CHAPTER V

DISCUSSION

A. Studies on a suitable liquid culture medium for the preparation of *Lentinus edodes* mycelia for DNA extraction.

The cultivation of *L. edodes* mycelia in liquid culture was planned to decrease the uniformity of DNA material and to target only on the ability to generate the mycelia suitable for DNA extraction. Their efficiency was compared when some amount of starting material was used.

The content of polysaccharides increased with mycelial age (Schneiderbauer *et al.*, 1991), therefore it is easier to isolate DNA from young mycelia. The mycelial growth studies aimed at obtaining a good starting material for DNA extraction via suitable liquid culture selection.

The result revealed that when mycelia were cultured for 20 days, the liquid culture formulation 11 showed significant difference in mycelial growth among each of the formulations (Appendices II-1, II-2). Superior growth rate in terms of fresh and dry weight gained 10 to 15 days after inoculation indicated the suitability of culture medium formulation 11. This culture medium contained 2% yeast extract, 2% glucose and 0.1% peptone, other culture media contained 2% malt extract and various percent of glucose and peptone (Appendix I-7), both yeast extract and glucose helped enrich the formulation. Glucose is an important monosaccharide which can be readily absorbed during mycelial growth, while malt extract is a source of carbon and amino acids in complex carbohydrate and protein forms. Glucose positively promotes rapid mycelial growth. This result agreed with those of Khanna and Garcha (1985) that referred to 2% glucose in culture medium as the best carbon source for rapid mycelial growth. N-source in culture medium came from yeast extract. The rapid growth promoted by the use of 2% glucose and yeast extract was

also reported (Jittra Kanchanaprayudh, 1996). The mycelia obtained balanced nutrients and growth factors from this culture medium because of the C-source and N-source difference. From this reason, the liquid culture formulation 11 is a suitable for culture mycelia to yield good starting material within a short time, while other culture media that contain malt extract are suitable for culturing for long-term survival.

Although PDB had an effect on mycelial growth in some mushrooms, in this experimental it was not suitable for rapid cultivation of *L. edodes* strains; may be because of nutrient source difference, e.g. C-source, N-source or growth factors. However, each of cultural conditions depended on the suitability to specific kind of mushrooms.

Shaking liquid medium also increased O_2 level and disperse the nutrient. Cultivation of mushroom by shaking gave better growth than surface culturing (Solomko, 1978).

Comparison of growth among all culture media indicated that liquid culture formulation 11 was the best to provide good starting material for further DNA extraction.

B. A simple and rapid method to obtain *L. edodes* genomic DNA isolation for RAPD analysis.

Most common problems encountered in DNA preparation are the relatively high production of polysaccharides (Möller *et al.*, 1992), fungal nuclease and pigment impurities from insufficient extraction of DNA from filamentous fungi (Reader and Broda, 1985). A few published protocols included steps for the removal of polysaccharides (Do and Adams, 1991). Moreover, most protocols contain phenol-chloroform treatment more than one precipitations (Sambrook *et al.*, 1989; Gaber and Yoder, 1983; Blaiseau *et al.* 1992); three precipitations (M.L.Saovaros Svasti, 1989); two precipitations (Khush, Becker and Wach, 1992); three precipitations (Solaya

Suksa-Ard, 1995). The last two steps were a probable cause of shearing (Möller *et al.*, 1992).

The study led to the development of a simple and rapid DNA isolation procedure so that DNA template for PCR amplification having pure genomic DNA of high molecular weight can be routinely obtained. These methods may be applicable to other filamentous fungi or other mushrooms.

In this study, 3 methods (standard method, SDS method and Glass bead method) provided better DNA quality that would yield PCR products with high reproducibility. The standard method required less operating time and provided good quality and yield of DNA. However, this method needed several Phenol Chloroform extraction steps, resulting in handling difficulty. These methods can be used as a standard reference to develop a better method.

The first step of the SDS method required a long period of time but after boiling, it took less time with high quality and reproducibility of DNA amplified once again by PCR. This method have range phenolchloroform extraction step shorter than the standard one. This helped reduce the phenolchloroform waste and less operating with such a strong oxidizing agent like phenol and phenolchloroform.

Glass bead method required a shorter time for each extraction step. The method in long lab take knows laborious hence accessible to a large number of samples isolated at one time.

The hemerit and demerit of each method can be weighted on the chemicals available in each lab and the ability took access the samples in this study. Both standard method and SDS method were preferred because they gave a relatively good quality of DNA at a relatively low cost. Moreover, all reagents used were common to all laboratories. When the amount of sample were excessive, the capability to extract with the standard and the Glass bead methods could be applied,

mycelia were disrupted by the glass beads, the DNA would dissolve in appropriate buffer.

These studies would be greatly facilitated if DNA preparation yields were obtained in milligram quantities (Specht *et al.*, 1981). It could be carried out in Eppendorf tubes, which was especially useful in DNA extraction from many different samples simultaneously (Reader and Broda, 1985), e.g. in the analysis of different strains. The result showed that the Glass bead method gave dark and smear bands of DNA similar to the standard extraction method described in Sambrook *et al.* (1989). However, the standard method gave the DNA yield much more than other protocols, e.g. CTAB, Chelex, and NaOH. Thus, these methods were appropriate for simultaneous processing of many samples because all steps could be done in Eppendorf tubes. It was possible to process hundreds of individual samples in a single working day (Edward *et al.*, 1991). The DNA yield was in a quality enough as DNA template for PCR amplification and distinction with RAPD analysis using random primers. The standard method was also used and turn out to be a generally useful method to purify nucleic acid from other mushrooms (Schneiderbauer *et al.*, 1991).

C. RAPD analysis

The analysis of random amplified polymorphic DNA yielded approximately 25 to 28 when all patterns were scored simultaneously, a total of 101 polymorphic DNA could be obtained as standard polymorphisms. Among 101 polymorphisms detected, more than 50% was polymorphic and the less are monomorphic. Distance among the morphs can be calculated using Jaccard's similarity coefficients. The calculated distance varied from 0.193548 to 0.646341 based on the genetic distance value between two groups of *L. edodes* (Fig. 5). The first group was A that included MuL2, MuL4, MuL5, MuL9/2, MuL9/4, MuL11 and the Japanese cultivar. The second group was B that included MuL12. However, based on this genetic distance, the original geographic origin could not be grouped together. In this study, the RFLP pattern as well as the isozyme pattern of the *L. edodes* strain were not available, thus this formed the genetic distance, though falling into two groups, could not be

concluded without comparison to the result from RFLP. The phylogenetic dendrogram presented here could be used as a preliminary study on *L. edodes* classification. More work is needed to identify and collaborate the available RFLP, AFLP, isozyme, and RAPD data. This data however could be used to predict the relationships between isolates and as a preview for differentiation and relationship studies of *L. edodes*.

Finally, the results indicated that it is possible to distinguish and recognize each isolate of *L. edodes*. Furthermore, it seems to make a prediction of phylogenic relationships among *L. edodes*.