UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND CHARACTERIZATION OF FUNGAL CONTAMINATION ON SPENT MUSHROOM SUBSTRATE

NUR NATASHA BINTI ANTONG IBRAHIM

FP 2017 53

IDENTIFICATION AND CHARACTERIZATION OF FUNGAL CONTAMINATION ON SPENT MUSHROOM SUBSTRATE.

BY

NUR NATASHA BINTI ANTONG IBRAHIM

FACULTY OF AGRICULTURE

UNIVERSITI PUTRA MALAYSIA

2016/2017

IDENTIFICATION AND CHARACTERIZATION OF FUNGAL CONTAMINATION ON SPENT MUSHROOM SUBSTRATE.

BY

NUR NATASHA BINTI ANTONG IBRAHIM

A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

Faculty of Agriculture

Universiti Putra Malaysia

2016/2017

CERTIFICATION PAGE

This project report entitled "Identification and Characterization of Fungal Contamination on Spend Mushroom Substrate" is prepared by Nur Natasha binti Antong Ibrahim and submitted to the Faculty of Agriculture in fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science.

Student's name: Student's signature:

NUR NATASHA BINTI ANTONG IBRAHIM .....

Certified by:

.....

(DR. SUMAIYAH BINTI ABDULLAH)

Project Supervisor

Department of Plant Protection

Faculty of Agriculture

Universiti Putra Malaysia

Date: .....

ACKNOWLEDGEMENT

First of all, I would like to express my thankfulness to Almighty Allah S.W.T because of His bless fullness and guidance to me from the beginning of this project until now. The successful of this project not only depends on me also support from many other people. I want to take this opportunity to express my appreciation to the person who has been helping me along the completion of this project.

I would like to thanks my project supervisor, Dr.Sumaiyahbinti Abdullah for helping me through the completion of this final project by giving advice, support, encouragement and collection of samples for mushroom house UPM until I can finish my project successfully. Besides that, I would to express my thankfulness to Dr.SitiIzera for guide me during the molecular identification of fungus process and also to laboratory staffs, EncikRazali and PuanJunainah for helping me during the preparation for the experiment in the laboratory.

Also thank to my family especially my parent by giving morale support, pray and encouragements to me for finishing the final year project. Last but not least, thanks to all my friends those help me directly or indirectly during this project are running.

ABSTRACT

Fungal contamination on spent mushroom substrate is the main problems faced by the mushroom growers in Malaysia. Among many pest and diseases in oyster mushroom cultivation, the most serious crop losses due to fungal contamination by *Trichoderma sp., Aspergillus spp.,Penicillium sp. and Fusarium sp.* that lead to green mould infection or other mould contamination. Many researchers have undergone the experiment in order to detect the fungal species that attack on mushroom substrates. The objectives of this experiment are (1) to isolate pure culture of fungal isolation causing fungal contamination on spent mushroom substrate (2) to identify fungal pathogens to species level based on morphology characteristics and Polymerase Chain Reaction (PCR) protocol using ITS1 and ITS4 (3) to construct internal transcribed spacer (ITS) phylogeny of fungal species using Maximum Likelihood method. Fungal strains were isolated from diseased mushroom bags from mushroom houseUniversiti Putra Malaysia. The pure fungi isolated from spent mushroom substrate were identified by in vitro morphological and molecular characteristics. Based on morphological characteristics, *Aspergillus oryzae* had caused fungal contamination on the mushroom substrate by the formation of ampulliformphialide and green coloured conidia also supported by the result of DNA sequencing and amplification using ITS1 and ITS4. Data obtained also had been analyzed to identify phylogeny of fungal species using Maximum Likelihood method.

Keywords:*Trichoderma, Aspergillus, Penicillium, Fusarium,* fungal contamination, oyster mushroom

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Mushroom is one of the agricultural plants that are grown by the farmers as source of income in Malaysia. The most commercial mushrooms planted by the growers are oyster mushroom and button mushroom. In Malaysia, most growers planted in the mushroom bags as a media. Mushroom bags contains sawdust, rice bran, agricultural lime and water. Rice bran contains amino acid, phosphorus, omega 3, and vitamins (B3, B6, B12 and B22) that suitable are for mushroom growth. In Malaysia, consumption of mushroom is the major increasing. Pulau Pinang has been identified mushroom cultivation in Malaysia.

Recently, mushroom growers faced major problems that attack mushroom industry. Fungi or contaminants are organisms that attack mushroom cultivation and theyaffects the growth and development of mushroom crops. Contaminants are also known as competitor weeds primarily consist of mould, bacteria, viruses and insects (Wiafe-Kwagyan, 2015). Among these fungi, harmful fungi that encountered mushroom substrate include *Coprinus logopus, Aspergillus* spp., *Mucor* species, *Pencillium* spp., *Sclerotium* spp. and *Trichoderma* spp. that inhibit spawn run (Maurya et al., 2014). However information on diseases and competitor moulds occurring in or on oyster mushrooms is scarse compared to that on button mushrooms. Variations of the fungi that attack on mushroom substrates vary depending on the variety of substrates used, the method of the substrate being prepare and the conditions of container used for cultivation (Sharma et al., 2007). Arevato et.al (1996) stated that most contaminants during spawn are caused by the

Penicillium spp., *Aspergillus ochraceus, Aspergillus flavus, Streptomycin* spp. and Trichoderma *viride.*

The characteristics of the fungus which attacked can be determined through morphological and molecular characteristic mechanisms. According to Kebeish and El-Sayed (2012), *Aspergillus oryzae* can be identified by the green-yellow colonies, conidial head radiate with uniseriate to biseriate prominent during maturity, conidia globuse and the conidial surfaces that are slightly ornamented and echinulate under high magnification of microscope. Molecular approaches to identify *Aspergillus* spp. can be done using 18S rRNA gene, mitochondrial DNA, the intergenic spacer region and internal transcribed spacer (ITS) region. It is also stated that using ITS region give advantages over other molecular approaches by the increase sensitivity due to the existence of approximately 100 copies per genome (Henry et al., 2000).

The objectives of this study are to isolate pure culture of fungus that cause contamination on spent mushroom substrate, identify fungal pathogens to species level based on morphological characteristics and Polymerase Chain Reaction (PCR) using ITS1 and ITS4 and to construct internal transcribed spacer (ITS) phylogeny of fungal species using Maximum Likelihood method.

REFERENCES

Accensi, F., Cano, J., Figuera, L., Abarca, M. L., &Cabanes, F. J. (1999). New PCR method to differentiate species in the Aspergillus niger aggregate. *FEMS Microbiology Letters*, *180*(2), 191-196.

Arvanitis, M., Anagnostou, T., Fuchs, B. B., Caliendo, A. M., &Mylonakis, E. (2014).Molecular and nonmolecular diagnostic methods for invasive fungal infections. *Clinical microbiology reviews*, *27*(3), 490-526.

Aamir, S., Sutar, S., Singh, S. K., &Baghela, A. (2015). A rapid and efficient method of fungal genomic DNA extraction, suitable for PCR based molecular methods.

Alwakeel, S. S. (2016). Molecular identification of fungi isolated from coastal regions of Red Sea, Jeddah, Saudi Arabia. *Journal of the Association of Arab Universities for Basic and Applied Sciences*.

Chaverri, P., & Samuels, G. J. (2003). *Hypocrea/Trichoderma (ascomycota, hypocreales, hypocreaceae): species with green ascospores*. Utrecht: CentraalbureauvoorSchimmelcultures.

Colavolpe, M. B., Mejía, S. J., &Albertó, E. (2014). Efficiency of treatments for controlling Trichodermaspp during spawning in cultivation of lignicolous mushrooms. *Brazilian Journal of Microbiology*, *45*(4), 1263-1270

Dias, E. S. (2010). Mushroom cultivation in Brazil: challenges and potential for growth. *Ciência e Agrotecnologia*, *34*(4), 795-803.

El-Fadaly, H. M., El-Kadi, S. M., Hamad, M. N., & Habib, A. A. (2015).Isolation and Identification of Egyptian Ras Cheese (Romy) Contaminating Fungi during Ripening Period. *Journal of Microbiology Research*, *5*(1), 1-10.

Gary E. Harman, Charles R. Howell, Ada Viterbo, Ilan Chet & Matteo Lorito (2004). Trichoderma species – opportunistic, avirulent plant symbionts.*Nature Reviews Microbiology* **2**, 43-56 doi:10.1038/nrmicro797

Ha, T. N. (2010). Using Trichoderma species for biological control of plant pathogens in Vietnam. *Journal ISSAAS*, *16*, 17-21.

Hassan, G.M., El-Feky,Z.A.,Hemada,N.F.,& Sayed, M.A (2015). Isolation and molecular characterization of Egyptian Trichoderma and assessment of their antagonistic potential against Rhizotoniasolani. The Journal of Microbiology, Biotechnology and Food Science, 4(6),495.

Jin, B., van Leeuwen, J. H., & Patel, B. (1999).Mycelial morphology and fungal protein production from starch processing wastewater in submerged cultures of Aspergillus oryzae. *Process Biochemistry*, *34*(4), 335-340.

Jia, Y., Wamishe, Y. A., & Zhou, B. (2014).An expedited method for isolation of DNA for PCR from Magnaportheoryzae stored on filter paper. *The Crop Journal*, *2*(5), 267-271.

Klich, M. A. (2006). Identification of clinically relevant aspergilli. *Medical Mycology*, *44*(sup1), 127-131.

Kebeish, R. M., & El-Sayed, A. S. (2012).Morphological and molecular characterization of L-methioninase producing Aspergillus species. *African Journal of Biotechnology*, *11*(87), 15280-15290.

Kullnig-Gradinger CM, Szakacs G, Kubicek CP (2002) Phylogeny and evolution of the genus *Trichoderma*: a multigene approach. Mycol Res 106: 757-767

Martin, K. J., &Rygiewicz, P. T. (2005). Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC microbiology*, *5*(1), 1.

Maurya, S., Kumar, R., Kumari, A., Choudhary, J. S., & Kumar, S. (2014). Substrate Decomposing Fungi of Mushroom and their Management by Some Common Fungicides. *Vegetos-An International Journal of Plant Research*, *27*(2), 240-244.

Ospina-Giraldo, M. D., Royse, D. J., Chen, X., & Romaine, C. P. (1999). Molecular phylogenetic analyses of biological control strains of Trichoderma harzianum and other biotypes of Trichoderma spp. associated with mushroom green mold. *Phytopathology*, *89*(4), 308-313.

Obire, O., &Ogechi, A. A. (2015). Cultivation of mushroom (Pleurotusostreatus) and the microorganisms associated with the substrate used.

Park, M. S., Seo, G. S., Bae, K. S., & Yu, S. H. (2005). Characterization of Trichoderma spp. associated with green mold of oyster mushroom by PCR-RFLP and sequence analysis of ITS regions of rDNA. *The Plant Pathology Journal*, *21*(3), 229-236.

Parmar, H. J., Hassan, M. M., Bodar, N. P., Lakhani, H. N., Umrania, V. V., &Golakiya, B. A. (2015). Development of SCAR marker for Specific Detection of Trichoderma harzanium and Trichoderma viride. American Journal of Microbiological Research, 3(1), 45-49.

Sharma, S. R., Kumar, S., & Sharma, V. P. (2007). *Diseases and competitor moulds of mushrooms and their management*. National Research Centre for Mushroom, Indian Council of Agricultural Research.

Sharma, K.K., & Singh, U.S. (2014).Cultural and morphological characterization of rhizospheric isolates of fungal antagonist Trichoderma. Journal of Applied and Natural Science,1(6),451-456.

Thom, C., & Church, M. (1921).Aspergillus flavus, A. oryzae, and Associated Species. *American Journal of Botany, 8*(2), 103-126. Retrieved from http://www.jstor.org/stable/2435149

Toju, H., Tanabe, A. S., Yamamoto, S., & Sato, H. (2012). High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PloS one*, *7*(7), e40863.

Wiafe-Kwagyan, M., Odamtten, G. T., &Obodai, M. (2015). Possible Antibiosis Effect of the Metabolites of Three Fungal Species Resident in Rice Straw and Husk Compost on the in vitro Radial and Vegetative Growth by Pleurotusostreatus Strain EM-1and P. eous Strain P-31. *Int. J. Curr. Microbiol. App. Sci*, *4*(8), 525-538.

Wang, G., Cao, X., Ma, X., Guo, M., Liu, C., Yan, L., &Bian, Y. (2016). Diversity and effect of Trichoderma spp. associated with green mold disease on Lentinula edodes in China. *MicrobiologyOpen*.