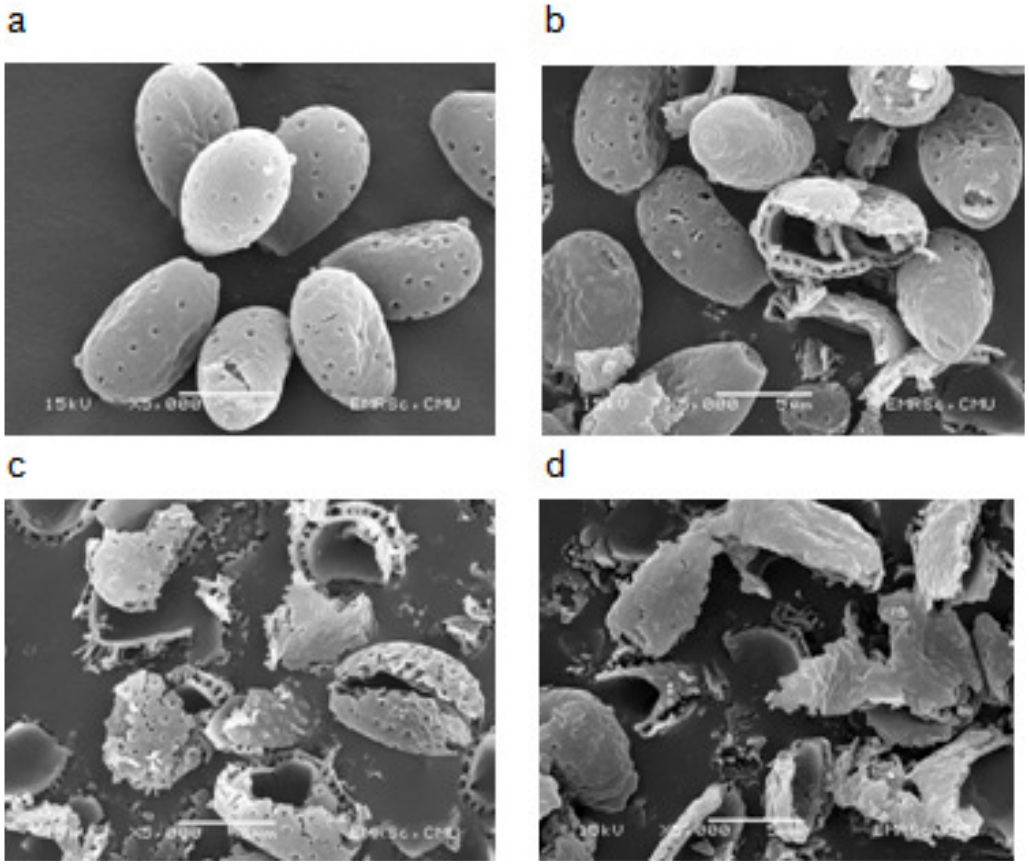

Figure 2. SEM images of fermented *G. lucidum* spores with *Lactobacillus plantarum* at: (a) day 0 (b) day 3 (c) day 5 (d) day 7.

Whereas, no broken spores of *G. lucidum* was observed when they were incubated with MRS broth media as shown in Figure 1a. The SEM images of incubated *G. lucidum* spores with lactic acid, acetic acid and combination of both acids at studied concentration did not show any broken spores during the studied period (Figures 1b - d). These acids could not break the spores. The acid concentrations that used were concentrations of occurred lactic acid and acetic acid during fermentation process of lactic acid bacteria at day 0, 3, 5 and 7, respectively. Sporoderm of *G. lucidum* consisted of silica (19.01%), calcium (19.01%) and chitin (52.08 - 57.64%) (Jungjing et al., 2007). This could make sporoderm resistant to acid. Therefore, some other metabolites present, rather than organic acid, during the fermentation of *L. plantarum* had contributed to the splitting of the *G. lucidum* spores. The pH value, within the range of 3.5 - 7.0, did not degrade the ganoderic acid and polysaccharide of *G. lucidum* (Fang and Zhong, 2002). Therefore, fermentation pH in this experiment, within the range of 4.0 - 5.8, would have less effect on the stability of ganoderic acid and polysaccharide, which are the main active bio-substances of *G. lucidum*.

Fermentation with lactic acid bacteria, *L. plantarum*, has shown the efficiency in encouraging *G. lucidum* spores to break down within 3 to 7 days at 37°C. The fermentation is a simple process with low cost. Expensive tools with high technology and harmful chemicals are not required. This is safe towards the consumption without any purification steps. Aside from the benefits of bioactive substances from *G. lucidum* spores, consumption of this fermented product will gain more benefits from selected lactic acid bacteria in terms of probiotics. Further study is to investigate the mechanism and the metabolite that take responsibility for the broken process of the *G. lucidum* spores.

ACKNOWLEDGEMENTS

We are thankful to Mr. Sutad Pintasen from Huai Hong Khrai Royal Development Study Center for *G. lucidum* spores.

REFERENCES

Eo SK, Kim YS, Lee CK, Han SS (1999a). Antiviral activities of various water and methanol soluble substances isolated from *Ganoderma*

lucidum. J. Ethnopharmacol. 68: 129-136.
Eo SK, Kim YS, Lee CK, Han SS (1999b). Antiherpetic activities of various protein bound polysaccharides isolated from *Ganoderma lucidum*. J. Ethnopharmacol. 68: 175-181.
Fang QH, Zhong JJ (2002). Effect of pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. Process Biochem. 37: 769-774.
Fu YJ, Liu W, Shi XG, Liu ZG, Schwarz G, Thomas E (2009). Breaking the spores of the fungus *Ganoderma lucidum* by supercritical CO_2. Food Chem. 112: 71-76.
Gan KH, Fann YF, Hsu SH, Kuo KW, Lin CN (1998). Mediation of the cytotoxicity of lanostanoids and steroids of *Ganoderma tsugae* through apoptosis and cell cycle. J. Nat. Prod. 61: 485-487.
Jungjing MA, Zhengyi FU, Peiyan MA, Yanli SU, Qingjie Z (2007). Breaking and characteristics of *Ganoderma lucidum* spores by high speed centrifugal shearing pulverizer. J. Wuhan Univ. Tech-Mater. Sci. Ed. 22: 617-621
Liu X, Yuan JP, Chung KC, Chen XJ (2002). Antitumor activity of the sporoderm-broken germinating spores of *Ganoderma lucidum*. Cancer Lett. 182: 155-161.
Mohammed A, Adelaiye AB, Abubakar MS, Abdurahman EM (2007). Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of normoglycemic and alloxan-induced diabetic wistar rats. J. Med. Plant Res. 1(2): 34-37.
Ping CX, Yan C, Bing LS, Guo CY, Yun LJ, Ping LL (2009). Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats. Carbohydr. Polym. 77: 389-393.
Russell A, Paterson M (2006). Ganoderma-A therapeutic fungal biofactory. Phytochemistry, 67: 1985-2001.
Wasser SP, Weis A (1999). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: Current perspectives (Review). Int. J. Med. Mushroom, 1: 31-62.
Xie YZ, Li SZ, Yee A, La Pierre DP, Deng Z, Lee DY, Wu QP, Chen Q, Li C, Zhang Z, Guo J, Jiang Z, Yang BB (2006). *Ganoderma lucidum* inhibits tumor cell proliferation and induces tumour cell death. Enzyme Microb. Technol. 40: 177-185.
Yen GC, Wu JY (1999). Antioxidant and radical scavenging properties of extracts from *Ganoderma tsugae*. Food Chem. 65: 375-379.
Zhu HS, Yang XL, Wang LB, Zhao DX, Chen L (2000). Effects of extracts from sporoderm-broken spores of *Ganoderma lucidum* on HeLa cells. Cell Biol. Toxicol. 16: 201-206.