CHAPTER I

INTRODUCTION

Shiitake is a well-known mushroom that has been cultivated in Asian countries including Japan, China, Korea, Hong Kong and Thailand. Beside its good taste and aroma, it also has high nutritional value and medicinal properties. The fruiting body and its extracts are used as medicine that aides in cholesterol reduction, high blood pressure reduction, allergy prevention, and also possess compounds with antitumor, anti cancer and antiviral properties against stomach cancer, colds, measles and polio.

At present, shiitake mushroom has become a popular mushroom. Japanese cultivars, receive the highest price because of their high quality when compared with those from other sources. Thailand has cultivated this mushroom at both commercial and industrial scales in the north. Local shiitake mushrooms have a low market share because of its lower quality, insufficient amount, uncertainty of the product availability. Most consumers prefer to consume import products. It's essential to create tolerant strain which is suitable to the country environment and has a high yield. Therefore, studies on strain improvement, strain collection and hybrid strain aimed at developing shiitake mushroom varieties for better fruiting body, high yield with all the desirable characteristics are important. Usually, shiitake mushroom was classified based on morphological characteristic of the fruiting body. It cannot be classified at spore formation stage or mycelium stage. However, the problems in morphological classification arise from the short fruiting body formation stage and quick decay. Some isolates may take a long time in fruiting body development growth or may be easily affected by the environment, that it cannot form fruiting body or produce deformed fruiting bodies. These cases make classification difficult. Therefore, it is essential to develop techniques for classification shiitake

mushroom that are simple, precise and independent the environment and growth stages.

The first technique that had been used was based on protein analysis technique, also known as isozyme analysis. However, it was limited by a specific growth stage. Later, the development of technology based on DNA analysis techniques such as RFLP and PCR were employed. This led to the development of several novel genetic assays. The RFLP method was laborious, expensive, required a large amount of DNA and suitable specific probes, and may involve the use of radioactive which all made it unpopular. Recently random amplified polymorphic DNA (RAPD) developed from PCR analysis using a single short synthetic primer of the arbitrary oligonucleotide sequences and ethidium bromide staining has been a method of choice. The RAPD analysis was supplemented with the use of polyacrylamide gel and silver staining and became DNA amplification fingerprint (DAF) which a better resolution. The major advantage of RAPD/DAF analysis was that the procedure was less expensive, quicker and no need of southern transfer, required only a small quantity (ng) of DNA and a universal set of primers, and can be applied to use on all species using genetic markers. RAPD/DAF was used in identification and phylogenetic studies such as identification of distinct genotypes among rice strains, fungi, microorganisms and/or medical research such as the detection of an abnormally human disease. However, the use of RAPD technique *Lentinus edodes* in the analysis has not been reported.

In this study, one of the RAPD analyses, DAF was used to differentiate *L. edodes* isolates for simultaneous processing of many samples. When this method was combined with the modified rapid DNA preparation, the results could be obtained within 3-4 days.

Objective of the present study

The objective of this study is to develop techniques based on DNA technology to differentiate shiitake mushroom isolates of *Lentinus edodes* (Berk.) Sing, especially the PCR-based RAPD. The result constitute a basis for genetic classification.