MORPHOLOGICAL, YIELD AND PROTEIN PROFILING ANALYSIS AMONG *Schizophyllum commune* NATURAL STRAINS, HYBRIDS AND HYBRIDS OF GAMMA-IRRADIATED MONOKARYOTIC MYCELIA

KHAIRUNNISA BINTI ABDUL RASHID

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

MORPHOLOGICAL, YIELD AND PROTEIN PROFILING ANALYSIS AMONG *Schizophyllum commune* NATURAL STRAINS, HYBRIDS AND HYBRIDS OF GAMMA-IRRADIATED MONOKARYOTIC MYCELIA

KHAIRUNNISA BINTI ABDUL RASHID

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF BIOTECHNOLOGY

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

UNIVERSITY MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **KHAIRUNNISA BINTI ABDUL RASHID**

Matric No: **SGF160004**

Name of Degree: **MASTER OF BIOTECHNOLOGY**

Title of Thesis ("this Work"):

MORPHOLOGICAL, YIELD AND PROTEIN PROFILING ANALYSIS AMONG SCHIZOPHYLLUM COMMUNE NATURAL STRAINS, HYBRIDS AND HYBRIDS OF GAMMA-IRRADIATED MONOKARYOTIC MYCELIA

Field of Study: **MYCOLOGY**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation

MORPHOLOGICAL, YIELD AND PROTEIN PROFILING ANALYSIS AMONG *Schizophyllum commune* NATURAL STRAINS, HYBRIDS AND HYBRIDS OF GAMMA-IRRADIATED MONOKARYOTIC MYCELIA

ABSTRACT

Cultivation of edible mushroom is rising due to their culinary, nutritional and medical value. The application of genetic diversity in mushroom breeding is crucial to search for new species and to enhance the existing species in terms of high yield and improved quality of fruiting bodies to meet the increasing demands from the society. The aims of this study are to evaluate sporophores of novel hybrids of *Schizophyllum commune* obtained by hybridisation and mutation and to profile proteome of hybrids by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In this study, three native strains of *S. commune* were selected as the parental strains where two strains are from Malaysia (w1 and w2) and another from Thailand (R). Traditional cross-breeding (hybridisation) and induce mutation by gamma radiation were applied as the method for the development of new strains with novel characteristics. Thirteen hybrids were produced by hybridisation between monokaryotic w1 and w2, while two hybrids were generated by the hybridisation between w1 and R. Ten strains were developed by the method of gamma radiation. All fifteen new hybrids were evaluated based on several aspects, which includes mycelial growth rate, mycelial density, the width of sporophore, yield, biological efficiency, and protein profiling by SDS-PAGE. By using the protein profile, the electrophoretic protein pattern and similarity coefficient of each strain were compared with the parental strains. Hybrids showed various diversity at the morphological characteristics and protein profiling. The results obtained in this study proved that hybrid w1bw2f, w1dw2f, w1Lw2a, Ir-w1dRa, Ir-w1dw2d, Ir-w1ew2a, Ir-w1ew2d and Ir-w1gw2a exhibited high potential to be commercialised. Moreover, the biological efficiency of w1bw2f, w1dw2f, w1Lw2a, Ir-w1dRa, Ir-w1dw2d, Ir-w1ew2a,

Ir-w1ew2d and Ir-w1gw2a were 34.74 ± 1.54 %, 27.38 ± 0.93 %, 33.84 ± 1.12 %, 29.07 ± 2.46 %, 29.14 ± 0.56 %, 28.72 ± 0.61 %, 28.67 ± 1.21 %, and 32.87 ± 0.91 % respectively. Most of the strains were able to be distinguished from one another except w1aw2a and w1bw2a. This enabled finger printing of the strains to protect breeder's rights. The similarity calculated on the basis of the presence and absence of bands ranged from 14 % to 92 %. Hybridisation and gamma induction were able to enhance the characteristics of the strains and alter the protein profile of the hybrids. The good features of these hybrids may contribute to the variety in the mushroom industry and better commercialisation value.

Keywords: Split-gill mushroom, cross-breeding, mutation, strain improvement, proteome.

ANALISIS MORFOLOGI, HASIL DAN PROFIL PROTEIN ANTARA STRAIN *Schizophyllum commune* INDUK, HIBRID DAN HIBRID MISELIA MONOKARYOTIK TERIRADIAT-GAMMA

ABSTRAK

Perusahaan cendawan semakin giat meningkat disebabkan kepentingan dalam masakan, khasiat dan nilai perubatan. Penggunaan kepelbagaian genetik dalam pembiakan cendawan adalah penting untuk menerokai spesies baru dan menambah baik spesies sedia ada dari segi hasil yang tinggi dan peningkatan kualiti cendawan bagi memenuhi permintaan yang semakin meningkat daripada masyarakat. Tujuan kajian ini dijalankan adalah untuk menghasilkan hibrid *Schizophyllum commune* yang mempunyai ciri-ciri novel dengan menggunakan teknik penghibridan dan mutasi, untuk mengoptimumkan dan menganalisis profil protein hibrid yang diperolehi melalui teknik SDS-PAGE untuk membezakan antara hibrid strain. Tiga strain induk *S. commune* (cendawan kukur) dipilih di mana dua strain adalah dari Malaysia (w1 dan w2) dan satu strain adalah dari negara Thailand (R). Lazimnya pembiakan silang (penghibridan) dan mutasi oleh radiasi gamma digunakan sebagai kaedah untuk menghasilkan variasi baru dengan ciri-ciri yang unik. Tiga belas hibrid dihasilkan melalui teknik penghibridan antara w1 dan w2, manakala dua hibrid dihasilkan melalui teknik penghibridan antara w1 dan R. Sepuluh strain kacukan baru dihasilkan melalui kaedah radiasi gamma. Kesemua lima belas hibrid dibandingkan dalam beberapa aspek, termasuk kadar pertumbuhan miselia, kepadatan miselia, lebar janabuah, hasil, kecekapan biologi (BE) dan profil protein dengan menggunakan kaedah SDS-PAGE. Dengan menggunakan profil protein, corak protein elektrolisis dan pekali persamaan setiap hibrid dibandingkan dengan strain induk. Strain hibrid menunjukkan kepelbagaian pada ciri-ciri morfologi dan profil protein. Hasil kajian yang diperolehi menunjukkan bahawa hibrid w1bw2f, w1dw2f, w1Lw2a, Ir-w1dRa, Ir-w1dw2d, Ir-w1ew2a, Ir-w1ew2d dan Ir-w1gw2a menunjukkan potensi yang berharga untuk

dikomersialkan. Tambahan lagi, kecekapan biologi bagi hibrid w1bw2f, w1dw2f, w1Lw2a, Ir-w1dRa, Ir-w1dw2d, Ir-w1ew2a, Ir-w1ew2d dan Ir-w1gw2a adalah 34.74 ± 1.54 %, 27.38 ± 0.93 %, 33.84 ± 1.12 %, 29.07 ± 2.46 %, 29.14 ± 0.56 %, 28.72 ± 0.61 %, 28.67 ± 1.21 %, and 32.87 ± 0.91 %. Kebanyakan strain dapat dibezakan antara satu sama lain kecuali w1aw2a dan w1bw2a. Persamaan dikira berdasarkan kehadiran dan ketiadaan band adalah dari 14 % dan 100 %. Hibridasi dan gamma induksi dapat meningkatkan ciri-ciri hibrid dan mengubah profil protein. Ciri-ciri yang baik sesuatu hibrid ini boleh menyumbang kepada kepelbagaian dalam industri cendawan dan nilai pengkomersialan yang lebih baik.

Kata kunci: Cendawan kukur, pembiakan silang, mutasi, penambahbaikan strain, proteome.

ACKNOWLEDGEMENTS

I would like to acknowledge the Ministry of Higher Education for funding through Fundamental Research Grant Scheme (FRGS) with project number of FP045-2016 that supported my study. I would like to express my sincere gratitude to the University of Malaya for letting me fulfill my dream of being a student here. I would also like to thank the Institute of Science and Biology (ISB) for giving me the opportunity to write a thesis.

I wish to thank my supervisor, Prof Dr Noorlidah Abdullah for introducing me to this project. It was only due to her valuable guidance, continuous support, and guidance that I was able to complete this thesis. I am thankful to Assoc Prof Dr Adawiyah Suriza Shuib for her advises and opinions especially in proteome part.

I also take this opportunity to express my special gratitude towards all lab members especially to Norjuliza Mohd Khir Johari, Erlina Binti Abdullah, Sivanathan Manoharan and Rushitha Sadasevevam for helping me out in all aspects during my project in Mycology Laboratory. They have given a great deal of time, valuable advice and continuous guidance from the beginning until the final revision of this thesis.

Many thanks go to all staffs in High Impact Research (HIR) especially Miss Nur Syairah Azimi, Mrs Rahimah Ismail and Mr Amirul Fatih Japri for their technical assistance provided during my proteome work. Lastly, special words of appreciations and sincere gratitude to my entire course mate and my friends who struggled together to finish their projects and for their happiness, supports and suggestions during my hard and happy times.

TABLE OF CONTENTS

Abstract iv

Abstrak vi

Acknowledgements viii

Table of Contents ix

List of Figures xiv

List of Tables xvii

List of Symbols and Abbreviations xix

List of Appendices xxii

CHAPTER 1: INTRODUCTION 1

1.1 Objectives 3

CHAPTER 2: LITERATURE REVIEW 4

2.1 World Mushroom Production 4

2.2 Mushroom Artificial Cultivation 6

2.2.1 Fungal Media 8

2.2.2 Spore Printing and Mushroom Tissue Culture 8

2.2.3 Preservation of Mycelial Cultures 10

2.2.4 Spawning 12

2.2.5 Preparation of Substrate for Cultivation 13

2.2.6 Harvest and Cropping 14

2.3 Growth Condition 15

2.3.1 Nutritional Requirements 15

2.3.1.1 Carbon sources ……. 15

2.3.1.2 Nitrogen sources ……... 17

2.3.2 Physical Requirements …….. 19

2.3.2.1 Temperature …….. 19

2.3.2.2 Light ……. 20

2.3.2.3 Moisture …… 21

2.4 Genetic Diversity in Mushroom Breeding …… 22

2.4.1 Hybridisation …….. 23

2.4.2 Mutation ……..... 26

2.4.3 Protoplast Fusion …… 28

2.5 Characterisation of Mushroom Strains …….. 30

2.5.1 Morphological characterisation of *S. commune* ……. 30

2.5.2 Molecular Identification ……..... 32

2.5.3 Protein Profiling ……..... 34

2.5.4 Metabolite Profiling …… 36

2.6 *Schizophyllum commune* ……... 38

2.6.1 Morphology of *Schizophyllum commune* ……... 40

2.6.2 Life cycle of *Schizophyllum commune* ……... 41

2.6.3 Nutritional Value and Medicinal Properties ……... 42

CHAPTER 3.0 METHODS AND MATERIALS ……... 45

3.1 Preparation of Mycelia Culture ……. 45

3.2 Fruiting Trials …… 45

3.2.1 Preparation of the Spawn …… 46

3.2.2 Preparation of Fruiting Substrate Bag and Spawn Inoculation …….. 47

3.2.3 Determination of the Mycelial Growth Rate and Mycelial Density ... 48

3.2.4 Determination of Sporophore Yield and Biological Efficiency …..... 49

3.3 Protein Profiling …… 50

3.3.1 Protein Extraction ……... 50

3.3.2 Protein Estimation using Bradford Assay …….. 52

3.3.3 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis …….. 53

3.3.4 Standard Silver Nitrate Staining Method ……... 53

3.3.5 Analysis of SDS-PAGE Gel Image …… 54

3.4 Statistical Analysis …… 54

CHAPTER 4.0 RESULTS AND DISCUSSION …… 55

4.1 Mycelial Growth Rate and Density of *S. commune* Strains ……. 55

4.1.1 Hybrids of Malaysian Strains Compared to the Parental Strains (w1 and w2) …….. 55

4.1.2 Hybrids between Malaysian and Thailand Strains Compared to the Parental Strains …….. 58

4.1.3 Irradiated Hybrids ……... 60

4.1.4 Comparison of Mycelial Growth Rate and Density of Parental Strains (w1 and w2) …… 62

4.2 Morphology of Sporophores ……. 64

4.2.1 Hybrids of Malaysian Strains Compared to the Parental Strains (w1 and w2) ……... 64

4.2.2 Hybrids between Malaysian and Thailand Strains Compared to the Parental Strains ……... 68

4.2.3 Irradiated Hybrids ……... 70

4.2.4 Comparison of the Sporophore Yield and Biological Efficiency of Parental Strains (w1 and w2), hybrid w1ew2a and monokaryon-irradiated hybrid (Ir-1ew2a) ……... 74

4.3 Yield and Biological Efficiency ……... 76

4.3.1 Hybrids of Malaysian Strains Compared to the Parental Strains (w1 and w2) ……... 76

4.3.2 Hybrids between Malaysia and Thailand Strains Compared to the Parental Strains ……... 80

4.3.3 Irradiated Hybrids ……... 82

4.3.4 Comparison of the Sporophore Yield and Biological Efficiency of Parental Strains (w1 and w2), Hybrid w1ew2a and Monokaryon-Irradiated Hybrid (Ir-w1ew2a) ……... 84

4.4 Protein Profiling …… 86

4.4.1 Hybrids of Malaysia Strains Compared to the Parental Strains (w1 and w2) ……... 86

4.4.2 Hybrids between Malaysia and Thailand Strains Compared to the Parental Strains …….. 91

4.4.3 Irradiated Hybrids …….. 92

4.4.4 Comparison between Parental Strains (w1 and w2), Hybrid w1ew2a and Monokaryon-Irradiated Hybrid Ir-w1ew2a ……. 96

4.5 Similarity between Parental Strains, Hybrids and Monokaryon-Irradiated Hybrids ……. 98

CHAPTER 5.0 CONCLUSIONS ……. 105

References ……... 106

Appendix A: Media Preparation ……. 126

Appendix B: Protocols for SDS-PAGE stock solution and buffers and preparation of stacking and resolving gel ……. 127

Appendix C: Protocols for silver staining solution …… 129

Appendix D: Protein content of Malaysia native strains, Thailand native strain, hybrids and monokaryon-irradiated hybrids ……... 130

Appendix E: Densitometric profiles of protein bands and calibration curve of protein marker ……... 132

Appendix F: Densitometric profiles of protein bands of each strain …….. 136

Appendix G: Relative mobility (Rf) parental strains, hybrids, and irradiated monokaryon hybrids based on protein data ……... 145

LIST OF FIGURES

Figure 2.1	:	World mushroom production by genera	5
Figure 2.2	:	General steps of mushroom production	7
Figure 2.3	:	Mushroom spore printing	9
Figure 2.4	:	Procedure of tissue culture mushroom	10
Figure 2.5	:	Preserving mushroom mycelia in the mineral oil	11
Figure 2.6	:	Effect of carbon sources on the mycelial biomass and EPS production in *S. commune*	15
Figure 2.7	:	Effect of nitrogen sources on the mycelial biomass and EPS production in *S. commune*	17
Figure 2.8	:	Effect of temperature on mycelium growth of *S. commune*	19
Figure 2.9	:	The growth of *S. commune* in submerged shake culture at different temperature	20
Figure 2.10	:	Tetrapolar mating system	24
Figure 2.11	:	Hybridisation of two compatible monokaryon of *Pleurotus pulmonarius*	24
Figure 2.12	:	Crossbreeding of *Sparassis latifolia* strains	25
Figure 2.13	:	Yield between parent and mutant isolates of *Volvariella volvacea*	28
Figure 2.14	:	Protoplast of *Pleurotus floridae* fuse with protoplast of *Pleurotus cystidiosus*	29

Figure 2.15 : Somatic hybrid sporophores between *Calocybe indica* var. APK2 and *Pleurotus florida* 29

Figure 2.16 : Upper and lower surface of *Schizophyllum commune* 31

Figure 2.17 : Cultural characteristics of *S. commune* on malt agar medium 31

Figure 2.18 : Common morphological characteristics of basidiomycetes including chlamydoconidia, arthroconidia, spicules and clamp connections 32

Figure 2.19 : Random amplification of polymorphic DNA profiles of 12 strains of *Schizophyllum commune* with primer OPA-1 33

Figure 2.20 : Protein profile of albumin fractions of 29 accessions of *Amaranth* seeds 36

Figure 2.21 : HPLC profiling of metabolites of fruiting bodies of *Antrodia cinnamomea* and *Antrodia salmonea* 37

Figure 2.22 : Distribution of *Schizophyllum commune* in Peninsular Malaysia 39

Figure 2.23 : Distribution of *Schizophyllum commune* in Sabah 39

Figure 2.24 : Sporophore of *Schizophyllum commune* 40

Figure 2.25 : Microscopic morphology of *S. commune* 41

Figure 2.26 : Life cycle of *Schizophyllum commune* 42

Figure 3.1 : Spawn 47

Figure 3.2 : Spawn running 48

Figure 3.3 : The evaluation of mycelia density 49

Figure 3.4 : Steps in protein extraction 51

Figure 4.1	:	Intensity of mycelial density of Malaysia strains, and their hybrids	57
Figure 4.2	:	Intensity of mycelia density of Malaysia parental strain (w1), Thailand parental strain, and their hybrids	59
Figure 4.3	:	Intensity of mycelia density of irradiated hybrids	62
Figure 4.4	:	Intensity of mycelial density of Malaysia parental strains, hybrid w1ew2a, and monokaryon-irradiated hybrid Ir-w1ew2a	64
Figure 4.5	:	Example of contaminations that occur in the substrate bag during fructification of Malaysia parental and hybrid strain	79
Figure 4.6	:	Example of contaminations that occur in the substrate bag during fructification of parental strains w1 and R	81
Figure 4.7	:	Example of contaminations that occur in the substrate bag during fructification Malaysia parental and hybrid strains	85
Figure 4.8	:	SDS-PAGE profile gel of Malaysia parental strains (w1 and w2) and the hybrids	87
Figure 4.9	:	SDS-PAGE profile gel of Malaysia and Thailand strains with the hybrids	91
Figure 4.10	:	SDS-PAGE profile gel of irradiated hybrids	93
Figure 4.11	:	SDS-PAGE gel profile of Malaysia parental strains (w1 and w2), hybrid w1ew2a and monokaryon-irradiated hybrid Ir-w1ew2a	96
Figure 4.12	:	Dendogram of the electrophoretic patterns of parental strains, hybrids, and monokaryon-irradiated hybrids, based on UPGMA analysis of the similarity coefficient matrix	104

LIST OF TABLES

Table 2.1	:	Various types of sawdust	14
Table 2.2	:	Lists of carbon sources and effect on fruiting in *S. commune*	16
Table 2.3	:	Lists of nitrogen sources and the effect on fruiting in *S. commune*	18
Table 2.4	:	Nutrient contents of wild and cultivated *S. commune*	43
Table 3.1	:	List of selected hybrids dikaryon (through hybridisation and hybridisation of gamma monokaryotic cultures) with their respective monokaryon cultures from w1, w2 and R.	46
Table 3.2	:	Volume and concentration of BSA for preparation of standard	52
Table 4.1	:	Average mycelial growth rate and mycelia density between parental strains (w1 & w2) and the hybrids	56
Table 4.2	:	Average mycelial growth rate and mycelia density between Malaysia parental strain (w1), Thailand parental strain (R), and their hybrids, w1eRa and w1jRc	59
Table 4.3	:	Average of mycelial growth rate and mycelia density among irradiated strains	61
Table 4.4	:	Average of mycelial growth rate (mm/day) and mycelia density of Malaysian parental strains (w1 and w2), hybrid w1ew2a, and monokaryon-irradiated hybrid Ir-w1ew2a	63
Table 4.5	:	Average size and morphology of the sporophores of the hybrids of Malaysian strains compared to the parental strains (w1 & w2)	66
Table 4.6	:	Average of width and morphology of sporophores of the hybrids between Malaysia and Thailand strains compared to the parental strains	69

Table 4.7	:	Average of width and morphology of the sporophores of the irradiated hybrids	71
Table 4.8	:	Average of width and morphology of the sporophores between parental strains (w1and w2), hybrid w1ew2a and monokaryon-irradiated hybrid (Ir-w1ew2a)	75
Table 4.9	:	Average of sporophores yield, biological efficiency (BE), percentage of contamination, and number of flushes between Malaysia parental strains (w1 & w2) and the hybrids.	77
Table 4.10	:	Average of sporophores yield, biological efficiency (BE), percentage of contamination, and number of flushes between Malaysia parental strain (w1), Thailand parental strain (R), and hybrids, w1eRa and w1jRc	81
Table 4.11	:	Average of sporophores yield, biological efficiency (BE), percentage of contamination, and number of flushes among irradiated hybrids	83
Table 4.12	:	Average of sporophores yield, biological efficiency (BE), number of flushes and percentage of contamination between parental strains (w1 and w2), hybrid w1ew2a and monokaryon-irradiated hybrid (Ir-w1ew2a)	84
Table 4.13	:	Molecular weight and number of protein bands of Malaysia parental strains (w1 and w2) and their hybrids	90
Table 4.14	:	Molecular weight and number of protein bands produce by Malaysia and Thailand strains with the hybrids	92
Table 4.15	:	Molecular weight and number of protein bands produced by irradiated hybrids	95
Table 4.16	:	Molecular weight and number of protein bands produced by Malaysia parental strains (w1 and w2), hybrid w1ew2a and monokaryon-irradiated hybrid Ir-w1ew2a	97
Table 4.17	:	The similarity coefficient (%) of the parental strains, non-irradiated hybrids, and irradiated hybrids based on protein data	100

LIST OF SYMBOLS AND ABBREVIATIONS

°C	:	Degree Celsius
%	:	Percentage
±	:	Plus-minus sign
µg	:	Microgram
µg/mL	:	Microgram per milliliter
µL	:	Microliter
µm	:	Micrometer
$CaCO_3$	:	Calcium carbonate
cm	:	Centimeter
g	:	Gravitational constant
g	:	Gram
kDa	:	Kilodalton
kGy	:	Kilogray
kg/cm^2	:	Kilogram per square centimeter
M	:	Molar concentration
mA	:	Milliampere
mg	:	Milligram
mg/mL	:	Milligram per milliliter
mL	:	Milliliter
mM	:	Millimolar
mm/d	:	Millimeter per day
nm	:	Nanometer
Rf	:	Relative mobility

V	:	Voltan
v/v	:	Volume per volume
w/v	:	Weight per volume
^{1}H NMR	:	Proton nuclear magnetic resonance
AFLP	:	Amplified fragment length polymorphism
ANOVA	:	Analysis of Variance
BE	:	Biological efficiency
BSA	:	Bovine Serum Albumin
CE-MS	:	Capillary electrophoresis coupled to MS
CHAPS	:	3-[(3-cholamidopropyl)dimethylammonio}-1-propanesulfonate
DNA	:	Deoxyribonucleic acid
DPPH	:	1,1-diphenyl-2-picryl-hydrazyl-hydrate
DTT	:	Dithiothreitol
EMS	:	Ethyl methanesulfonate
EPS	:	Exopolysaccharides
FT-IR	:	Fourier transform infrared spectroscopy
GC-MS	:	Gas chromatography coupled with mass spectrometry
GCxGC-MS	:	Two-dimensional GC coupled to MS
GMO	:	Genetically modified organism
HPLC	:	High performance liquid chromatography
LC-MS	:	Liquid chromatography coupled to MS
MEA	:	Malt extract agar
MS	:	Mass spectrometry
PCR	:	Polymerase chain reaction
PDA	:	Potato dextrose agar
PEG	:	Polyethylene glycol

RAPD	:	Random amplified polymorphic DNA
SDS-PAGE	:	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	:	Scanning electron microscopy
SPG	:	Schizophyllan
SPSS	:	Statistical Package for the Social Sciences
TCA	:	Trichloroacetic acid
UV	:	Ultraviolet

LIST OF APPENDICES

Appendix A	:	Protocols for SDS-PAGE stock solution and buffers and preparation of stacking and resolving gel	127
Appendix B	:	Protocols for silver staining solution	128
Appendix C	:	Graphical representation of protein bands and calibration curve of protein marker	130
Appendix D	:	Graphical representation of protein bands of each strain	131
Appendix E	:	Relative Mobility (Rf) parental strains, hybrids, and irradiated monokaryon hybrids based on protein data	133
Appendix F	:	Distance of the parental strains, hybrids, and irradiated monokaryon hybrids based on protein data	137
Appendix G	:	Relative Mobility (Rf) Parental Strains, Hybrids, and Irradiated Monokaryon Hybrids based on Protein Data.	146

CHAPTER 1: INTRODUCTION

The edible mushroom in industry is becoming more important every year because mushrooms are an effective low-fat protein source, capable to be cultivated in a wide range of substrates and very useful in many industrial and medical applications (Gharehaghaji *et al.*, 2007). Numerous studies have demonstrated the effectiveness of multiple compounds in mushroom towards the human health, such as antifungal properties (Ye *et al.*, 1999), antioxidation (Roupas *et al.*, 2012), anti-hypertensive, immunostimulation (Vaz *et al.*, 2011), and hypocholesterolaemic activity (Han *et al.*, 2011).

Schizophyllum commune is one of the main edible mushroom which is cultivated in Malaysia (Zainol, 2016). *Schizophyllum commune* is able to grow on decaying woods under natural conditions and thrive during the rainy season (Dasanayaka & Wijeyaratne, 2017). This mushroom can be isolated in every continent except Antarctica (Khatua *et al*., 2013). *Schizophyllum commune* is recognised as a great source of proteins, lipids, vitamins, and mineral elements (Adejoye *et al.*, 2007). Schizophyllan is a water-soluble polysaccharide which is extracted from *S. commune* and shows immunomodulatory, antineoplastic and antiviral activities which are important in the pharmaceutical industry (Kumari *et al.*, 2007).

The quality and productivity of cultivated mushrooms largely rely on the genetic makeup of the strain (Kaur & Sodhi, 2012). Therefore, various new strains have constantly been cultivated which aim at the greater yield, pathogen resistance, faster maturation and improved quality characteristics (Kumara & Edirimanna, 2009). Cultivation and introduction of improved strains could improve the marker lower the costs of cultivating and accelerate the farmers' revenue (Avin *et al.*, 2014). In addition,

the production of intraspecific which assemble the desired traits from particular strains can generate pre-eminent strains (Avin *et al.*, 2016).

Genetic variation is the main component which breeders require to develop new and improved cultivars (Suprasanna *et al.*, 2015). Mushroom breeding can be achieved through the various methods such as mycelial mating (hybridisation) (Lee *et al.*, 2011) and induced mutation. Hybridisation begins with the selection of lines which represent the desired traits. The parents are crossed and the progenies are selected based on the desired combination of traits (Sonnenberg *et al.*, 2008). Mutation induction is defined as the exposure or treatment of biological samples to one or more mutagens that elevate the frequency of mutation above the natural spontaneous rate (Bado *et al.*, 2015). Classical breeding (hybridisation) methods are still employed effectively for the generation of new species and to acquire expected superior characteristics. However, this method results in the contraction in the gene. Therefore, some breeder prefers to apply mutation breeding as an alternative method (Ulukapi & Nasircilar, 2015).

SDS-PAGE can be used to differentiate between parental strains and hybrids. The protein electrophoretic separation produces the characteristic banding pattern which has been utilised with good outcome to estimate the genetic diversity in various crops such as rice, cotton and wheat (Iqbal *et al.*, 2014). The banding pattern also known as protein electrophore gram or electrophoretic protein is a reproducible technique and can be considered as a 'fingerprinting' of the strain (Ehlers & Cloete, 1999). These protein electrophoretic patterns are directly associated with the genetic background of the protein and can be applied to verify the genetic composition of wild, cultivated, or newly derived organism (Gorinstein *et al.*, 1999).

Thus, the objective of this study is to develop *Schizophyllum commune* hybrids with improved traits in terms of the morphology of sporophores, mycelial growth rate, yield and biological efficiency through cross-breeding (hybridisation) and induce

mutation. The different traits are analysed through proteomic approaches. The protein profiling of different traits is obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

1.1 OBJECTIVES

The specific objectives of the current study are:

1. To evaluate the sporophore morphology and yield of novel hybrids of *Schizophyllum commune* obtained by hybridisation and mutation.
2. To determine the protein profiling of parental and hybrids strains of *Schizophyllum commune* by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

CHAPTER 2: LITERATURE REVIEW

2.1 World Mushroom Production

During the earliest history, records have shown that mushrooms have been consumed by the human population. For example, the ancient Greeks believed that mushrooms supplied strength for warriors in battle. The Romans recognised mushrooms as the 'Food of the Gods'. The Chinese culture has perceived mushrooms as a health food, an 'elixir of life' (Valverde *et al.*, 2015). Mushrooms are fungi which consist of high protein, carbohydrate, multivitamins, and minerals that are valuable for the health and has a high source of folic acid. Scientific studies proved that vitamins and minerals in mushrooms are suited for nutraceutical, pharmaceutical, and cosmetic product. Human consumes mushroom due to several reasons which are for their nutritional, medicinal and recreational activities or for religious purposes (Amin *et al.*, 2013).

The worldwide mushroom industry has expanded in terms of production and addition of new types of edible mushrooms at a fast rate since the late 1990s (Royse, 2014). China is the leading country that produce mushroom globally, followed by United States of America (USA) and European Union Countries such as France, Germany, The Netherlands, Italy, Poland, Spain, Hungary and others (Dhar, 2014). Five major genera of mushrooms which are mainly cultivated are *Agaricus* (primarily *Agaricus bisporus* with some *Agaricus brasiliensis* which constitutes approximately 30 % of the world's cultivated mushroom. Secondly, *Pleurotus* spp contributing about 27 % of the world's production while *Lentinula edodes* (Shiitake) generate 17 % of the total world's output. Genera of *Auricularia* and *Flammulina* contributing 6 % and 5 % respectively. The figure of the world mushroom production by genera is shown in the Figure 2.1 (Royse, 2014).

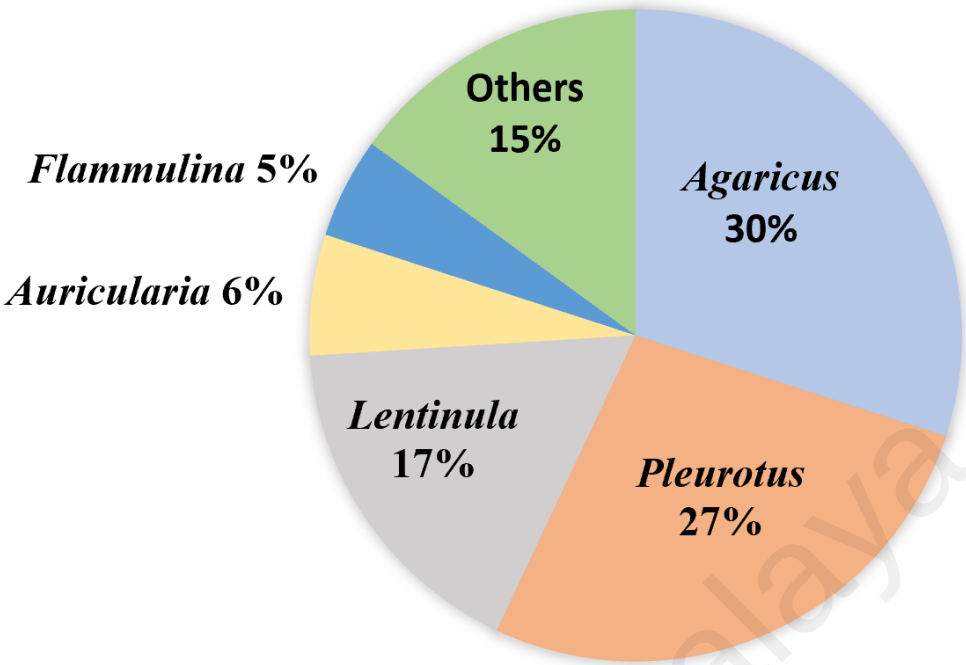


Figure 2.1: World mushroom production by genera (Cited from: Royse (2014).

Malaysia has agro-climatic conditions which are applicable for the cultivation of mushrooms during the whole year. Hence, Malaysia has the capability to become a large mushroom producer in the world market (Amin & Harun, 2015). The production of mushroom in Malaysia has increased from RM49.1 million in 2007 to RM110 million in 2014 (Zainol, 2016). *Pleurotus pulmonarius* is the most cultivated mushroom species in Malaysia (Rashid *et al*., 2016). The huge increase of mushroom production in Malaysia is contributed by the increasing number of growers, productivity, land area and application of mushroom in both medicine and culinary (Ibrahim *et al.*, 2017). Recently, research studies in Malaysia are more focused on identifying new mushroom strains with nutritious and medical properties for commercial purpose (Avin *et al.*, 2014).

2.2 Mushroom Artificial Cultivation

Mushroom cultivation technology is environmentally-friendly whereby mushroom mycelia secretes various extracellular enzymes which can degrade and manipulate the lignocellulosic wastes and hence reducing pollution. In addition, mushroom cultivation also requires a number of workers, thus can help to provide income and employment, especially for women and youth in developing countries (Girmay *et al.*, 2016).

Mushroom cultivation includes several processes which must be operated properly. Miscalculation or inaccuracy at any step can cause a decline in total yield or total loss (Royse, 1997). Figure 2.2 shows the main steps for mushroom depending on the species cultivated. The mycelium can be acquired from a small piece of the certain mushroom, or from several germplasm suppliers such as American Type Culture Collection and National Center for Agricultural Utilization Research). The mycelium is then propagated onto a medium consisting of grains, sawdust or liquid nutrients known as spawn (Sánchez, 2004).

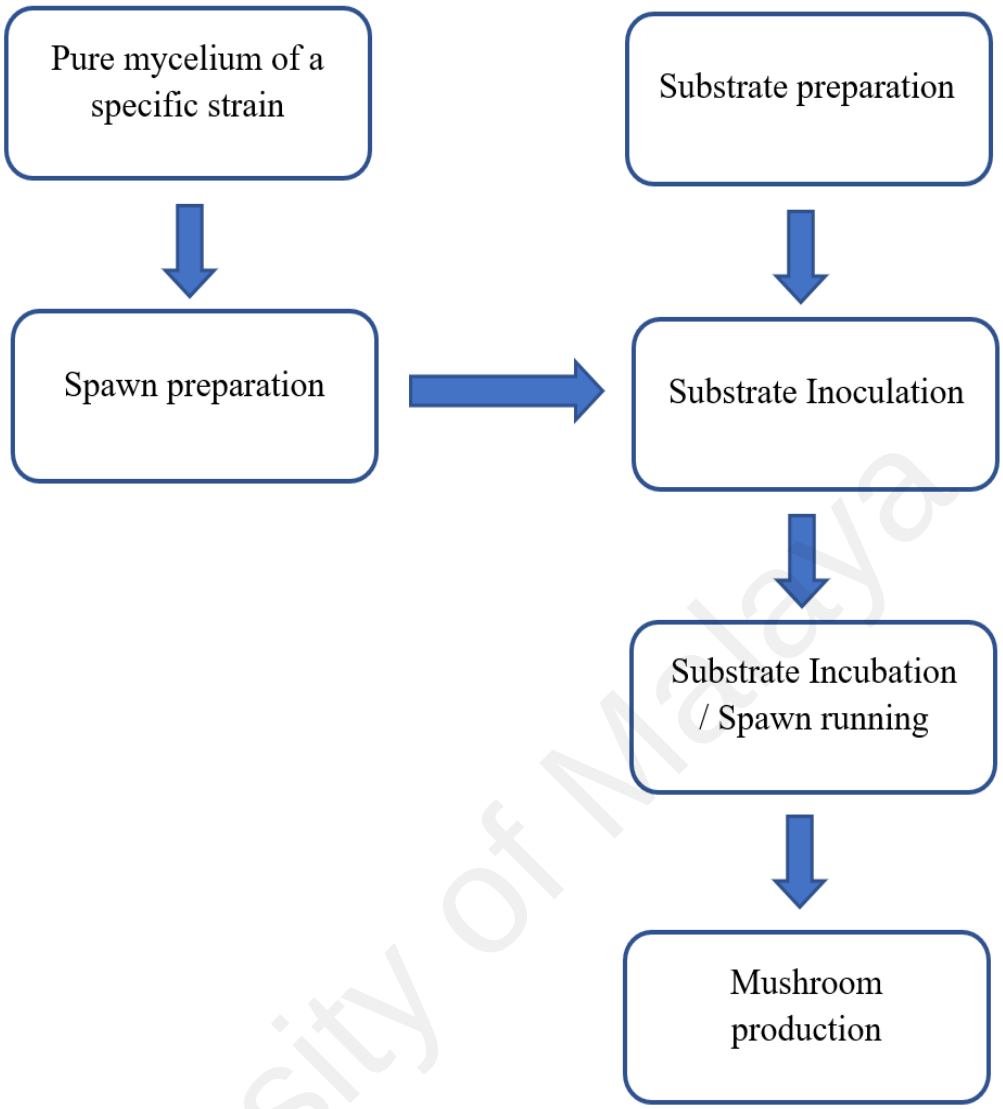


Figure 2.2: General steps of mushroom production (Cited from: Sánchez (2004).

The mycelium growing on spawn will grow through the substrate during the spawn run phase. The period of the spawn run is varied between species and rely on the size of the bag, the quantity of spawn and the temperature. Then, the bags are located in the incubation rooms. Within two to three weeks, the mycelium will colonise the substrate and begin to produce small fruiting bodies. Harvesting is carried out by slowly pulling or twisting the mushrooms from the substrate (van Nieuwenhuijzen, 2005).

2.2.1 Fungal Media

Agar is a seaweed-derived substance that solidifies water. Nutrients are included into the agar solution which after sterilisation, include the growth of a healthy mushroom mycelium (Stamet, 1993a). Media containing plenty of nitrogen and carbon sources are essential for the cultivation of fungi at pH range of 5 to 6. There are two main types of fungal culture which are natural media and synthetic media. Natural media contains natural components such as herbaceous or woody stems, leaves, seeds, corn meal, wheat germ, oatmeal and others while synthetic media composed of substrates of known composition (Basu *et al.*, 2015). Many agar media are easy and simple to prepare (Stamet, 1993a). There are many formulas for preparing enriched agar media for mushroom culture. The commonly used agar media for mushroom culture are potato dextrose agar (PDA) and malt extract agar (MEA) in which yeast is frequently added as a nutritional supplement (Stamets & Chilton, 1983a). Antibacterial agents such as sodium azide, potassium tellurite and strepromycin may be added to the agar media to prevent the growth of certain bacteria which might retard the development of fungi (Littman, 1947).

2.2.2 Spore Printing and Mushroom Tissue Culture

The first step in mushroom culture can be done in two ways which are spore printing and mushroom tissue culture (Stamets & Chilton, 1983a). Spore print is a technique to photograph the print of the shape and size of mushroom with pores or gills. The colour of the print provides information about the species of the mushroom (Storey, 2005). For example, *Amanita* spp has white spore color, *Gymnopilus* spp has rusty orange spore while *Psilocybe* spp has purplish or purple brown spore colour (Menser, 1996). Spore print also help in supplying the clean sample of mature spore for microscopy and culturing. The main fundamental of spore printing is to place the fruiting body with gills,

pores or spines facing downward on the appropriate surface by covering it under glass container to capture the spores (Storey, 2005). After a few hours, the spores will form a print that resembles the pattern of the gills of the mushroom as shown in Figure 2.3 (Thomas, 1928).

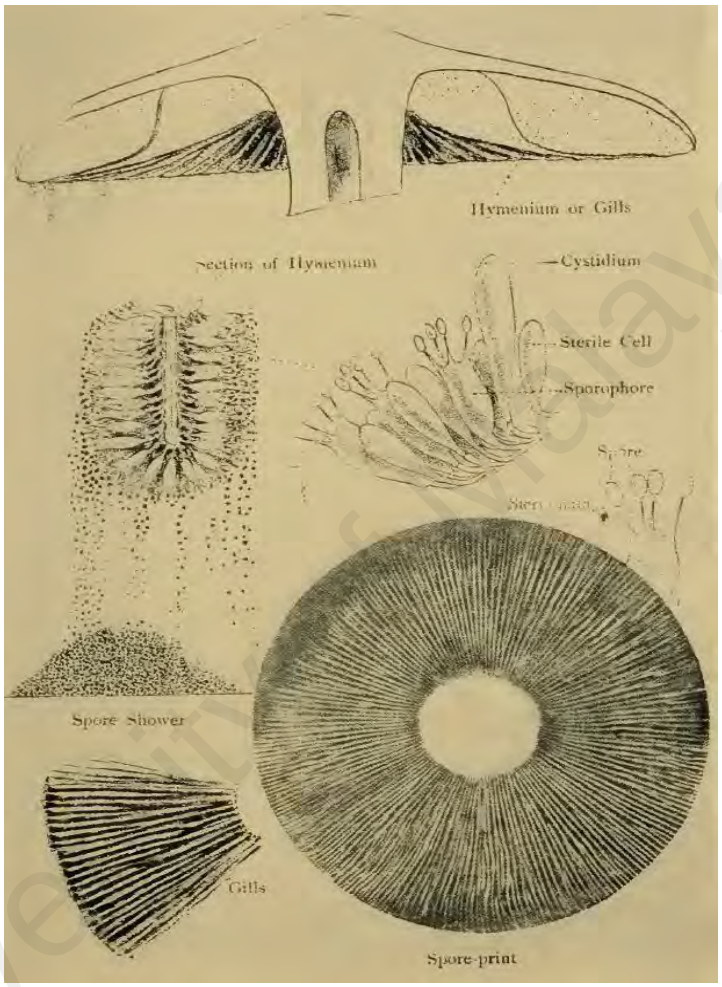


Figure 2.3: Mushroom spore printing (Cited from: Thomas (1928).

Mushroom tissue culture or cloning is a method where a piece of pure, living flesh is removed from the mushroom and transfer into a sterilised agar medium as shown in Figure 2.4. This technique allows the cultivators to retain the unique strain which express the particular phenotype. Young mushroom is preferable compare to the old mushroom as young mushroom has a very active cell division. However, old mushroom can still be cloned but have a larger risk of contamination and are slower to grow (Stamet, 1993a).

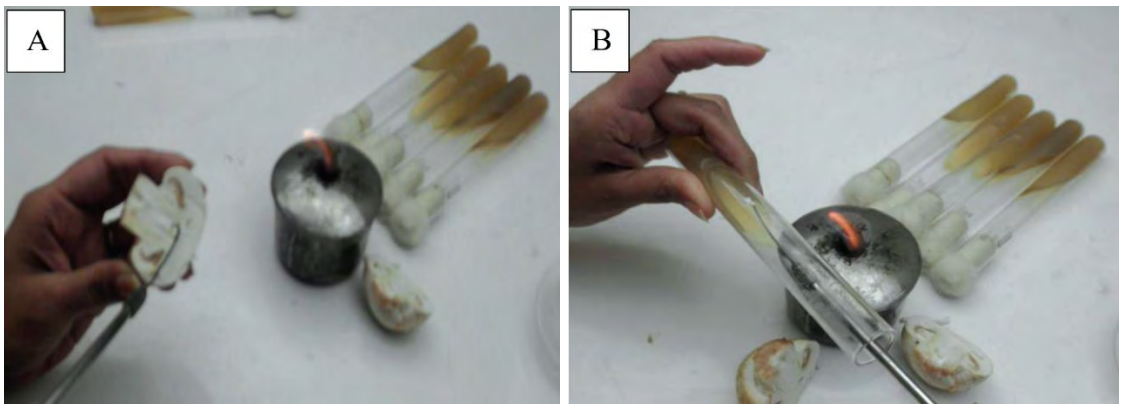


Figure 2.4: Procedure of tissue culture mushroom (A) Mushroom tissue culture (B) Inoculation of mushroom tissue culture into a tube with medium agar (Cited from: Upadhyay (2011).

2.2.3 Preservation of Mycelial Cultures

Proper culture preservation is an essential factor for successful mushroom cultivation. The primary objective of culture preservation is to maintain cultures in viable and stable condition for the long term without reducing phenotypic, genotypic and physiological characteristics (Veena & Meera, 2010). There are several methods to preserve the sample, which include the subculturing method, using liquid nitrogen, and the storage under sterile water, mineral oil, or glycerol (Karaduman *et al.*, 2012). The method of preserve depends on the species, the resources available and the objectives of the project (Nakasone *et al.*, 2004).

The simplest methods of preservation are continuous growth, drying, and freezing. Each culture is grown on agar and stored at the temperature from 5 °C to -20 °C, or the cultures may be frozen. This method is cost-effective because no specialised equipment is used (Nakasone *et al.*, 2004). Preserving culture under the sterile mineral oils such as liquid paraffin or medical paraffin is one of the oldest methods for long-term storage. The oil acts by maintaining the sterile condition and eliminating gas exchange, thus decreasing fungal metabolism. This method can preserve the sample for decades

(Humber, 1997). The example of mycelia preserved in mineral oil is shown in Figure 2.5 (Upadhyay, 2011).

Figure 2.5: Preserving mushroom mycelia in the mineral oil (Cited from: Upadhyay (2011).

Storage under sterile distilled water is an economic and low-maintenance technique for oomycetes, ascomycetes, basidiomycetes, ectomycorrhizal, plant pathogenic fungi, Hyphomycetes, stramenopiles (water molds), aerobic actinomycetes, yeasts and human pathogen. In this method, small square agar blocks are cut from the sample and placed in sterile distilled water. This allows the sample to remain viable for two to three years (Mehrotra, 2009).

In addition, freeze-drying which also known as lyophilisation is a low-cost method of permanent preservation (Nakasone *et al.*, 2004). Spores and conidia are more suitable for lyophilization compared to mycelium (Hwang, 1966). Lyophilization enables to stabilise the mechanism of the cell for a long period of time. However, this method is complicated and requires high-cost equipment (Ingroff *et al.*, 2004). In this method, the samples are kept at a very low temperature and placed under a high vacuum. The water content in the sample will undergo sublimation, from the frozen solid state to the gaseous state. Thus, the sample will dry without disturbing them (Mehrotra, 2009).

Preservation using liquid nitrogen is an effective technique for the most organism which includes those that cannot be lyophilized. These methods are successfully preserved dictyostelids, amoebae, Entomophthorales, Zygomycetes, oomycetes, phytopathogenic fungi, and yeasts. This technique will cease cell division completely and totally stop metabolism, while still remaining viability (Nakasone *et al.*, 2004).

2.2.4 Spawning

Spawn is generally known as the mushroom seed. Spawns are produce by growing the mycelium of mushrooms on a base of the sterilised substrate such as cereal grain **(**Royse, 1997) with the aim to propagate the vegetative mycelium of mushroom (Rosado *et al.*, 2001). This involves production of pure culture of mushroom from spores or tissue that is generally grow on any agar medium, followed by transferring on sterilised grains and then multiplied on grains. Thus, the spawn consists of mycelium of the mushroom and medium which supplying nutrition to the mycelia during theirs growth (Sharma & Kumar, 2011).

The grain spawn can be prepared by submerging the grains in a boiling water for one hour, removed the water and sterilise the grains. Moisture content exhibits an important role in the successful colonisation by mushroom mycelium of sterilised grain. If the grain is too dry, mycelia with form fine threads, grow at a slow pace and can lead to the growth retardation. However, if the grain is too moist, it will enhance the growth of other parasitic microorganisms. The ideal moisture content for grain spawn is in the range of 45 % to 55 % (Stamets, 1993b).

2.2.5 Preparation of Substrate for Cultivation

A substrate is the substance or material that supply 'food' for the mycelium for mushroom production (Kashangura *et al.*, 2004). The standard criteria of substrates for mushroom cultivation includes absence of pests and diseases, specific accessibility of nutrients for the cultivated species, suitable pH value and a bulk density enabling gas exchanges and avoiding excess moisture content (Zied *et al.*, 2011). Mushrooms rely on the substrate for nutrition. Substrate usually contains lignocellulose material which promotes growth, development, and fruiting of mushrooms. *Schizophyllum commune* normally grows on the decaying wood (Dasanayaka & Wijeyaratne, 2017).

Several wood substrates are used for the cultivation of *S. commune* and Table 2.1 shows the various types of sawdust used in this study. In this study, *Artocarpus heterophyllus* (Jackfruit) shows the highest yield of *S. commune* while *Alstonia macrophylla* (Thungfaa) shows the lowest yield (Dasanayaka & Wijeyaratne, 2017). Different kinds of mushrooms utilise different types of substrates (Miles & Chang, 2004). Rice straw, sawdust, and wheat straw are commonly used for the cultivation of oyster mushroom in Asia, Southeast Asian and Europe respectively (Uddin *et al.*, 2013). Paddy straw and cotton waste are the suitable substrates for the cultivation of paddy straw mushroom, *Volvariella volvacea* (Rajapakse, 2011).

The properties of appropriate substrate containers are the containers should encase the substrate during the spawn run, not easily break, avoid too much loss of moisture and self-heating and enable the highest production in space utilised. In the developing countries, the commonly used substrate containers are plastic bags which are made up from polyethylene (for pasteurised substrates), polypropylene or polyvinyl chloride (for sterilised substrate) (Mamiro *et al.*, 2014). Prior to mushroom cultivation, the substrates are sterilised to produce a medium which is exclusive to the mushroom without allowing the growth of other organisms, thus reducing competition (Kortei & Kwagyan, 2014).

Table 2.1: Various types of sawdust (Cited from: Dasanayaka & Wijeyaratne (2017).

English Name	Local Name	Scientific Name	Family
Thungfaa	Hawari Nuga	*Alstonia macrophylla*	Apocynaceae
Jackfruit	Kos	*Artocarpus heterophyllus*	Moraceae
Tulipwood tree	Na Imbul	*Harpullia arborea*	Sapindaceae
Mango	Amba	*Mangifera indica*	Dilleniaceae
Elephant apple	Honda para	*Dillenia indica*	Sapindaceae
Rambutan	Rambutan	*Nephelium lappaceum*	Combretaceae
Country almond	Kottamba	*Terminalia catappa*	Anacaraceae

2.2.6 Harvest and Cropping

Harvesting is accomplished by gently twisting or using a tool like knife or scissor to remove the mushrooms from the substrate. In generals, three or four flushes can be harvested depends on the environmental conditions and the species of the mushroom (Oei, 2005). Young mushrooms should be harvest as the aged mushrooms bear more spores on the top of gills and this accelerates the deterioration in term of quality. For most species, the ideal phase for harvesting is when the caps are still in convex position and before flattening out (Stamets, 2000). Carelessness during harvesting can increase the cost of mushroom production that are endure by breeders (Stamets & Chilton, 1983b).

2.3 Growth Condition

2.3.1 Nutritional Requirements

2.3.1.1 Carbon Sources

Carbon sources supply energy and structural support for the fungus (Miles & Chang, 2004). Simple, defined medium which consists of D-glucose, L-asparagine and thiamine are suitable for *S. commune* to grow and fruits. Various carbon sources are applicable for *S. commune*, including certain monosaccharides, disaccharides, sugar alcohols, xylose and ethyl alcohol. However, lactose, L-sorbose, and inositol are found to be a poor carbon source as shown in Table 2.2 (Niederpruem & Wessels, 1969). Based on Figure 2.6, carbon source xylose shows the maximum yield of mycelial biomass and EPS while glycerol shows the lowest mycelial biomass and exopolysaccharides (EPS) (Joshi *et al.*, 2013).

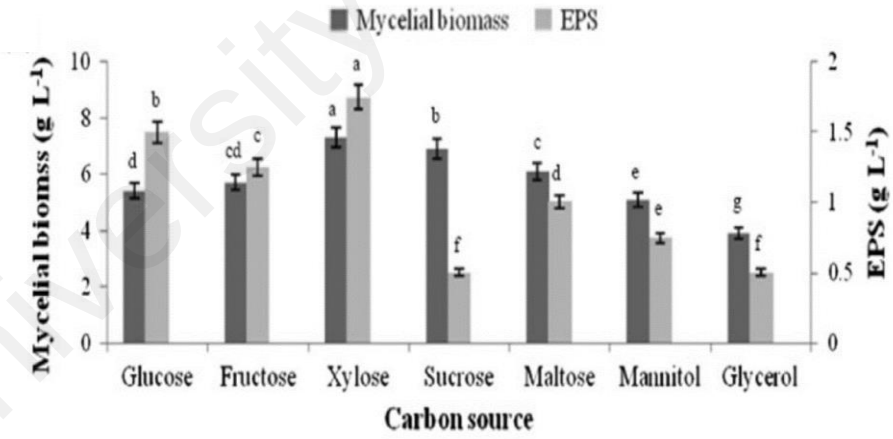


Figure 2.6: Effect of carbon sources on the mycelial biomass and EPS production in *S. commune* (Cited from: Joshi *et al.*, (2013).

Alternative substrates such as bagasse, molasses and sawdust can be used to supply the carbon sources for the cultivation of mushroom. Bagasse and molasses are by-product from the extraction of sugar process. The main composition of sawdust and rice bran are cellulose and hemicellulose. Glucose and cellobiose are produce from the

digestion of cellulose while xylose and other sugars such as mannose, galactose, pyranose, and arabinose are generated from the digestion of hemicellulose. These sugars provide the carbon sources for the growth of mycelia, initiation of primordium and fruiting body formation (Vetayasuporn *et al.*, 2006).

Table 2.2: Lists of carbon sources and effect on fruiting in *S. commune* (Cited from: Niederpruem *et al.*, (1964).

Good	Fair	Poor
Sucrose	Raffinose	Lactose
Maltose	Sorbitol	L-Rhamnose
Trehalose	Ribose	L-Sorbose
Cellobiose	L-Arabinose	Arabinose
Glucose		i-Inositol
Fructose		Succinate
Mannose		Citrate
Galactose		Acetate
Mannitol		
Xylose		
Glycerol		
Ethanol		

In Malaysia, the abundant of the paddy straw which are generated seasonally as solid waste can be utilise as an alternative substrate for mushroom cultivation. In addition, empty fruit bunches (EFB) and palm pressed fiber (PPF) are the wastes produce from the oil palm industry (Harith *et al.*, 2014). These by-products contain high amount of cellulose, hemicellulose and lignin that able to provide carbon source for mushroom cultivation. Specifically, EFB contains 48.4 % carbon and 0.2 % nitrogen while PPF contain 47.2 % carbon and 1.4 % nitrogen (Tabi *et al.*, 2008).

2.3.1.2 Nitrogen Sources

Nitrogen is crucial in producing proteins, pyrimidines, and purines (Miles & Chang, 2004). Nitrogen also an important element for the formation of the cell wall of the fungus which composed of β(1-4)-linked unit of N-actylglucosamine (Hoa & Wang, 2015). Nitrogen is also used in the formation of enzymes which are involved in the synthesis of both primary and secondary metabolites. Yeast extract (nitrogen source) produced the highest mycelial biomass and EPS in *S. commune* as shown in Figure 2.7 (Joshi *et al.*, 2013). Study done by Debnath *et al.* (2017) also reported that yeast extract was the best nitrogen sources for *S. commune*. Yeast extract can be obtained from the by-product of the brewery industry consists of high level of protein and vitamin B (Harith *et al.*, 2014). There is various nitrogen which gives a good effect on the fruiting of *S. commune* as shown in Table 2.3 (Niederpruem *et al.*, 1964). Ammonium chloride is an excellent source of nitrogen for the cultivation of fungus of *Pleurotus florida*, *Pleurotus ostreatus*, *Villosiclava virens* and *Cryphonectria parasitica*. However, excessive concentration of nitrogen leading to a very low carbon/nitrogen ratio, which restrained the growth of mycelium (Hoa & Wang, 2015).

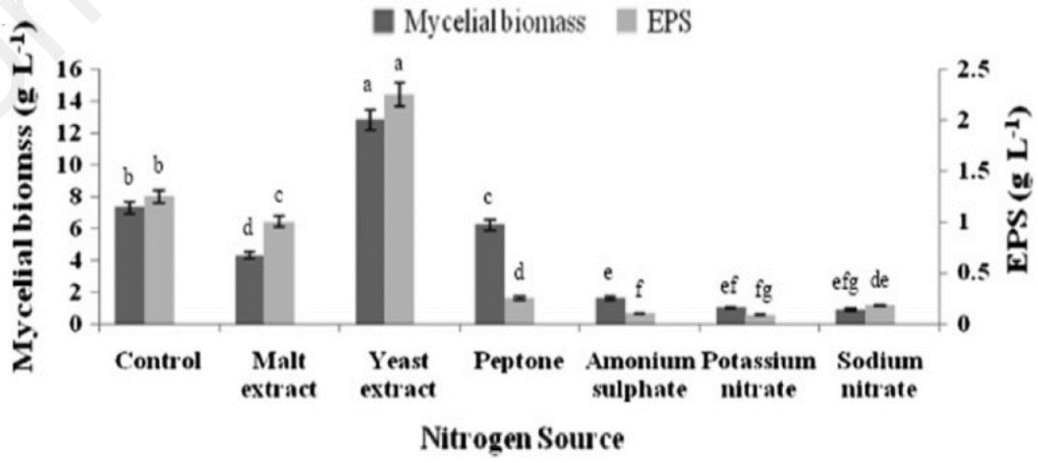


Figure 2.7: Effect of nitrogen sources on the mycelial biomass and EPS production in *S. commune* (Cited from: Joshi *et al.*, (2013).

The substrates like sawdust, cereal straw, cottonseed straw and corncob can also be added with additional nitrogen sources such as oat bran, wheat bran, rice bran, millet or sorghum to improve the quality of the mushroom (Masevhe *et al.*, 2015). In addition, alternative substrate such as amaranth flour can be used to promote the mycelial growth of *S. commune*. Amaranth flour is a product after carbon dioxide extraction of *Amananthus* grains to produce squalene and oils, which contains high amounts of amino acids such as glutamic acid, argenine, leucine and glycine that supplies nitrogen sources for the growth of *S. commune* (Krupodorova & Barshteyn, 2015). Moreover, soybean meal can be used as another alternative substrate due to its high level of protein content especially for *Agaricus* species (Zeid *et al.*, 2011).

Table 2.3: Lists of nitrogen sources and the effect on fruiting in *S. commune* (Cited from: Niederpruem *et al.*, (1964).

Good	Fair	Poor
Peptone	Threonine	Cysteine
Tryptone	Leucine	Histidine
Casitone	Glycine	*β*-Alanine
Yeast extract	Proline	D-Alanine
Asparagine	Valine	D-Lysine
Glutamine	Phenylalanine	KNO_3
Glutamic acid	Isoleucine	Tryptophan
Serine	Methionine	KNO_2
Alanine		Lysine
Arginine		
Urea		
di-Ammonium hydrogen phosphate		
Ammonium chloride		
Ammonium nitrate		
Ammonium sulphate		

2.3.2 Physical Requirements

2.3.2.1 Temperature

Temperature is one of the most crucial factors that will affect the fungal growth and product production (Teoh *et al.*, 2017). Temperature can influence the rate of enzyme-catalysed processes, and extreme temperatures can denature the proteins (Tang *et al.*, 2015). Most of the higher fungi thrive at a temperature between 24 °C and 40 °C. However, some species can grow at high temperature and some can grow at low temperature (0 °C to 15 °C (Teoh *et al.*, 2017). The mycelial growth and density of *S. commune* grow at the optimum temperature between 30 °C to 35 °C. At 15 °C, no growth was recorded in one of the strains (Imtiaj *et al.*, 2008). Based on Figure 2.8, 35 °C was the optimum temperature for mycelial growth and the spawn running required 11 days after inoculation to completely cover the culture bag. However, the time taken for spawn running were longer for temperature 20 °C, 25 °C and 30 °C. At the temperature of 15 °C and 45 °C, no mycelial growth was recorded (Dasanayaka & Wijeyaratne, 2017).

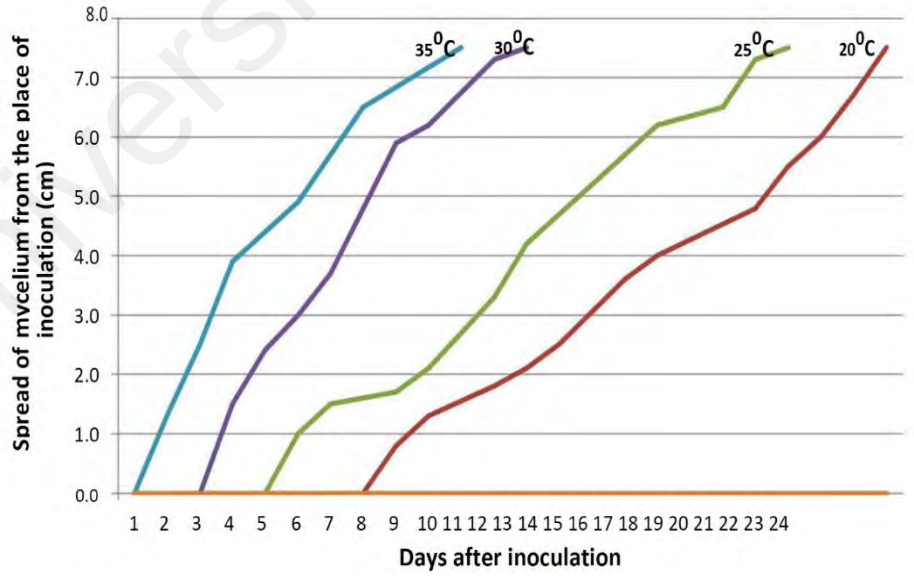


Figure 2.8: Effect of temperature on mycelium growth of *S. commune* (Cited from: Dasanayaka & Wijeyaratne (2017).

When *S. commune* was cultured in submerged shake culture, the highest biomass was formed at 30 °C as shown in Figure 2.9. High temperature can affect the denaturation of fungal internal structure (Teoh *et al.*, 2017). Fruiting commonly occurs at 20 °C to 25 °C, however, fruiting is inhibited by increasing temperature (30 °C to 37 °C). During fruiting, high temperature can cause a decrease in susceptibility of glucan in the cell wall towards enzymatic attack (Niederpruem & Wessels, 1969).

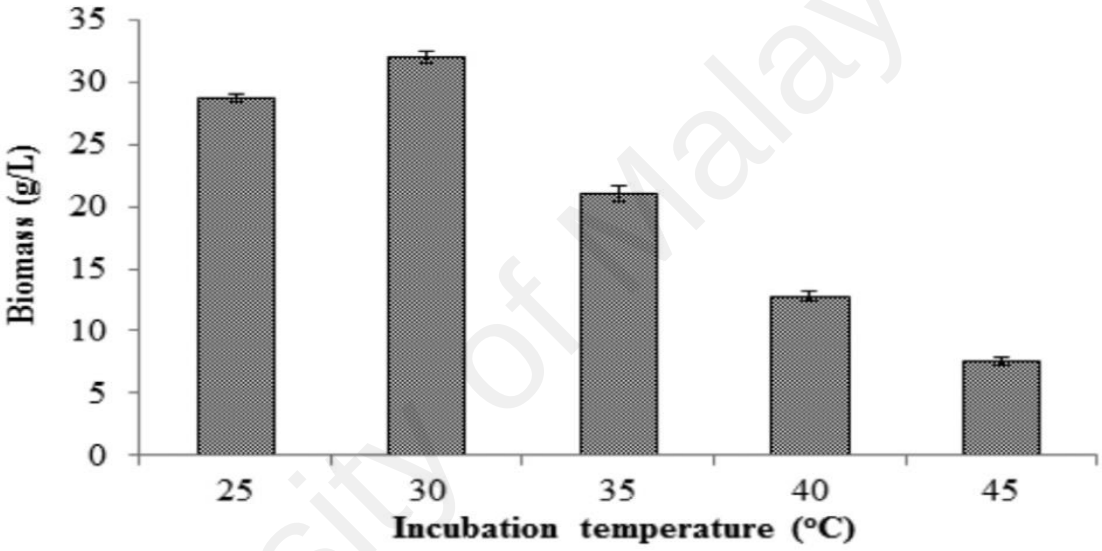


Figure 2.9: The growth of *S. commune* in submerged shake culture at different temperature (Cited from: Teoh *et al.*, 2017).

2.3.2 Light

The growth of many fungi is not affected by light. However, strong light may prevent or even kill the fungi because strong light can eliminate certain vitamins. The most important function of light is in the phototrophic responses of reproductive structures, production of reproductive structures and formation of fruit body primordia. Ultraviolet light between 200 nm to 300 nm can cause a lethal effect or it may trigger mutations (Miles & Chang, 2004). However, this effect can be reversed through a process known as photoreactivation, where the sample is exposed to visible light between the

range 360 nm to 420 nm. This wavelength able to trigger an enzyme which separates the thymine and cytosine dimers those allow the normal DNA synthesis to take place (Miles & Chang, 1997).

In *S. commune*, the production of primordia and the initial stages of fruiting body development requires light. During the early stages of the fruiting body, small cylindrical stipes with terminal apical pits are developed. However, light is not needed during the formation of gills and the continuous growth of the mature fruiting body. In addition, the spore germination will cease when the mature fruiting body is placed in the dark. This situation can be reversed by exposing the mature fruiting body to the light for five to six hours (Bromberg & Schwalb, 1976).

2.3.3 Moisture

The moisture content of the substrate and the relative humidity of the atmosphere are an important factor in the mushroom cultivation (Miles & Chang, 1997). A constant moisture flow is necessary to transport nutrients from the mycelium to the fruiting bodies of the mushroom. Very high moisture content in the substrate can lead to the difficult breathing for the mycelium, preventing perspiration, interrupt the development of the fruiting body and enhancing the growth of non-desired organisms such as bacteria and nematodes. Low moisture content can cause the death of the fruiting body (Bellettini *et al*., 2016). However, different species and different phase of growth may require different humidity (Miles & Chang, 1997). Generally, most fungi need high moisture levels, which 95 % to 100 % for the relative humidity and 50 % and 75 % for the moisture content of the substrate (Miles & Chang, 2004). However, there is an exception for the *Serpula lacrymans*, whose mycelial can grow in the low moisture substrates by the mechanism of translocation of nutrients and the generation of 'metabolic water' (Miles & Chang, 1997).

2.4 Genetic Diversity in Mushroom Breeding

Despite an increasing demand, mushroom industry encounters many challenges including lack of the quality seed, high contamination, and reduced mushroom yields. Therefore, new mushrooms varieties strain with favourable features such as high yields, fast colonisation, and resistance to disease are required to maintain the development and sustainability of the industry (Rashid *et al.*, 2016). The main objective of breeding is to combine the desirable features from different strains and generate variability in the existing germplasm. Morphological features including prominent interaction in the contact zone, high rate of mycelium growth, better colony morphology have been applied as the morphological markers in the previous report for breeding purposes (Gupta *et al.*, 2011; Gharehaghaji *et al.*, 2007).

The strain used for cultivation is important for the success of mushroom production and marketing (Sánchez, 2009). One strain can establish a various range of morphologies depending on the maturity of the colonies, the medium and the external conditions such as temperature and light (Hansen *et al*., 2018). Genetic variation is the main cause of phenotypic diversity and is the source of evolutionary diversification (Cieslak *et al.*, 2017). In order to introduce new traits, wild strains which is unrelated strains are employed as they vary in the genetic base and differ in one or a number of traits. However, the wild strains still restrain their common agronomic values. Hence, interbreeding of these wild strains will lead to the formation of new combinations of trait without the loss of essential traits such as yield and quality (Sonnenberg *et al.*, 2008). The culture and generation of new species of mushrooms are rising. The culturing and improving new strains has significantly enhanced, allowing the formation of strains which produce high yield and resistance to diseases, increasing productivity, eliminating the use of chemicals for pest control (Singh *et al.*, 2007) and lower the production costs (Amin *et al.*, 2014).

2.4.1 Hybridisation

Hybridisation is a method for exchange of genetic information between two compatible nuclei to generate a recombinant genome with a probable expression for a desirable trait. This method able to generate various changes at the genetic levels, resulting in altered phenotypic expression of the characters, such as fruiting efficiency and variability in sporophores characteristics (Kaur *et al.*, 2008). The first cross-breeding had occurred in 1983 which develop two *Agaricus bisporus* hybrid known as hybrid Horst U1 and Horst U3 (Fan *et al.*, 2006). Hybridisation is not limited to the mushroom only and can be applied to other crops. Exchange of genes between two nuclei during cross hybridisation is known to develop at random to produce a cross-over set of genes (Kaur *et al.*, 2008).

This technique will produce dikaryotic mycelial cells when both mycelia consist of compatible mating type genes. In the tetrapolar mating system, mushroom generates four different haploid basidiospore and their mating is controlled by mating type genes in two independent loci, A and B (Ha *et al.*, 2015). Loci A determine the pairing of the haploid nuclei from the parental strains, the production, and septation of clamp cells and mitotic division of the paired nuclei. Loci B controls the migration of nuclei, the fusion of the A-induced clamp cell with the subapical hyphal cell and disintegration of the septum during nuclear migration within another mycelium (Au *et al.*, 2014). Successful mating between monokaryons requires different allelic specificity (x and y) in factor A and B. The rate of actual mating is 25 %, which is due to the facts that only one mating interaction is compatible while the other three interactions are not fertile. This situation can be explained in Figure 2.10 (Kothe, 2001).

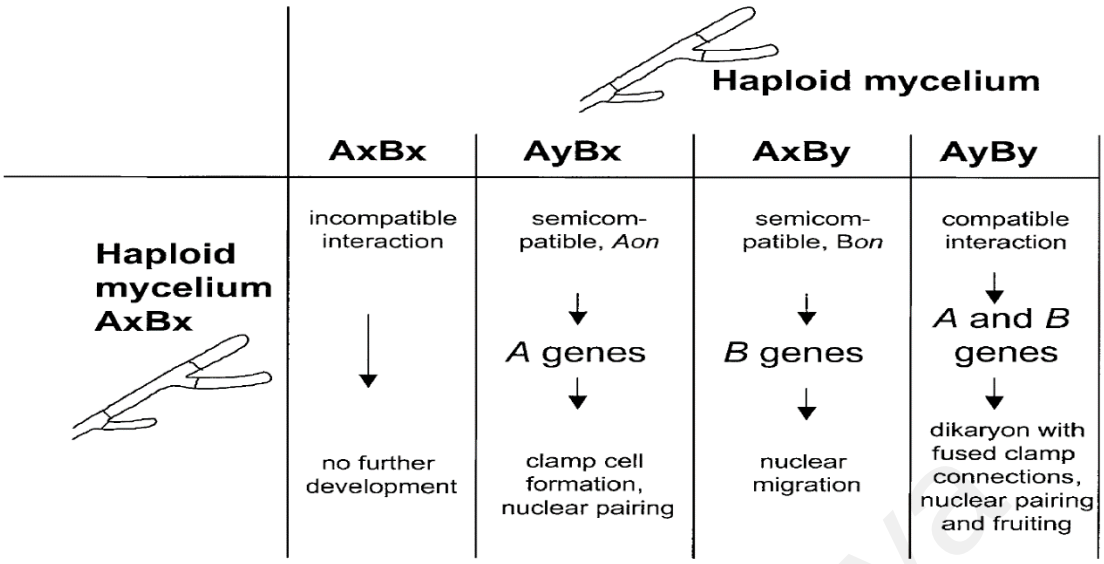


Figure 2.10: Tetrapolar mating system (Cited from: Kothe (2001).

Crosses between monokaryotic mycelia were done by inoculating the mycelia plugs apart from each other. The mycelia were allowed to grow for several days until the presence of a conspicuous contact zone can be observed clearly. A plug from this zone was transferred to the new plate as shown in Figure 2.11 (Avin *et al.*, 2016). When the newly produced dikaryotic mycelial strains are available, they are cultivated to generate sporophores, which are then subjected to screening to determine the strains with good commercial and cultivation traits (Kothe, 2001).

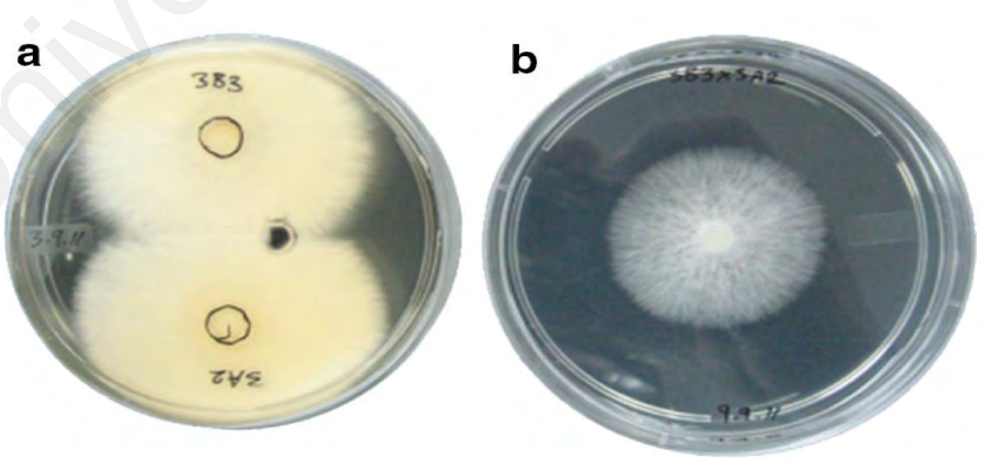


Figure 2.11: Hybridisation of two compatible monokaryon of *Pleurotus pulmonarius*. (A) a plug from the contact zone was transferred to the other plate. (b) the dikaryon was allowed to grow on the agar plate (Cited from: Avin *et al.*, (2016).

Study done by Hernández and Salmones, (2008) reported that interbreeding among *Pleurotus ostreatus* strains produced hybrid which can grow at the warm regions and has a high lignin enzyme activity which can be used in substrate delignification. Besides that, the cross-breeding between *Pleurotus eryngii* strains generate a new hybrid that has a longer shelf-life than the parental strains which can improved storability after harvesting (Kim *et al.*, 2013). Research done by Sou *et al.* (2013) by hybridising two strains of *Sparassis latifolia* (cauliflower mushroom) produce hybrid with different morphology in term of size of marginal and basidiocarp colour as shown in Figure 2.12. In addition, 3 hybrids produced higher yield than their parental strains.

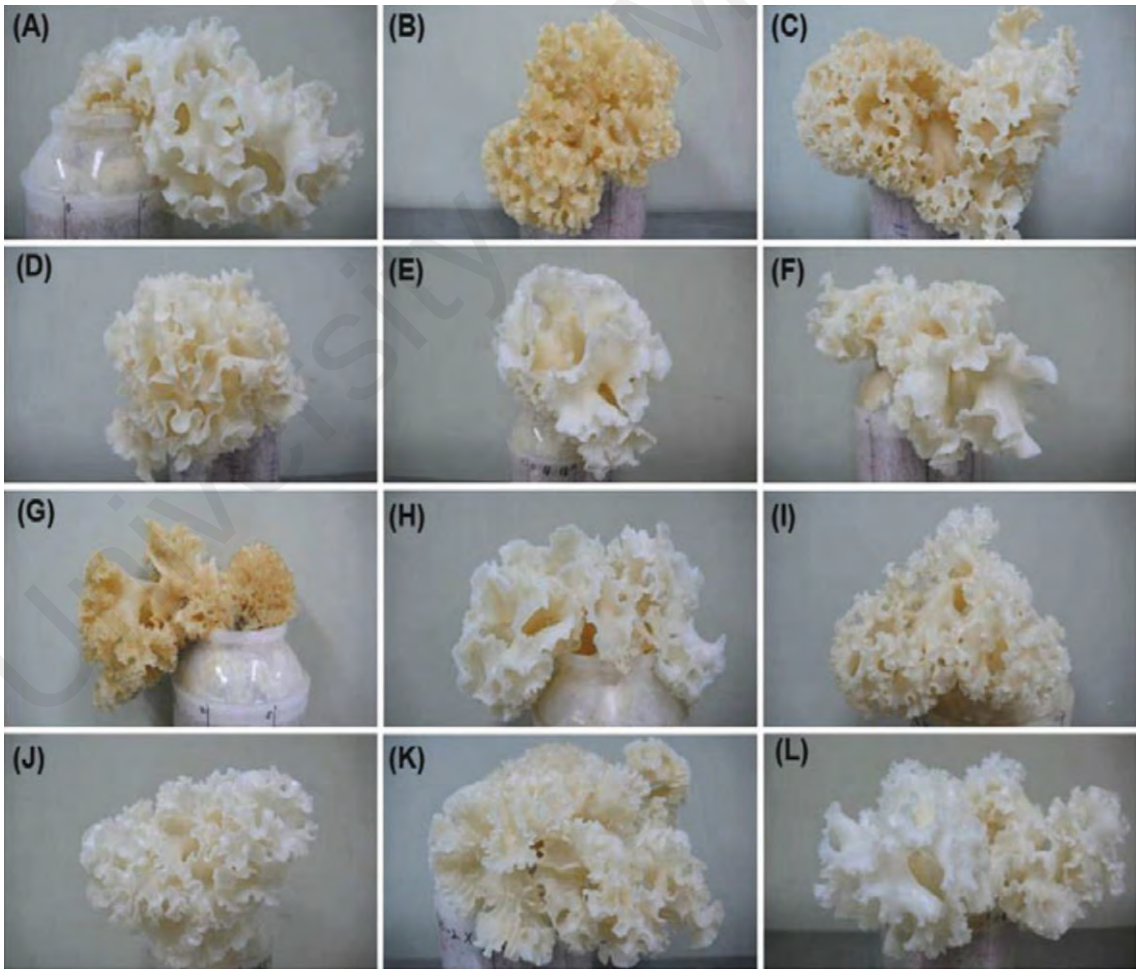


Figure 2.12: Crossbreeding of *Sparassis latifolia* strains. (A) and (B) are parent strains and (C-L) are hybrids (Cited from Sou *et al.*, (2013).

2.4.2 Mutation

The technology of mushroom breeding has become greatly sophisticated since the days of simple selection among natural populations and sexual recombination. Modern day, mushroom breeding is mainly on constructing variation, selection, evaluation and multiplication of favourable genotypes. However, mutagenesis is applied to make breeding fast and increase efficiency (Ahloowalia & Maluszynski, 2001). Mutation breeding is a technique by which mutant variants with desirable characteristics are produced without changing the remaining genotype through physical, chemical and biological mutagenic agents. The term 'Mutation Breeding' was initially used in 1944 by Freisleben and Lein to describe the application of induction for product development on plant and generation of mutant lines (Ulukapi & Nasircilar, 2015). Mutations may enhance one or more changes in the features of the mutated organisms at the gene level, which will transfer to the next generation (Sathesh-Prabu & Lee, 2016).

The mutation has been applied in crop breeding programs to enhance both productivity and quality. Many techniques of inducing mutation can be used, which include chemical, biological and physical agents like gamma and ultraviolet (UV) irradiations (Teimoori *et al.*, 2014). A study in China has reported that induced mutations are the easiest and fastest method to create newly desired genotypes and improve genetic diversity (Liu *et al.*, 2004). In addition, mutation breeding has become an alternative technique for breeders as it produces the possibility of obtaining desired features which do not exist naturally or disappeared during the evolution (Ulukapi & Nasircilar, 2015). In contrast to the genetically modified organism (GMO), mutation breeding is easy to performs, no transferring of foreign genes were involved, affordable, and safe (Jain, 2010).

Mutagen is a natural or artificial agent where it serves to change the sequence, structure, and function of deoxyribonucleic acid (DNA) (Sathesh-Prabu & Lee, 2016) and

living things that carry the permanent hereditary changes caused by mutagen are called mutants (Ulukapi & Nasircilar, 2015). Gamma radiation is a powerful ionizing radiation with high energy that is capable to penetrate into the cell wall of mushroom mycelia. This radiation has the ability to break the structure of DNA molecules and alter the purine and pyrimidine bases. Gamma ray can be obtained through several sources, which are Cobalt-60, Cesium-137, and technetium-99 (Djajanegara & Harsoyo, 2008). Cesium-137 is more used as it has a longer half-life compared to the Cobalt-60 (Çelik & Atak, 2017).

Chemical mutagenesis is recognised as an important supplement to the conventional breeding techniques for crop improvement (Dhanavel *et al.*, 2008). The most commonly used chemical mutagens are the alkylating agent, with ethyl methanesulfonate (EMS) being the most famous due to its simplicity, effectiveness and its ability to detoxify through hydrolysis for disposal (Pathirana, 2011). However, chemical mutagens are less acceptable because they possess several disadvantages such as poor reproducibility, uneven penetration and health risk upon handling (Sathesh-Prabu & Lee, 2011).

The application of mutation can be presented by research done by Djajanegara and Harsoyo, (2008) where *Pleurotus florida* was induced by gamma radiation and produced PO-5 hybrid with higher productivity in term of number of fruit bodies, fresh weight and dry weight yield of three successive flush periods than the control strain. Besides that, PO-4 hybrid has higher anti-oxidant activity than the control strain. In addition, study done by Sathesh-Prabu and Lee, (2016) proved that gamma radiation able to induce genetic variation in the *Pleurotus florida* where the genetic similarity between the wild species and mutant is 22.30 %. In addition, the mutant hybrid has higher cellulolytic activity than the wild species. Moreover, research conducted by Sermkiattipong and Charoen, (2014) on the effect of different dosage of radiation at 0.25 kGy, 0.50 kGy, 0.75 kGy, 1.00 kGy, 1.25 kGy and 1.5 kGy on the mycelia of *Volvariella*

volvacea as shown in Figure 2.13. Out of 153 hybrids, 59 hybrids have higher yield than the parent strain. The hybrid which was irradiated at 0.25 kGy generate the highest productivity.

Figure 2.13: Yield between parent and mutant isolates of *Volvariella volvacea* (Cited from: Semkiattipong & Charoen (2014).

2.4.3 Protoplast Fusion

Protoplast is the organism cell where the cell wall is dissolved and covered by a cytoplasmic membrane as the outmost layer. Protoplast fusion is the physical phenomenon where two or more protoplasts connect and adhere to one another (Verma *et al.*, 2008) as shown in Figure 2.14, where protoplasts from *Pleurotus floridae* and *Pleurotus cystidiosus* fuse together (Djajanegara & Masduki, 2010). Protoplast fusion technology is a useful tool for breeders to induce crosses between sexually incompatible species for transferring the nuclear and cytoplasmic traits. This technology is capable to generate crosses within species (intraspecies), within genera (intrageneric) and between genera (intergeneric) which has a greater potentiality than their parental strains. Through this method, improved strains with increased potential for the manufacturing of antibiotics, enzymes, valuable myco products and high yielding mushrooms could effectively be produced (Bengochea & Dodds, 1986).

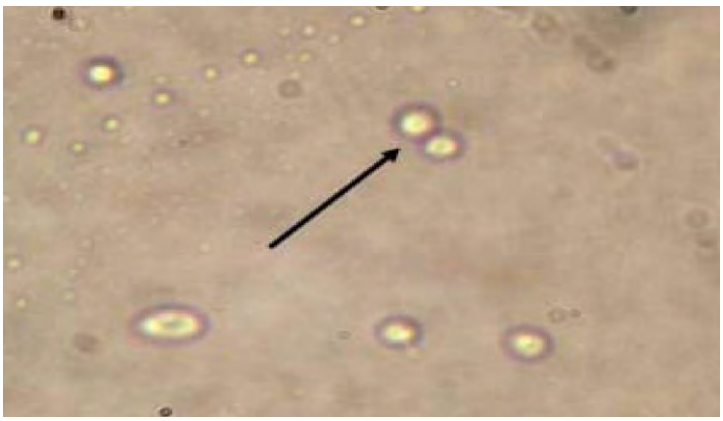


Figure 2.14: Protoplast of *Pleurotus floridae* fuse with protoplast of *Pleurotus cystidiosus* (Cited from: Djajanegara & Masduki (2010).

There are many experimental methods which can be used to induced protoplast fusion, such as by using polyethylene glycol (PEG), calcium and high pH, sodium nitrite, immunological technique, mechanical fusion and electrical fusion (Bengochea & Dodds, 1986). A successful intergeneric protoplast fusion has been performed between two edible mushrooms *Calocybe indica* (milky mushroom) and *Pleurotus florida* where the hybrid has a larger size as shown in Figure 2.15, higher biological efficiency and a greater proportion of unsaturated fatty acid than their parental strains (Chakraborty & Sikdar, 2010). Selvakumar *et al.* (2015) have reported the interspecific fusion protoplasts between *Pleurotus ostreatus* var. *florida* and *Pleurotus djamor* var *roseus*, where the hybrids show greater biological efficiency and increased energy values, vitamins and mineral contents in the hybrids.

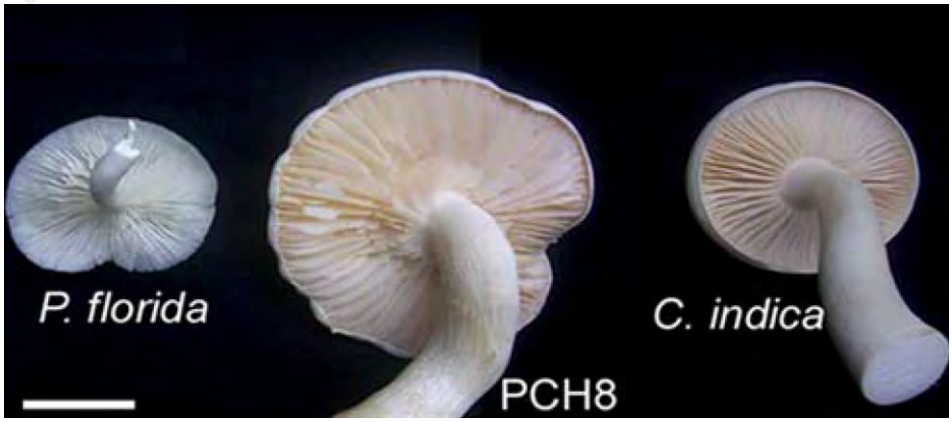


Figure 2.15: Somatic hybrid sporophores between *Calocybe indica* var. APK2 and *Pleurotus florida* (Cited from: Chakraborty & Sikdar (2010).

2.5 Characterisation of Mushroom Strains

Accurate taxonomic characterisation and phylogenetic classification of mushroom are very beneficial in diverse mushroom species and applying this information for genetic engineering or commercial cultivation of valuable species in the future. In addition, precise identification of fungi can avoid misidentification of mushroom species in future (Inyod *et al.*, 2017). Characterisation of mushroom strains will also benefit the researcher who developed an improved new strain of mushroom and invested in the breeding programs to protect from unauthorised propagation of line (Royse *et al.*, 2016).

There are several criteria that are implemented by The Seeds and Seedlings Division of the Ministry of Agriculture in Japan to register the new strains which is based on genetic properties, physiological quality, cultivation properties and morphological characteristics of the strain. Genetic properties include the ability of the strains to form an inhibition zone in dual culture on sawdust medium while physiological quality includes the unique mycelial growth and the mycelia density. Cultivation properties include the time of the strain to fruiting, environmental factors of the area where cultivation occurs, and yield of sporophores, while morphological characteristics covered on the cap morphology, sporophore size, colour, firmness, scaliness, and gill and stipe morphology (Royse *et al.*, 2016).

2.5.1 Morphology Characterisation of *S. commune*

Macroscopic characteristics are crucial for recognising fungi and determining an initial identification (Elnaiem *et al.*, 2017). Generally, the fruiting bodies of *S. commune* were scattered or grouped on hardwood timbers and branches. The size of fruiting bodies usually between 1 cm to 4 cm and laterally attached to the substratum, stipeless or irregular to shell shaped. The upper surface of the fruiting bodies contains tiny white to

grayish hairs as shown in Figure 2.16. Fruiting bodies consist of sporiferous parts which are located underneath the cap. The hymenophore was gilled and the gills were folded and split down at end of the gills. Hence, this mushroom was also known as split fungi or split gilled fungi (Padhiar *et al.*, 2009).

Figure 2.16: Upper and lower surface of *Schizophyllum commune* (Cited from: Padhiar *et al.*, (2009).

Microorganisms display various culture characters and the variation depending on the type of medium utilised for culturing. *Schizophyllum commune* exhibits white and odourless mycelial mat on the malt agar medium (Padhiar *et al.*, 2009) while sometime appear cream to golden, orange, or slightly brownish on potato dextrose agar (PDA) (Romanelli *et al.*, 2010). Moreover, the mycelial mat of this mushroom has a smooth texture as shown in Figure 2.17 (Padhiar *et al.*, 2009).

Figure 2.17: Cultural characteristics of *S. commune* on malt agar medium (Cited from: Padhiar *et al.*, (2009).

Microscopically, fungi that were grouped in basidiomycetes may exhibit hyphae only or hyphae with chlamydoconidia (Figure 2.18A). However, most of the fungi are arthroconidia (Figure 2.18B) or condensed clusters of anthroconidia, as examined in some of *Hormographiella* species. Besides that, one of the main characteristics of basidiomycetes that can be useful for identification is the present of clamp connections. In addition, some of the fungi produce spicules along the sides of hyphae with or without clamp connection (Figure 2.18C) (Romanelli *et al.*, 2010). Morphology of hyphae of *S. commune* includes the presence of hyaline, septate hyphae with clamp connections and small spicules (Tullio *et al.*, 2008).

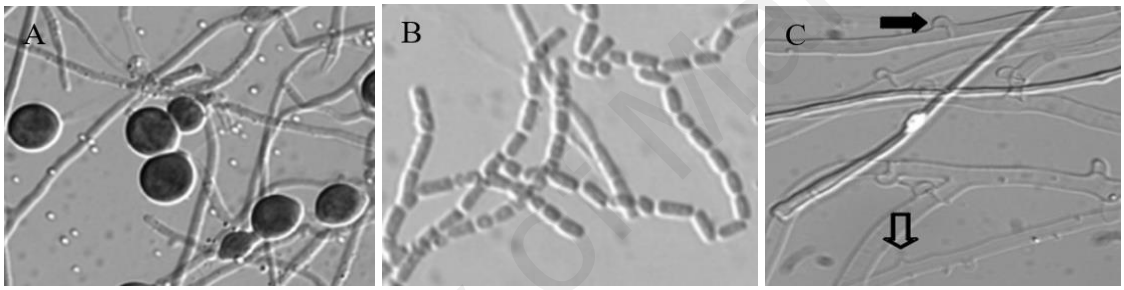


Figure 2.18: Common morphological characteristics of basidiomycetes including chlamydoconidia (A), arthroconidia (B), spicules (open arrow) and clamp connections (solid arrow) (Cited from: Romanelli *et al.*, (2010).

2.5.2 Molecular Identification

Molecular approaches have been proven to be a powerful means in the categorisation of complex fungal taxonomic groups, including mushrooms (Elnaiem *et al.*, 2017). There is an immediate demand of advance technique apply for species identification beyond morphological examination as this characteristic are greatly influenced by cultivating conditions (Khan *et al.*, 2011). The expression of a certain gene is a cumulative outcome of the genetic makeup of a species or a strain and environment conditions (Mehmood *et al.*, 2008). Molecular methods also express evolutionary relationships between organisms, remove the old practical systems which produced dubious information (Muruke *et al.*, 2002).

One of the technique is the random amplified polymorphic DNA (RAPD). This method is depended on the polymerase chain reaction (PCR) has been one of the most frequently utilised molecular techniques to generate DNA markers (Kumar & Gurusubramanian, 2011). The advantages of RAPD are due to the speed, cost-effective and efficiency of the technique to form huge numbers of markers in a short period (Kumar & Gurusubramanian, 2011). Alam *et al.* (2010) had conducted a study to differentiate 12 different strains of *S. commune* that were obtained from Korea and China by using RAPD and the RAPD profiles are shown in Figure 2.19. Three strains which are IUM-0202, IUM-0137 and IUM-0395 show significant different in band patterns while the others show 90 % to 100 % similarity. Therefore, RAPD is an effective technique to study the genetic relationships among strains (Alam *et al.*, 2010).

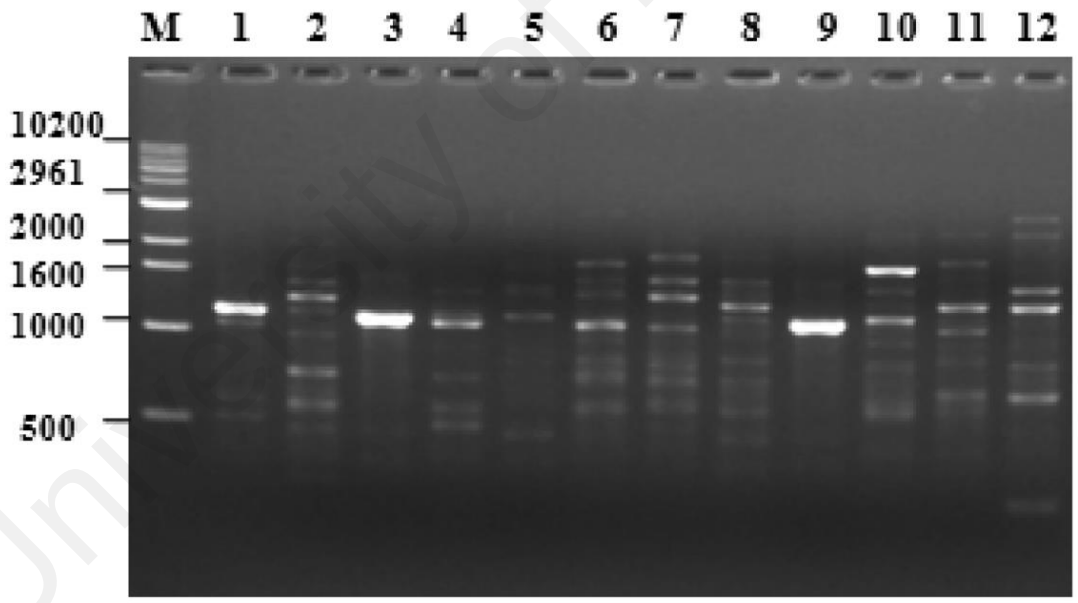


Figure 2.19: Random amplification of polymorphic DNA profiles of 12 strains of *Schizophyllum commune* with primer OPA-1. M, molecular size marker; lane 1, IUM-0137; lane 2, IUM-0157; lane 3, IUM-0202; lane 4, IUM-0395; lane 5, IUM-0548; lane 6, IUM-1763; lane 7, IUM-1768; lane 8, IUM-2324; lane 9, IUM-2650; lane 10, IUM-2659; lane 11, IUM-3353; lane 12, IUM-3566 (Cited from: Alam *et al.*, (2010).

Amplified fragment length polymorphism (AFLP) is another molecular approach that is widely used to determine the polymorphisms among individuals, populations and independently evolving lineages (Mueller & Wolfenbarger, 1999). AFLP was reported to

be highly specific and able to distinguish 21 *Pleuotus* isolates of Asian and European origin (Pawlik *et al.*, 2012). Mukhopadhyay *et al.* (2012) have applied AFLP to study the genetic similarity and geographical diversity 30 different *Lentinula* species. In addition, AFLP analysis was conducted to analyse the genetic diversity of 15 isolates of the white oyster mushroom (*Pleurotus ostreatus*) that grow from various locations in Indonesia (Java, Bali, Sumatra, and Kalimantan) and Thailand. Besides that, Terashima *et al.* (2002) analysed genetic diversity among the cultivated Shiitake in Japan. Therefore, AFLP is very useful tool to analyse genetic diversity as this technique able to detect slight genetic differences between the strains (Terashima *et al.*, 2002).

2.5.3 Protein Profiling

Generally, proteome means 'PROTein complement of a genOME'. The broad definition of the proteome is the entire set of protein species that are present in a biological unit which includes organelle, cell, tissue, organ, individual, species, and ecosystem at any developmental phase and under specific environmental conditions (Fernández & Novo, 2010). Proteomics is an effective tool in profiling, determining, and identifying proteins which are synthesised due to a changing cellular environment (Chen *et al.*, 2012). There are several factors that influenced the protein content of the mushroom, which includes compost composition, flush number, type of strains and harvest time (Braaksma & Schaap, 1996). The proteomic studies in mushroom involve research in functional protein content, mushroom developmental phases, emulsifier reaction on mycelium growth, cell wall proteins, mushroom medical properties (Al-Obaidi, 2016).

Proteomics quantification has the ability to detect small variations in protein and peptide abundance as a consequence to an altered state (Wasinger *et al.*, 2013). Normally, a cell controls the activities and levels of its proteins due to the reaction to some internal

and external changes. Hence, variation in the proteome can produce a clear illustration of a cell in action (Mumtaz *et al.*, 2017). Analysis of gene expression in the study of proteins contains in the biological samples can be performed to generate 'reference maps' of all detectable proteins. The reference maps provide the patterns of normal and abnormal gene expression in the organism and enable the examination of post-translational protein modifications which are functionally necessary for many proteins. In addition, reference maps can be exploited to screen the protein, thus able to establish their identities (Wilkins *et al.*, 1996). Moreover, total protein profiles and their expression amount indicate the life phase of the organism (Horie *et al.*, 2008).

The classical identification depends on the morphological, biochemical and physiological characterisation. However, this approach is time-consuming and frequently generates ambiguous results. Thus, the application and development of new methods that promote an efficient identification and detection is advisable (Das *et al.*, 2005). Protein fingerprinting is a valuable technique used for identification, preliminary characterisation, and comparison of the protein (Nikodem & Fresco, 1979). Figure 2.20 shows the protein fingerprinting of albumin fractions of *Amaranth seeds* by SDS-PAGE (Akin-Idowu *et al.*, 2013). Protein electrophoretic separation has been applied for the last 20 years (Debelian *et al.*, 1996). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a convenient tool to investigate genetic diversity in a short duration of time (Akhbar *et al.*, 2012). SDS-PAGE can be used to study the similarity and the different within species and genera. In addition, protein electrophoresis exhibits even smaller genetic between strains compared to the DNA hybridisation (Eribe & Olsen, 2002).

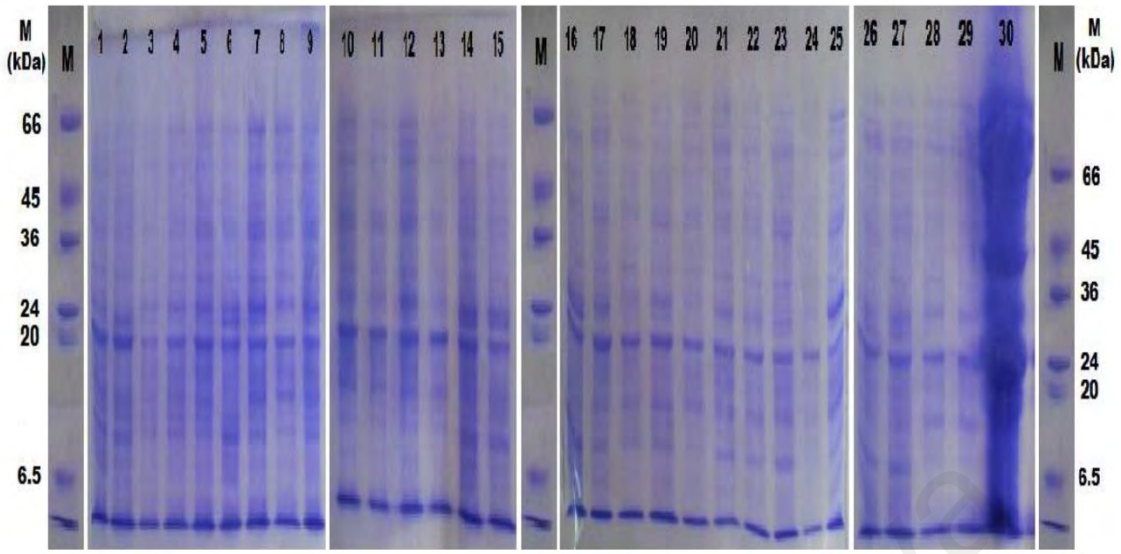


Figure 2.20: Protein profile of albumin fractions of 29 accessions of *Amaranth* seeds. M marker, Lane 1-4 *A. caudatus*, Lane 5-12 *A. cruentus*, Lane 13-19 *A. hybrid*, Lane 20-27 *A. hypochondriacus*, Lane 28-29, *A. hybridus*, Lane 30 soybean (TGX 1448-2E) (Cited from: Akin-Idowu *et al.*, (2013).

2.5.4 Metabolite Profiling

Metabolites are low molecular weight (in relation to proteins and nucleic acids) organics and inorganics substances that are the reactants, intermediates or products of biochemical reactions (Dunn *et al.*, 2011). Metabolomics is defined as the quantitative and qualitative analysis of entire metabolite that is available in an organism at a particular time (Mumtaz *et al.*, 2017). Metabolomics is an important approach that generates metabolite profiles for examining biochemical networks which contain the set of metabolites, enzymes, reactions and their interactions (Tagore *et al.*, 2014). Metabolite profiling can be classified into two group which are endogenous and exogenous metabolites. Endogenous metabolite consists of primary and secondary metabolites. Primary metabolites such as amino acids or glycolysis metabolites are involved in the basic life processes such as growth and reproduction. Secondary metabolites such as hormones and alkaloids are species-specific, and their synthesis is depending on the specific biological function (Roux *et al.*, 2011).

A range of analytical platforms is applied for metabonomic/metabolomic study, including proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, direct infusion mass spectrometry (MS), gas chromatography coupled with mass spectrometry (GC-MS), Fourier transform infrared (FT-IR) spectroscopy, capillary electrophoresis coupled to MS (CE-MS), two-dimensional GC coupled to MS (GCxGC-MS) and liquid chromatography coupled to MS (LC-MS) (Theodoridis *et al.*, 2008). However, no single analysis is effective in metabolite study as the diversity and various concentration of metabolites produces large ranges of physicochemical properties including molecular weight, hydrophobicity/hydrophilicity, acidity/basicity and boiling point (Dunn *et al.*, 2011). Therefore, a combination of more than one analytical method is necessary for the reliable outcome (Theodoridis *et al.*, 2008). Example of metabolite profiling of fruiting bodies of *Antrodia cinnamomea* and *Antrodia salmonea* are illustrated in Figure 2.21.

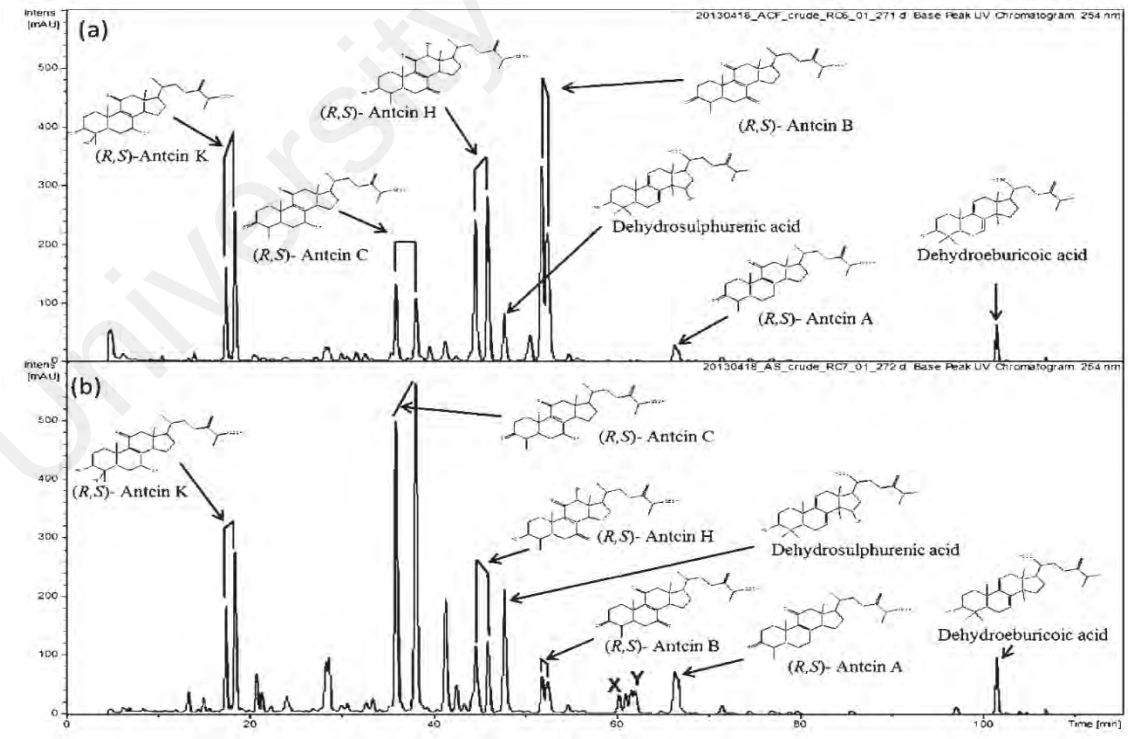


Figure 2.21: HPLC profiling of metabolites of fruiting bodies of (a) *Antrodia cinnamomea* and (b) *Antrodia salmonea* (Cited from: Chen *et al.*, (2016).

2.6 *Schizophyllum commune*

Schizophyllum commune is one of the most commonly distributed mushrooms (Chowdhary *et al.*, 2013). This mushroom can be found throughout the entire world due to its endurance towards habitat hardiness (Rahilah *et al.*, 2012) except in Antartica, where there is no substrate to be used for growing (Imtiaj *et al.*, 2008). It is grouped in the phylum of Basidiomycota, subphylum Agaricomycotina, class of Agaricomycetes, subclass Agaricomycetidae, an order of Agaricales, the family of Schizophyllaceae (Hanafusa *et al.*, 2016). In the genus *Schizophyllum*, species commune is the only species which is distributed throughout the world, while *S. fasciatum* and *S. umbrinum* are restricted to a few countries like Mexico, Central America and the Caribbean (Cooke, 1961).

This mushroom grows widely during the rainy season and is very popular in certain region such as Africa (Ohm *et al.*, 2010), Thailand (Preecha *et al.*, 2016), Vietnam, Southern China (Imtiaj *et al.*, 2008), Mexico and Malaysia as edible mushroom (Takemoto *et al.*, 2010). Distribution of this mushroom in the Malaysia include in the Peninsular Malaysia such as in the area of FRIM, Pasoh, Kemasul, Ulu Sedili, Mata Ayer and Jeram Lenang as shown in the Figure 2.22 (Ujang *et al.*, 2002) and in Sabah as shown in the Figure 2.23 (Fui *et al.*, 2018). In Malaysia, this mushroom is popularly known as 'Cendawan Kukur / Sisir' (Rahilah *et al.*, 2012) and 'Kulat Kodop' (Fui *et al.*, 2018). This species thrives in dead wood of deciduous trees (Kumar *et al.*, 2013).

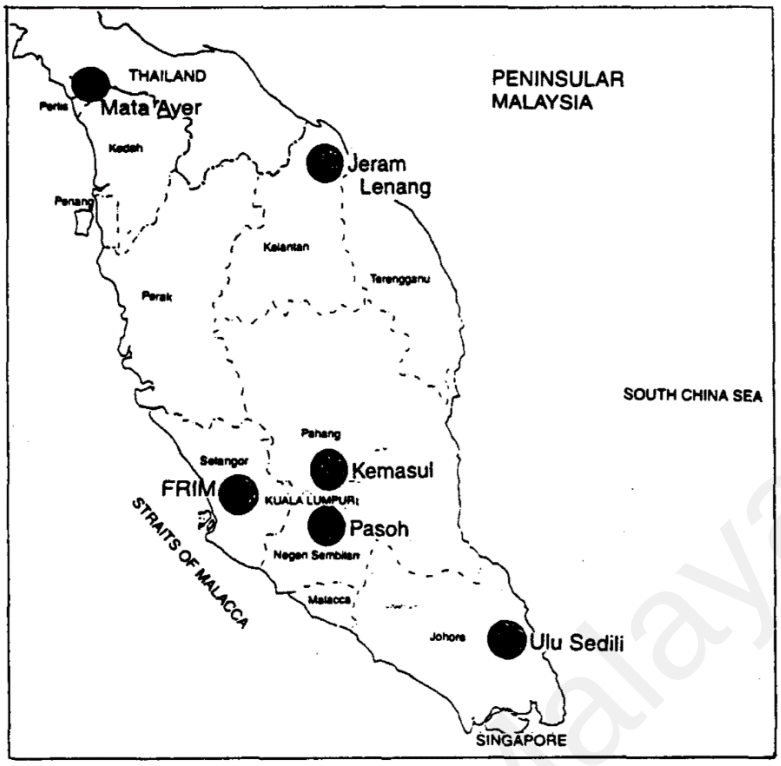


Figure 2.22: Distribution of *Schizophyllum commune* in Peninsular Malaysia (Cited from: Ujang *et al.*, (2002).

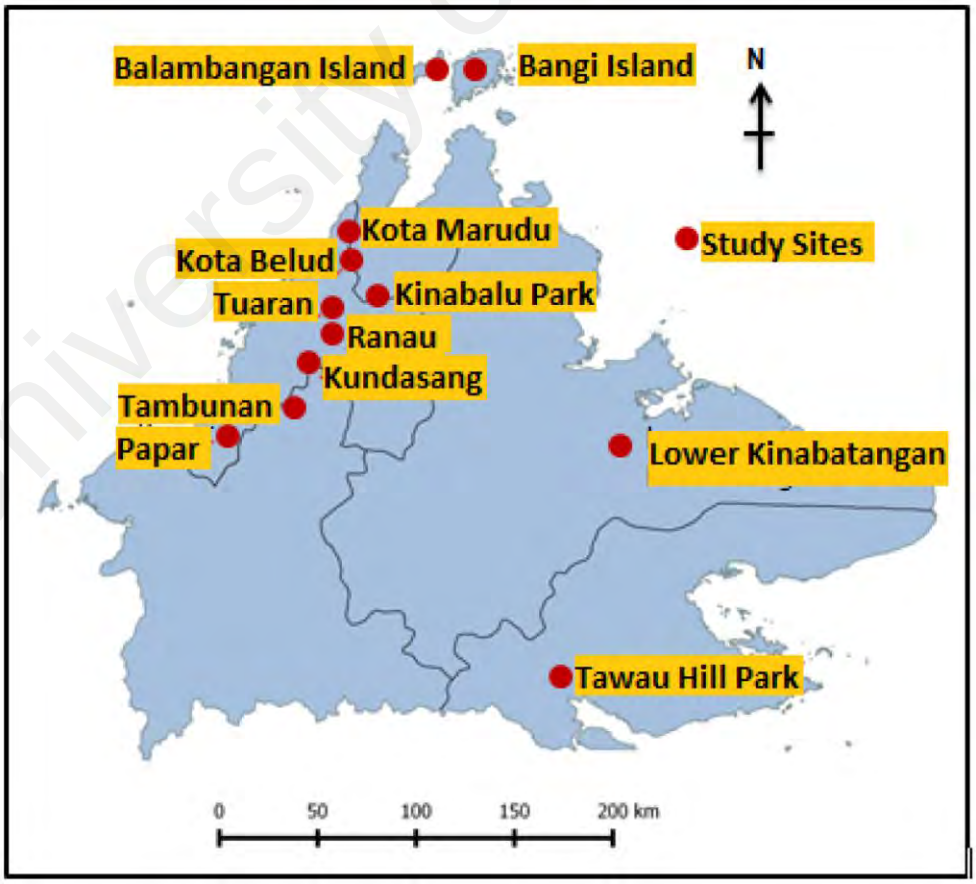


Figure 2.23: Distribution of *Schizophyllum commune* in Sabah (Cited from: Fui *et al.*, (2018).

2.6.1 Morphology of *Schizophyllum commune*

The macroscopic morphology of this mushroom is shown in Figure 2.24 whereby the fruiting body is 1 cm to 5 cm wide and has a fan-shaped with small pileus on the upper surface. The colour of the fruiting body can vary from white to greyish (Kumar *et al.*, 2013). The fruiting body commonly laterally adheres to the substratum, stipeless or irregular to shell-shaped. Hymenophore has gills. The gills were located under the surface, folded, and split down the middle. Therefore, these fungi are known as split-gilled fungi. The split was shallow, and the feature looks like a groove (Amee *et al.*, 2009). The function of gills is to form basidiospores on their surfaces. The colour of these spore print is white (Chandrawanshi *et al.*, 2017), and the size is approximately 3 – 4 x 1 – 1.5 µm. The shape of the spore can vary between cylindrical to elliptical and smooth (Matavuly *et al.*, 2013). In addition, hyphae wall is thin and the dikaryotic mycelium has clamp connection as shown in Figure 2.25 (Amee *et al.*, 2009).

Figure 2.24: Sporophore of *Schizophyllum commune* (Cited from: Palmer & Horton (2016).

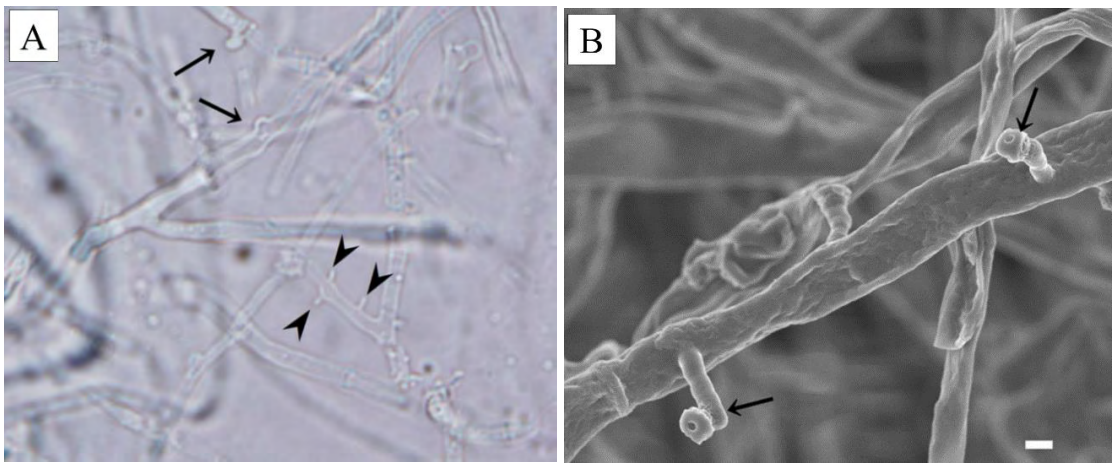


Figure 2.25: Microscopic morphology of *S.commune* (A) Staining using lactophenol (B) Scanning electron microscopy (SEM) (Cited from: Hanafusa *et al.*, (2016).

2.6.2 Life Cycle of *Schizophyllum commune*

The life cycle of *Schizophyllum commune* as shown in Figure 2.26 begins with the germination of haploid spore which will form homokaryotic mycelium. Homokaryotic mycelium consists of uninucleate, haploid cells (Stankis & Specht., 2007). *Schizophyllum commune* is heterothallic where the mating between homokaryotic mycelia is depended on the bifactorial incompatibility factors which are *A* and *B* (Raper & Miles, 1958). Two homokaryons are compatible when each of them has different alleles of A and B genes as shown in Figure 2.26 as *AiBi* and *AjBj*. Heterokaryotic cells will be formed when two compatible homokaryotic mycelia fused together. Then, the heterokaryotic cell will be developed into dikaryon mycelium (Stankis & Specht, 2007).

The difference between hypha of *S. commune* dikaryon and monokaryon (primary mycelium) is the presence of clamp connection. Clamp connection only formed at dikaryon mycelium while monokaryon mycelium has no clamp connection (Clark & Anderson, 2004). Generation of the clamp is generally used as an evidence of sexual compatibility. Formation of the clamp connection occurs during the conjugate division of nuclei in the arising hyphae tip (Gharehaghaji *et al.*, 2007). Under appropriate conditions, fruiting body formation begins with the aggregation of aerial dikaryotic hyphae which

eventually will develop into fruiting body primordia. Primordia will differentiate to form mature fruiting bodies (Ohm *et al.*, 2010).

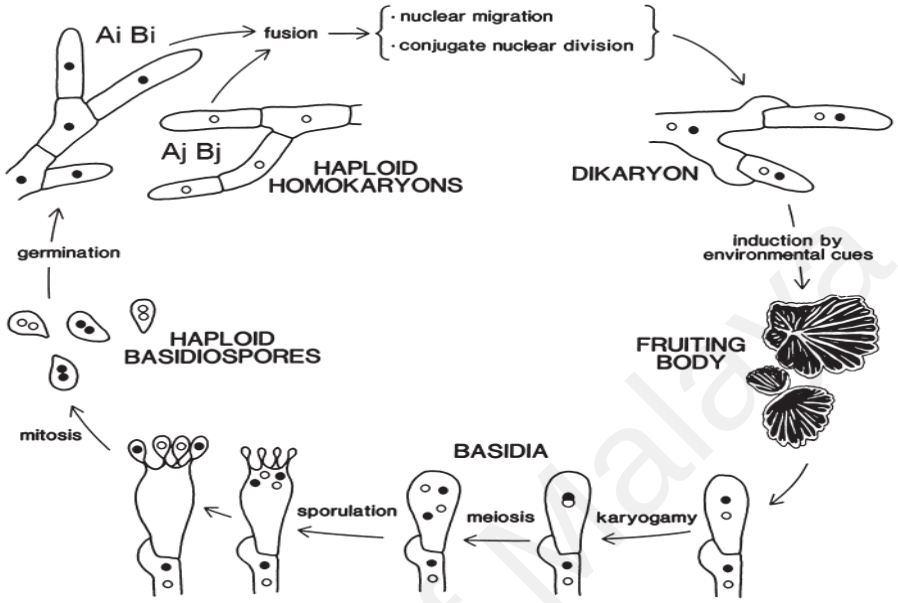


Figure 2.26: Life cycle of *Schizophyllum commune* (Cited from: Stankis & Specht (2007).

2.6.3 Nutritional Value and Medical Properties

In general, mushroom consists of 90 % water and 10 % dry matter. Nutritional values of the mushroom are comparable to that of eggs, milk, and meat. In addition, protein in mushroom is intermediate between animals and plants, and the quality is greater due to the presence of all amino acids (Hoa & Wang, 2015). Moreover, mushroom also has high in minerals (Ingale & Ramteke, 2010), low in calories and fat (Dasanayaka & Wijeyaratne, 2017) and contains no cholesterol (Rajapakse, 2011). Edible mushrooms are very good as an alternative protein source for vegetarian diets because they contain the essential amino acids for adult consumption (Valverde *et al.*, 2015). Table 2.4 shows that the nutrient compounds of wild and cultivated *S. commune* (Herawati *et al.*, 2016). *Schizophyllum commune* also contains high in phosphorus (P), magnesium (Mg), potassium (K), and selenium (Se) (Dasanayaka & Wijeyaratne, 2017).

Table 2.4: Nutrient contents of wild and cultivated *S. commune* (Cited from: Herawati *et al.*, (2016).

Parameter	Result of	
	Wild mushroom (%)	**Cultivated mushroom (%)**
Moisture content	52.0	50.0
Ash	2.0	1.94
Fat	4.5	4.5
Protein	6.1	6.13
Fiber	0.002	0.002
Carbohydrate	35.39	37.42

Schizophyllum commune is a filamentous fungus which synthesises exopolysaccharide (EPS) and secretes *β*-glucan as main molecular structure (Joshi *et al.*, 2013). Schizophyllan (SPG) is a polysaccharide which consists of the main chain of the 3-*β*-D-glucopyranosyl unit in which every third unit has a (1→6)-branched *β*-D-glucopyranosyl substituent. It is proven that SPG can be used in vaccines and anticancer therapies, in oxygen-impermeable films which are used for food preservation and to increase petroleum recovery (Zhong *et al.*, 2013). SPG enables to recover and increases cellular immunity in the ill organism by enhancing macrophages (Hilszczańska, 2012). This *β*-glucan is also known as Sonifilan, which can be applied for the treatment of stomach and neck cancer. In addition, it is also introduced during radiotherapy because of its radioprotective properties (Lemieszek & Rzeski, 2012).

In addition, dichloromethane extraction of *S. commune* has proven to be an effective antibacterial and antifungal properties against some species of the gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Enterobacter faecalis*, and *Staphylococcus aureus*), gram negative bacteria (*Escheria coli*, *Plesiomonas sigelloides*, *Pseudomonas*

aeruginosa, *Proteus vulgaris*, *Salmonella typhi*, *Shigell* sp., *Shigella fexneri*, *Streptococcus mitis*, *Streptococcus mutans* and *Streptococcus sanguis*) and fungi (*Candida albicans*, *Candida parapsilosis* and *Saccharomyces pombe*) (Mirfat *et al.*, 2014). Research done by Jayakumar *et al.* (2010) resulted that the oxidised schizophyllan (scleraldehyde) of *S. commune* is an efficient antibacterial against ten strains of gram positive bacteria and gram-negative bacteria.

Research performed by Mirfat *et al.* (2010) reported that compound extracted from *S. commune* shown the highest antioxidant and scavenging activity, which able to reduce the stable radical 1,1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH) to yellow-coloured diphenyl-1-picrylhydrazine. Study done by Chandrawanshi *et al.* (2017) also shown that *S. commune* may be used as potential sources of natural antioxidant due to its scavenging activity and high content of phenolic compounds.

CHAPTER 3: MATERIALS AND METHODS

3.1 Preparation of Mycelial Culture

Cultures of *Schizophyllum commune*, consisting of natural strains from Malaysia (w1 and w2) and commercial Thailand strain (R) were obtained from Mycology Laboratory, University of Malaya. Also 15 strains of dikaryon culture obtained through hybridisation (cross-breeding) and 10 strains obtained through hybridisation of gamma-irradiated monokaryon culture were selected based on the compatibility between strains in experiments previously conducted in the laboratory (Bakar, 2017; Rohizad, 2017). The list of selected strains is shown in the Table 3.1.

The mycelial culture of each strain was cultured on malt extract agar (MEA) as prepared in Appendix A to be used as inoculum for spawn. The culture was incubated for 7 days at 25 °C in the darkness for the preparation in the fruiting trials.

3.2 Fruiting Trials

During the fruiting trials, all strains were maintained and cultured in a uniform environmental condition in terms of light, temperature, humidity and aeration to minimise the effect of environmental factors. Each parental strain and hybrid strains were assessed by growing on substrate in polyethylene bags with 10 replicates. Mycelial growth rate and density were assessed. Then, all the bags were transferred to the mushroom house for fructification. During fructification, several parameters such as size of the sporophores, yield (total fresh weight of mushroom), biological efficiency and the occurrence of contamination were determined.

Table 3.1: List of selected hybrids dikaryon (through hybridisation and hybridisation of gamma monokaryotic cultures) with their respective monokaryon cultures from w1, w2 and R.

Genetic variation of hybrid strains					
Genetic variation through hybridisation			**Genetic variation through hybridisation of gamma monokaryotic culture**		
w1a	w2a	w1aw2a	w1d	Ra	Ir-w1dRa
w1b	w2a	w1bw2a	w1d	Rc	Ir-w1dRc
w1b	w2f	w1bw2f	w1d	w2d	Ir-w1dw2d
w1c	w2a	w1cw2a	w1e	w2a	Ir-w1ew2a
w1d	w2a	w1dw2a	w1e	w2d	Ir-w1ew2d
w1d	w2f	w1dw2f	w1g	Rc	Ir-w1gRc
w1e	Ra	w1eRa	w1g	w2a	Ir-w1gw2a
w1e	w2a	w1ew2a	w1h	w2d	Ir-w1hw2d
w1e	w2f	w1ew2f	w1j	Ra	Ir-w1jRa
w1f	w2a	w1fw2a	w1L	w2f	Ir-w1Lw2f
w1g	w2a	w1gw2a			
w1h	w2a	w1hw2a			
w1j	Rc	w1jRc			
w1j	w2a	w1jw2a			
w1L	w2a	w1Lw2a			

3.2.1 Preparation of the Spawn

Spawn was prepared by washing and soaking the wheat grains for 1 hour using hot water until the grains became soft and 1 % calcium carbonate ($CaCO_3$) was added into the water. The wheat grains were filtered, and excess water were removed. One hundred grams of the wheat grains were filled in polypropylene bags and autoclaved at 121°C for 60 minutes at pressure of 1.2 kg/cm². The autoclaved grains were left at room temperature to cool for 30 minutes. Once cooled, five mycelial plugs of different strains were transferred into the spawn substrate under aseptic condition. The spawns were kept

at 25 °C for seven days to allow the mycelia to grow. Figure 3.1 (A) shows the first day of inoculation while Figure 3.1 (B) shows seventh day of inoculation.

Figure 3.1: Spawn (A) Before inoculation (B) Spawn colonised by mycelia on day 7.

3.2.2 Preparation of Fruiting Substrate Bag and Spawn Inoculation

Fruiting substrate bag was prepared by mixing the sawdust with rice bran and $CaCO_3$ in the ratio of 100:20:1. One liter of water was added into the mixtures to obtain a moisture content of 70 %. Next, 600 grams of the fruiting substrate was packed into polypropylene bag. The bag was sealed by using plastic cap. The substrate bags were autoclaved at 121°C for 1 hour and allowed to cool to room temperature. Then, one full spoon of grain spawn of each strain was transferred into the substrate bag. Each strain was inoculated into ten bags. The bags were kept in the dark chamber at room temperature until full colonisation.

3.2.3 Determination of the Mycelial Growth Rate and Mycelial Density

Average mycelial growth rate was determined by measuring the mycelial growth at four equidistant points around the circumference of each bags. Mycelial progression as shown in Figure 3.2 was calculated as millimetre per day (mm/d) and measurements were taken every two days. The growth rate of mycelial of each strain was determined by plotting the graph of average reading against time (day).

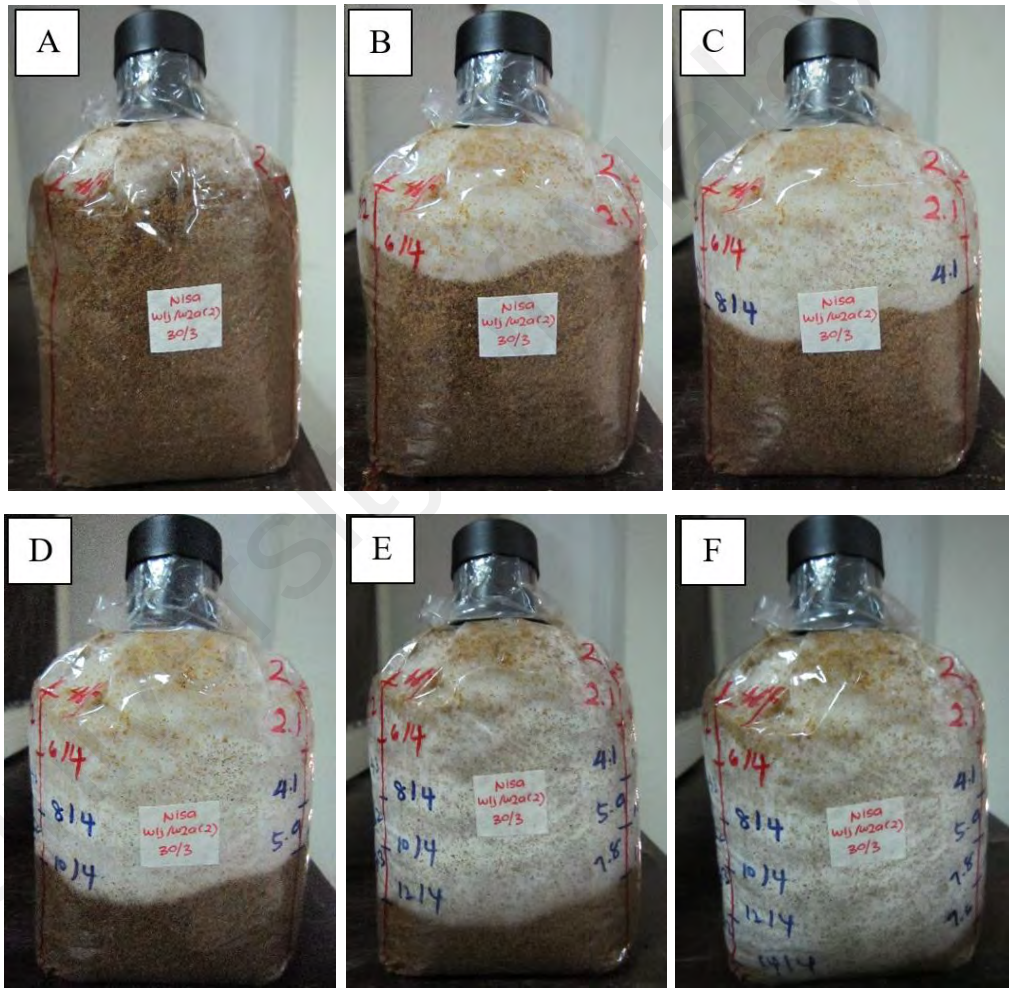


Figure 3.2: Spawn running (A) Day 2 (B) Day 4 (C) Day 6 (D) Day 8 (E) Day 10 (F) Day 12.

The thickness of the mycelia of each strain was observed and recorded with a digital camera. The intensity of the mycelia when the mycelia fully colonises the substrate bag was grouped into three categories where (+) poor running growth and has low density,

(++) mycelia grows throughout the substrate bag is not uniformly white and has moderate density, and (+++) mycelia grows throughout the whole bag and is uniformly white and has intense density as referring to the study done by Obodai *et al.* (2003) as shown in Figure 3.3.

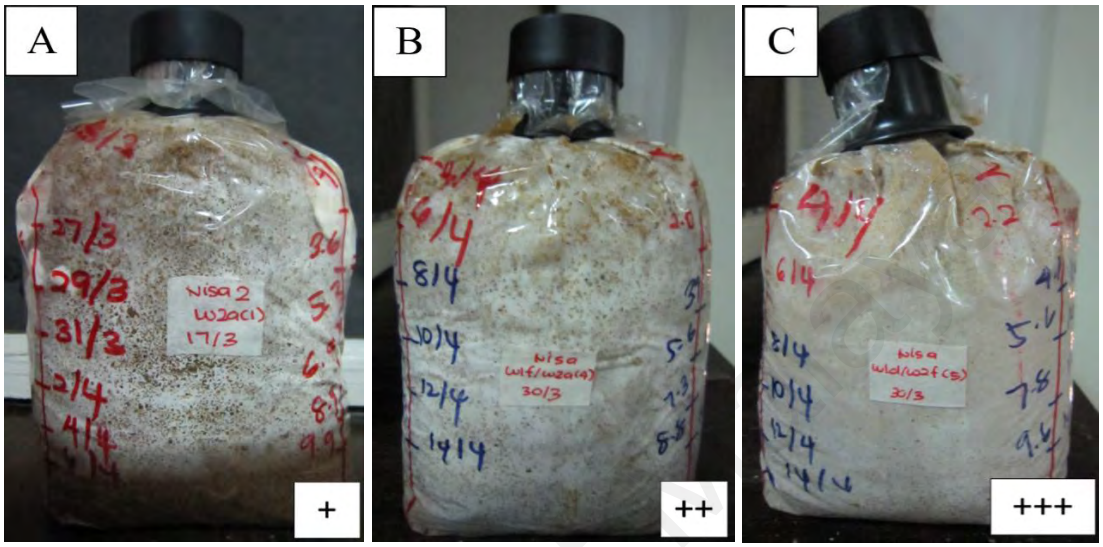


Figure 3.3: The evaluation of mycelia density (A) low density (B) moderate density (C) intense density.

3.2.4 Determination of Sporophore Yield and Biological Efficiency (BE)

Once fully colonised, the fruiting bags were slit at the side to allow primordia formation and maturation. Six slits were made per bag. The bags were then transferred to the mushroom house with higher humidity (>90 %) and aeration to allow fructification. Primordia formed were allowed to develop into mature sporophores. The sporophores of different strains were harvested on day 7 and day 14 for first and second flushes respectively. Flushes or break is when the mushroom produce the yield of mushroom sporophores (Burton & Noble, 1992). Morphological characterisation of mature sporophore was determined by direct measurement of width. Width of 20 mature sporophores from each strain was measured by using a ruler. The productivity of each strain was evaluated based on the sporophore weight (yield) and biological efficiency (BE). The formula of yield and biological efficiency are as follow:

Yield (g / bag) = Total fresh weight of harvested sporophore of each strain for 2 flushes (Average of 10 replicate bags) (Islam *et al.*, 2017).

Biological efficiency (%) = (Weight of fresh sporophores harvested / weight of dry substrate) x 100 (Peng *et al.*, 2001).

3.3 Protein Profiling

Sporophores of every strain were extracted for proteins and SDS-PAGE procedures were performed to obtain protein profiling for all strains.

3.3.1 Protein Extraction

Protein extraction is performed by using the trichloroacetic acid (TCA) / acetone-phenol / methanol method with a few modifications (Fernández & Novo, 2013). Fresh sporophore of all strains of *Schizophyllum commune* were cleaned and blended into powder. Next, 100 mg of the powdered mushroom were grinded with addition of 1 mL of 10 % (w/v) TCA / acetone by using mortar and pestle. The grinding process as conducted on ice to maintain the low temperature. The mixture was transferred to the 2 mL microcentrifuge tube. The tube is then filled with 10 % (w/v) TCA / acetone and mixed by vortexing. The tube then was centrifuge at 16000 x *g* for 5 minutes and the supernatant was removed.

The tube was then filled with 0.1 M ammonium acetate in 80 % (v/v) methanol and was vortexed and centrifuged again. The supernatant was removed and filled 80 % (v/v) acetone was added and vortexed. It was then centrifuge at 16000 x g and the supernatant was discarded. The pellet obtained was air-dry at room temperature to evaporate the residual acetone. 1.0 mL of 1:1 phenol / SDS buffer was added into the tube

and vortexed. The tube was incubated in ice for 5 minutes. The tube was centrifuged and the upper phenol phase as shown in Figure 3.4A was transferred into a new 2 mL tube. The tube was filled with 0.1 M ammonium acetate in 100 % (v/v) methanol, vortexing in few seconds and incubated overnight at -20 °C to allow precipitation to take place.

Next day, the tube was centrifuged, and the supernatant were removed. The white coloured pellet as shown in Figure 3.4B was washed with 100 % methanol and vortexed. The tube was centrifuge and the supernatant were removed. The pellet was washed again by using 80 % (v/v) acetone and vortexed. The tube was centrifuge and the supernatant were discarded. Finally, the pellet was dried at room temperature. The pellet was dissolved in solubilisation solution which contain 9 M urea, 2 M thiourea, 4 % (w/v) CHAPS, 0.5% (v/v) Tritón-X100 and 20 mM Dithiothreitol (DTT).

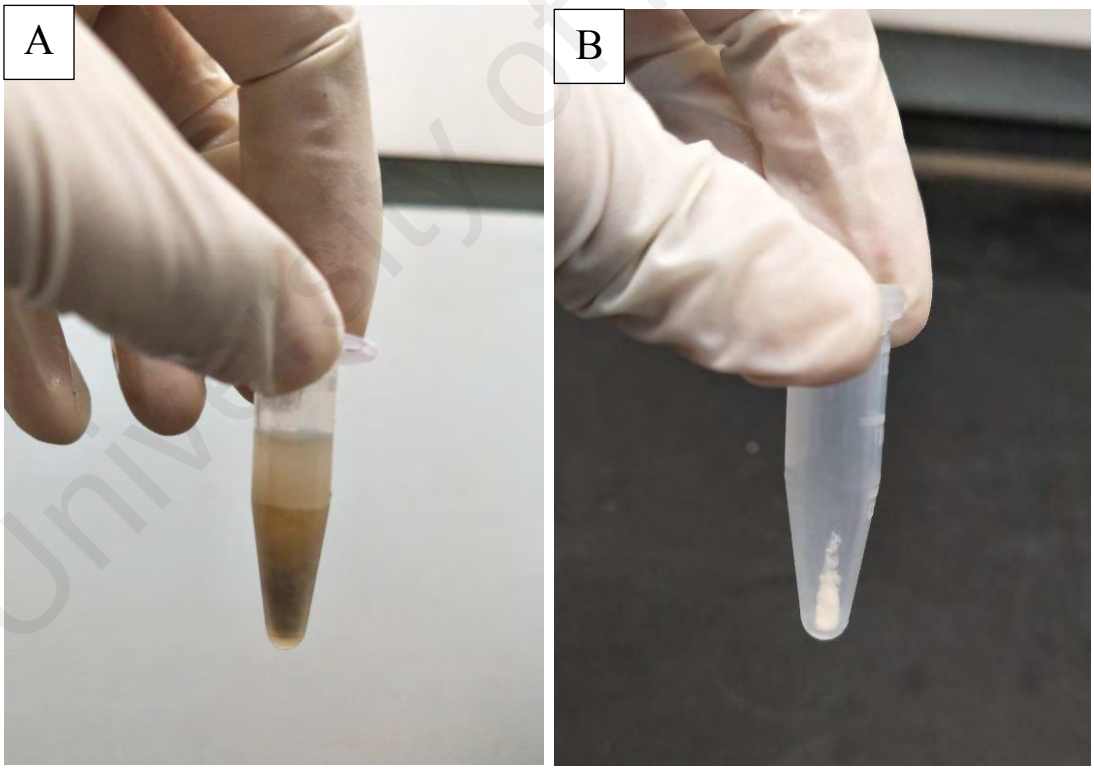


Figure 3.4: Steps in protein extraction. (A) Upper phase of phenol (B) White pellet.

3.3.2 Protein Estimation using Bradford Assay

Bradford assay can be used to estimate the protein content in the sample. This method is based on the binding of Coomassie Brilliant Blue G-250 to the protein which are measured at the absorbance of 595 nm (Bradford, 1976). The colour change is due to the interaction of dye with the hydrophobic interaction and certain amino groups such as arginine, histidine, phenylalanine, tryptophan and tyrosine residue (Noble & Bailey, 2009). Protein concentration standard was prepared by using Bovine Serum Albumin (BSA) as shown in the Table 3.2 Bradford assay was carry out using microplate assay where 10 µL of sample are mixed with 200 µL of Bradford reagent. The mixture was incubated for 5 minutes at room temperature. The absorbances was recorded at 595 nm.

Table 3.2: Volume and concentration of BSA for preparation of standard.

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)
A	0	300 of stock	2000
B	125	375 of stock	1500
C	325	325 of stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 = Blank

3.3.3 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Protein profiling was done by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in a vertical slab gel apparatus. BLUtra prestained protein ladder marker kit, ranges from 6.5 kDa to 270 kDa, manufactured by GeneDireX was used as standard marker. Each strain with different protein content as shown in Appendix D was standardised to contain 50 µg of protein estimated by using Bradford assay. Protein samples were prepared by mixing 40 µL of protein sample with 40 µL of sample buffer. Then the solution was heated at 95 °C for 5 minutes. Next, 15 µL of the solution was loaded into the wells of the stacking gel. Electrophoresis was performed at a constant current 25 mA, 60 V for stacking gel (4 % polyacrylamide) and 80 V for resolving gel (16 % polyacrylamide). After electrophoresis, the gel was stained by standard silver nitrate staining methods.

3.3.4 Standard Silver Nitrate Staining Method

After electrophoresis was conducted, the gel was fix in 50 % (v/v) methanol, 12 % (v/v) acetic acid and 0.05 % (v/v) formaldehyde for two hours or overnight. The gel was rinsed with 35 % (v/v) ethanol for three times and sensitised by soaking in a solution containing 0.025 % (w/v) of sodium thiosulphate for three minutes. Then, the gel was rinsed three times with ultrapure water. The gel was immersed in silver nitrate solution for 20 minutes. The gel was rinsed with ultrapure water before soaking in the developing solution which contain 6 % (w/v) sodium carbonate, 0.05 % (v/v) formaldehyde, and 2 % (v/v) sensitising solution for 5 minutes. The solution was immersed with stop solution for 5 minutes or until adequate degree of staining has been achieved. Finally, the gel was washed with ultrapure water.

3.3.5 Analysis SDS-PAGE Gel Image

Gels were analysed with GelAnalyzer software. The densitometric analysis of electrophoretic bands of polypeptide subunits was performed to determine the number of peak produce by each strain. The densitometric profile is plotted based on the average intensity of each row of pixels across the specified width of the lane. The molecular masses were calculated according to the standard curves, constructed by plotting the migrating distance (in mm) of each relative mobility (Rf) and logarithm of molecular mass of corresponding standard. Relative mobility (Rf) is the movement of a type of polypeptide through a gel relative to other protein bands in the gel.

The banding pattern of each strains were scored as bands for presence or absence of the band. The computer software DendroUPGMA was used to calculate the similarity coefficients and generating the similarity matrix for all strains. The strains were considered the same isolates when the Dice coefficient of correlation was above 60 % (Santos *et al.*, 2011). The dendrogram was constructed by calculating similarity coefficients into distances and makes a clustering using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm.

3.4 Statistical Analysis

All experiments were conducted in 10 replicates except protein contents where the experiments were performed in 3 replicates. Data obtained on mycelial growth rate, width of sporophores, mushroom yield, biological efficiency and protein content of all strains were subjected to analysis of variance (one-way ANOVA), where significant differences were obtained. A p-value <0.05 was considered significant. Duncan multiple range test was performed to determine the highest producing strains by using Statistical Package for the Social Sciences (SPSS) version 16 for Windows (Avin *et al.*, 2016).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Mycelial Growth Rate and Density of *S. commune* strains

4.1.1 Hybrids of Malaysian Strains compared to Parental Strains (w1 and w2)

Comparison between the rate of mycelial growth and density between Malaysia parental strains and their hybrids are shown in Table 4.1. The fastest mycelial growth rates were recorded for hybrid strains of w1ew2a and w1jw2a having the similar value of 9.9 mm/day while hybrid strains of w1ew2f showed the slowest mycelial growth rate of 7.8 ± 0.90 mm/day. Adebayo *et al.* (2013) reported similar findings whereby hybrid LL910 which was obtained from interbreeding between *Pleurotus pulmonarius* and *Pleurotus ostreatus* had the highest mycelial growth rate. Ten strains of the hybrids showed similar significant different with the Malaysian native strains. In addition, Isikhuemhen *et al.* (2000) also produced a hybrid (Pt-Omon9) which showed higher mycelial growth rate than the parental strains of *Pleurotus tuberregium* from different ecological regions which were from Nigeria and Papua New Guinea. Moreover, Ying *et al.* (2017) also successfully produced hybrids of *Pleurotus sahor-caju* (Fr.) Sings which have higher mycelial growth rate than their parents.

Mycelial growth rate is an important element in producing high yield of mushroom (Royse, 1985). Fast mycelial growth rates elevate the rate of penetration of the substrate by the mushroom mycelia and decrease the length of the production cycle (Zharare *et al.*, 2010). Thus, rapid mycelial growth rate is able to reduce mushroom grower expenses as they could assume up to twice the rate of production as compared to the strain with lower mycelial growth rate (Royse, 1985). Moreover, a strain that is able to colonise the substrate rapidly can simply exclude contaminant microorganisms which

will lead to high production yield (Zharare *et al.*, 2010) as it will increase the production of enzymes present in the substrate (Royse, 1985).

Table 4.1: Average mycelial growth rate and mycelia density between parental strains (w1 & w2) and the hybrids.

Strains	Average of mycelial growth rate (mm/day)	Average of mycelial density
w1	9.0 ± 0.09^{bc}	++
w2	9.3 ± 0.12^{bcde}	++
w1aw2a	8.9 ± 0.04^{bc}	+++
w1bw2a	9.1 ± 0.04^{bcd}	++
w1bw2f	8.9 ± 0.04^{bc}	++
w1cw2a	9.3 ± 0.14^{bcde}	++
w1dw2a	9.3 ± 0.04^{bcde}	+++
w1dw2f	9.3 ± 0.03^{bcde}	++
w1ew2a	9.9 ± 0.04^{e}	++
w1ew2f	7.8 ± 0.09^{a}	++
w1fw2a	9.6 ± 0.04^{de}	++
w1gw2a	9.6 ± 0.03^{cde}	++
w1hw2a	9.6 ± 0.04^{cde}	++
w1jw2a	9.9 ± 0.05^{e}	++
w1Lw2a	8.9 ± 0.02^{b}	+++

Values are the means of 10 replicates. Intensity of mycelial density when the mycelia fully colonises the substrate bag where + poor running growth and has low density, ++ mycelia grows throughout the substrate bag is not uniformly white and has moderate density, and +++ mycelia grows throughout the whole bag and is uniformly white and has intense density.

Both parental strains have moderate mycelial density. However, 3 hybrid strains, which were w1aw2a, w1dw2a, and w1Lw2a showed very abundant mycelial density. The other strains produced similar mycelial density with the parental strains. None of the hybrid strain had low mycelial density. The intensity of mycelial density of these strains can be observed in Figure 4.1. Research published by (Guadarrama-Mendoza *et al.*, 2014)

reported that the hybrid of *Pleurotus* spp. which has rapid mycelia growth rate produced intense mycelia density. However, the inference may not be suitable to apply to this study as the hybrid w1ew2f which had the slowest mycelial growth rate only produced moderate mycelial density.

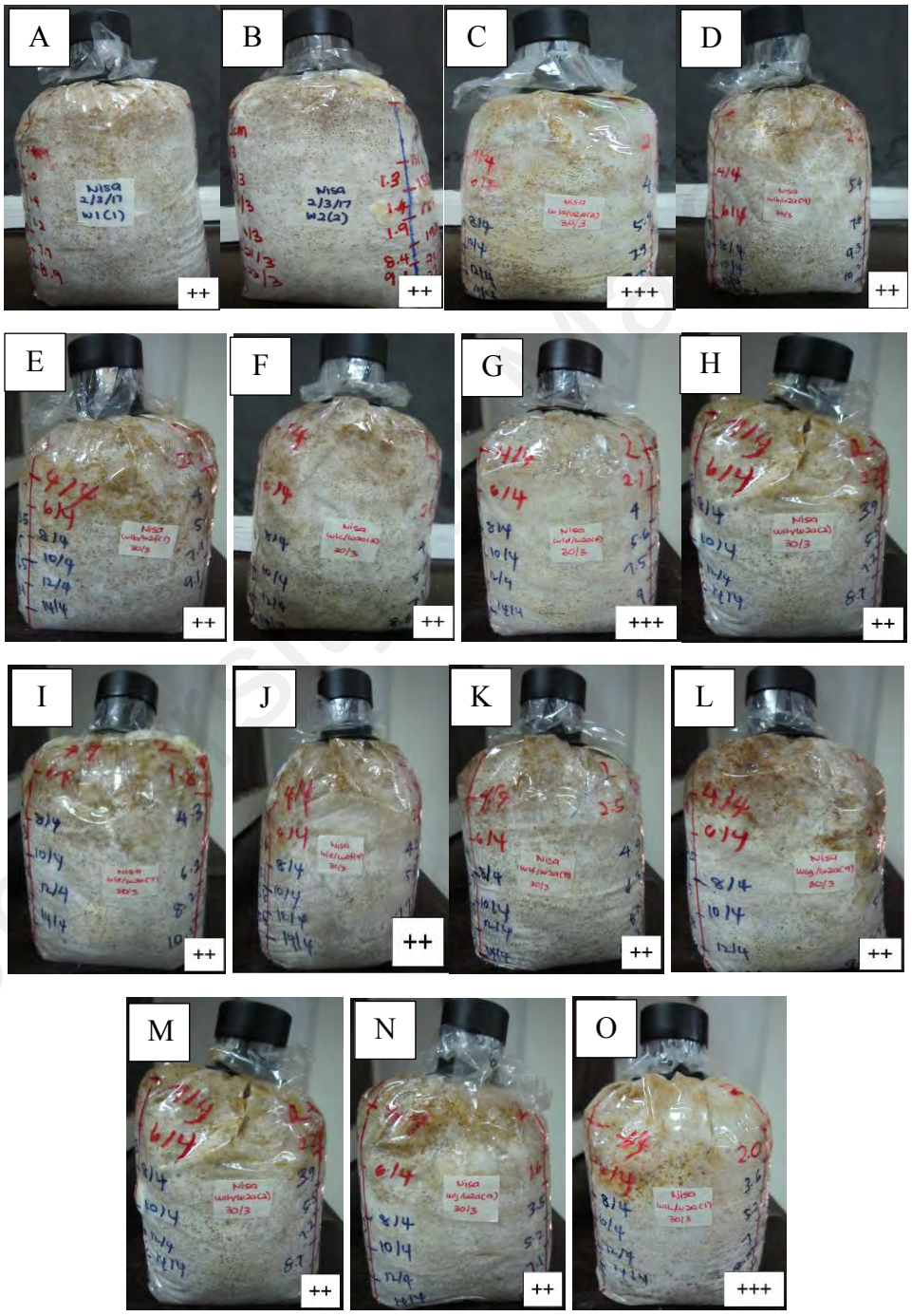


Figure 4.1: Intensity of mycelial density of Malaysia strains, and their hybrids (A) w1 (B) w2 (C) w1aw2a (D) w1bw2a (E) w1bw2f (F) w1cw2a (G) w1dw2a (H) w1dw2f (I) w1ew2a (J) w1ew2f (K) w1fw2a (L) w1gw2a (M) w1hw2a (N) w1jw2a (O) w1Lw2a.

4.1.2 Hybrids between Malaysian and Thailand Strains compared to the Parental Strains

Table 4.2 summarised the results of mycelial growth rate and mycelia density between Malaysia parental strain (w1), Thailand parental strain (R), and their hybrids w1eRa and w1jRc. The most rapid mycelial growths are obtained from both parental strains, w1 and R with measurement 9.0 ± 0.86 mm/day and 8.6 ± 0.80 mm/day respectively. No significant difference ($p<0.05$) of mycelial growth rate between parental strains from two different countries may due to the location of both countries as they experience almost similar environmental condition while study done by Sher *et al.* (2010) reported that different mycelial growth rate of the oyster mushroom was observed from two different regions in Pakistan as both regions have different temperature and humidity.

In contrast, both hybrid strains, w1eRa and w1jRc have significantly slow mycelial growth rate which is 7.0 ± 0.40 mm/day and 7.1 ± 0.30 mm/day respectively. Results obtained agreed with the mycelial growth rate reported by Llarena-Hernández *et al.*, (2013) where intercontinental hybrid of *Agaricus subrufescens* produced a lower mycelial growth rate than the parental strains. The mycelial growth rate has been proven to be an accurate and relevant measure to differentiate different strains (Clark & Anderson, 2004). The mycelial growth rate can be different due to many factors including genetic composition within strains (Kumara & Edirimanna, 2009). The mycelial growth rate is the primary quality to be selected, describing the higher values in cultivars than in wild isolates (Llarena-Hernández *et al.*, 2013). The hybridisation between Malaysia strain and Thailand strain produced lower mycelial growth rate than the parental strains. Thus, w1eRa and w1jRc can be removed in term of mycelial growth rate for the commercial purpose.

Table 4.2: Average mycelial growth rate and mycelia density between Malaysia parental strain (w1), Thailand parental strain (R), and their hybrids, w1eRa and w1jRc.

Strains	Average of mycelial growth rate (mm/day)	Average of mycelial density
w1	9.0 ± 0.90^{b}	++
R	8.6 ± 0.80^{b}	++
w1eRa	7.0 ± 0.40^{a}	+++
w1jRc	7.1 ± 0.30^{a}	+++

Values are the means of 10 replicates. Values are the means of 10 replicates. Intensity of mycelial density when the mycelia fully colonises the substrate bag where + poor running growth and has low density, ++ mycelia grows throughout the substrate bag is not uniformly white and has moderate density, and +++ mycelia grows throughout the whole bag and is uniformly white and has intense density.

Both hybrids w1eRa and w1jRc developed abundant mycelia density. In contrast, the parental strains produced moderate mycelia density. The mycelial density of these four strains can be observed in Figure 4.2. The relationship between mycelial growth rate and mycelia density was reported by Royse (1985) that the low mycelial growth rate may allow increased in mycelial density of *Lentinus edodes* to develop as it increased the amounts of hyphae in contact with the substrate. Thus, this may explain the different of mycelia density between parental strains and the hybrids.

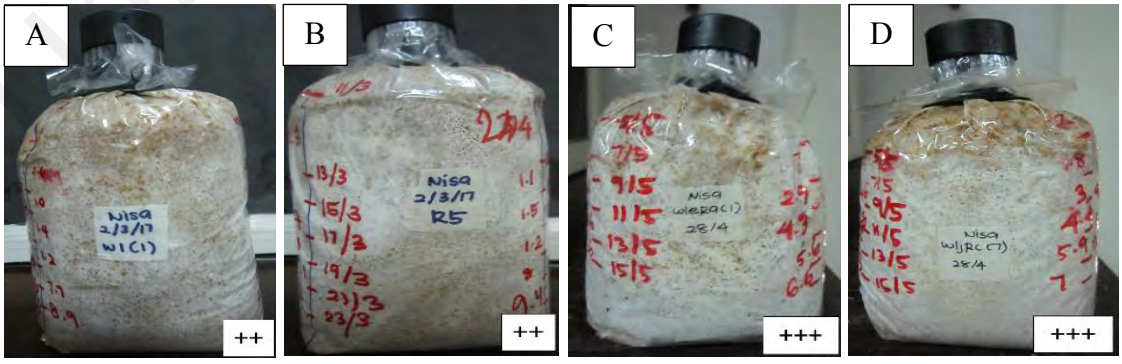


Figure 4.2: Intensity of mycelia density of Malaysia parental strain (w1), Thailand parental strain, and their hybrids. (A) w1 (B) R (C) w1eRa (D) w1jRc.

4.1.3 Irradiated Hybrids

The influence of 2 kGy gamma irradiation of monokaryon culture and hybridisation on the mycelial growth rate is shown in Table 4.3. Ten strains of monokaryotic-irradiated hybrids were selected, whereby 6 strains are hybrids between Malaysia native strain and 4 strains are the hybrids between Malaysia and Thailand native strain. ANOVA analysis resulted that the mycelial growth rate is significantly different among the irradiated strains.

Strain Ir-w1ew2a showed the fastest mycelial growth rate which is 8.7 ± 0.50 mm/day however it is lower than the natural parental strains w1 (9.0 ± 0.90 mm/day) and w2 (9.3 ± 0.12 mm/day) while strain Ir-w1jRa shows the slowest mycelial growth rate which is 6.9 ± 0.30 mm/day. Moreover, all the four strains hybridised between Malaysia strain and Thailand strain showed lower mycelial growth rate than w1 and R. Similar results were obtained by Majolagbe *et al.* (2013) where all the mutant hybrids of *Lentinus subnudus* have significantly similar and lower mycelial growth rate than the wild strain. This result also can be supported by a study done by Rashid *et al.* (2016) as the mycelial growth rate of the hybrid of *Pleurotus sajor-caju* decrease when exposed to the gamma radiation. Thus, this indicate that irradiation able to affect the mycelia growth rate of the mushroom hybrids and producing new strain of mushroom that show variant properties than the wild strains.

Table 4.3: Average of mycelial growth rate and mycelia density among irradiated strains**.**

Strains	Average mycelial growth rate (mm/day)	Average mycelial density
w1	9.0 ± 0.90^{fg}	++
w2	9.3 ± 0.12^{g}	++
R	8.6 ± 0.80^{ef}	++
Ir-w1dRa	7.1 ± 0.04^{ab}	++
Ir-w1dRc	7.8 ± 0.05^{bcd}	+++
Ir-w1dw2d	8.1 ± 0.02^{cde}	++
Ir-w1ew2a	8.7 ± 0.05^{efg}	++
Ir-w1ew2d	8.4 ± 0.07^{def}	++
Ir-w1gRc	7.3 ± 0.06^{ab}	++
Ir-w1gw2a	8.5 ± 0.05^{ef}	+++
Ir-w1hw2d	7.5 ± 0.03^{abc}	++
Ir-w1jRa	6.9 ± 0.03^{a}	++
Ir-w1Lw2f	7.6 ± 0.05^{bc}	+++

Values are the means of 10 replicates. Intensity of mycelial density when the mycelia fully colonises the substrate bag where + poor running growth and has low density, ++ mycelia grows throughout the substrate bag is not uniformly white and has moderate density, and +++ mycelia grows throughout the whole bag and is uniformly white and has intense density.

Table 4.3 presented the average mycelial density between irradiated hybrid strains. Three strains of irradiated hybrid produce the abundant mycelial density, which are Ir-w1dRc, Ir-w1gw2a and Ir-w1Lw2f. This result is accordance to the report by Kortei *et al.* (2014) where very abundant mycelial density of *Pleurotus eous* (Berk.) Sacc. strain P-31 was recorded when exposed by gamma irradiation. These results revealed that gamma irradiation could be used as an alternative method for the production of new strain with intense mycelia density. However, the other strains show moderate mycelial density. None of the irradiated hybrid strains show very little mycelial density. The mycelia density among irradiated hybrid strains can be observed in Figure 4.3.

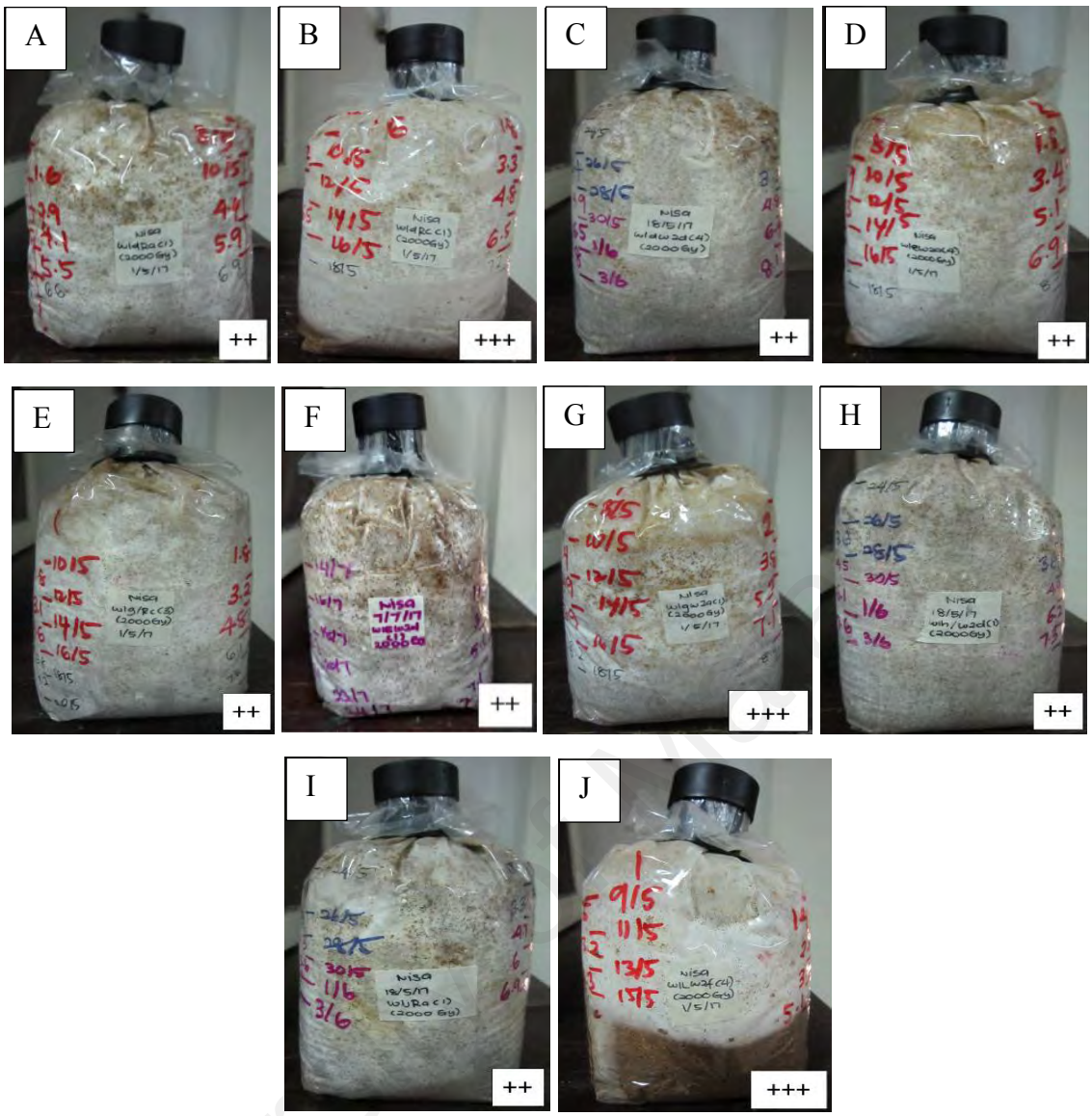


Figure 4.3: Intensity of mycelia density of irradiated hybrids (A) Ir-w1dRa (B) Ir-w1dRc (C) Ir-w1dw2d (D) Ir-w1ew2a (E) Ir-w1ew2d (F) Ir-w1gRc (G) Ir-w1gw2a (H) Ir-w1hw2d (I) Ir-w1jRa (J) Ir-w1Lw2f.

4.1.4 Comparison of Mycelial Growth Rate and Density of Parental Strains (w1 and w2), Hybrid (w1ew2a) and Monokaryon-Irradiated Hybrid (Ir-w1ew2a)

This comparison is made to determine whether hybridisation alone or irradiation and hybridisation have the potential to enhance growth rate or density. Table 4.4 shows the average mycelial growth rate between Malaysia parental strains (w1 and w2), their hybrids w1ew2a and the respective Ir-w1ew2a. The ability to colonise the cultivation substrate is recognised as a criterion for strain preferences of the cultivated mushroom

(Llarena-Hernández *et al.*, 2013). The graph exhibits that the average mycelial growth rate were significant differences ($p<0.05$) among these four strains. The mycelia growth of the hybrid w1ew2a showed significant ($p<0.05$) increase of 9.9 ± 0.40 mm/day compared to both parental strains w1 and w2 with 9.0 ± 0.86 mm/day and 9.3 ± 1.30 mm/day respectively. The growth rates of strains isolated from natural populations have a narrow range yet hybridises made from wild-collected strains displayed an improved range of growth rate (Clark & Anderson, 2004).

The irradiated hybrid Ir-w1ew2a shows a non-significant ($p>0.05$) reduction in the mycelial growth rate of 8.7 ± 0.50 mm/day. This result was in accordance with Rashid *et al.* (2014), where the study mycelial growth rate of *Pleurotus sajor-caju* decreased as the level of dosage of gamma radiation increase. It implies that the gamma radiation can cause changes in the growth rate of mycelium. The mycelial growth rate could affect the duration of colonisation of mycelium on a substrate. The strains which have high mycelial growth rate were able to colonise the substrate in a short period (Rashid *et al.*, 2016). However, mycelial growth rate had no significant effect on the yield of sporophores, time to fruiting and sporophore mean weight (Llarena-Hernández *et al.*, 2011).

Table 4.4: Average of mycelial growth rate (mm/day) and mycelia density of Malaysian parental strains (w1 and w2), hybrid w1ew2a, and monokaryon-irradiated hybrid Ir-w1ew2a.

Strains	Average mycelial growth rate (mm/day)	Average density of mycelium
w1	9.0 ± 0.09^{a}	++
w2	9.3 ± 0.13^{a}	++
w1ew2a	9.9 ± 0.04^{ab}	++
Ir-w1ew2a	8.7 ± 0.05^{b}	++

Values are the means of 10 replicates. Intensity of mycelial density when the mycelia fully colonises the substrate bag where + poor running growth and has low density, ++ mycelia grows throughout the substrate bag is not uniformly white and has moderate density, and +++ mycelia grows throughout the whole bag and is uniformly white and has intense density.

All the strains showed the similar moderate mycelial density. The intensity of the mycelial density of these four strains can be observed in Figure 4.4. This comparison shows that hybridisation and gamma irradiation may not affect the mycelial density for the strain w1ew2a and Ir-w1ew2a.

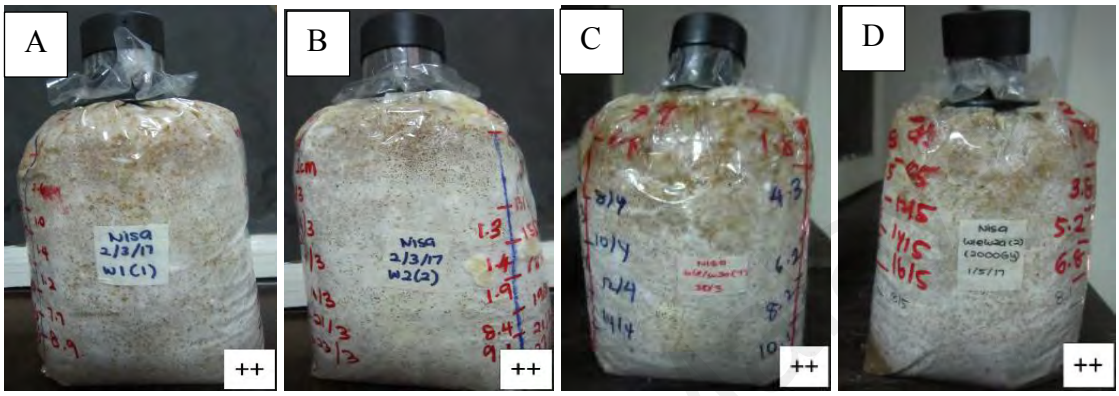


Figure 4.4: Intensity of mycelial density of Malaysia parental strains, hybrid w1ew2a, and monokaryon-irradiated hybrid Ir-w1ew2a (A) w1 (B) w2 (C) w1ew2a (D) Ir-w1ew2a.

4.2 Morphology of Sporophore

4.2.1 Hybrids of Malaysian Strains compared to Parental Strains (w1 and w2)

Although mycelia growth rate is important trait to the phenotypic variability of a crop, quality of the harvested mushrooms is also influential. The shape and colour are the primary benchmark for quality of fresh mushroom for market. Quality of mushroom strains might be influenced by both the genetic composition and the environmental condition (Llarena-Hernández *et al.*, 2013). Under our controlled fructification condition, the morphology and average of the width sporophores between Malaysia parental strains (w1 and w2) and hybrids are presented in the Table 4.5. Different colour of sporophores were observed between w1 and w2, where w1 had greyish white colour while w2 has yellowish brown colour. This proved that wild strains showed variation among the same species and due to these variations, wild strains act as huge collection of gene pool in

nature available for strain breeding (Xiao *et al.*, 2016). Three of the hybrids which are w1aw2a, w1ew2f and w1gw2a appeared in greyish white while the others appeared in yellowish brown. However, no considerable morphological differences in terms of sporophores margin were observed between the parental strains and the hybrids. Similar result was produced by study conducted by Kumara and Edirimanna (2009) where four hybrids of *Pleurotus* sp. have similar margin with their parental strains generated by hybridisation.

Interestingly, w1 has larger sporophores than w2. This signify that there is genetic variation among the parental strains even from the same region. The width of the sporophores also shown variant among hybrid and parental strains. Hybrid w1fw2a exhibited significantly ($p<0.05$) biggest sporophore size of 35 ± 5.6 mm. This is followed by parental w1 and the hybrid w1cw2a and hybrid w1jw2a. Hybrid w1hw2a had the significantly smallest sporophore size of 16 ± 2.0 mm. Kumara and Edirimanna (2009) also produced hybrid with smaller size sporophores compared to the parental strains which was called hybrid A_3L_3. Sporophore size of all other hybrids were significantly ($p<0.05$) smaller than the parental w1. Based on these results, hybridisation process may affect the width of sporophores while remained some of the characteristics of the parental strains. Besides genetic composition, size of mushroom also can be affected by ecological factors such as temperature, humidity, fresh air and substrate materials. The lowest temperature and drought environment decrease the size of mushroom (Sher *et al.*, 2010).

Table 4.5: Average size and morphology of the sporophores of the hybrids of Malaysian strains compared to the parental strains (w1 & w2).

Strains	Morphology of the sporophore	Average of width of sporophore (mm)	Width of Sporophores	Fruitification Density
w1	Greyish white colour with lobed margin sporophore	32 ± 2.7^{f}		
w2	Yellowish brown colour with lobed margin sporophore	26 ± 3.1^{cde}		
w1aw2a	Greyish white colour with lobed margin sporophore	25 ± 3.5^{cde}		
w1bw2a	Yellowish brown colour with lobed margin sporophore	24 ± 3.8^{c}		
w1bw2f	Yellowish brown colour with lobed margin sporophore	24 ± 4.6^{cd}		
w1cw2a	Yellowish brown colour with lobed margin sporophore	32 ± 3.7^{f}		

Values of the width of sporophore are the means of 20 mature sporophores from 10 fruiting bags of each strains

Table 4.5, continued

Strains	Morphology of the sporophore	Average of width of sporophore (mm)	Width of Sporophores	Fruitification Density
w1dw2a	Yellowish brown colour with lobed margin sporophore	27 ± 5.8^{de}		
w1dw2f	Yellowish brown colour with lobed margin sporophore	27 ± 6.2^{e}		
w1ew2a	Yellowish brown colour with lobed margin sporophore	27 ± 3.3^{de}		
w1ew2f	Greyish white colour with lobed margin sporophore	30 ± 2.7^{f}		
w1fw2a	Yellowish brown colour with lobed margin sporophore	35 ± 5.6^{g}		
w1gw2a	Greyish white colour with lobed margin sporophore	19 ± 3.4^{b}		

Values of the width of sporophore are the means of 20 mature sporophores from 10 fruiting bags of each strains

Table 4.5, continued

Strains	Morphology of the sporophore	Average of width of sporophore (mm)	Width of Sporophores	Fruitification Density
w1hw2a	Yellowish brown colour with lobed margin sporophore	16 ± 2.0[a]		
w1jw2a	Yellowish brown colour with lobed margin sporophore	32 ± 4.4[f]		
w1Lw2a	Yellowish brown colour with lobed margin sporophore	21 ± 2.2[b]		

Values of the width of sporophore are the means of 20 mature sporophores from 10 fruiting bags of each strains

4.2.2 Hybrids between Malaysian and Thailand Strains compared to the Parental Strains

The morphology and average sporophore sizes of Malaysia parental strain, Thailand parental strain and their hybrid strains are shown in Table 4.6. Both hybrids appeared in the similar colour as the parental strains. Interestingly, hybrid strain w1eRa has the distinguishable morphology of sporophore with wavy margin compared to the smooth margin of the hybrid w1jRc and the parental strains. Kumara and Edirimanna (2009) also produced hybrid of *Pleurotus* sp. with wavy appearance through hybridisation. Significantly larger sporophores which is 32 ± 2.7 mm were obtained by strain w1.

Table 4.6: Average of width and morphology of sporophores of the hybrids between Malaysia and Thailand strains compared to the parental strains (A) w1 (B) R (C) w1eRa (D) w1jRc.

Strains	Morphology of the sporophore	Average of width of sporophore (mm)	Width of Sporophores	Fructification Density
w1	Greyish white colour with lobed margin sporophore	32 ± 2.7[c]		
R	Yellowish brown colour with lobed margin sporophore	18 ± 2.9[a]		
w1eRa	Yellowish brown colour with irregularly wavy margin sporophore	24 ± 4.3[b]		
w1jRc	Yellowish brown colour with lobed margin sporophore	25 ± 2.4[b]		

Values of the width of sporophore are the means of 20 mature sporophores from 10 fruiting bags of each strains

The hybrids, w1eRa and w1jRc showed the significantly bigger size of sporophores with measurement 24 ± 4.3 mm and 25 ± 2.4 mm compared to the Thailand parental strain, R. R has significantly the smallest width of sporophores with the measurement 18 ± 2.9 mm. Even though R has rapid mycelia growth rate, yet R produced the smallest sporophore. This shows that mycelial growth rate does not influence the size

of mushroom. In addition, relatively smaller size of mushroom sporophores is an undesirable feature for marketable value (Yang *et al.*, 2013). Hybridisation of *S. commune* from two countries produced a moderate size of sporophores. This indicate that hybridisation between two different ecological strains may produce improved strains that expressed the combined quality traits obtained from the parental strains (Llarena-Hernández *et al.*, 2013). Moreover, strains displayed individual characteristics irrespective of their origin (Llarena-Hernández *et al.*, 2013).

4.2.3 Irradiated Hybrids

The results of the morphology and width of the sporophores of the irradiated hybrids are presented in the Table 4.7. A total of 10 strains of irradiated hybrid are tested in this experiment, where 6 strains are hybridised between the irradiated Malaysia strains and four strains are hybridised between irradiated Malaysia strain and Thailand strain. All irradiated hybrids produced sporophores with lobed margins. The sporophore colour clearly separated three irradiated monokaryon hybrids that are Ir-w1dRc, Ir-w1gRc and Ir-w1hw2d that exhibited greyish white sporophores while others appeared in the yellowish brown sporophores. In addition, hybrid Ir-w1jRa has wavy margin of sporophores that was different margin morphology than the parental strains and other hybrids. This result is in agreement with the research done by Rashid *et al.* (2014) where different morphology is observed after irradiated by gamma ray. This indicate that the irradiation by gamma ray is a reliable technique for generating new varieties of mushroom species.

Two strains produced the longest sporophores, which are w1 and Ir-w1dw2d with measurement of 32 ± 2.7 mm and 31 ± 3.0 mm respectively with no significant difference between these strains. Ir-w1hw2d and Ir-w1ew2d produced sporophore which are larger than parental strain w2 with measurement of 30 ± 2.5 mm and 29 ± 4.1 mm. On the other

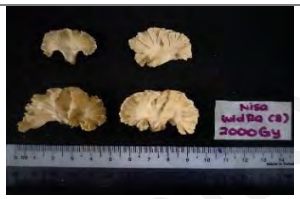
hand, Ir-w1gRc has the smallest width of sporophore with the measurement of 24 ± 3.3 mm. Despite that, hybrid Ir-w1gRc has larger sporophore than the parental strain R with only 18 ± 2.9 mm. Moreover, the length of sporophore of all irradiated hybrids are larger compared to the study done by Preecha *et al.* (2016) which produced sporophore with length of 17 mm.

Based on the United Nations Economic Commission for Europe, the minimum concerning sizing for mushroom sporophore should be 20 mm or more for the purposes of marketing and commercial quality control of cultivated mushrooms (UNECE, 2017). Although parental strain R has the smallest sporophores, all irradiated monokaryon hybrids produced follow the standard provided by UNECE. Thus, irradiation method able to generate new strains of hybrids that has improved quality for commercial purposes.

Table 4.7: Average of width and morphology of the sporophores of the irradiated hybrids.

Strains	Morphology of the sporophore	Average width of sporophores (mm)	Width of Sporophores	Fructification Density
w1	Greyish white colour with lobed margin sporophore	32 ± 2.7^{f}		
w2	Yellowish brown colour with lobed margin sporophore	26 ± 3.1^{cd}		

Table 4.7, continued

Strains	Morphology of the sporophore	Average width of sporophores (mm)	Width of Sporophores	Fructification Density
R	Yellowish brown colour with lobed margin sporophore	18 ± 2.9[a]		
Ir-w1dRa	Yellowish brown colour with lobed margin sporophore	26 ± 4.0[d]		
Ir-w1dRc	Greyish white colour with lobed margin sporophore	26 ± 2.9[d]		
Ir-w1dw2d	Yellowish brown colour with lobed margin sporophore	31 ± 3.0[f]		
Ir-w1ew2a	Yellowish brown colour with lobed margin sporophore	26 ± 2.0[cd]		
Ir-w1ew2d	Yellowish brown colour with lobed margin sporophore	29 ± 4.1[e]		

Values of the width of sporophore are the means of 20 mature sporophores from 10 fruiting bags of each strains

Table 4.7, continued

Strains	Morphology of the sporophore	Average width of sporophores (mm)	Width of Sporophores	Fructification Density
Ir-w1gRc	Greyish white colour with lobed margin sporophore	24 ± 3.3^{bc}		
Ir-w1gw2a	Yellowish brown colour with lobed margin sporophore	26 ± 3.2^{d}		
Ir-w1hw2d	Greyish white colour with lobed margin sporophore	30 ± 2.5^{ef}		
Ir-w1jRa	Yellowish brown colour with wavy margin sporophore	26 ± 3.4^{d}		
Ir-w1Lw2f	Yellowish brown colour with lobed margin sporophore	23 ± 3.2^{b}		

Values of the width of sporophore are the means of 20 mature sporophores from 10 fruiting bags of each strains

4.2.4 Comparison of Sporophores Size of the Parental Strains (w1 and w2), Hybrid w1ew2a and Monokaryon-Irradiated Hybrid (Ir-w1ew2a)

Table 4.8 shows the morphology and width of sporophore between Malaysia parental strain and their hybrids, w1ew2a, and Ir-w1ew2a. w1 had greyish white sporophore while R and the hybrids have yellowish grey sporophore. However, both hybrids produced from hybridisation and irradiation of gamma ray produced similar morphology with the parental strains that have lobed margin. The sporophores of both hybrids are indistinguishable from one another and towards their parental strains. This may be presumed that hybridisation and gamma radiation show no affect or minimal affect in term of morphology of the sporophores.

In terms of the width of sporophores, w1 produced the longest sporophore with measurement of 32 ± 2.7 mm while the three strains, w2, w1ew2a, and Ir-w1ew2a produce sporophore with no significant difference among themselves with measurement of 26 ± 3.1 mm, 27 ± 3.3 mm, and 26 ± 2.0 mm respectively. From this experiment, it can be pronounced in two ways. Firstly, the hybrids might express the genetic information retrieved from w2 that are responsible for the colour and size of the sporophores. Besides that, this result can be explained in a way that gamma ray does not affect the size of sporophore.

Table 4.8: Average of width and morphology of the sporophores between parental strains (w1and w2), hybrid w1ew2a and monokaryon-irradiated hybrid (Ir-w1ew2a).

Strains	Morphology of the sporophore	Average width of sporophores (mm)	Width of Sporophores	Fructification Density
w1	Greyish white colour with lobed margin sporophore	32 ± 2.7[b]		
w2	Yellowish brown colour with lobed margin sporophore	26 ± 3.1[a]		
w1ew2a	Yellowish brown colour with lobed margin sporophore	27 ± 3.3[a]		
Ir-w1ew2a	Yellowish brown colour with lobed margin sporophore	26 ± 2.0[a]		

Values of the width of sporophore are the means of 20 mature sporophores from 10 fruiting bags of each strains

4.3 Yield and Biological Efficiency

4.3.1 Hybrids of Malaysian Strains compared to the Parental Strains (w1 and w2)

The average values of the sporophores yield and biological efficiency of Malaysia parental strain (w1 and w2) and the hybrids are shown in Table 4.9. The greatest number of fresh weight of mushroom are obtained in the strains of w1bw2f and w1Lw2a with the yield of 63.60 ± 2.91 g / bag, and 64.44 ± 2.12 g / bag respectively. On the other hand, hybrid w1aw2a generates the lowest yield which is 29.30 ± 2.68 g / bag. Six out of thirteen hybrid strains have a better yield than the parental strains while seven strains have significantly same or lower than the parental strains. The seven hybrid strains are w1bw2f, w1dw2f, w1gw2a, w1hw2a, w1jw2a, and w1Lw2a. The yield obtained by these six strains are higher than the result obtained by Ediriweera *et al.* (2015), where in this study the yield collected was 3.726 ± 0.63 g / bag.

Biological efficiency, which is applied to measure the efficiency of substrate conversion for every strains in mushroom cultivation, was estimated as ratio of the biological yield harvested to the dry weight of each substrate (Girmay *et al.*, 2016). Significantly, the highest biological efficiency was observed in the hybrid strains of w1bw2f and w1Lw2a with 34.74 ± 1.54 % and 33.84 ± 1.12 %. Six hybrid strains which are w1bw2f, w1dw2f, w1gw2a, w1hw2a, w1jw2a and w1lw2a had greater biological efficiency than the parental strains. The performances of each strain in term of biological efficiency is influenced by genetic composition of individual strain (Royse & Bahler, 1986). This result indicated that the hybridisation method is a useful technique to produced new strains with improved biological efficiency. However, hybrid w1aw2a resulted in the significantly the lowest biological efficiency with 17.36 ± 1.41 %.

Table 4.9: Average of sporophores yield, biological efficiency (BE), percentage of contamination, and number of flushes between Malaysia parental strains (w1 & w2) and the hybrids.

Strain	Number of flushes	Average of sporophores yield (g / bag)	Average of biological efficiency (%)	Percentage of contamination (%)
w1	2	47.91 ± 4.74^{e}	25.28 ± 2.50^{o}	30.0
w2	2	41.82 ± 4.72^{d}	23.99 ± 3.14^{o}	30.0
w1aw2a	2	32.90 ± 2.68^{a}	17.36 ± 1.41^{L}	100.0
w1bw2a	2	38.89 ± 3.72^{cd}	20.51 ± 1.96^{n}	80.0
w1bw2f	2	65.85 ± 2.91^{h}	34.74 ± 1.54^{r}	10.0
w1cw2a	2	34.22 ± 1.27^{ab}	18.05 ± 0.67^{Lm}	30.0
w1dw2a	2	37.68 ± 2.20^{bc}	19.88 ± 1.16^{mn}	0.0
w1dw2f	2	51.90 ± 1.76^{f}	27.38 ± 0.93^{p}	30.0
w1ew2a	2	46.73 ± 1.33^{e}	24.65 ± 0.70^{o}	30.0
w1ew2f	2	46.08 ± 2.25^{e}	24.31 ± 1.19^{o}	0.0
w1fw2a	2	38.25 ± 2.12^{cd}	20.18 ± 1.12^{n}	10.0
w1gw2a	2	57.75 ± 2.18^{g}	30.46 ± 1.15^{q}	30.0
w1hw2a	2	57.82 ± 3.01^{g}	30.50 ± 1.59^{q}	60.0
w1jw2a	2	53.07 ± 2.19^{f}	28.00 ± 1.15^{p}	30.0
w1Lw2a	2	64.14 ± 2.12^{h}	33.84 ± 1.12^{r}	20.0

Observation pertaining to the percentage of contamination between Malaysia parental strains with their hybrid strains are present in the Figure 4.5. Both parental strains w1 and w2 have the similar percentage of contamination that is 30 %. Among thirteen hybrid strains, only two strains show no contamination that are hybrid w1dw2a and w1ew2f, while w1bw2f and w1fw2a has lower rate of contamination than the parental strains. Five hybrid strains (w1cw2a, w1dw2f, w1ew2a, w1gw2a, and w1jw2a) have the same percentage with the parent strains. This shows that hybridisation method between Malaysia parental strains produce low resistance strains towards green mould fungus. Even though most of the hybrids have a high rate of contamination, yet the strains were still able to produce sporophores. Contrary, the report by Alananbeh *et al.* (2014) where

no sporophores were produced when *Pluerotus ostreatus* bag which culture on date palm wastes were affected by *Trichoderma* sp.

The different of yield and biological efficiency produce by parental strains and hybrid strains may be due to the genotype of mushroom strains that were affect by the hybridisation process (Moonmoon *et al.*, 2010). This means that hybridisation method between Malaysia strains improved the yield of hybrid strains, which is the evidence of strain improvement. The number of flushes for the Malaysia native strains and their non-irradiated hybrids are reported in Table 4.9. All the strains show the same number of flushes which is two times.

Contamination by green mould can be observed in Figure 4.6. The main agents of green mould epidemics of mushroom cultivation are *Trichoderma spp.* (*T. asperellum, T. atroviride, T. citrinoviride, T. hazianum, T. longibrachiatum, T. pleurotum, T. pleuroticola,* and *T. virens*) (Bellettini *et al.*, 2019). Green mould competes for nutrient and space with mushroom mycelia by secreting secondary toxic metabolites, extracellular enzymes and many volatile organic compounds that are harmful to the mushroom. Thus, depleting the yield production of the mushroom (Hatvani *et al.*, 2012).

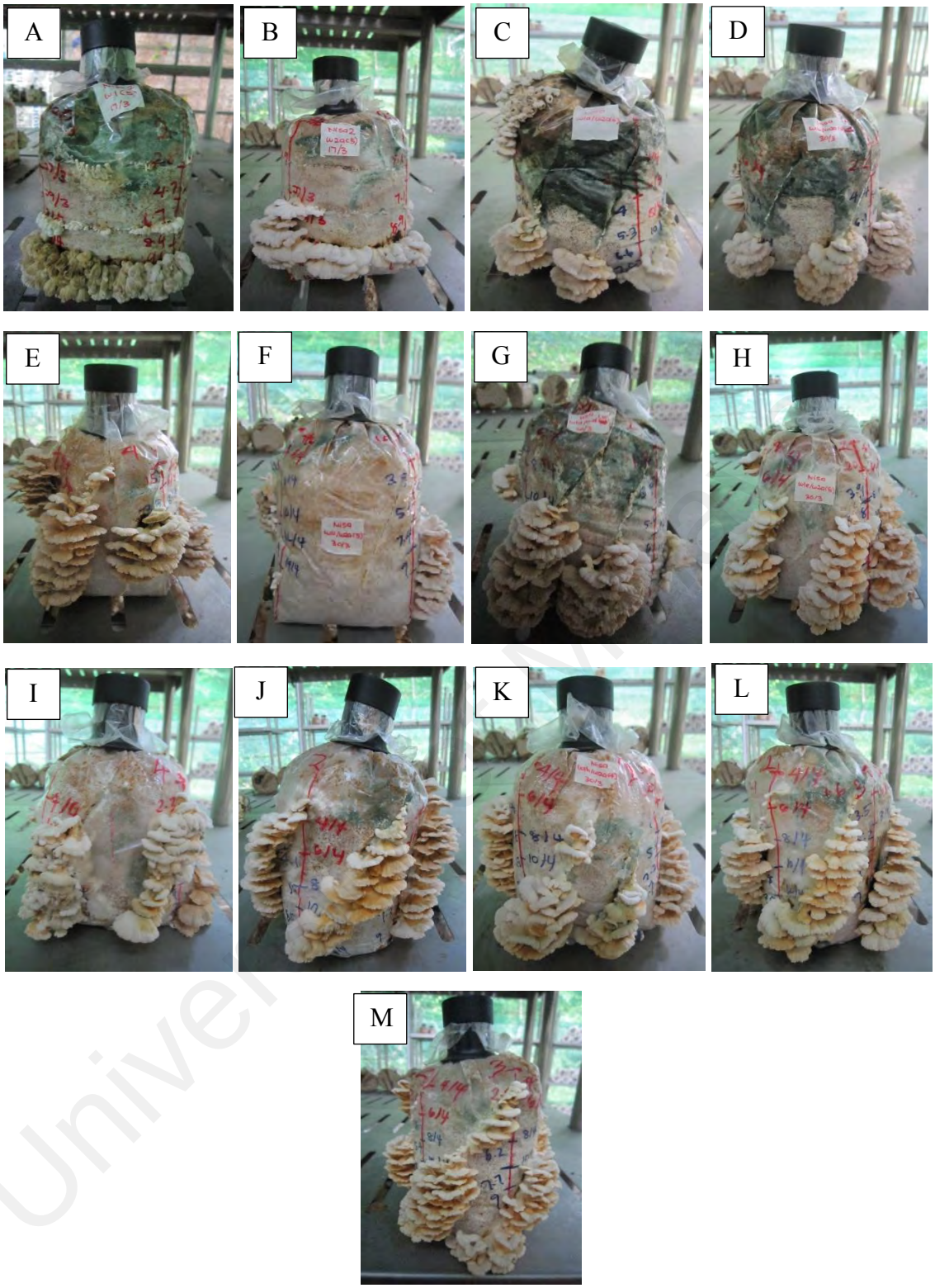


Figure 4.5: Example of contaminations that occur in the substrate bag during fructification of Malaysia parental and hybrid strain (A) w1 (B) w2 (C) w1aw2a (D) w1bww2a (E) w1bw2f (F) w1cw2a (G) w1dw2f (H) w1ew2a (I) w1fw2a (J) w1gw2a (K) w1hw2a (L) w1jw2a (M) w1Lw2a.

4.3.2 Hybrids between Malaysia and Thailand Strains compared to the Parental Strains

Sporophore yield and biological efficiency between Malaysia parental strain, w1, Thailand parental strain, R, and the hybrids are shown in Table 4.10. Despite R produced small sporophores, R shows significantly the highest sporophores yield capacity that is 60.48 ± 5.23 g / bag while the other three strains (w1, w1eRa, and w1jRc) produced similar significant yield capacities that are 47.91 ± 4.74 g / bag, 47.49 ± 1.10 g / bag, and 51.43 ± 2.00 g / bag. This result was consistent with Llarena-Hernández *et al.* (2011) where hybrid of *Agaricus subrufescens* that were hybridised between strains from Brazil and France has sporophore yield that fell between the yields of its two parents. The results presented here may be explained as both of the hybrid from the hybridisation method between Malaysia strain and Thailand strain may receive and express the genetic information regarding the production of sporophores from the Malaysia strain. Moreover, both hybrid strains have the minimum number of flushes.

Biological efficiency (BE) can be defined as the performance of each strain of mushroom by the ability to convert the percentage from dry substrate to fresh sporophores (Chang *et al.*, 1981). Thailand native strain, R produced biological efficiency significantly surpassing all the other strains, which is 31.91 ± 2.76 %. In term of yield and number of flushes, the hybridisation technique does not produce hybrid strains that has improved quality.

Table 4.10: Average of sporophores yield, biological efficiency (BE), percentage of contamination, and number of flushes between Malaysia parental strain (w1), Thailand parental strain (R), and hybrids, w1eRa and w1jRc.

Strains	Number of flushes	Average of sporophores yield (g / bag)	Average of biological efficiency (%)	Percentage of contamination (%)
w1	2	47.91 ± 4.74[a]	25.28 ± 2.50[c]	30.0
R	2	60.48 ± 5.23[b]	31.91 ± 2.76[d]	10.0
w1eRa	1	47.49 ± 1.10[a]	25.05 ± 0.58[c]	0.0
w1jRc	1	51.43 ± 2.00[a]	27.13 ± 1.06[c]	0.0

The percentage of contamination of these two native strains and their hybrids are tabulated in the Table 4.10. Both hybrids show zero percentage of contamination while the Malaysia parental strain (w1) had the highest percentage of contamination by green mould with 30 %. Only 10 % of the substrate bag of the Thailand strain was infected with green mould. Even though the quality of hybrid strains w1eRa and w1jRc are improved in term of, the rate of contamination, yet the yield generates, and the number of flush are still unsatisfactory. The presence of the green mould in w1 and R can be observed in Figure 4.6. Green mould disease not only affecting *S. commune*, but also affecting other species of mushroom such as *Agaricus bisporus* (Hatvani *et al.*, 2007), *Pleurotus ostreatus* (Hatvani *et al.*, 2012), and *Lentinula edodes* (Wang *et al.*, 2016).

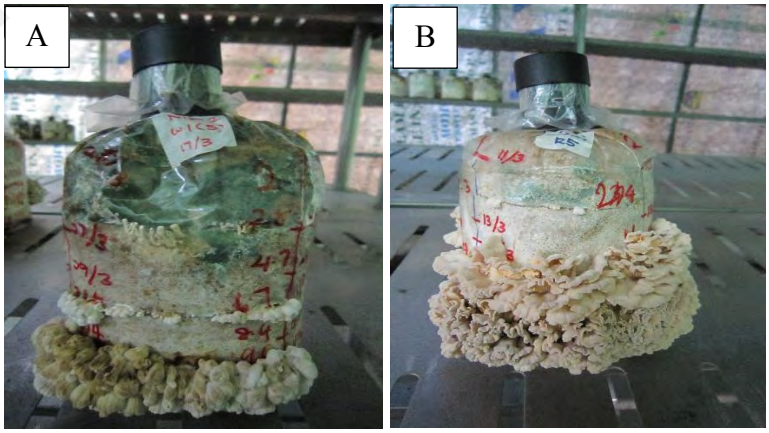


Figure 4.6: Example of contaminations that occur in the substrate bag during fructification of parental strains w1 and R (A) w1 (B) R.

4.3.3 Irradiated Hybrids

Table 4.11 represented the results of sporophore yield and biological efficiency among irradiated hybrid strains. At the end of experiment, despite their low mycelial growth rate, some of the irradiated monokaryon hybrids produced high yield of sporophores. Ir-w1gw2a has the highest yields that was 62.30 ± 1.72 g / bag. However, strain Ir-w1gRc produced significantly the lowest yield with 15.16 ± 1.21 g / bag. The number of flushes also influences the sporophores harvested. The minimum number of flushes are seen on strains Ir-w1dRa, Ir-w1gRc, and Ir-w1hw2d.

The biological efficiency indicated the utilisation of substrate nutrients to the sporophores of the mushroom and characterises the mushroom growth (Myronycheva *et al.*, 2017). The best biological efficiency was observed in strain Ir-w1gw2a with 32.87 ± 0.91 % that is significantly higher than other strains. In case of the percentage of contamination, all strains of irradiated hybrid showed zero contamination. The similar report was obtained by a study done by Kortei & Kwagyan (2014) where zero contamination in substrate bag was observed. This indicates that gamma radiation caused improvement in the strain of mushroom

Table 4.11: Average of sporophores yield, biological efficiency (BE), percentage of contamination, and number of flushes among irradiated hybrids.

Strains	Number of flushes	Average sporophores yield (g / bag)	Average of biological efficiency (%)	Percentage of contamination (%)
w1	2	47.91 ± 4.74^{e}	25.28 ± 2.50^{c}	30.0
w2	2	41.82 ± 4.72^{d}	23.99 ± 3.14^{c}	30.0
R	2	60.48 ± 5.23^{f}	31.91 ± 2.76^{e}	10.0
Ir-w1dRa	1	55.11 ± 4.66^{e}	29.07 ± 2.46^{c}	0.0
Ir-w1dRc	2	44.78 ± 2.22^{f}	23.62 ± 1.17^{de}	0.0
Ir-w1dw2d	2	55.24 ± 1.06^{b}	29.14 ± 0.56^{de}	0.0
Ir-w1ew2a	2	54.44 ± 0.91^{f}	28.72 ± 0.61^{d}	0.0
Ir-w1ew2d	2	54.34 ± 2.29^{f}	28.67 ± 1.21^{de}	0.0
Ir-w1gRc	1	15.16 ± 1.21^{a}	8.00 ± 0.64^{a}	0.0
Ir-w1gw2a	2	62.30 ± 1.72^{bc}	32.87 ± 0.91^{f}	0.0
Ir-w1hw2d	1	31.40 ± 1.20^{bc}	16.56 ± 0.61^{b}	0.0
Ir-w1jRa	2	39.66 ± 3.58^{bc}	20.92 ± 1.89^{b}	0.0
Ir-w1Lw2f	2	34.91 ± 1.52^{c}	18.42 ± 0.80^{b}	0.0

No green moulds were presented in all replicates of the strains. All strains were consistent with no percentage of contamination. Similar result was obtained by a study conducted by Staněk (1978) where the number of microorganisms colonising the surface of sporophores reduced when irradiated with gamma rays. Gamma ray cause retardation of the growth of microorganisms by preventing the production of numerous components that were essential for their development (Staněk, 1978). Hence, mutation by gamma radiation produced a high resistance strains towards competition by green moulds. Green moulds have caused significant mushroom losses in many countries, which can indicate worldwide threat (Hatvani *et al.*, 2008). Many alternative prevention steps have been taken, including by utilising disinfectants such as chlorine (household bleach) and a particular fungicide, which associated with high costs. In addition, the application of

chemical had been found to leaves undesired residues (Bellettini *et al.*, 2016). Therefore, a high resistance strains of mushroom towards green mould must be generated.

4.3.4 Comparison of the Sporophore Yield and Biological Efficiency of Parental Strains (w1 and w2), Hybrid w1ew2a and Monokaryon-Irradiated Hybrid (Ir-w1ew2a)

The number of fresh weight referred to the harvested sporophores of the mushroom and it is directly proportional towards the yield. Table 4.12 illustrates the average of yield provide by Malaysia parental strains (w1 and w2) and their non-irradiated hybrid (w1ew2a) and irradiated hybrid (Ir-w1ew2a). The maximum number of harvested fresh weight was produced by irradiated hybrid Ir-w1ew2a which was 56.81 ± 0.91 g / bag. Malaysia parental strain, w1 produce yield with no significant difference with hybrid w1ew2a. However, Malaysia parental strain, w2 provide the least yield which was 41.82 ± 4.72 g / bag. All strains were harvested for two flushes.

Table 4.12: Average of sporophores yield, biological efficiency (BE), number of flushes and percentage of contamination between parental strains (w1 and w2), hybrid w1ew2a and monokaryon-irradiated hybrid (Ir-w1ew2a).

Strains	Number of flushes	Average of sporophores yield (g / bag)	Average biological efficiency (%)	Percentage of contamination (%)
w1	2	47.91 ± 4.74^{b}	25.28 ± 2.50^{e}	30.0
w2	2	41.82 ± 4.72^{a}	22.06 ± 2.49^{d}	30.0
w1ew2a	2	46.73 ± 1.33^{b}	24.65 ± 0.70^{e}	30.0
Ir-w1ew2a	2	56.81 ± 0.91^{c}	29.97 ± 0.48^{f}	0.0

The capability of the mushroom strains to utilised substrate materials can be measured through biological efficiency (Stamets, 2000). The effect of Malaysia parental strains (w1 and w2) and their hybrids (w1ew2a and Ir-w1ew2a) towards the biological efficiency can be examined based in Table 4.12. The irradiated hybrids Ir-w1ew2a produced significantly the highest biological efficiency which is 29.97 ± 0.48 %. However, the native Malaysia strain w2 produces significantly the lowest biological efficiency which is 22.06 ± 2.49 %. The biological efficiency of non-irradiated hybrids, w1ew2a has a similar significant difference with the Malaysia parental strain, w1. This result can be interpreting in a way that gamma radiation shows an improvement towards the biological efficiency of the strain.

Based on the Table 4.12, the percentage of contamination can be compared between these four strains. Irradiated hybrid strain w1ew2a shows no contamination while the others exhibit a similar percentage of contamination which is 30.0 %. Figure 4.7 presented the contamination that affected w1, w2, and w1ew2a. The pattern of contamination by both Malaysia native strains were followed by the hybrid w1ew2a. However, the irradiated hybrid strain Ir-w1ew2a show no contamination. Thus, in this aspect, gamma radiation produced high resistance strains to the competitive microbiota under the studied cultivation conditions. This is a valuable information for the selection of strain for the commercial purpose.

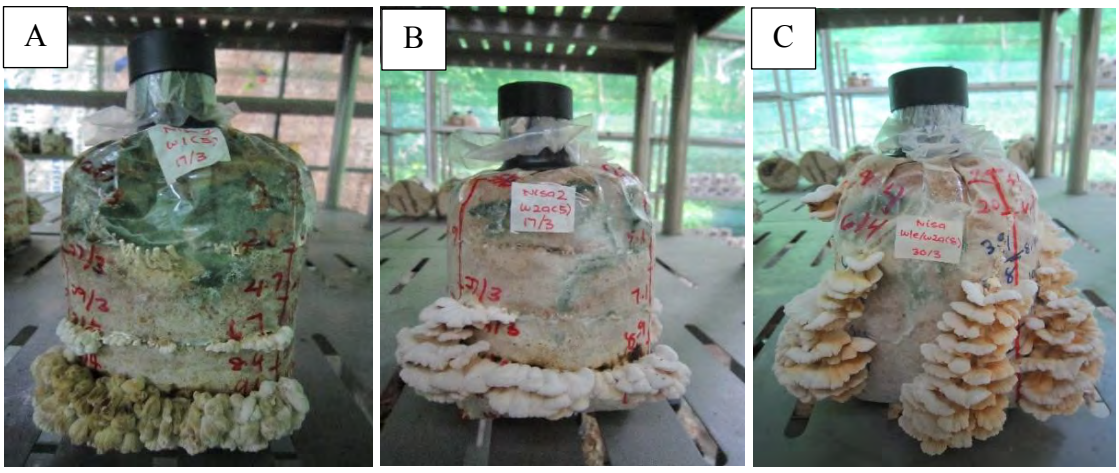


Figure 4.7: Example of contaminations that occur in the substrate bag during fructification Malaysia parental and hybrid strains (A) w1 (B) w2 (C) w1ew2a.

4.4 Protein Profiling

The identification of mushroom strains based on the morphological features can be supported by protein profiling. In addition, the combination of both morphological and protein profiling can generate a clear picture of the diversity of the hybrid strains. The protein content of each strain as shown in Appendix D was standardised and SDS-PAGE method separates proteins based on the molecular weight independently of charge. Hence, the proteins are separated down the gel in decreasing order of size. The protein pattern of bands displays after staining represent the distribution of variously sized proteins contained in the extract of samples. The difference in protein patterns of different strains are due to the variation in protein content between the samples (Gardiner *et al.*, 2012).

4.4.1 Hybrids of Malaysia Strains compared to Parental Strains (w1 and w2)

Protein electrophoresis is an efficient method, generating valuable information on the similarity or dissimilarity amongst various strains. SDS-PAGE of whole-cell soluble proteins, prepared under standard procedure, generated a reproducible and complex banding pattern that is called protein electrophore gram or electrophoretic protein pattern,

that can be recongnised as a 'fingerprint' of the samples investigated (Ehlers & Cloete, 1999). The protein patterns between Malaysia parental strains (w1 and w2) and their non-irradiated hybrids obtained by electrophoresis are in Figure 4.8 (a-c). The electrophoretic bands on these three gels were distinct and no artifacts were observed. Based on these figures, protein subunit for w1 and w2 were concentrated between 66 kDa to 30 kDa, while protein subunits for hybrids were concentrated at the various molecular weight. w1aw2a, w1bw2a, w1bw2f, w1dw2a, w1dw2f, and w1ew2a have protein subunit intensify between 66 kDa to 30 kDa. Four hybrids which are w1fw2a, w1gw2a, w1hw2a, and w1jw2a have protein pattern concentrated between 130 kDa to 30 kDa. However, w1cw2a, w1ew2f, and w1Lw2a show protein fraction dominant at 66 kDa to 37 kDa.

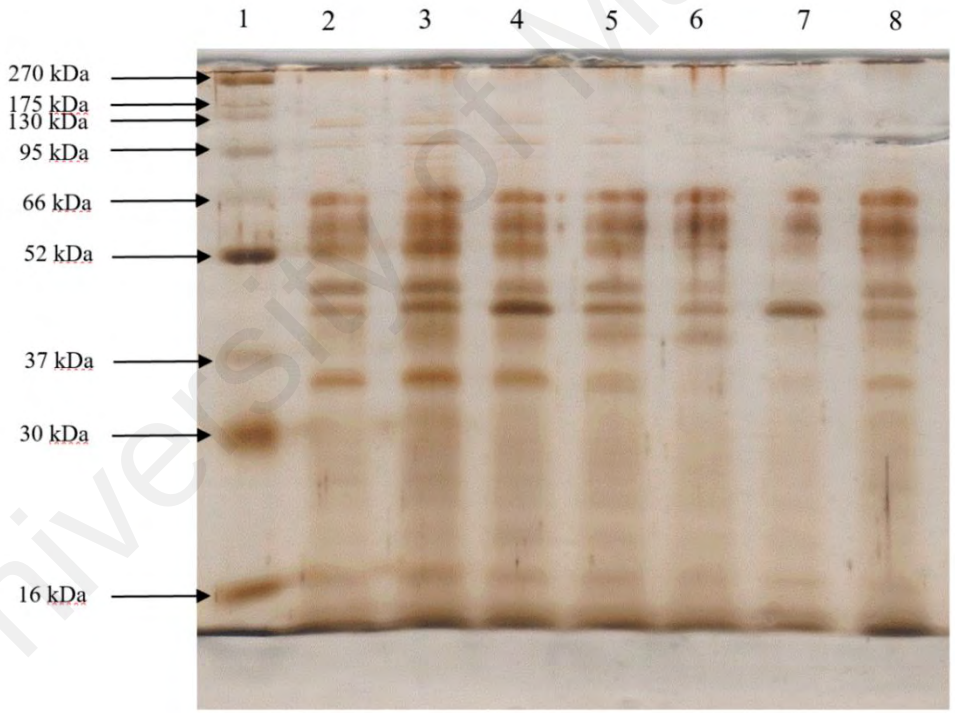


Figure 4.8A: SDS-PAGE profile gel of Malaysia parental strains (w1 and w2) and the hybrids (1) Marker (2) w1 (3) w2 (4) w1aw2a (5) w1bw2a (6) w1bw2f (7) w1cw2a (8) w1dw2a.

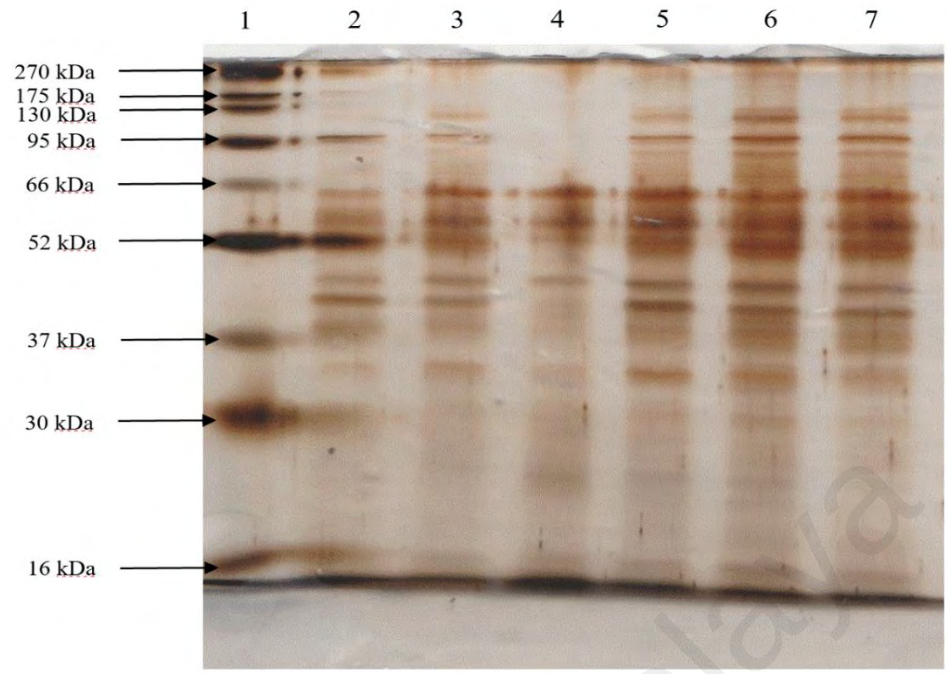


Figure 4.8B: SDS-PAGE profile gel of Malaysia parental strains (w1 and w2) and the hybrids (1) Marker (2) w1dw2f (3) w1ew2a (4) w1ew2f (5) w1fw2a (6) w1gw2a (7) w1hw2a.

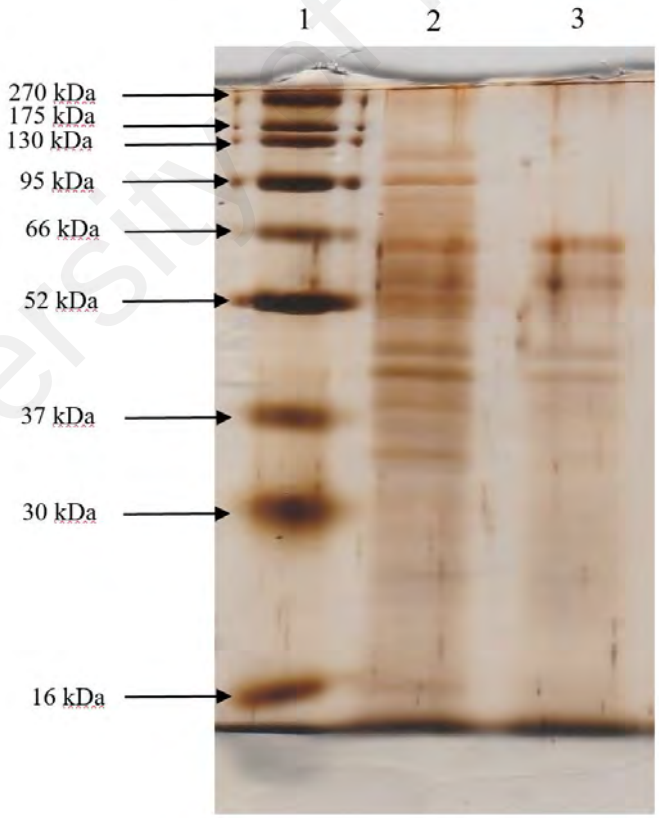


Figure 4.8C: SDS-PAGE profile gel of SDS-PAGE profile gel of Malaysia parental strains (w1 and w2) and the hybrids (1) Marker (2) w1jw2a (3) w1Lw2a.

The protein bands were analysed further to obtain more information about the number of protein bands and estimated molecular weight of every band. Genetic variability estimation between strains was performed based on the presence and absence of polypeptide bands (Iqbal *et al.*, 2014). Table 4.13 presented the number of protein bands and molecular weight of protein subunit produced by w1, w2 and their hybrids. Protein fractions display by w1 and w2 show the similar number of protein bands which are 8 protein bands and in the same range of molecular weight. Hybrid w1gw2a has the highest number of protein bands which are 11 bands, while w1cw2a, w1ew2f, and w1Lw2a have the least number of protein bands which are 4 bands. Only two hybrids which are w1bw2a and w1hw2a that produce the same number of protein bands as their parental strains. However, hybrid w1hw2a show more similar protein pattern and comparable molecular weight with parental strains than hybrid w1bw2a.

Based on these results, parental strains are crucial sources of breeding component to restore or develop genetic variability as well as to improve the quality of commercially cultivated varieties (Llarena-Hernández *et al.*, 2013). Even though both parental strains have similar number of protein bands, the molecular weight of individual proteins are slightly different, where the present of high molecular weight protein with 128.8 kDa were detected in w1. Hybridisation of these parental strains produced hybrid strains with unique molecular weight that different from other strains. Large molecular weight protein with 168.7 kDa was discovered in hybrid w1dw2f while small molecular weight protein ranges from 12.9 kDa to 13.7 kDa were identified in six hybrid strains that were absent in protein profiling of both parental strains.

Table 4.13: Molecular weight and number of protein bands of Malaysia parental strains (w1 and w2) and their hybrids.

Strain	Molecular Weight (kDa)											Number of Protein Bands
	1	2	3	4	5	6	7	8	9	10	11	
w1	128.8	114.2	85.6	75.5	67.1	54.3	47.8	33.8				8
w2	114.8	86.0	75.5	72.1	65.3	53.6	48.0	34.0				8
w1aw2a	90.0	78.9	69.3	57.7	51.9	36.8	13.7					7
w1bw2a	90.7	78.1	71.2	57.9	52.0	45.6	36.4	13.4				8
w1bw2f	91.5	79.2	58.8	51.7	45.6							5
w1cw2a	90.2	77.7	50.9	13.3								4
w1dw2a	88.3	76.0	55.8	49.8	35.7							5
w1dw2f	168.7	119.2	88.5	77.4	68.8	56.2	50.3	43.3	27.3	13.2		10
w1ew2a	84.2	72.1	65.5	52.8	46.0	32.6	19.5					7
w1ew2f	88.8	76.0	69.6	55.7								4
w1fw2a	119.2	88.8	76.2	68.7	55.6	49.4	34.6					7
w1gw2a	131.2	118.5	109.4	88.5	76.8	68.5	55.9	49.5	35.5	27.6	13.1	11
w1hw2a	131.9	119.2	89.0	76.4	68.1	55.3	48.7	35.5				8
w1jw2a	113.6	87.2	74.0	68.5	55.3	49.9	43.5	35.3	20.9	12.9		10
w1Lw2a	86.2	74.0	54.1	48.8								4

4.4.2 Hybrids between Malaysia and Thailand Strains compared to the Parental Strains

The variety of strains with diverse genetic composition is one of the purpose of mushroom breeding for commercial purposes. Proteins have been applied as markers to estimate the genetic different in various crop species (Iqbal *et al.*, 2014). The expressed protein are the decoded information from DNA which can be applied to differentiate the genetic diversity in crops (Wilson, 1985). In the present study, protein separation of Malaysia parental strain (w1), Thailand parental strain (R), and their hybrids (w1eRa and w1jRc) are shown in Figure 4.9. No artifacts are found in all lanes except lane 3, where streaking is observed at the right side of the lane. There are several steps which can be used to avoid these problems, including centrifuging the sample to eliminate precipitates, diluting the sample to avoid overloading, slower the voltage by 25 %, lower the concentration of protein and decrease the volume loaded (James & Milos, 2007). Hybrid w1eRa and hybrid w1jRc produce the similar pattern with w1 than R.

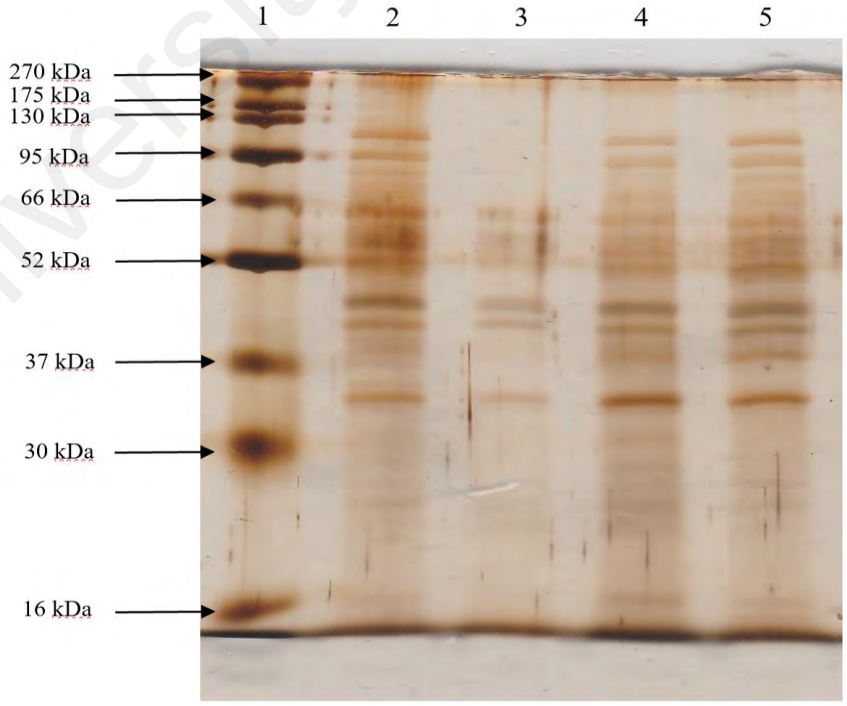


Figure 4.9: SDS-PAGE profile gel of Malaysia and Thailand strains with the hybrids (1) Marker (2) w1 (3) R (4) w1eRa (5) w1jRc.

Table 4.14 presented the number of protein bands produced by w1, R, and their hybrids. w1 has a higher number of protein which has 8 protein bands than R which only has 4 bands. This result indicate that the protein composition of Thailand parental strain R strain differs from that of the Malaysia parental strain w1. R lack large molecular weight protein and small molecular weight protein. R only produce medium size protein bands. Interestingly, both hybrids exhibit an identical number of protein bands with w1. In addition, the protein variation may be small between the parental w1 and hybrid w1jRc judging from the slight difference in the protein molecular weight. Despite that having similar number of protein bands with w1, hybrid w1eRa exhibited small molecular weight protein with 13.1 kDa that are absent in both parental strains. These results suggest that hybridisation between intercontinental wild strains generated variety in the new hybrids with combine traits and unique strain with low molecular weight protein.

Table 4.14: Molecular weight and number of protein bands produce by Malaysia and Thailand strains with the hybrids.

Strain	Molecular Weight of Protein Bands (kDa)								Number of Protein Bands
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
w1	128.8	114.2	85.6	75.5	67.1	54.3	47.8	33.8	8
R	86.7	55.8	50.7	34.9					4
w1eRa	126.2	113.4	86.7	68.6	56.3	50.4	35.4	13.1	8
w1jRc	127.6	114.3	85.8	68.6	56.1	50.4	44.4	35.9	8

4.4.3 Irradiated Hybrids

Figure 4.10A and Figure 4.10B exhibit the electrophoretic protein pattern of irradiated hybrids. Streaking are clearly observed at the lane 2 (Ir-w1dRa), lane 3 (Ir-w1dRc) and lane 5 (Ir-w1ew2a) in Figure 4.10A while no streaking is seen in Figure 4.10B. Based on these SDS-PAGE gel profile, Ir-w1dRa, Ir-w1dRc, Ir-w1dw2d, and Ir-

w1gRc produce protein bands which are intensified between 66 kDa to 30 kDa. Protein bands of Ir-w1ew2a and Ir-w1ew2d are concentrated at 66 kDa to 37 kDa while protein bands of Ir-w1gw2a, Ir-w1hw2d, Ir-w1jRa, and Ir-w1Lw2f are concentrated at 130 kDa to 30 kDa.

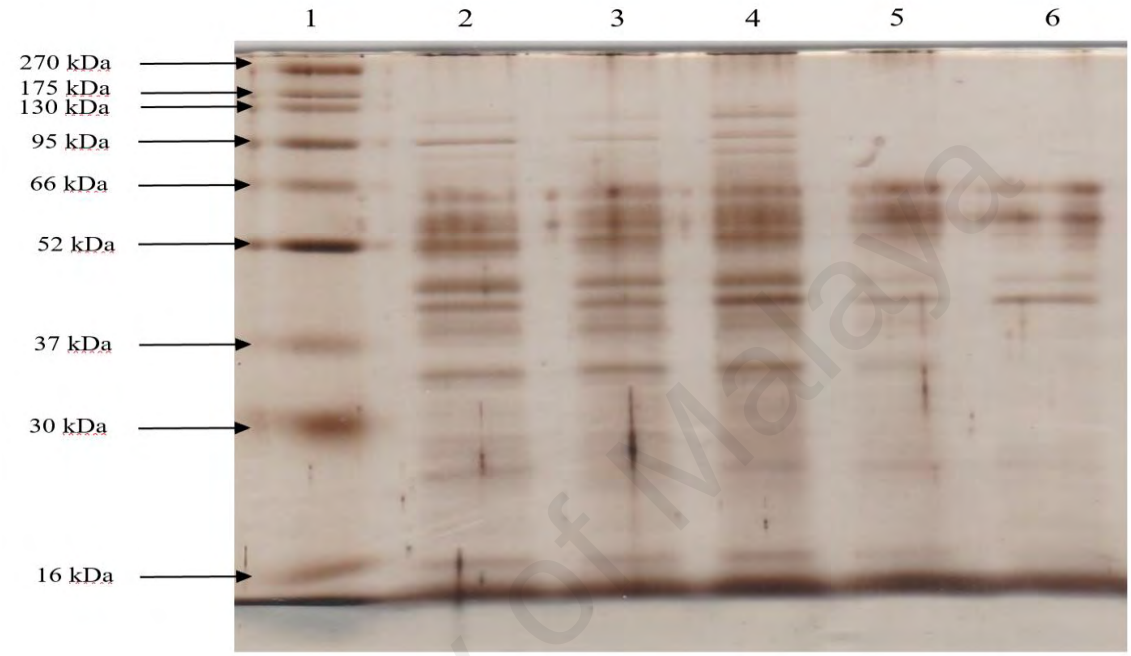


Figure 4.10A: SDS-PAGE profile gel of irradiated hybrids (1) Marker (2) Ir-w1dRa (3) Ir-w1dRc (4) Ir-w1dw2d (5) Ir-w1ew2a (6) Ir-w1ew2d.

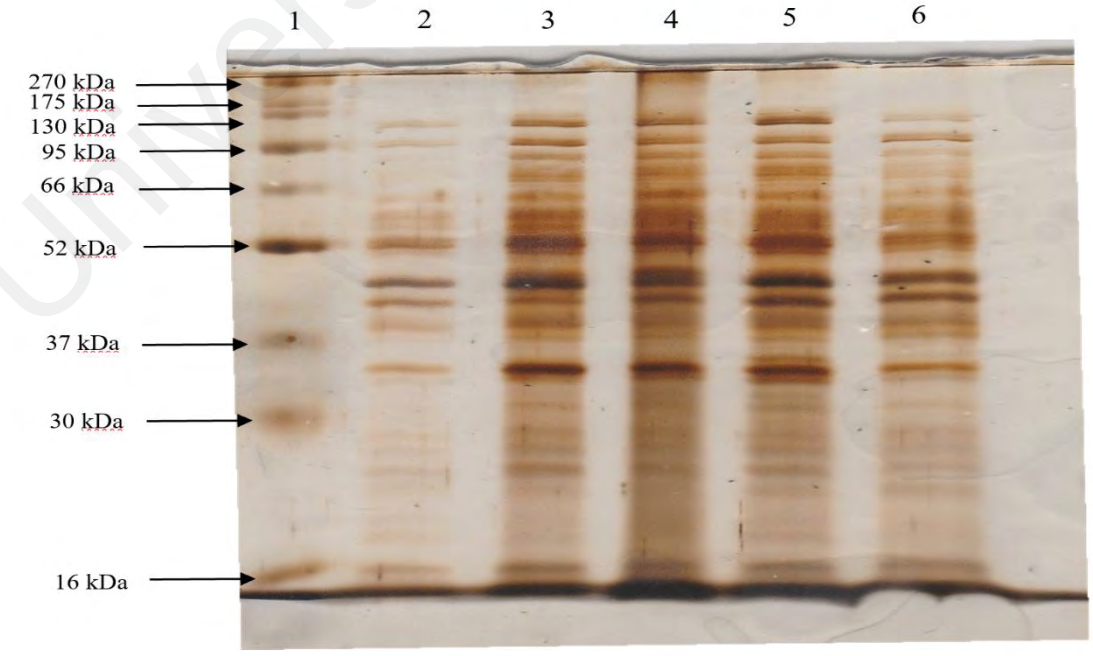


Figure 4.10B: SDS-PAGE profile gel of irradiated hybrids (1) Marker (2) Ir-w1gRc (3) Ir-w1gw2a (4) Ir-w1hw2d (5) Ir-w1jRa (6) Ir-w1Lw2f.

Using SDS-PAGE electrophoresis of total proteins, Ir-w1gw2a generate the highest number of protein bands (17 bands) while Ir-w1ew2d produce the lowest number of protein bands (4 bands) as shown in Table 4.15. In addition, four hybrids develop 12 protein bands, including Ir-w1dRa, Ir-w1gRc, Ir-w1hw2d, and Ir-w1Lw2f. Among these four hybrids, Ir-w1dRa and Ir-w1hw2d has the similar range of molecular weight of protein bands. Large size protein subunit (100 kDa to 135 kDa) and small size protein subunit (12 kDa to 13 kDa) are produced by most of the irradiated hybrids except Ir-w1ew2a and Ir-w1ew2d. From these data, gamma radiation induction has affected the number of protein bands produce by the hybrids.

Table 4.15: Molecular weight and number of protein bands produced by irradiated hybrids.

Strain	Molecular Weight of protein bands (kDa)																	Number of Protein Bands
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Ir-w1dRa	128.7	114.9	106.6	87.5	74.6	66,8	54.8	49.4	43.8	35.0	21.2	13.4						12
Ir-w1dRc	116.8	88.7	75.6	69.7	55.7	50.2	44.0	35.7	13.3									9
Ir-w1dw2d	132.3	118.4	109.8	89.9	77.7	70.5	56.7	50.7	35.6	21.2	13.4							11
Ir-w1ew2a	89.2	76.9	70.7	56.2	50.9	21.6												6
Ir-w1ew2d	88.0	75.8	55.7	50.0														4
Ir-w1gRc	129.1	116.8	89.0	79.8	76.5	68.9	56.8	51.2	45.0	36.5	21.3	12.8						12
Ir-w1gw2a	127.8	116.2	109.6	98.4	88.3	80.5	75.3	68.7	56.0	51.2	45.5	35.8	29.9	25.8	23.8	21.4	12.7	17
Ir-w1hw2d	126.8	115.3	106.8	97.9	87.6	68.0	56.8	51.1	41.7	35.5	21.0	12.2						12
Ir-w1jRa	127.5	115.6	107.1	97.6	87.3	68.3	56.5	50.5	45.0	41.6	35.2	29.1	21.1	12.9				14
Ir-w1Lw2f	126.1	114.1	107.9	86.7	68.5	56.0	50.1	44.3	34.7	28.8	18.4	12.4						12

4.4.4 Comparison of Protein Profiling of Parental Strains (w1 and w2), Hybrid w1ew2a and Monokaryon-Irradiated Hybrid Ir-w1ew2a

SDS-PAGE gel profile of w1, w2, hybrid w1ew2a, and monokaryon-irradiated hybrid Ir-w1ew2a are shown in Figure 4.11. No streaking is observed in the gel. All four strains produce protein bands concentrated at 66 kDa to 30 kDa. The number of protein bands produces by these strains are presented in Table 4.16. Both Malaysia parental strain produces the identical number of protein bands which are 8 bands. This finding suggests that both parental strains used in this study have little protein variation. However, one factor in this finding may be that the cultivated strains used in this study were gathered from a similar area. Both hybrids generate lower protein bands than the parental strains.

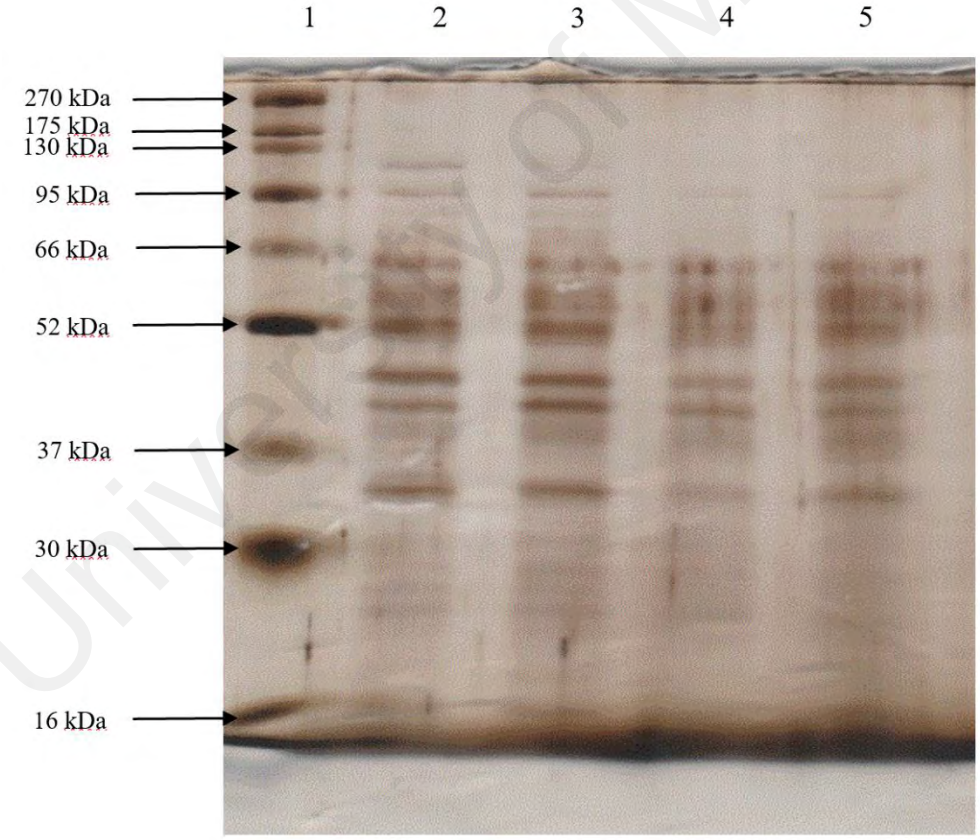


Figure 4.11: SDS-PAGE gel profile of Malaysia parental strains (w1 and w2), hybrid w1ew2a and monokaryon-irradiated hybrid Ir-w1ew2a (1) Marker (2) w1 (3) w2 (4) w1ew2a (5) Ir-w1ew2a.

Hybrid w1ew2a produce a slightly higher number of protein which is 7 bands than Ir-w1ew2a which only produce 6 bands. Analysis of molecular weight and number of protein based on SDS-PAGE method provided a useful information on the protein profiling which were found that hybrid w1ew2a contains a unique small molecular weight protein with 19.5 kDa that were absent in both parental strains and hybrid Ir-w1ew2a. Table 4.16 shows the molecular weight of protein bands produce by w1, w2, hybrid w1ew2a, and Ir-w1ew2a. Medium size protein, and small size protein are produced by w1ew2a while small size protein is absent in hybrid Ir-w1ew2a. These results indicated that hybridisation and gamma radiation method able to produce new strains with different protein electrophoretic profiles. Proteins are relatively express gene products and can be recognised as markers of these genes, and diverse in the electrophoretic profiles are presumably proportional to the genetic divergence among the organisms being studied (Rogl *et al.*, 1996).

Table 4.16: Molecular weight and number of protein bands produced by Malaysia parental strains (w1 and w2), hybrid w1ew2a and monokaryon-irradiated hybrid Ir-w1ew2a.

Strain	**Molecular Weight of Protein Bands (kDa)**								**Number of Protein Bands**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
w1	128.8	114.2	85.6	75.5	67.1	54.3	47.8	33.8	8
w2	114.8	86.0	75.5	72.1	65.3	53.6	48.0	34.0	8
w1ew2a	84.2	72.1	65.5	52.8	46.0	32.6	19.5		7
Ir-w1ew2a	85.3	75.9	66.6	52.1	46.8	32.4			6

4.5 Similarity between Parental Strains, Hybrids, and Monokaryon-Irradiated Hybrids.

Similarity coefficients were calculated between all pairs of the strains based on the presence or absence of individual protein bands as shown in Table 4.17. Similarity coefficient analysis usually used to estimate or clarify taxonomic data based usually on the morphological characterisation (Drzewiecki *et al.*, 2003). In the present study, SDS-PAGE analysis was performed to examine the different in protein profiling among three parental strains, fifteen hybrids and ten monokaryons irradiated hybrids of *S. commune*, and useful information was obtained about the protein variation among them. Most of the strains examined can be distinguished from one another. This indicates a high level of polymorphism of protein pattern between these hybrids. However, w1aw2a has 100 % similar to w1bw2a. Similarity coefficients for non-irradiated hybrids vary from 14 % to 67 % with w1. Among the non-irradiated hybrids, w1jRc show the highest similarity with w1. However, non-irradiated hybrids show higher similarity with w2 than w1. The non-irradiated hybrids produce similarity between 31 % to 67 %. Five hybrid strains such as w1dw2a, w1fw2a, w1jw2a, w1eRa, and w1jRc have the similarity above 60 % with w2. Both hybrids, w1eRa and w1jRc show identical similarity coefficient with R which is 67 %. This indicates that hybridisation cause variation in protein profile in hybrids.

Based on the Table 4.17, all monokaryon-irradiated hybrids can differentiate from one another except for the hybrid w1aw2a and w1bw2a. Irradiated hybrids have higher similarity coefficient with w1 compared to the non-irradiated hybrids. Similarity coefficient of radiated hybrids varies from 22 % to 77 % with w1. Ir-w1dw2a show the lowest similarity with 22 % while Ir-w1ew2a shows the highest similarity with 77 % with w1. Besides that, irradiated hybrids have slightly higher similarity with w2 than non-irradiated hybrids which in the range of 40 % to 71 %. Both hybrids, Ir-w1dRc and Ir-

w1ew2a have the highest similarity with w2 which is 71 %. In addition, irradiated hybrids also have high similarity coefficient with R which are in the range of 40 % to 75 %. Among the irradiated hybrids, Ir-w1hw2d exhibits the highest similarity (92 %) with Ir-w1dRa and Ir-w1jRa. This indicates that Ir-w1hw2d has protein pattern closely related to the Ir-w1dRa, and Ir-w1jRa and these hybrids.

Table 4.17: The similarity coefficient (%) of the parental strains, non-irradiated hybrids, and irradiated hybrids based on protein data. (1) w1 (2) w2 (3) R (4) w1aw2a (5) w1bw2a (6) w1bw2f (7) w1cw2a (8) w1dw2a (9) w1dw2f (10) w1ew2a (11) w1ew2f (12) w1fw2a (13) w1gw2a (14) w1hw2a (15) w1jw2a (16) w1Lw2a (17) w1eRa (18) w1jRc (19) Ir-w1dRa (20) Ir-w1dRc (21) Ir-w1dw2d (22) Ir-w1ew2a (23) Ir-w1ew2d (24) Ir-w1gRc (25) Ir-w1gw2a (26) Ir-w1hw2d (27) Ir-w1jRa (28) Ir-w1Lw2f.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	100	53	18	14	14	17	18	33	35	14	36	43	35	40	35	36	40	67	53	50	22	77	36	53	63	42	57	53
2		100	50	53	53	31	33	62	44	53	50	67	56	50	67	50	63	63	50	71	53	71	50	60	70	40	46	50
3			100	55	55	44	25	89	43	55	50	73	43	33	57	50	67	67	50	62	40	40	75	50	50	50	44	50
4				100	100	50	73	67	47	86	55	57	59	53	59	36	53	40	53	75	78	31	55	53	42	42	38	42
5					100	50	73	67	47	86	55	57	59	53	59	36	53	40	53	75	78	31	55	53	42	42	38	42
6						100	44	40	27	50	22	33	27	46	27	44	31	46	24	43	38	36	44	47	47	24	32	35
7							100	44	43	55	25	36	43	33	43	0	33	17	38	46	53	40	50	38	25	25	22	25
8								100	53	67	67	83	53	46	67	44	62	62	59	71	50	55	89	59	59	47	42	47
9									100	47	43	71	60	44	70	29	56	44	64	53	38	50	57	55	46	55	50	55
10										100	55	71	47	67	47	36	40	40	42	63	67	31	55	42	42	32	29	32
11											100	55	29	33	43	75	33	33	38	62	40	40	75	38	38	25	22	25
12												100	59	67	71	36	67	67	63	63	44	62	73	63	63	53	48	53

Table 4.17, continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
13													100	56	70	14	67	56	64	63	76	38	43	73	64	55	50	64
14														100	44	33	38	50	40	47	53	43	33	60	50	30	36	40
15															100	29	78	56	82	63	67	50	57	73	64	73	67	55
16																100	33	50	25	62	27	40	50	38	38	25	33	38
17																	100	75	70	59	53	43	50	60	70	70	64	70
18																		100	70	71	42	57	50	70	80	70	73	80
19																			100	67	61	44	50	75	67	92	85	75
20																				100	70	53	62	76	67	57	61	67
21																					100	24	40	70	52	52	48	44
22																						100	60	56	56	33	50	44
23																							100	50	50	38	33	38
24																								100	75	67	69	67
25																									100	67	69	67

Table 4.17, continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
26																										100	92	75
27																											100	77
28																												100

In the present study, the dendrogram was constructed based on the similarity coefficient between parental strains, hybrids, and monokaryon-irradiated hybrids by using unweighted pair-group method with arithmetic clustering (UPGMA) analysis. The dendogram as shown in Figure 4.12 that all the strains can be divided into three clusters. The first cluster consists of hybrid w1Lw2a and w1ew2f while the second cluster consists of 6 hybrids strains which are w1bw2f, w1hw2a, w1cw2a, w1ew2a, w1aw2a and w1bw2a. The other strains were grouped into the third cluster.

Based on this result, Ir-w1ew2a has the closest protein pattern with w1. Four hybrids exhibit the closer protein pattern with w2, including Ir-w1dRc, Ir-w1gRc, w1jRc, and Ir-w1gw2a. Interestingly, hybrid w1dw2a display the closest protein pattern with R. Hybrid w1aw2a and hybrid w1bw2a are located in the same position as their similarity is 100 %. Irradiated monokaryon Ir-w1ew2a exhibit higher similarity towards the Malaysia parental strains than the hybrid w1ew2a. This shows that breeding method using hybridisation and gamma irradiation produced variety of strains in term of protein profiling.

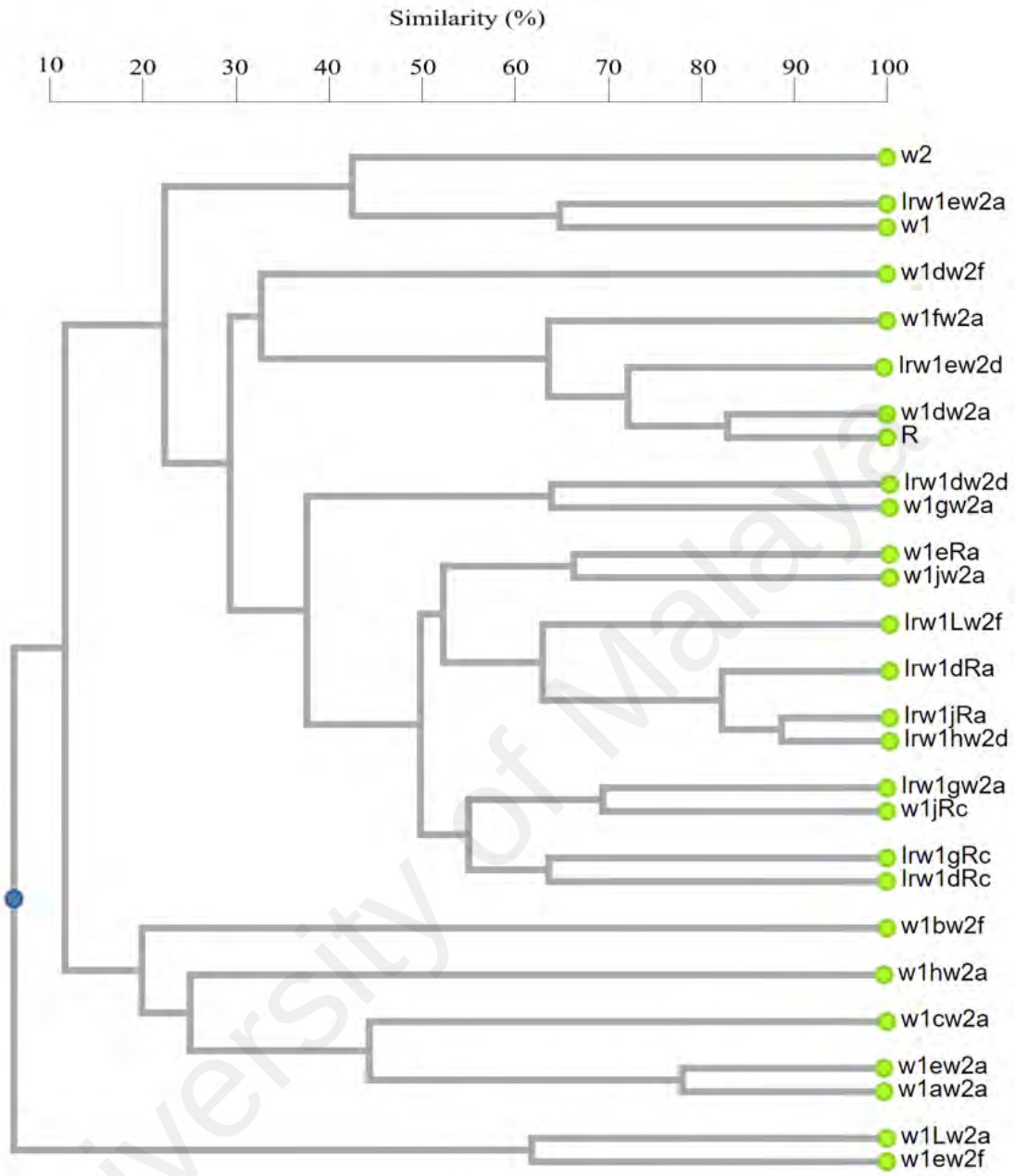


Figure 4.12: Dendogram of the electrophoretic patterns of parental strains, hybrids, and monokaryon-irradiated hybrids, based on UPGMA analysis of the similarity coefficient matrix.

CHAPTER 5: CONCLUSIONS

Combined data of mycelial growth rate, mycelial density, width of sporophores, yield, percentage of contamination and biological efficiency are essential for selecting candidate strains for commercial mushroom production. This study provides fundamental aspects of the cultivation of new strains of *Schizophyllum commune* obtained by method of cross-breeding and gamma radiation. Our investigation showed that there is a good potential for improving native *S. commune* by using these methods. There were three potential strains obtained through cross-breeding which are hybrid w1bw2f, w1dw2f and w1Lw2a while through induced gamma mutation, there are five potential strains which are Ir-w1dRa, Ir-w1dw2d, Ir-w1ew2a, Ir-w1ew2d and Ir-w1gw2a. Further work is needed to carry out large scale cultivation for few generation. This study proved that hybridisation and gamma radiation could lead to strains with high hybrid vigour. The identification of mushroom strains based on the morphological features can be supported by protein profiling. In addition, the combination of both morphological and protein profiling can generate a clear picture of the diversity of the hybrid strains. These experiment analyses on *Schizophyllum commune* are very crucial to elucidate that the methods of genetic improvement of the strain, which in this case are cross-breeding and induction of gamma radiation do affect the characteristics and features of the strains. SDS-PAGE is an effective tool which can be used to discriminate strains within similar species. By utilising this method, a database can be developed for a strategic breeding programme to enhance mushroom cultivation, thus benefits the agribusiness. Therefore, generating new varieties of strains and novelty features is really demanded for development of Malaysian mushroom industry.

REFERENCES

Adebayo, E. A., Oloke, J.K., Yadav, A., Barooah, M., & Bora, T.C. (2013). Improving yield performance of *Pleurotus pulmonarius* through hyphal anastomosis fusion of dikaryons. *World Journal Microbial Biotechnology*, *29*, 1029-1037.

Adejoye, O.D., Adebayo-Tayo, B.C., Ogunjobi, A.A., & Afolabi, O.O. (2007). Physicochemical studies on *Schizophyllum commune* (Fries) a Nigerian edible fungus. *World Applied Sciences Journal, 2*(1), 73-76.

Ahloowalia, B. S., & Maluszynski, M. (2001). Induced mutations - a new paradigm in plant breeding. *Euphytica, 118*(2), 167–173.

Akhbar, F., Yousaf, N., Rabbani, M.A., Shinwari, Z.K., & Masood, M.S. (2012). Study of total seed proteins pattern of sesame (*Sesamum Indicum L.*) landraces via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). *Pakistan Journal of Botany*, *44*(6), 2009-2014.

Akin-Idowu, P.E., Odunola, O.A., Adunni, O., Gbadegesin, M.A., Oke, A., & Orkpeh, U. (2013). Assessment of the protein quality of twenty nine grain amaranth (*Amaranthus* spp. L.) accessios using amino acid analysis and one-dimensional electrophoresis. *African Journal of Biotechnology, 12*(15), 1802-1810.

Al-Obaidi, J. R. (2016). Proteomics of edible mushrooms: A mini-review. *Electrophoresis, 37*(10), 1257–1263.

Alam, N., Cha, Y.J., Shim, M.J., Lee, T.S., & Lee, U.Y. (2010). Cultural conditions for mycelial grwth and molecular phylogenetic relationship in different wild strains of *Schizophyllum commune*. *Mycobiology, 38*(1), 17-25.

Alananbeh, K.M., Bouqellah, N.A., & Al Kaff, A.S. (2014). Cultivation of oyster mushroom *Pleurotus ostreatus* on date-palm leaves mixed with other agro-wastes in Saudi Arabia. *Saudi Journal of Biological Sciences*, *21*, 616-625.

Amee, P., Nagadesi, P., Susy, A., & Arya, A. (2009). Morphology, anatomy and cultural characters of two wood decaying fungi *Schizophyllum commune* and *Flavadon flavus*. *Journal of Mycology and Plant Pathology, 39*(1), 27–31.

Amin, M. M. Z., & Harun, A. (2015). Competitiveness of the mushroom industry in Malaysia. *FFTC Agricultural Policy Platform (FFTC-AP)*. Retrieved 4th December 2019 from http://ap.fftc.agnet.org /ap_db.php?id=481.

Amin, M. M. Z., Harun, A., & Wahab, M. A. M. A. (2014). Status and potential of mushroom industry in Malaysia. *Economic and Technology Management Review*, *9*, 103–111.

Amin, M. M. Z., Mohd, Y. S., & Harun, A. (2013). Viability of oyster mushroom industry in peninsular Malaysia. *Economic and Technology Management Review*, *8*, 13–25.

Au, C. H., Wong, M. C., Bao, D., Zhang, M., Song, C., Song, W., … Kwan, H. S. (2014). The genetic structure of the A mating-type locus of *Lentinula edodes*. *Gene*, *535*(2), 184-190.

Avin, F.A., Bhassu, S., Tan, Y.S., & Sabaratnam, V. (2014). DNA pedigree tracking to identify compatible mating partners of *Pleurotus pulmonarius*. *Journal of Animal and Plant Sciences*, *24*(1), 89-97.

Avin, F.A., Bhassu, S., Rameeh, V., Tan, Y.S., & Sabaratnam, V. (2016). Genetics and hybrid breeding of *Pleurotus pulmonarius*: Heterosis, heritability and combining ability. *Euphytica, 209*(1), 85-102.

Bado, S., Forster, B.P., Nielen, S., Ali, A.M., Lagoda, P.J.L., Till, B.J., & Laimer, M. (2015). Plant mutation breeding: current progress and future assessment. *Plant Breeding Reviews, 39*, 23-88.

Bakar, A.N.A (2017). *Genetic analysis of hybridized Schizophyllum commune strains (split-gill mushroom) by inter-simple sequence repeats (issr) markers*. (Unpublished bachelor degree's thesis). University of Malaya, Kuala Lumpur, Malaysia.

Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Das, J., Pal, M., & Khurana, S. (2015). Evolution of bacterial and fungal growth media. *Biomedical Informatics*, *11*(4), 182-184.

Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., … Ribani, R. H. (2019). Factors affecting mushroom *Pleurotus* spp. *Saudi Journal of Biological Sciences*, *26*(4), 633-646.

Bengochea, T., & Dodds, J. H. (1986). Protoplast fusion. In W.J. Brammar, M. Edidin & J. Hopkins (Eds.), *Plant Protoplasts — a biotechnological tool for plant improvement* (pp. 44-57). London, New York: Chapman and Hall.

Braaksma, A., & Schaap, D. J. (1996). Protein analysis of the common mushroom *Agaricus bisporus*. *Postharvest Biology and Technology, 7*(1), 119–127.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Bromberg, S. K., & Schwalb, M. N. (1976). Studies on basidiospore development in *Schizophyllum commune*. *Microbiology, 96*(2), 409–413.

Burton, K. S., & Noble, R. (1992). The influence of flush number, bruising and storage temperature on mushroom quality. *Postharvest Biology and Technology*, *3*, 39–47.

Çelik, Ö., & Atak, Ç. (2017). Applications of ionizing radiation in mutation breeding. In A. M. Maghraby (Ed.), *New insights on gamma rays* (pp. 111–132). Rijeka: InTech.

Chakraborty, U., & Sikdar, S. R. (2010). Intergeneric protoplast fusion between *Calocybe indica* (milky mushroom) and *Pleurotus florida* aids in the qualitative and quantitative improvement of sporophore of the milky mushroom. *World Journal of Microbiology and Biotechnology, 26*(2), 213–225.

Chandrawanshi, N.K., Tandia, D.K., & Jadhav, S.K. (2017). Nutraceutical properties evaluation of *Schizophyllum commune*. *Indian Journal of Scientific Journal*, *13*(2), 57-62.

Chang, S.T., Lau, O.W., & Cho, K.Y. (1981). The cultivation and nutritional value of *Pleurotus sajor-caju*. *European Journal of Applied Microbiology and Biotechnology*, *12*, 58-62.

Chen, C. Y., Chien, S. C., Tsao, N. W., Lai, C. S., Wang, Y. Y., Hsiao, W. W., … Wang, S. Y. (2016). Metabolite profiling and comparison of bioactivity in *Antrodia cinnamomea* and *Antrodia salmonea* fruiting bodies. *Planta Medica, 82*(3), 244–249.

Chen, L., Zhang, B. B., & Cheung, P. C. K. (2012). Comparative proteomic analysis of mushroom cell wall proteins among the different developmental stages of *Pleurotus tuber-regium*. *Journal of Agricultural and Food Chemistry, 60*(24), 6173–6182.

Chowdhary, A., Kathuria, S., Singh, P. K., Agarwal, K., Gaur, S. N., Roy, P., … Meis, J. F. (2013). Molecular characterization and in vitro antifungal susceptibility profile of *Schizophyllum commune*, an emerging basidiomycete in bronchopulmonary mycoses. *Antimicrobial Agents and Chemotherapy, 57*(6), 2845–2848.

Cieslak, J. J., Mba, C., & Till, B. J. (2017). Mutagenesis for crop breeding and functional genomics. In J. Jankowicz-Cieslak, T. H. Tai, J. Kumlehn, & B. J. Till (Eds.), *Biotechnologies for plant mutation breeding: protocols* (pp. 3–18). Switzerland: Springer International Publishing.

Clark, T. A., & Anderson, J. B. (2004). Dikaryons of the basidiomycete fungus *Schizophyllum commune*: Evolution in long-term culture. *Genetics*, *167*(4), 1663–1675.

Cooke, W. B. (1961). The genus *Schizophyllum*. *Mycologia*, *53*(6), 575–599.

Das, B.K., Samal, S.K., Samantaray, B.R., & Meher, P.K. (2005). Protein fingerprinting profiles in different strains of *Aeromonas hydrophila* isolated from diseased freshwater fish. *World Journal of Microbiology & Biotechnology, 21*(4), 587-591.

Dasanayaka, P., & Wijeyaratne, S. (2017). Cultivation of *Schizophyllum commune* mushroom on different wood substrates. *Journal of Tropical Forestry and Environment, 7*(1), 65–73.

Debelian, G.J., Olsen, I., & Tronstad, L. (1996). Electrophoresis of whole-cell soluble proteins of microoganisms isolated from bacteremias in endodontic therapy. *European Journal of Oral Sciences*, *104*(5-6), 540-546.

Debnath, S., Saha, A.K., & Das, P. (2017). Biological activities of *Schizophyllum commune* Fr.: A wild edible mushroom of Tripura, North East India. *Journal of Mycopathological Research*, *54*(4), 469-475.

Dhanavel, D., Pavadai, P., Mullainathan, L., Mohana, D., Raju, G., Girija, M., & Thilagavathi, C. (2008). Effectiveness and efficiency of chemical mutagens in cowpea (*Vigna unguiculata* (L.) Walp). *African Journal of Biotechnology*, *7*(22), 4116–4117.

Dhar, B. (2014, Nov). *Changing global scenario in mushroom industry.* Paper presented at the Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8), New Delhi, India.

Djajanegara, I., & Harsoyo (2008). White oyster mushroom (*Pleurotus florida*) mutant with altered antioxidant contents. *Biotropia*, *15*(1), 65–73.

Djajanegara, I., & Masduki, A. (2010). Protoplast fusion between white and brown oyster mushrooms. *Indonesian Journal of Agricultural Science*, *11*(1), 16–23.

Drzewiecki, J., Delgado-Licon, E., Haruenkit, R., Pawelzik, E., Martin-Belloso, O., Park, Y.S., … Gorinstein, S. (2003). Identification and differences of total proteins and their soluble fractions in some pseudocereals based on electrophoretic patterns. *Journal of Agricultural and Food Chemistry, 51*, 7798-7804.

Dunn, W. B., Broadhurst, D. I., Atherton, H. J., Goodacre, R., & Griffin, J. L. (2011). Systems level studies of mammalian metabolomes: The roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chemical Society Reviews, 40*(1), 387–426.

Ediriweera, S.S., Wijesundera, R. L. C., Nanayakkara, C. M ., & Weerasena, O. V. D. S. J. (2015). Comparative study on growth and yield of edible mushrooms, *Schizophyllum commune*, Fr., *Auricularia polytricha* (Mont.) Sacc. And *Lentinus squarrosulus* Mont. on lignocellulosic substrates. *Mycosphere, 6*(6), 760-765.

Ehlers, M. M., & Cloete, T. E. (1999). Comparing the protein profiles of 21 different activated sludge systems after SDS-PAGE. *Water Research*, *33*, 1181-1186.

Elnaiem, M. H. E., Mahdi, A. A., Badawi, G. H., & Attitalla, I. H. (2017). Morphological and molecular characterisation of wild mushrooms in Khartoum North, Sudan. *International Journal of Research in Ayurveda and Pharmacy*, *8*(3), 187–194.

Eribe, E.R.K., & Olsen, I. (2002). SDS-PAGE of whole-cell proteins and random amplified polymorphic DNA (RAPD) analyses of *Leptotrichia* isolates. *Microbial Ecology in Health and Disease*, *14*(4), 193-202.

Fan, L., Pan, H., Soccol, A. T., Pandey, A., & Soccol, C. R. (2006). Advances in mushroom research in the last decade. *Food Technology and Biotechnology, 44*(3), 303–311.

Fernández, R. G., & Novo, J. V. J. (2010). Proteomics of fungal plant patogens: the case of *Botrytis cinerea*. In A. Méndez-Vilas (Ed.), *Current research, technology and education topics in pplied microbiology and microbial biotechnology* (pp. 205–217). Badajoz, Spain: Formatex Research Center.

Fernández, R. G., & Novo, J. V. J. (2013). Proteomic protocols for the study of filamentous fungi. In V. K. Gupta, M. G. Tuohy, M. Ayyachamy, K. M. Turner, & A. O'Donovan (Eds.), *Laboratory protocols in fungal biology: current methods in fungal biology* (pp. 299–308). New York, NY: Springer New York.

Fui, F. S., Saikim, F. H., Kulip, J., & Seelan, J. S. S. (2018). Distribution and ethnomycological knowledge of wild edible mushrooms in Sabah (Northern Borneo). Malaysia. *Journal of Tropical Biology and Conservation*, *15*, 203-222.

Gharehaghaji, A. N., Goltapeh, E. M., Masiha, S., & Gordan, H. R. (2007). Hybrid production of oyster mushroom *Pleurotus ostreatus* (Jacq: Fries) Kummer. *Pakistan Journal of Biology Sciences, 10*(14), 2334–2340.

Girmay, Z., Gorems, W., Birhanu, G., & Zewdie, S. (2016). Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. *AMB Express, 6*(1), 87.

Gorinstein, S., Jaramillo, N.O., Medina, O.J., Rogriques, W.A., Tosello, G., & Paredes-Lopez, O. (1999). Evaluation of some cereals, plants and tubers through protein composition. *Journal of Protein Chemistry*, *18*(6), 687-693.

Guadarrama-Mendoza, P.C., Toro, G.V.D., Ramírez-Carrillo, R., Robles-Martínez. F., Yáñez-Fernández, J., Garín-Aguilar, M.E., … Bravo-Villa, G. (2014). Morphology and mycelial growth rate of *Pleurotus* spp. Strains from the Mexican mixtec region. *Brazilian Journal of Microbiology*, *45*(3), 861-872.

Gupta, B., Niranjan Reddy, B. P., & Kotasthane, A. S. (2011). Molecular characterization and mating type analysis of oyster mushroom (*Pleurotus spp*.) using single basidiospores for strain improvement. *World Journal of Microbiology and Biotechnology, 27*(1), 1–9.

Ha, B, S., Kim, S., & Ro, H.S. (2015). Isolation and characterisation of monokaryotic strains of *Lentinula edodes* showing higher fruiting rate and better fruiting body production. *Mycobiology*, *43*(1), 24-30.

Han, E.H., Hwang, Y.P., Kim, H.G., Choi, J.H., Im, J.H., Yang, J.H. … Jeong, H.G. (2011). Inhibitory effect of *Pleurotus eryngii* extracts on the activities of allergic mediators in antigen-stimulated mast cells. *Food and Chemical Toxicology*, *49*(6), 1416-1425.

Hanafusa, Y., Hirano, Y., Watabe, H., Hosaka, K., Ikezawa, M., & Shibahara, T. (2016). First isolation of *Schizophyllum commune* in a harbor seal (*Phoca vitulina*). *Sabouraudia, 54*(5), 492–499.

Hansen, M. E., Hansen, P. W., Landvik, S., Sasa, Mikako., Nielsen, K. F. & Michael, J. (2018, Aug). *Identification of basidiomycetes using image analysis of colony morphology and pigmentation*. Paper presented at the 7th International Mycology Congress, Oslo, Norway.

Harith, N., Abdullah, N., & Sabaratnam, V. (2014). Cultivation of *Flammulina velutipes* mushroom using various agro-residues as a fruiting substrate. *Pesquisa Agropecuária Brasileira*, *49*(3), 181-188.

Hatvani, L., Antal, Z., Manczinger, L., Szekeres, A., Druzhinina, I.S., Kubicek, C.P., … Kredics, L. (2007). Green mold diseases of *Agaricus* and *Pleurotus* spp. are caused by related but phylogenetically different *Trichoderma* species. *Ecology and Epidemiology, 97*(4), 532-537.

Hatvani, L., Kocsubé, S., Manczinger, L.., Antal, Z., Szekeres, A., Druzhinina, I.S., … Kredics, L. (2008, May). *The green mould disease global threat to the cultivation of oyster mushroom (Pleurotus ostreatus): a review*. Paper presented at the Proceedings of the 17th Congress of the International Society for Mushroom Science, Cape Town, South Africa.

Hatvani, L., Sabolić, P., Kocsubé, S., Kredics, L., Czifra, D., Vágvölgyi, C., … Kosalec, I. (2012). The first report on mushroom green mould disease in Croatia. *Archives of Industrial Hygiene and Toxicology, 63*(4), 481-487.

Herawati, E., Arung, E. T., & Amirta, R. (2016). Domestication and nutrient analysis of *Schizopyllum commune*, alternative natural food sources in east Kalimantan. *Agriculture and Agricultural Science Procedia, 9*, 291–296.

Hernández, R.G., & Salmones, D. (2008). Obtaining and characterizing *Pleurotus ostreatus* strains for commercial cultivation under warm environmental conditions. *Scientia Horticulturae, 118*(2), 106-110.

Hilszczańska, D. (2012). Medicinal properties of macrofungi. *Forest Research Papers, 73*(4), 347–353.

Hoa, H. T., & Wang, C. L. (2015). The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology, 43*(1), 14–23.

Horie, K., Rakwal, R., Hirano, M., Shibato, J., Nam, H.W., Kim, Y.S., … Yonekura, M. (2008). Proteomics of two cultivated mushrooms *Sparassis crispa* and *Hericium erinaceum* provides insight into their numerous functional protein components and diversity. *Journal of Proteome Research*, *7*(5), 1819–1835.

Humber, R. A. (1997). Fungi: Identification. In L. A. Lacey (Ed.), *Manual of techniques in insect pathology* (pp. 153–185). San Diego, California: Academic Press.

Hwang, S. W. (1966). Long-term preservation of fungus cultures with liquid nitrogen refrigeration. *Applied Microbiology, 14*(5), 784–788.

Ibrahim, R., Jamil, A. A. I. M., Hasan, S. M. Z., Arshad, A. M., & Zakaria, Z. (2017, April). *Enhancing growth and yield of grey oyster mushroom (Plearotus sajor caju) using different acoustic sound treatments*. Paper presented at the 3rd Electronic and Green Matrials International Conference, Krabi, Thailand.

Imtiaj, A., Jayasinghe, C., Lee, G. W., Kim, H. Y., Shim, M. J., Rho, H. S., … Lee, U. (2008). Physicochemical requirement for the vegetative growth of *Schizophyllum commune* collected from different ecological origins. *Mycobiology, 36*(1), 34–39.

Ingale, A., & Ramteke, A. (2010). Studies on cultivation and biological efficiency of mushrooms grown on different agro-residues. *Innovative Romanian Food Biotechnology, 6*, 25–28.

Ingroff, E. A., Montero, D., & Martin-Mazuelos, E. (2004). Long-term preservation of fungal isolates in commercially prepared cryogenic microbank vials. *Journal of Clinical Microbiology, 42*(3), 1257–1259.

Inyod, T., Sassanarakit, S., Payapanon, A., & Keawsompong, S. (2017). Morphological characteristics and molecular identification of a wild Thai isolate of the tropics mushroom Hed Taen Rad (*Macrocybe crassa*). *Biodiversitas*, *18*(1), 221–228.

Iqbal, J., Shinwari, Z. K., Rabbani, M. A., & Khan, S. A. (2014). Genetic variability assessment of maize (*Zea mays* L.) germplasm based on total seed storage proteins bading patterns using SDS-PAGE. *European Academic Research, 2*(2), 2144-2160.

Isikhuemhen, O.S., Nerud, F., & Vilgalys, R. (2000). Cultivation studies on wild and hybrid strains of *Pleurotus tuberregium* (Fr.) Sing. on wheat straw substrate. *World Journal of Microbiology & Biotechnology*, *16*, 431-435.

Islam, M.T., Zakaria, Z., Hamidin, N., & Ishak M.A.M. (2017). Selection and cultivation of oyster mushroom for the indoor controlled environment in Malaysia and similar ecological region. *MAYFEB Journal of Agricultural Science*, *1*, 14-22.

Jain, S. (2010). Mutagenesis in crop improvement under the climate change. *Romanian Biotechnological Letters*, *15*(2), 88–106.

James, T.L., & Milos, N.C. (2007). Elimination of vertical streaking in polyacrylamide gel electrophoresis. *Analytical Biochemistry, 367*(1), 134-136.

Jayakumar, G.C., Kanth, S.V., Chandrasekaran, B., Rao, J.R., & Nair, B.U. (2010). Preparation and antimicrobial activity of scleraldehyde from *Schizophyllum commune*. *Carbohydrate Research*, *345*(2), 2213-2219.

Joshi, M., Patel, H., Gupte, S., & Gupte, A. (2013). Nutrient improvement for simultaneous production of exopolysaccharide and mycelial biomass by submerged cultivation of *Schizophyllum commune* AGMJ-1 using statistical optimization. *3 Biotech, 3*(4), 307–318.

Kashangura, C., Kunjeku, E., Mabveni, A., Chirara, T., Mswaka, A., & Dalu, M. V. (2004). Introduction: background to mushrooms. In C.J. Chetsanga (Ed.), *Manual for development workers (mushroom cultivation)* (pp. 9–14). Harare, Zimbabwe: Biotechnology Trust of Zimbabwe 2004.

Karaduman, A. B., Atli, B., & Yamac, M. (2012). An example for comparison of storage methods of macrofungus cultures: *Schizophyllum commune*. *Turkish Journal of Botany, 36*(2), 205–212.

Kaur. J., & Sodhi, H.S. (2012). Molecular characterization of mutant strains of *Calocybe indica* using RAPD-PCR. *The Bioscan*, *7*(3): 527-532.

Kaur, J., Sodhi, H. S., & Kapoor, S. (2008). Breeding of *Pleurotus florida* (oyster mushroom) for phenotypic pigmentation and high yield potential. *Journal of Science of Food and Agriculture*, *88*, 2676–2681.

Khan, S. M., Nawaz, A., Malik, W., Javed, N., Yasmin, T., Rehman, M. U., … Khan, A.A. (2011). Morphological and molecular characterization of oyster mushroom (*Pleurotus* spp.). *African Journal of Biotechnology*, *10*(14), 2638–2643.

Khatua, S., Paul, S., & Acharya, K. (2013). Mushroom as the potential source of new generation of antioxidant: a review. *Research Journal of Pharmacy and Technology, 6*(5), 496-505.

Kim, M.K., Ryu, J.S., Lee, Y.H., & Kim, H.R. (2013). Breeding of a long shelf-life strain for commercial cultivation by mono-mono crossing in *Pleurotus eryngii*. *Scientia Horticulturae, 163*, 265-270.

Körkoca, H., & Boynukara, B. (2003). The characterisation of protein profiles of the *Aeromonas hydrophila* and *A. caviae* strains isolated from gull and rainbow trout feces by SDS-PAGE. *Turkish Journal of Veterinary and Animal Sciences, 27*(5), 1173-1177.

Kortei, N. K., & Kwagyan, W. M. (2014). Evaluating the effect of gama radiation on eight different agro-lignocellulose waste materials for the production of oyster mushrooms *(Pleurotus eous* (Berk,)Sacc.strain P-31). *Croatian Journal of Food Technology, Biotechnology and Nutrition*, *9*(3–4), 83–90.

Kothe, E. (2001). Mating-type genes for basidiomycete strain improvement in mushroom farming. *Applied Microbiology Biotechnology*, *56*, 602−612.

Krupodorova, T.A., & Barshteyn, V.Y. (2015). Amaranth flour as a new alternative substrate for *Schizophyllum commune* Fr.: Fr. and *Cordyceps sinensis* (Berl) Sacc. growth. *Journal of Siberian Federal University*, *8*, 32-44.

Kumar, N. S., & Gurusubramanian, G. (2011). Random amplified polymorphic DNA (RAPD) markers and its applications. *Science Vision*, *11*(3), 116−124.

Kumar, R., Tapwal, A., Pandey, S., Borah, R. K., Borah, D., & Borgohain, J. (2013). Macro-fungal diversity and nutrient content of some edible mushrooms of Nagaland, India. *Nusantara Bioscience, 5*(1), 1−7.

Kumara, K.L.W., & Edirimanna, I.C.S. (2009). Improvement of strains of two oyster mushroom cultivars using duel culture technique. *World Applied Scieces Journal*, *7*(5), 654-660.

Kumari, M., Survase, S.A., & Singhal, R.S. (2007). Production of schizophyllan using *Schizophyllum commune* NRCM. *Bioresource Technology*, *99*(5), 1036-1043.

Lee, J., Kang, H.W., Kim, S.W., Lee, C.Y., & Ro, H.S. (2011). Breeding of new strains of mushroom by basidiospore chemical mutagenesis. *Mycobiology, 39*(4), 272-277.

Lemieszek, M., & Rzeski, W. (2012). Anticancer properties of polysaccharides isolated from fungi of the basidiomycetes class. *Contemporary Oncology, 16*(4), 285.

Littman, M.L. (1947). A culture medium for the primary isolation of fungi. *Science, 106*(2744), 109-111.

Liu, L., Zabten, L. V., Shu, Q. Y., & Maluszynski, M. (2004). Officially released mutant varieties in China. *Mutation Breeding Review*, *14*, 1−62.

Llarena-Hernández, R.C., Largeteau, M.L., Farnet, A.M., Foulongne-Oriol, M., Ferrer, N., Regnault-Roger, C., & Savoie, J.M. (2013). Potential of european wild strains of *Agaricus subrufescens* for productivity and quality on wheat straw based compost. *World Journal of Microbiology and Biotechnology, 29*(7), 1243-1253.

Llarena-Hernández, R.C., Largeteau, M., Farnet, A.M., Minvielle, N., Regnault-Roger, C., & Savoie, J.M. (2011). Phenotypic variability in cultivars and wild strains of *Agaricus brasiliensis* and *Agaricus subrufescens*. *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom products*, *2*, 38-49.

Majolagbe, O.N., Oloke, J.K., Deka-Boruah, H.O., & Aina, D.A. (2013). Effects of mutagenesis on mycelial growth rate (Mgr). total protein, total phenolics and total sugar contents of *Lentinus subnudus* using ultra-violet radiation as mutagen. *International Journal of Current Microbiology and Applied Sciences*, *2*(5), 18-27.

Mamiro, D.P., Mamiro, P.S., & Mwatawala, M.W. (2014). Oyster mushroom (*Pleurotus* spp.) cultivation technique using re-usable substrate containers and comparison of mineral contents with common leafy vegetables. *Journal of Applied Biosciences*, *80*, 7060-7070.

Masevhe, M.R., Soundy, P., & Taylor, N.J. (2015). Alternative substrates for cultivating oyster mushrooms (*Pleurotus ostreatus*). *South African Journal of Plant and Soil*, *33*(2), 1-8.

Matavuly, M., Lolić, S., Vujčić, S., Milovac, S., Rakić, M., & Karaman, M. (2013). *Schizophyllum commune*: the main cause of dying trees of the Banja Luka arbored walks and parks. *Matica Srpska Journal for Natural Siences*, *2013*(124), 367–377.

Mehmood, S., Bashir, A., Ahmad, A., Akram, Z., Jabeen, N., & Gulfraz, M. (2008). Molecular characterization of regional *Sorghum bicolor* varieties from Pakistan. *Pakistan Journal of Botany*, *40*(5), 2015–2021.

Mehrotra (2009). Growth, culture and maintenance of microorganisms. In G. Sumbali & R. S. Mehrotra (Eds.), *Principles of microbiology* (pp. 71-124). New Delhi, India: Tata McGraw-Hill Education Pvt. Ltd.

Menser, G.P. (1996). How to collect, identify and dry. In W. Griffith (Ed.), *Hallucinogenic and poisonous mushroom – field guide* (pp. 6-14). Berkeley, California: Ronin Publishing, Inc.

Miles, P. G., & Chang, S. T. (1997). Biology of fungi. In P. G. Miles (Ed.), *Mushroom biology: concise basics and current developments* (pp. 14–85). Singapore: World Scientific.

Miles, P. G., & Chang, S. T. (2004). Substrate and mycelial growth. In P. G. Miles (Ed.), *Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact* (pp. 93–101). Boca Raton, Florida: CRC press.

Mirfat, A.H.S., Abdullah, N., & Subramaniam, V. (2010). Scavenging acticity of *Schizophyllum commune* extracts and its correlation to total phenolic content. *Journal of Tropical Agriculture and Food Science*, *38*(2), 231-238.

Mirfat, A.H.S., Abdullah, N., & Subramaniam, V. (2014). Antimirobial activities of split gill mushroom *Schizophyllum commune* Fr. *American Journal of Research Communication*, *2*(7),113-124.

Moonmoon, M., Uddin, M.N., Ahmed, S., Shelly, N.J., & Khan, M.A. (2010). Cultivation of different strains of king oyster mushroom (*Pleurotus eryngii*) on saw dust and rice straw in Bangladesh. *Saudi Journal of Biological Sciences*, *17*, 341-345.

Mueller, U. G., & Wolfenbarger, L. L. (1999). AFLP genotyping and fingerprinting. *Trends in Ecology and Evolution, 14*(10), 389–394.

Mukhopadhyay, K., Haque, I., Bandopadhyay, R., Covert, S., & Porter, D. (2012). AFLP based assessment of genetic relationships among shiitake (*Lentinula* ssp.) mushrooms. *Molecular Biology Reports, 39*(5), 6059–6065.

Mumtaz, M. W., Hamid, A. A., Akhtar, M. T., Anwar, F., Rashid, U., & Al-Zuaidy, M. H. (2017). An overview of recent developments in metabolomics and proteomics — Phytotherapic research perspectives. *Frontiers in Life Science, 10*(1), 1–37.

Muruke, M. H. S., Kivaisi, A. K., Magingo, F. S. S., & Danell, E. (2002). Identification of mushroom mycelia using DNA techniques. *Tanzania Journal of Science*, *28*(1), 115–128.

Myronycheva, O., Bandura, I., Bisko, N., Gryganskyi, A.P., & Karlsson, O. (2017). Assessment of the growth and fruiting of 19 oyster mushroom strains for indoor cultivation on lignocellulosic wastes. *Bioresources, 12*(3), 4606-4626.

Nakasone, K. K., Peterson, S. W., & Jong, S. C. (2004). Preservation and distribution of fungal cultures. In M. S. Foster & G. F. Bills, (Eds.), *Biodiversity of fungi: inventory and monitoring methods* (pp. 37–48). London, United Kingdom: Elsevier Academic Press.

Niederpruem, D. J., Hobbs, H., & Henry, L. (1964). Nutritional studies of development in *Schizophyllum commune*. *Journal of Bacteriology, 88*(6), 1721–1729.

Niederpruem, D. J., & Wessels, J. (1969). Cytodifferentiation and morphogenesis in *Schizophyllum commune*. *Bacteriological Reviews, 33*(4), 505.

Nikodem, V., & Fresco, J.R. (1979). Protein fingerprinting by SDS-gel electrophoresis after partial fragmentation with CNBr. *Analytical Biochemistry*, *97*(2), 382-386.

Noble, J.E., & Bailey M. J. A. (2009). Quantitation of protein. In J. Albelson (Ed.), *Methods in enzymology* (pp. 73-95). United Kingdom: Elseviar Inc.

Obodai, M., Cleland-Okine, J., & Vowotor, K.A. (2003). Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-product. *Journal of Industrial Microbiology & Biotechnology*, *30*, 146-149.

Oei, P. (2005). Growing oyster mushrooms on pasteurised or 'sterilised' substrates. In Oei, P.(Eds.), *Small-scale mushroom cultivation: oyster, shiitake and wood ear mushrooms* (pp. 37–57). Wageningen, The Netherlands: Agromisa Foundation and CTA.

Ohm, R. A., de Jong, J. F., Lugones, L. G., Aerts, A., Kothe, E., Stajich, J. E., … Wosten, H. A. (2010). Genome sequence of the model mushroom *Schizophyllum commune. Nature Biotechnology, 28*(9), 957–963.

Padhiar, A., Nagadesi, P.K., Albert, S., & Arya, A. (2009). Morphology, anatomy and cultural characters of two wood decaying fungi *Schizophyllum commune* and *Flavadon flavus*. *Journal of Mycology and Plant Pathology, 39*(1), 27-31.

Palmer, G. E., & Horton, J. S. (2006). Mushrooms by magic: making connections between signal transduction and fruiting body development in the basidiomycete fungus *Schizophyllum commune*. *FEMS Microbiology Letters*, *262*(1), 1–8.

Pathirana, R. (2011). Plant mutation breeding in agriculture. *CAB Reviews: Perspectives in Agriculture, Veterinary Science Nutrition and Natural Resources*, *6*(32), 1–20.

Pawlik, A., Janusz, G., Koszerny, J., Malek, W., & Rogalski, J. (2012). Genetic diversity of the edible mushroom *Pleurotus* sp. by amplified fragment length polymorphism. *Current Microbiology*, *65*, 438-445.

Peng, J.T., Dai, M.C., Tsai, Y.F., Chen, M.H., & Chen, J.T. (2001). Selection and breeding of king oyster mushroom. *Journal of Agricultural Research of China, 50*(4), 43-58.

Preecha, C., Wisutthiphaet, W., Seephueak, P., & Thongliumnak, S. (2016). Bag cultivation of split gill mushroom (*Schizophyllum commune*) by application coconut meal substitute rice bran. *International Journal of Agricultural Technology, 12*(7.2), 2073–2077.

Rahilah, A.R.N., Raha, A.R., & Maizirwan, M. (2012). Cytotoxicity study of *Schizophyllum commune* extract towards DF-1 cell line. *4th International Conference on Chemical and Bioprocess Engineering (ICCBPE 2012) in conjunction with 26th Symposium of Malaysian Chemical Engineers*, 21st – 23rd November 2012: Kota Kinabalu, Sabah, Malaysia.

Rajapakse, P. (2011). New cultivation technology for paddy straw mushroom *(Volvariella volvacea)*. *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7) 1*, 446-451.

Raper, J. R., & Miles, P. G. (1958). The genetics of *Schizophyllum commune*. *Genetics, 43*(3), 530-546.

Rashid, R.A., Daud, F., Senafi, S., Awang, M.R., Mohamad, A., Mutaat, H.H., & Maskom, M.M. (2014). Radiosensitivity study and radiation effects on morphology characterization of grey oyster mushroom *Pleurotus sajor-caju*. *AIP Conference Proceedings 1614*, 570-574.

Rashid, R. A., Daud, F., Awang, M. R., Mutaat, H. H., Senafi, S., Mohamad, A., Maskom, M. M., & Rahim, K. A. (2016). Evaluation of mycelia growth, morphology and yield for low dose gamma irradiated grey oyster mushroom *Pleurotus sajor-caju*. *International Journal of Inovation and Scientific Research*, *24*(2), 332–336.

Rogl. S., Javornik, B., Sinkovič, T., & Batič, F. (1996). Characterization of oak (*Quetcus* L.) seed proteins by electrophoresis. *Phyton Annales Rei Botanicae, 36*(3), 159-162.

Rohizad, N.N. (2017). *Breeding of new strains of Schizophyllum commune (split-gill mushroom) obtained by hybridization of gamma-irradiated monokaryotic culture and dna fingerprinting by intersimple sequence repeat (issr) markers*. (Unpublished bachelor degree's thesis). University of Malaya, Kuala Lumpur, Malaysia.

Romanelli, A.M., Sutton, D.A., Thompson, E.H., Rinaldi, M.G., & Wickes, B.L. (2010). Sequence-based identification of filamentous basidiomycetous fungi from clinical specimens: a cautionary note. *Journal of Clinical Microbiology, 48*(3), 741-752.

Rosado, F.R., Kemmelmeier, C., & Costa, S.M.D. (2001). Alternative method of inoculum and spawn production for the cultivation of the edible Brazilia mushroom *Pleurotus ostreatoroseus* Sing. *Journal of Basic Microbiology*, *42*(1), 37-44.

Roupas, P., Keogh, J., Noakes, M., Maretts, C., & Taylor, P. (2012). The role of edible mushrooms in health: evaluation of the evidence. *Journal of Functional Foods, 4*(4), 687-709.

Roux, A., Lison, D., Junot, C., & Heilier, J. F. (2011). Applications of liquid chromatography coupled to mass spectrometry-based metabolomics in clinical chemistry and toxicology: a review. *Clinical Biochemistry, 44*(1), 119–135.

Royse, D.J. (1985). Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom. *Mycologia, 77*(5), 756-762.

Royse, D. J. (1997). Speciality mushrooms and their cultivation. In J. Janick (Ed.), *Horticultural reviews, volume 19* (pp. 59–97). United States: John Wiley & Sons, Inc.

Royse, D. J. (2014). A global perspective on the high five: *Agaricus, Pleurotus, Lentinula, Auricularia* & *Flammulina*. *Proceeding of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8) 1*, 1-6.

Royse, D.J., & Bahler, C.C. (1986). Effects of genotype, spawn run time, and substrate formulation on biological efficiency of shiitake. *Applied and Environmental Microbiology*, *52*(6), 1425-1427.

Royse, D.J., Schisler, L.C., & Diehle, D.A. (2016). Shiitake mushrooms consumptions, production and cultivation. *Interdisciplinary Science Reviews, 10*(4), 329-335.

Sánchez, C. (2004). Modern aspects of mushroom culture technology. *Applied Microbiology and Biotechnology, 64*(6), 756–762.

Sánchez, C. (2009). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology*, 85, 1321–1337.

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology, 85*(5),1321–1337.

Santos, O. D., Resende, M. C. C. D., Mello, A. L. D., Frazzon, A.P.G., & D'Azevedo, P.A. (2011). The use of whole-cell protein profile analysis by SDS-PAGE as an accuratetool to identify species and subspecies of coagulase-negative staphylococci. *Acta Pathologica, Microbiologica Et Immunologica Scandinavica, 120*, 39-46.

Sathesh-Prabu, C., & Lee, Y.K. (2011). Mutation breeding of mushroom by radiation. *Journal of Radiation Industry, 5*(4), 285-295.

Sathesh-Prabu, C., & Lee, Y. K. (2016). Genetic variability and proteome profiling of a radiation induced cellulase mutant mushroom *Pleurotus florida*. *Polish Journal of Microbiology*, *65*(3), 271–277.

Selvakumar, P., Rajasekar, S., Babu, A. G., Periasamy, K., Raaman, N., & Reddy, M. S. (2015). Improving biological efficiency of *Pleurotus* strain through protoplast fusion between *P. ostreatus* var. *florida* and *P. djamor* var. *roseus*. *Food Science and Biotechnology*, *24*(5), 1741–1748.

Sermkiattipong, N., & Charoen, S. (2014). Development of straw mushroom strain for high yield by gamma radiation. *Journal of Agricultural Technology, 10*(5), 1151-1164.

Sharma, V., & Kumar, S. (2011). Spawn production technology. In M. Singh, V. Bhuvnesh, K. Shwet, & G. C. Wakchaure (Eds.), *Mushrooms cultivation, marketing and consumption* (pp. 31–42). Chambaghat, Solan: Directorate of Mushroom Research (ICAR).

Sher, H., Al-Yemeni, M., Bahkali, A.H.A., & Sher, H. (2010). Effect of environmental factors on the yield of selected mushroom species growing in two different agro ecological zones of Pakistan. *Saudi Journal of Biological Sciences*, *17*, 321-326.

Singh, R. I., Aarti, K., & Singh, S. S. (2007). Formation of interspecies fusants of *Agaricus bisporus* and *Agaricus bitorquis* mushroom by protoplast fusion. *Indian Journal of Microbiology, 47*(4), 369–372.

Sonnenberg, A. S. M., Baars, J. J. P., & Kerrigan, R. W. (2008). Mushroom breeding: hurdles and challenges. *Proceedings of the 6th International Conference on Mushroom Biology and Mushroom Products, 2008*, 96-103.

Sou, H.D., Roo, R., Ryu, S.R., Ka K.H., Park, H., & Joo, S.H. (2013). Morphological and genetic characteristics of newly crossbred cauliflower mushroom (*Sparassis latifolia*). *Journal of Microbiology, 51*(5), 552-557.

Stamets, P. (1993a). Culturing mushroom mycelium on agar media. In Stamets, P. (Eds.), *Growing gourmet and medicinal mushrooms*, (pp. 117-126). Berekely, California: Ten Speed Press.

Stamets, P. (1993b). Generating grain spawn. In P. Stamets (Ed.), *Growing gourmet and medicinal mushrooms* (pp. 423–430). Berekely, California: Ten Speed Press.

Stamets, P. (2000). Harvesting, storing and packing the crop for market. In P. Stamets (Ed.), *Growing gourmet and medicinal mushrooms* (pp. 423–430). Berekely, California: Ten Speed Press.

Stamets. P., & Chilton, J.S. (1983). Sterile technique and agar culture. In P. Stamets & J. S. Chilton (Eds.), *The mushroom cultivator: a practical guide to growing mushrooms at home* (pp. 149-158). Olympia, Washington: Agarikon Press.

Staněk, M. (1978). Irradiation of fresh mushroom fruit-bodies. *Karstenis*, *18*, 85-86.

Stankis, M. M., & Specht, C. A. (2007). Cloning the mating-type genes of *Schizophyllum commune*: a historical perspective. In J. Heitman (Ed.), *Sex in fungi: molecular determination and evolutionary implications* (pp. 267–282). Washington, DC: American Society of Microbiology.

Storey, M. (2005). Making spore prints. *Field Mycology, 6*(3), 104-106.

Suprasanna, P., Mirajkar, S.J., & Bhagwat, S.G. (2015). Induced mutations and crop improvement. In B. Bahadur (Ed.), *Plant biology and biotechnology: volume I: plant diversity, organization, function and improvement* (pp. 593-617). Mumbai, India: Springer India.

Tabi, A.N.M., Zakil, F.A., Fauzai, W.N.F.M., Ali, N., & Hassan, O. (2008). The usage of empty fruit bunch (EFB) and palm pressed fibre (PPF) as substrates for the cultivation of *Pleurotus ostreatus*. *Jurnal Teknologi*, *49*(F), 189-196.

Tagore, S., Chowdhury, N., & De, R. K. (2014). Analyzing methods for path mining with applications in metabolomics. *Gene, 534*(2), 125–138.

Takemoto, S., Nakamura, H., Imamura, Y., & Shimane, T. (2010). *Schizophyllum commune* as a ubiquitous plant parasite. *Japan Agricultural Research Quarterly, 44*(4), 357–364.

Tang, W., Kueh, T.H., & Simcik, M.F. (2015). Effects of temperature, humidity and air flow on fungal growth rate on loaded ventilation filters. *Journal of Occupational and Environmental Hygiene*, *12*(8), 525-537.

Teimoori, B. B., Pourianfar, H. R., Moeini, M. J., & Janpoor, J. (2014). Chemically and physically induced mutagenesis in basidiospores of oyster mushroom *Pleurotus ostreatus* var. florida. *International Journal of Advanced Research, 2*, 915–921.

Teoh, Y. P., Don, M. M., & Ujang, S. (2017). Production of biomass by *Schizophyllum commune* and its antifungal activity towards rubberwood-degrading fungi. *Sains Malaysiana, 46*(1), 123–128.

Terashima, K., Matsumoto, T., Hasebe, K., & Nakai, Y.F. (2002). Genetic diversity and strain-typing in cultivated strains of *Lentinula edodes* (the shii-take mushroom) in Japan by AFLP analysis. *Mycological Research, 106*(1), 34-39.

Theodoridis, G., Gika, H. G., & Wilson, I. D. (2008). LC-MS-based methodology for global metabolite profiling in metabonomics/metabolomics. *TrAC Trends in Analytical Chemistry, 27*(3), 251–260.

Thomas, W. S. (1928). Gilled mushrooms, their propagation and structure. In W.S. Thomas (Ed.), *Field book of common gilled mushrooms: with a key to their identification and directions for cooking those that are edible* (pp. 7–17), New York, United States: G.P.Putnam's Sons.

Tullio, V., Mandras, N., Banche, G., Allizond, V., Gaido, E., Roana, J., … Carlone, N.A. (2008). *Schizophyllum commune*: an unusual agent of bronchopneumonia in an immunocompromised patient. *Medical Mycology*, *46*, 735-738.

Uddin, M., Haque, S., Haque, M., Bilkis, S., & Biswas, A. (2013). Effect of different substrate on growth and yield of button mushroom. *Journal of Environmental Science and Natural Resources, 5*(2), 177-180.

Ujang, S., Jones, E.B.G., & Watling, R. (2002). The distribution of wood-inhabiting fungi in peninsular Malaysia. *Journal of Tropical Science*, *14*(4), 433-440.

Ulukapi, K., & Nasircilar, A. G. (2015). Developments of gamma ray application on mutation breeding studies in recent years. *International Conference on Advances in Agricultural, Biological & Environmental Sciences*.

United Nations Economic Commission for Europe. (2017). UNECE standard FFV-24 concerning the marketing and commercial quality control of cultivated mushrooms. Retrieved 6 Mei 2018 from https://www.unece.org/fileadmin/DAM/trade/agr/standard/standard/fresh/FFV-Std/English/24_CultivatedMushrooms.pdf.

Upadhyay, R.C. (2011). Mushroom culture preparation and preservation techniques. In M. Singh, B. Vijay, S. Kamal, & G.C. Wakchaure (Eds.), *Mushrooms: cultivation, marketing and consumption* (pp. 23-30). New Delhi, India: Indian Council of Agricultural Research.

Valvarede, M. E., Pérez, T. H., & López, O. P. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology*, *2015*, 1–14.

van Nieuwenhuijzen, B. (2005). Biology of mushrooms. In P. Oei. (Ed.), *Small-scale mushroom cultivation*. Wageningen, The Netherlands: Agromisa Foundation.

Vaz, J.A., Barros, L., Martins, A., Santos-Buelga, C., Vasconcelos, M.H., & Ferreira, I.C.F.R. (2011). Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions. *Food Chemistry, 126*(2), 610-616.

Veena, S.S., & Meera, P. (2010). A simple method for culture conservation of some commercial mushrooms. *Mycosphere, 1*(3), 191-194.

Vetayasuporn, S., Chutichudet, P., & Cho-Ruk., K. (2006). Bagasse as a possible substrate for *Pleurotus ostreatus* (Fr.) Kummer cultivation for the local mushroom farms in the norteast of Thailand. *Pakistan Journal of Biological Sciences*, *9*(13), 2512-2515.

Verma, N., Bansal, M.C., & Kumar, V. (2008). Protoplast fusion technology and its biotechnological applications. *1st International conference on industrial biotechnology*, 14, 113-120.

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*, *5*(4), 709-718.

Wasinger, V. C., Zeng, M., & Yau, Y. (2013). Current status and advances in quantitative proteomic mass spectrometry. *International Journal of Proteomics, 2013*, 1–12.

Wilkins, M. R., Sanchez, J. C., Gooley, A. A., Appel, R. D., Humphery-Smith, I., Hochstrasser, D. F., & Williams, K. L. (1996). Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. *Biotechnology and Genetic Engineering Reviews, 13*, 19–50.

Wilson, C.M. (1985). Mapping of zein polypeptides after isoelectric focusing on agarose gels. *Biochemical Genetics, 23*(1), 115-124.

Xiao, B.L., Feng, B., Li, J., Yan, C., & Zhu, L.Y. (2016). Genetic diversity and breeding history of winter mushroom (*Flammulina velutipes*) in China uncovered by genomic SSR markers. *Gene*, *591*(1), 227-235.

Yang, W. J., Guo, F. L., & Wan, Z. J. (2013). Yield and size of oyster mushroom grown on rice / wheat straw basal substrate supplemented with cotton seed hull. *Saudi Journal of Biological Sciences*, *20*, 333-338.

Ye, X. Y., Wang, H.X., & Ng, T.B. (1999). First chromatographic isolation of an antifungal thaumatin-like protein from French bean legumes and demonstration of its antifungal activity. *Biochemical and Biophysical Research Communications, 263*(1), 130-134.

Ying, F.J.L., Bolhassan, M.H., & Yeo, F.K.S. (2017). Variations and hybridization compatibility of single basidiospore isolates of *Pleurotus sajor-caju* (Fr.) Sings. *Borneo Journal of Resource Science and Technology*, *7*(1), 47-55.

Zainol, M. R. (2016). Prospects for increasing commercial mushroom production in Malaysia: challenges and opportunities. *Mediterranean Journal of Social Sciences*, *7*(1), 406–415.

Zharare, G.E., Kabanda, S.M., & Poku, J.Z. (2010). Effects of temperature and hydrogen peroxide on mycelial growth of eight *Pleurotus* strains. *Scientia Horticulturae, 125*(2), 95-102.

Zhong, K., Liu, L., Tong, L., Wang, X., & Zhou, S. (2013). Rheological properties and antitumor activity of schizophyllan produced with solid-state fermentation. *International Journal of Biological Macromolecules, 62*(2013), 13-17.

Zied, D.C., Savoie, J.M., & Pardo-Giménez, A. (2011). Soybean the main nitrogen source in cultivation substrates of edible and medicinal mushrooms. In H. A. El-Shemy (Ed.), *Soybean and nutrition* (pp. 433-452). Rijeka, Croatia:InTech.