Pilot-scale production of *Boletus colossus* culture for promoting the growth of Para rubber trees

W Dechmahitkul$^{1,*}$, K Khumvongsa$^2$ and P Mekvichitsaeng$^2$

$^1$Biochemical Research and Pilot Plant Development Unit (BEC) at KMUTT, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand

$^2$Pilot Plant Development and Training Institute (PDTI), King Mongkut’s University of Technology, Thonburi (KMUTT), Thailand

*E-mail: wairuj.dec@mail.kmutt.ac.th

**Abstract.** *Boletus colossus* is an Ectomycorrhiza fungi. It grows on roots of big trees such as Rubber tree, Mango tree and etc. This mushroom grows by using the organic materials released from the roots. The moisture adsorbed on the fungi makes the roots resist the drought better. The mushroom can also turn some insoluble inorganic materials into the form that plants can make use of. This research is to study the production of the starter culture by using low-cost media. The result shows that the fungi can grow best in whey to 9.2 g/L (dry weight) in 10 days. The liquid broth can inhibit the growth of pathogens (*Alternaria brassicola*, *Alternaria pori*, and *Fusarium solani*) on Potato-Dextrose agar. The starter culture can also be produced in a 50L fermenter to the dry weight of 8.1 g/L in 6 days. Loam is the best as solid carrier followed by peat moss. The result in one year shows that the Para rubber trees grow with the fungi that have significantly longer stems, leaves, and roots.

1. Introduction

Bolete mushroom (*Boletus colossus*) is Ectomycorrhiza fungi that usually grow on roots of big trees. The growth is symbiosis. The plant provides nutrients such as sugar, amino acids, vitamins to the fungi [1] and in return, the fungi decompose some insoluble materials into the form that plant can utilize such as nitrogen and phosphorus [2]. The fungi also provide several other benefits to plants such as competition with pathogens. With the fungi, the plant produces secondary metabolites that can promote immunity against mold, bacteria, and viruses that may cause diseases [3]. Extract of Bolete spawn was proved to promote the growth of several crops such as Guava, Papaya, and Java Apple [4-5].

Para rubber tree is one of the important economic plants in Thailand. It is prone to many diseases caused by plant pathogens which can cause a lot of economic loss. Now a day, farmers use chemicals to solve this problem but it raises another issue of chemical residue and health problems of the users. The use of Ectomycorrhiza fungi in plant farming may be a sustainable solution to these problems and also good to the environment.

This study will explore the possibility of producing these fungi as biomass to promote growth on Para rubber tree cultivation. The microorganism was grown in liquid medium in a fermenter using a by-product from food industries. The product was tested for its activities and its performance in helping the plant grows.
2. Methods

2.1. Study of Liquid Media
The media using in this study are whey (a by-product from cheese factory), pineapple core and peel extract (a by-product from pineapple can factory) compared to potato dextrose broth. The fifty-milliliter culture was grown in 250 ml Erlenmeyer flask on a shaker at 30 °C.

2.2. Study of Pathogen Inhibition Capability
Inhibition capability was tested on three plant pathogens: Alternaria brassicola, Alternaria porri, and Fusarium solani by Dual culture plate technique [7]. B.colossus was cultured in Potato Dextrose agar plates for 7 days. The plates that the size of the colony size are the same (diameter around 3-5 cm) was chosen. Each pathogen was inoculated to triplicate plates by the loop at the same distance from the edge. The culture was incubated at 30 °C. Percentage of inhibition was calculated by

\[ L = \left( \frac{C - T}{C} \right) \times 100 \]  

L = Percentage of inhibition  
C = Colony radius of plant-pathogen in control plate  
T = Colony of plant-pathogen in tested plate  

Data were collected when the control plate showed fully grown Colony.

2.3. Study of Solid Media
Media studied which were loam, peat moss, coconut dust, and volcanic soil were put in each 250-ml Erlenmeyer flask. The concentration of inoculation were 10, 20 and 30%. After 15 days, the sample was observed and tested by the soil dilution spread plate method.

2.4. Pilot-scale culture
The culture was transferred from a plate to 50-ml Erlenmeyer flasks and put on shaker plate for 7 days. Then culture was transferred to 1-L Erlenmeyer flasks on a magnetic stir plate for 7 days. Finally, the culture was transferred to 50 L fermenters using 5% inoculation for another 7 days. The sample was collected every day to measure dry weight.

2.5. Study the effect on the growth of Para rubber trees
The culture was mixed with soil at the start at the concentration of 10 % and added again at 6 months. After a year, the height of the trees, the width of the leaves and the growth of the roots are observed compared with control. There are 30 trees in the experiment.

3. Results and Discussion
The culture was grown on potato dextrose agar plate (PDA). The average colony diameter after growing for 14 days is 9 cm as shown in Figure 1. Tree media used in this experiment cause significantly difference in dry weight as shown in Table 1. Whey is the media that gave higher dry weight than pineapple core extract and potato dextrose respectively (Table 1).

The fungi can inhibit some pathogens better than others as shown in Figure 2. The percentage of pathogen inhibition was also shown in Table 2. Alternaria porri were inhibited the most, following by Alternaria brassicola and Fusarium solani respectively. The observation was made on day 15 after inoculation (Figure 3). For coconut coir dust and peat moss little mycelia were observed at the surface of the carriers. The result from the dilution spread plate method on day 15 also showed that the best media is loam followed by peat moss, coconut coir dust, and zeolite respectively. (Table 3). There is no significant difference in cfu/g while using 20 and 30%. The growth was lower significantly at a percentage inoculation of 10%.
**Figure 1.** Growth of the culture on Potato Dextrose agar for 14 days.

**Table 1.** Effect of liquid media on growth.

| Media                    | Mycelial Dry Weight (g/L) |
|--------------------------|---------------------------|
| Potato Dextrose          | 4.8667^a                  |
| Whey                     | 9.2573^c                  |
| Pineapple core Extract   | 7.2953^b                  |

Letters show significant difference (P <0.05)

**Figure 2.** Growth of culture grown with plant pathogens Row 1 = *Alternaria porri* Row 2 = *Alternaria brassicola* Row3 = *Fusarium solani*, Column 1 = 5 days, Column 2 = 8 days Column 3 = 12 days.

The culture was grown in a 75-L fermenter (with 50-L working volume) successfully. The dry weight of culture in each day of cultivation was shown in Figure 4. The culture enters the exponential growth phase after day 1. The final dry weight is 8g/L on day 6. Which is believed to be improved more by media optimization and process improvement.
Table 2. Inhibition of Plant-Pathogen.

| Plant pathogen       | Inhibition (%) |
|----------------------|----------------|
| Alternaria pori      | 45 ± 1.6 a     |
| Alternaria brassicola| 33 ± 0.9 b     |
| Fusarium solani      | 28 ± 0.8 c     |

Letters show significant difference (P <0.05)

Figure 3. Growth of culture on various carriers for 15 days Row 1 = Peat moss, Row 2 = Coconut coir dust, Row 3 = Zeolite, Row 4 = Loam Column 1 = 10 % inoculation Column 2 = 20% inoculation Column 3 = 30 % inoculation.

Table 3. Effect of solid media on growth on day 15 Using 30 % inoculation.

| Growing Media          | CFU x 10^4 |
|------------------------|------------|
| Loam                   | 1.6 b      |
| Peat moss              | 1.0 ab     |
| Coconut coir dust      | 1.0 ab     |
| Zeolite                | 0.6 a      |

Letters show significant difference (P <0.05)

The liquid culture was mixed with the loam at the proportion of 20 % to grow Para rubber trees for a year. The trunk and the root of the treated plants were significantly longer compared with control (Figure 5). The average length of root, trunk, and leaves of the treated planted measured in a year was significantly higher than that of the control (Table 4).
Figure 4. The growth curve of *B. colossus* in 75 L-fermenter.

Figure 5. Comparison of trunks leaves and roots of a year-old Para rubber tree treat with Boletus culture and control (with a white bow).

Table 4. Effect on the growth of Para rubber tree.

|                          | Experimental sample | Control |
|--------------------------|---------------------|---------|
| The average height of the tree (cm.) | 104                 | 89.8    |
| The Average width of leave(cm.)     | 5.3                 | 4.5     |
| The average length of root (cm.)    | 16.0                | 13.3    |

Treatments show significant difference from control (P <0.05)

4. Conclusion

*Boletus colossus* can be cultured in a liquid submerged fermenter using a by-product from the food factories. The final concentration achieved using whey as carbon source was 8.1 g/L in 6 days. The liquid product can inhibit some plant pathogens well. It can also be used to promote growth of Para rubber trees significantly.

This study shows the possibility of producing *Boletus colossus* in a large scale liquid culture for used as a growth promoter of Para rubber trees instead of the conventional way when the culture was grown on solid culture which is laborious and difficult to scale-up by using engineering parameters. The production process can be further optimized to improve yield and productivity. The pilot-scale product can be tested further with other plants. It is a promising new product that provides a solution to the cultivation problems for the plants that are important for economic growth.
Acknowledgment
Thanks to the Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen Kasetsart University for Para rubber sapling and useful advices.

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