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# Expression analysis of glucan synthase in edible mushrooms *Lentinula edodes* and *Pleurotus eryngii* and their potential for wound-healing applications

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## Abstract

**Mushrooms are valued in traditional medicine for their healing properties. In particular, β-glucans found in edible fungi, grains, and yeast have been demonstrated to promote wound healing. In this study, we aim to characterize the expression of glucan synthase in *Lentinula edodes* (shiitake) and *Pleurotus eryngii* (eryngii), as well as the expression in organic and conventionally-raised shiitake. Through semi-quantitative RT-PCR, we found that organic shiitake showed the highest expression of glucan synthase, followed by conventionally-raised eryngii and shiitake. Our preliminary findings suggest that organic shiitake has the highest potential for wound healing applications.**

*Keywords: Lentinula edodes, Pleurotus eryngii, mushroom, wound healing*

## Introduction

Mushrooms, known for their umami (うま味), are an integral part of culture and cuisine. Some mushrooms have been used as an herbal medicine for decades. It has, for example, been used for a long time in China and Japan to strengthen the immune system. While most people are aware that mushrooms are valuable food for their low calories, carbohydrates, salt, and fat, many people are still unaware of the important role that mushrooms can play.

Mushrooms are important in traditional medicines although their concept of action is not fully understood. Many studies showed that various edible mushrooms have been reported to generate immunomodulatory and antineoplastic properties (Finimundy et al. 2014, Bożena et al. 2017, Fang et al. 2012, Ding et al. 2016). Studies have shown that certain chemicals in edible fungi have beneficial effects on cell activity and on the secondary production of chemical compounds that can strengthen the human immune system and promote the treatment of several diseases (Borchers et al. 2004).

In particular, shiitake and eryngii are found to have beneficial effects purported in other species. *Lentinula edodes*, commonly called shiitake (椎茸, シイタケ), is one of the most studied edible mushrooms. Studies have demonstrated the antitumor, antifungal, antibacterial, anti-inflammatory effects of substances in L. edodes (Bożena et al. 2017). On the other hand, *Pleurotus eryngii*, also known as king oyster mushroom, is one of the less-studied edible mushrooms. Studies conducted suggest that eryngii may contain substances that activate the immune system (Alam et al. 2011). Furthermore, studies show that extracts from *Pleurotus eryngii* have an effect on anticancer activity, immuno-stimulating activity, and antiviral activity (Fu et al. 2016). It also contains various compounds that have benefits in the treatment of different

diseases. Both shiitake and eryngii give a variety of health benefits, though their molecular mechanisms are yet to be fully elucidated.

$\beta$ -glucans are carbohydrate polymers present in the cell walls of several species, such as microbes, fungi, yeasts, and certain cereals such as barley and oat (Jurikova et al. 2009). They are among the most prevalent polysaccharides that activate the human immune system, protect against pathogenic bacteria, adverse effects of environmental chemicals, and carcinogens that have impaired immune systems. They also guard against infectious diseases and tumors and help people recover from chemotherapy and radiotherapy (Valverde et al. 2015).

Along with the antitumor, antifungal, and antibacterial effects, β-glucan possesses wound-healing effects (Majtan et al. 2018). In the course of wound healing, the human body undergoes complex processes such as haemostasis, inflammation, proliferation, and remodeling. Inflammation provides resistance to microbial infection; it happens nearly simultaneously with haemostasis. Failed management of any specific mechanism results in pathologically impaired wound healing, such as chronic wounds marked by protracted or excessive inflammatory phases, recurring infections, and delayed wound contraction (Yasuda et al. 2018).

Properties of β-glucan may be effectively used to accelerate the wound healing process. Specifically, β-glucan triggers immune and non-immune pathways, which in turn promote collagen deposition and re-epithelialization (Majtan et al. 2018).
Additionally, β-glucan has been shown to have wound-healing properties in vitro. β-glucan with varying physical and chemical characters are found to be potent inducers of wound closure, greatly influencing the migration and proliferation of cells involved in wound healing through many studies (Majtan et al. 2018). It has been used in the production of bioartificial skin due to its ability to bind to gelatin and collagen. It is known that β-glucan wound dressing enhances wound healing and decreases pain (Seo et al. 2019).

Here, we aim to characterize the expression of glucan synthase in *Lentinula edodes* (shiitake) and *Pleurotus eryngii* (eryngii), as well as the expression in organic and conventionally-raised shiitake.

## Methods

*Primer design*

We found the sequences of the housekeeping gene (18S) of *Lentinula edodes* and *Pleurotus eryngii* from (Yoo et al. 2019) and GenBank FJ572254.1, respectively. The sequence of β-1,3 glucan synthase in shiitake was obtained using transcript ID: 8225 from reference genome W1-26 and transcript ID: 1439750 from P. eryngii ATCC 90797 for eryngii. In addition to the 18S primers from (Yoo et al. 2019), the primers were designed using Primer3 with an annealing temperature of 58C and 175-225 base pair amplicon as conditions.

TABLE 1: List of primers used

| Primers | Sequence | Amplicon size(bp) |
|---|---|---|
| **18S (housekeeping gene):** | | |
| Shiitake18s_1F<br>Shiitake18s_1R | gcgctacactgacagagcca<br>gcggtgtgtacaaagggcag | 178 |
| Shiitake18s_2F<br>Shiitake18s_2R | agggctctttcgggtcttat<br>cagtcagacagtacacaccg | 201 |
| Eryngii18s_1F<br>Eryngii18s_1R | gtgcacgcttcactagtctt<br>gagagccaagagatccgttg | 222 |
| Eryngii18s_2F<br>Eryngii18s_2R | caacggatctcttggctctc<br>ccccaacaatccaaacatcac | 200 |
| **Glucan Synthase** | | |
| Shiitake_3F<br>Shiitake_3R | tacctcgaggaatgtctaaag<br>attggtaatactccgaatgttg | 366 |
| Eryngii_3F<br>Eryngii_3R | ctcggaggaatgcttgaaga<br>actgataatattccgagtgct | 361 |

To address the primer inefficiency, additional sets of primers of *Lentinula edodes* were found from (Reverberi et al. 2004). Partial sequences of shiitake and eryngii were obtained from Genbank AY158742.1 and AY254580.1, respectively. The sequence found was aligned using the blastn algorithm in BLAST. Other additional primers were designed using primer3 with the same conditions. The base pair length of *Lentinula edodes* β-1,3 glucan synthase and Pleurotus eyrngii β-1,3 glucan synthase is 420 bp and 483 bp respectively.

*Sample collection*

Fresh packs of different types of *Lentinula edodes* and *Pleurotus eryngii* were collected- shiitake, organic shiitake, and eryngii. The mushrooms were purchased at local supermarkets: the conventionally-raised shiitake were from the My Basket in Nishiazabu, Tokyo, and the organic shiitake and eryngii were from the Peacock store in Hamacho, Tokyo. Three biological replicates were taken from each pack.

*RNA isolation and cDNA synthesis*

RNA was isolated using the RNA Plant and Fungi isolation kit (Takara Bio Cat. #: U0949B) as described with minor modifications. Mushroom tissues were homogenized using a mortar and pestle without liquid nitrogen. Ethanol was substituted with isopropanol for the precipitation of RNA. We used 30µlof rDNAse in a buffered solution from the RNA Plant kit to digest the DNA on the column after filtration. 1st strand cDNA synthesized using PrimerScript 1st strand cDNA Synthesis Kit as described (Cat. # 6110A).

*Semi-quantitative gene expression analysis using RT-PCR*

PCR and gel electrophoresis was conducted using the mini16 thermal cycler and bluGel electrophoresis unit from MiniPCR. (QP-1500-01, QP-1016-01)

An amplification curve was created to determine the optimum number of cycles before RT-PCR. Table 1 shows the sequence of the primers used. All primers had a concentration of 10 uM. PCR conditions were as follows: 98℃ for 2 minutes of initial denaturation, 98℃ for 15 seconds, 60℃ for 30 seconds, and 72℃ for 1 minute, repeated 24 cycles. Reactions were as follows: 12.5µlEmeraldAmp 2X Master mix, 1µl of cDNA, 0.5µl of forward primer, 0.5µlof reverse primer, and 11µl of dH2O. Gel electrophoresis was used for semi-quantitative analysis. Band size was quantified from RAW photos of gels using the ImageJ Bio-Formats plug-in.

TABLE 2: RNA quality for each biological replicate. Samples S1, SO-1, SO-3, and E-2 were used for analysis

| RNA isolation | | | | |
|---|---|---|---|---|
| Sample | A260 | A280 | A260/A280 | Concentration of RNA (ng/l) |
| Shiitake-1 | 0.086 | 0.047 | 1.829 | 137.6 |
| S-2 | 0.067 | 0.05 | 1.34 | 107.2 |
| S-3 | 0.036 | 0.029 | 1.241 | 57.6 |
| Shiitake organic-1 | 0.155 | 0.075 | 2.066 | 248 |
| SO-2 | 0.009 | 0.011 | 0.818 | 14.4 |
| SO-3 | 0.108 | 0.006 | 18 | 172.8 |
| Eryngii-1 | 0.042 | 0.018 | 2.333 | 67.2 |
| E-2 | 0.265 | 0.082 | 3.231 | 424 |
| E-3 | 0.329 | 0.169 | 1.946 | 526.4 |

## Results and Discussion

Based on the results of semi-quantitative RT-PCR, we found that organic shiitake showed the highest expression of glucan synthase, followed by eryngii and conventional shiitake (Figure 1). Organic Shiitake sample 3 showed the highest expression of glucan synthase- 156.1% of the expression of housekeeping gene 18S- followed by Organic Shiitake sample 1 with 69.8%, Erygnii sample 2 with 31.7%, and Conventional Shiitake sample 1

with 0%. There was no glucan synthase expression detected for conventional shiitake although the housekeeping gene was present.

TABLE 3: RNA quality for each biological replicate. Samples: Conventional Shiitake Sample 1, Organic Shiitake Sample 1, Organic Shiitake Sample 3, and Conventional Eryngii Sample 2 were used for analysis.

| Sample | Normalized expression of glucan synthase (% of 18S expression) |
|---|---|
| Conventional Shiitake Sample 1 | 0 |
| Organic Shiitake Sample 1 | 69.8 |
| Organic Shiitake Sample 3 | 156.1 |
| Conventional Eryngii Sample 2 | 31.7 |

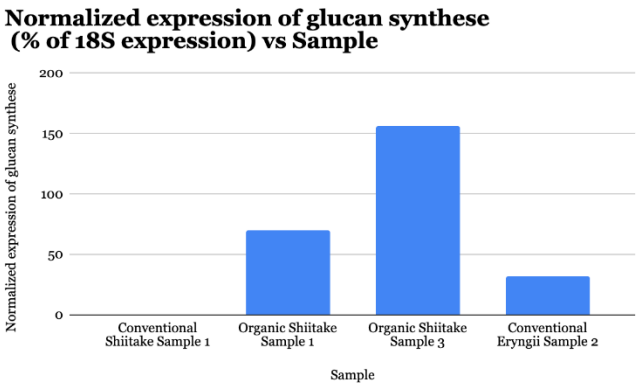


FIGURE 1: Normalized expression of glucan synthase in each sample relative to 18S expression (internal control).

Although this research could not determine the reason, there may be several possible explanations as to why conventional shiitake did not show any glucan synthase expression.
First, the difference in fertilizers or pesticides used between conventional and organic shiitake may affect the amount of glucan synthase being expressed in mature fruiting bodies (Brunner and Freed, 1994). However, mushroom cultivation practices were beyond the scope of this study. Future experiments should include more biological and technical replicates to more fully characterize the possible effect. Second, since the expression of glucan synthase is highest for organic shiitake, it could be the most useful for wound healing compared to the other samples tested. Furthermore, the effect of the expression of glucan synthase in wound-healing could be tested on animal models and synthetic skin (Bożena et al., 2017).

Additional testing is required to determine whether the RNA quality was a response to the lack of glucan synthase expression.
Also, it was tough finding and designing the primers; several primer pairs were ineffective. The primer efficiency of shiitake and eryngii should be assessed moving forward. If our hypothesis is truly supported by the data- that conventional shiitake does express less glucan synthase- it represents a novel insight into the differences between organic and conventional shiitake.

Even though this is preliminary research, we hope that our work can facilitate a greater understanding of glucan synthase expression found in edible fungi.

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