

## **β-glucan synthase gene expression in *Pleurotus sp.***

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### **Abstract**

*Pleurotus sp.* is a popular edible mushroom, containing various functional component, in particular, β-glucan. β-glucans is a part of glucan family of polysaccharides and supposedly contribute to medicinal and nutritional value of *Pleurotus.sp.* In order to understand the distribution of β-glucan in *Pleurotus.sp.*, the β-glucan synthase gene expression was determined and compared in different part of *Pleurotus*, namely mycelium, stipe and cap. The *Pleurotus.sp* RNA was extracted using commercial kit, employing Tissuelyser II (Qiagen, USA) to disrupt the cell walls. Then the RNA was quantified by Nanodrop (ThermoFisher, USA) and visualized using denaturing agarose gel. RNA with good OD 260/280 reading ( $\approx 2.0$ ) was chosen and converted to cDNA. Using Laccase synthase gene as homekeeping gene, β-glucan synthase gene expression was quantified using CFX 96 Real Time PCR detection system (Biorad, USA). Preliminary result shows that β-glucan synthase was relatively expressed the most in stipe, followed by mycelium and barely in cap.

Katakunci/keyword : gene expression, Beta Glucan, cloning

## Introduction

Edible mushroom was long used by human for its nutritional and medicinal value (Manzi and Pizzoferrato, 1999). One of the functional part of mushroom that responsible for its valuable properties is  $\beta$ -glucan. Together with chitin,  $\beta$ -glucan is a part of polysaccharide that form mushroom cell walls (Rop et al, 2009).

B-glucan synthase is a group of glucosyltransferase enzyme that involve in generation of  $\beta$ -glucan in fungi (Mio et al, 1997). This property makes  $\beta$ -glucan synthase a suitable target for monitoring and quantification of  $\beta$ -glucan expression in mushroom.

## Method

The edible mushrooms were collected fresh and its fruiting body was separated into caps and stipes. A bit of fruiting body was plated on standard potato dextrose agar (PDA) plates for mycelium growing and strain maintaining. The plates was incubated at room temperature in dark.

Total RNA was extracted from both caps and stipes directly after sampling. As for mycelium, total RNA was extracted from third sub-cultured mycelium. The extraction was done using commercial kit, DNA, RNA and protein purification kit (Macherey-Nagel, Germany) following kit manufacturer protocol. The integrity of the extracted RNA was tested on denaturing agarose gel and further quantified using Nanodrop 2000 Spectrophotometer (Thermo Scientific, USA). Next, the extracted RNA was converted directly into cDNA using ProtoScript II, First Strand cDNA Synthesis kit (New England Biolabs, USA).

The BGS expression was monitored and analysed using qPCR method. The qPCR was run on CFX96 Real-Time PCR Detection System (Biorad, USA) with qBGS primer as target and Lacc3 primer as reference gene (Castanera et al, 2012). Biorads' iQ SYBR Green Supermix was used as reaction mastermix. Each reaction was contained 1x iQ SYBR Green Supermix, 100nM of both primers and 10ng of cDNA, topped to 20 $\mu$ l with nucleases free water. The amplification program was standard CFX 96 amplification and melting curve program with annealing temperature of 55°C. Fluorescent data was monitored and analysed by CFX 96 native program, Biorad CFX Manager.

## Result and discussion.

The extracted RNA was of good quality and integrity. This quality makes the extracted RNA suitable for reverse transcriptase into cDNA and subsequent qPCR analysis.

The samples were ATCC, CJ and Mutant-K. ATCC was reference samples, sourced from ATCC, USA. CJ was local, commercial strain and Mutant-K was mutated strain from our previous study. Using the relative expression analysis mode on Biorad CFX Manager, it was revealed that BGS expression was relatively higher in stipe compared to mycelium and cap. This trend is consistent across the samples as shown in Figure 1. From the figure, we also able to observe that the expression pattern in ATCC and CJ was similar compared to Mutant-K.

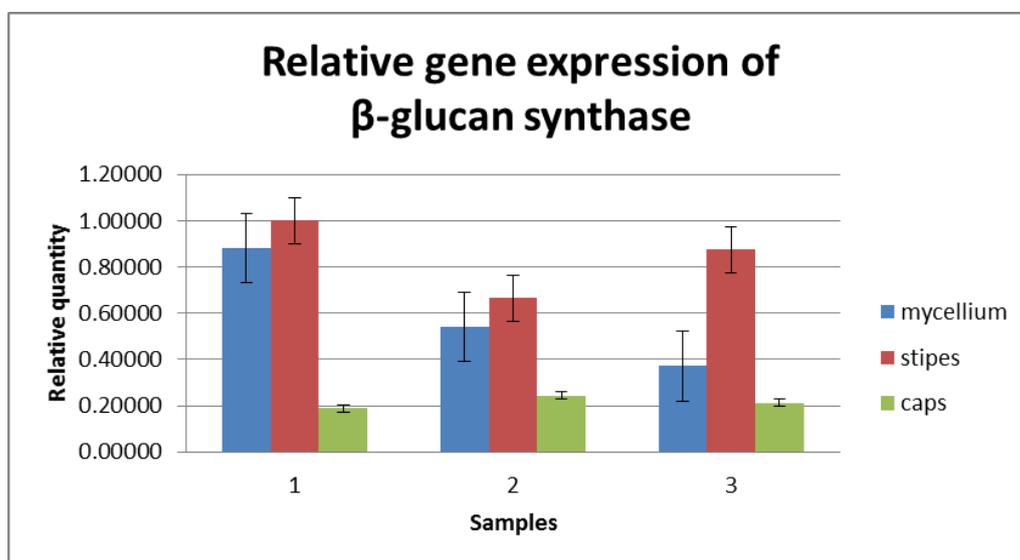


Figure 1. Relative BGS expression in selected samples. 1=ATCC, 2=CJ and 3=Mutant-K.

## Conclusion

B-glucan synthase gene was shown to express more in the stipes. This is due to its function in catalysing the generation of  $\beta$ -glucan, a part of polysaccharide that formed fungi cell walls. Stipe is a still-growing part of fruiting body, compared to cap that already matured, hence the needs for generation of  $\beta$ -glucan.

## References

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