

<b>Thesis title</b>	Optimizing growing conditions for the tropical mushrooms, <i>Clitopilus</i> species and <i>Agaricus subrufescens</i>
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## ABSTRACT

The aim of this study was to study taxonomy and phylogeny, optimal condition for mycelium growth, antibacterial, antioxidant, and enzyme activities of *Clitopilus doimaesalongensis* strain MFLUCC13-0806 and *C. chalybescens* strain MFLUCC13-0809 and to investigate the optimal conditions for the cultivation of *Agaricus subrufescens* hybrid strains in northern Thailand.

Collection of *Clitopilus* were made in the rainy season during the period 2012-2013 in northern Thailand. They were studied in terms of taxonomy and phylogeny. Two collections were identified as *Clitopilus chalybescens* and another as a new species, *C. doimaesalongensis*. *Clitopilus doimaesalongensis* (MFLUCC13-0806) and *C. chalybescens* (MFLUCC13-0809) were determined for optimal growth conditions; type of media, temperature and pH. The optimal conditions for *C. doimaesalongensis* were cultivation on malt extract agar (MEA), supplemented with sucrose and tryptone, pH can range from 5-9 at a temperature range from 27-29°C. Yeast extract agar (YEA), a pH of 5-8 and temperature range of 20-29°C were optimal conditions for *C. chalybescens* and this mushroom can use fructose, sucrose, starch, malt extract, yeast extract, tryptone and beef extract as carbon and nitrogen sources. The antibacterial activity from culture mycelium extracts and broth cultured extracts of

*C. doimaesalongensis* inhibited the growth of Gram positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus* and Gram negative bacteria; *Pseudomonas aeruginosa*. This activity, however, was not observed on dried mycelial extracts. *Clitopilus chalybescens* could inhibit the growth of Gram positive bacteria; *B. subtilis* and *S. aureus*, but not Gram negative bacteria. Antioxidant assay indicated that the crude extracts had noticeable scavenging activity on 2,2-diphenyl-1-picryl-hydrazyle (DPPH) radical about 21.41% and 26.45% DPPH scavenging activity for *C. chalybescens* and 29.14% and 40.62% for *C. doimaesalongensis* at 40 and 50 mg/mL, respectively. Cellulase, xylanase and amylase activities were detected in *C. doimaesalongensis*.

*Agaricus subrufescens* is an edible mushroom, one of the most important medicinal mushroom with high potential to treat many diseases. Several strains of *A. subrufescens* have been cultivated throughout the world, especially in Brazil. The *Agaricus subrufescens* hybrid strain used in this study has bred at INRA, France and successfully cultivated. The optimum conditions for the cultivation of *A. subrufescens* hybrid strains in northern Thailand were investigated in this study. Suitable temperatures and pH for mycelium growth were established on compost extract agar. The optimum temperature and pH for mycelial growth ranged from 25-30°C and pH 7-9. The four hybrid strains could be grown and produced fruiting bodies on rice straw compost. *Agaricus subrufescens* hybrid of Thai and Brazilian CA 918-076 × CA 454-4 (T2×B) produced the highest yield (56.92 g/kg of compost) among the studied strain.

**Keywords:** *Clitopilus*/ optimal condition/ antibacterial activity/ antioxidant activity/  
*Agaricus subrufescens* hybrid strain/ compost/ mushroom cultivation