A STUDY ON *Fusarium* ISOLATION, ITS MOLECULAR IDENTIFICATION, CLINICAL MANIFESTATIONS AND RISK FACTORS

DR. NOR RASIDAH BINTI RASID

DISSERTATION SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PATHOLOGY (MICROBIOLOGY)

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ACKNOWLEDGEMENTS

All praises to Allah S.W.T The Most Gracious and The Most Merciful for giving me strength and courage to complete my thesis on the time.

I would like to express my deeper gratitude and appreciation to my supervisor Associate Professor Dr Azian Harun for her guidance, unfailing support and for her kind patience and precious time given to me to complete the study. Also a special thanks to Dr Norlela Othman for her continuous support and guidance. My appreciation to HSNZ microbiology lab staffs especially Encik Zulazlan bin Taja Ariffin who help me a lot in learning and recognizing *Fusarium* and help me to collect isolates throughout in HSNZ. Not forget to HUSM mycology laboratory staffs Puan Roziawati Yusof and Puan Nur Athirah in helping me collecting and keeping the isolates needed throughout the study. My appreciation also goes to post-graduates students, Yasmin Khairani Ismadi and others that had helped me with the laboratory work especially the molecular part.

My sincere thanks to all my colleagues Zulkhairi, Syazwani, Aishah, Norjihan and Noorilham for lending hand and their continuous support, be it technically and morally from beginning till the end of this study.

My deepest appreciation goes to my husband Wan Mohd Aidil Fahmi W Nawang who gave me tremendous support throughout this journey. To my lovely daughter Wan Nur Amyra Alisya and my son Wan Muhammad Afiq Rizqi who were sacrifice a lot and inspired me to finish my study. My gratitude also goes to my parents, Rasid bin Salleh and Kamariah binti Yatim and my siblings whom always help me in the hardness journey of my study.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols/Abbreviations	**Definition**
%	Percentage
/	Per
~	Approximate
<	Less than
>	More than
±	Plus minus
ºC	Degree Celsius
µg	Microgram
µl	Microliter
BLAST	Basic local alignment search tool
Bp	Base pair
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
EF	Elongation factor
FSSC	*Fusarium solani* species complex
FOSC	*Fusarium oxysporum* species complex
FFSC	*Fusarium fujikuroi* species complex
G	Gram
IDSA	Infectious Disease Society of America
ITS	Internal Transcribed Spacer
HSNZ	Hospital Sultanah Nur Zahirah
HUSM	Hospital Universiti Sains Malaysia
M	Molarity
Min	Minute
Mm	Milimolar

Ml	Milliliter
MLST	Multilocus sequence typing
NCBI	National Center for Biotechnology Information
OR	Odds Ratio
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
RPB1	DNA-directed RNA polymerase II largest
RPB2	DNA-diected RNA polymerase II Second largest subunit
Rpm	Revolution per minute
SC	Species complex
SDA	Sabouraud Dextrose Agar
Taq	*Thermus aquaticus*
TEF/ TEF-1α	Translation elongation factor -1α
TBE	Tris-Borate EDTA
U	Unit
USA	United States of America
UV	Ultravoilet
V	Volt
X	Times or multiplication

ABSTRAK

Spesies *Fusarium* dijumpai di persekitaran tanah dan serpihan tumbuhan. Sejak kebelakangan ini, spesies *Fusarium* diakui sebagai patogen tumbuhan dan juga telah menjadi patogen kepada manusia yang mempunyai imuniti badan yang lemah, mahupun normal. Kaedah identifikasi secara konvensional sahaja tidak memadai kerana kaedah tersebut hanya dapat mengenalpasti sehingga tahap genus sahaja. Kaedah molekular seperti jujukan nukleotida adalah kaedah yang lebih tepat dan konsisten untuk identifikasi spesis *Fusarium*. Kajian ke atas 87 rekod perubatan pesakit yang menunjukkan kultur positif dalam pelbagai spesimen klinikal telah dijalankan di Hospital Sultanah Nur Zahirah, Terengganu dan Hospital Universiti Sains Malaysia, Kelantan dari tahun 2017 hingga 2019, di mana 43 kes daripadanya adalah kes yang disahkan jangkitan *Fusarium*, manakala 44 kes lagi dipilih dalam kalangan pesakit kultur positif selain *Fusarium*. Kami mengenalpasti perkadaran *Fusarium* berdasarkan spesies, manifestasi klinikal dan faktor risiko dari isolat klinikal. Dua puluh empat isolat klinikal telah dikenalpasti melalui proses jujukan di mana gen '*translation elongation factor 1-alpha* (*TEF-1a*)' digunakan untuk identifikasi spesies yang berlainan. Keputusan menunjukkan jangkitan pada kornea mata (n=17, 39.5%) dan kuku (n=16, 37.2%) mewakili fusariosis yang biasanya ditemui, diikuti dengan jangkitan kulat dalam darah (n=7, 16.3%). Jangkitan kulit pada lengan, radang paru-paru, jangkitan kulat pada sinus maksilari masing-masing menunjukan satu kes. Daripada 24 isolat kulat yang menjalani jujukan gen *TEF-1a*, 22 isolat terdiri daripada *Fusarium solani* kompleks spesies (FSSC), n=22; *Fusarium solani (n=10), Fusarium proliferatum* (n=7), dan *Fusarium keratoplasticum* (n=5). Dua daripadanya adalah *Fusarium pseudocircinatum* (n=1) di bawah kumpulan *Fusarium fujikuroi* kompleks spesies (FFSC) dan *Fusarium oxysporum* (n=1) termasuk dalam kumpulan *Fusarium oxysporum*

kompleks species (FOSC). Jangkitan *Fusarium* lebih banyak ditemui dalam kalangan lelaki (n=29, 67.4%), dan purata umur adalah 51 tahun. Faktor risiko penting yang ditemui dalam kajian ini adalah penyakit kencing manis, kanser, bilangan sel neutrofil rendah dalam darah, kecederaan mata dan tertusuk benda asing. Namun, analisis faktor risiko dalam kajian ini tidak menunjukkan kaitan yang signifikan secara statistik.

Kata kunci: *Fusarium*, *TEF-1α*, identifikasi, faktor risiko

ABSTRACT

Fusarium species are ubiquitous in soil and plant debris. *Fusarium* species are well recognized as plant pathogens and have emerged as human pathogens affecting both immunocompetent and immunocompromised hosts since recent years. Conventional methods of *Fusarium* identification are inadequate as it only identifies *Fusarium* to genus level. Molecular methods by nucleotide sequences are more accurate and consistent for species identification. We reviewed 87 medical records of patients for whom fungal culture was performed on various clinical specimens in Hospital Sultanah Nur Zahirah, Terengganu and Hospital Universiti Sains Malaysia, Kelantan from 2017 until 2019. Out of 87 cases, 43 cases were positive for *Fusarium* and 44 cases were non-*Fusarium* cases. We identified the proportion of *Fusarium* based on species, clinical manifestation, and risk factors from clinical isolates. Twenty four available clinical isolates were specifically identified by sequencing the translation elongation factor 1-alpha (*TEF-1α*) gene. Our results showed that keratitis (n=17, 39.5%) and onychomycosis (n=16, 37.2%) were the most common type of fusariosis, followed by fungaemia (n=7, 16.3%). Forearm skin infection, fungal pneumonia, and fungal maxillary sinusitis were presented in one case each. Based on *TEF-1α* sequencing, 22 of 24 isolates belong to *Fusarium solani* species complex (FSSC), n=22; which comprised *Fusarium solani* (n=10), *Fusarium proliferatum* (n=7), and *Fusarium keratoplasticum* (n=5). Another two were *Fusarium pseudocircinatum* (n=1) which belongs to *Fusarium fujikuroi* species complex (FFSC), and *Fusarium oxysporum* (n=1) which is a member in *Fusarium oxysporum* species complex (FOSC). *Fusarium* infection was more common in males (n=29, 67.4%), and the mean age was 51 years old. Important risk factors for *Fusarium* infection, including diabetes

mellitus, malignancies, neutropenia, eyes trauma, and embedded foreign body, were analysed but none were statistically significant.

Keywords: *Fusarium*, *TEF-1α*, identification, risk factors

CHAPTER 1:

INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Fusarium species are the well-known plant pathogens[1], now emerged as a new fungal pathogen in both immunocompetent and immunocompromised humans.[2-4] *Fusarium* species specifically infects certain parts of plants such as grains, seedlings, heads, roots or stem, cause various plant diseases thus will reduce commercial yield and product quality.[5] In human, *Fusarium* species cause broad clinical manifestation, superficial in immunocompetent host and locally invasive or disseminated infections occurring almost exclusively in the severely immunocompromised host.[3,6] Type of *Fusarium* infection clearly can be divided into four main types: (i) superficial infections, mainly onychomycosis and paronychia, (ii) keratitis and other eye infections, (iii) deep localised infections and (iv) disseminated infections.[4,6]

Fusarium is a large genus of filamentous fungi, often referred to as hyaline hyphomycetes, or also known as mould that commonly found in the environment, where it is isolated from soil, plants and water systems.[4,7] Several species and species complexes (SC) are related to human fusariosis which include *F. solani SC, F. oxysporum SC, F. fujikuroi SC, F. dimerum, F. chlamydosporum, F. incarnatum-equiseti* and *F. sporotrichoides.* [8-10]

Most species are harmless saprobes which were abundant in soil. However, 20 of the most common *Fusarium* species were evaluated associated with mycotoxin production[11] which is the core of mechanism of infection in humans and plants. Shi *et al*. evaluated the mycotoxin production from the *Fusarium* species and sorted them into the three following groups based on molecular characterization.[11] Group 1 mycotoxin production comprised fusaric acid producers which classified into two subgroups. Subgroup-I comprised *F. fujikuroi, F.solani, F. verticillioides* and *F. proliferatum* that

produce fusaric acid and fumonisins; subgroup-II comprised *F.musae, F. equiseti, F. temperatum, F. subglutinans, F. tricinctum, F. oxysporum, F. concentricum, F. sacchari* and *F. anadiyazi* that produce only fusidic acid. Group-II, produce type–A trichothecene mycotoxin comprises of *F. polyphialidicum, F. sporotrichioides* and *F. langsethiae* and group-III are type-B trichothecene mycotoxin producers comprising *F. meridionale, F. culmorum, F. graminearum* and *F. poae*.

1.1.1 History and taxonomy development

The genus of *Fusarium* was first introduced in 1809 by Link.[12] Since then, about a thousand distinct *Fusarium* species have been described for every host. However, *Fusarium* only received attention when ‘’Die Fusarien’’ was published in 1935 by Wollenweber and Reinking in Germany[13] in which this author offered order to a chaotic situation of *Fusarium* taxonomy. Sixteen sections, 65 species, 55 varieties and 22 forms of *Fusarium* were introduced, which separated by based on morphological differences. Since then, for the past 80 years, tremendous studies and investigations have been carried out on the taxonomy, biology and mycotoxins of *Fusarium* species. In 1950, a Russian Scientist, Raillo had published a taxonomic system based on microconidia shape, presence of microconidia and chlamydospores.[14] A significant development in the taxonomy then was made by Booth from England in 1960s and 1970s, in which Booth had revised “The Genus *Fusarium*” by Wollenweber and Reinking. Booth introduced the use of morphology of the conidiogenous cells, especially those producing macroconidia.[15] Gerlach and Nirenberg then have published their own *Fusarium* taxonomy in Germany in 1982.[16] Despite much criticism on their taxonomic system, their work still continued in the understanding of *Fusarium* species and, now

many of their suggested species are accepted. In 1968, Toussoun and Nelson from the United States also published a pictorial guide for *Fusarium* species identification in which nine species and ten cultivars where described.[17] However, in 1983 Nelson and Toussoun together with Marasas from South Africa published a definitive a more complicated taxonomy which described 46 species.[18] A unique agreement among *Fusarium* taxonomists, including Burgess and Summerell from Australia, Gerlach and Nirenberg from Germany, Marasas from South Africa, and Nelson and Toussoun from the US in the 1980s, in which they conclude that *Fusarium* taxonomy based on fungal morphological characteristics. In 1990s, phylogenic species concept applied which was based on DNA sequencing, resulting in introducing new species of *Fusarium* that often cannot be distinguished morphologically.[19] The latest taxonomy update was in the 2006, where Leslie from United States and Summerell from Australia integrated the morphological, biological and phylogenic species concepts and published "The *Fusarium* Laboratory Manual" with 70 species.[20]

As a conclusion, morphological characteristics combined with molecular data has minimizes the differences in identification of *Fusarium* isolates even though no unanimous consensus have been made from previous taxonomists.[19]

1.1.2 *Fusarium* taxonomy classification

Fusarium is one of the first fungal groups that use the term 'species complex' for the closely related species.[20] Geiser at al. in 2013 have concluded that terminal *Fusarium* clade comprises 20 strongly supported species complexes and nine monotypic lineages.[21] Application of phylogenetic species recognition based on genealogical concordance and non-discordance over the past 20 years, has resulted in its explosive

growth.[22,23] Based on O'Donnell *et al.* in 2015, current genus *Fusarium* comprises at least 300 phylogenetically distinct species, 20 species complexes and nine monotypic lineages.[24] However, the majority of the species are unnamed and many of these are undistinguishable morphologically.[24] (Figure 1.1)

Seven *Fusarium* species complexes (SC) associated with reported cases of human infections are: the *F. solani* species complex (FSSC), *F. dimerum* species complex (FDSC), *F. oxysporum* species complex (FOSC), *F. fujikuroi* species complex (FFSC, comprising *F. proliferatum* and *F.verticillioides*), *F. incarnatum-equiseti* species complex (FIESC) and the complex including *F. sporotrichioides* (FSAMSC). Only a few other than these species complexes have occasionally associated with human infection. Members of FSSC include *F. solani sensu latu, F.falciforme, F. keratoplasticum, F. lichenicola* and *F. petroliphilum.*[9,10,25]

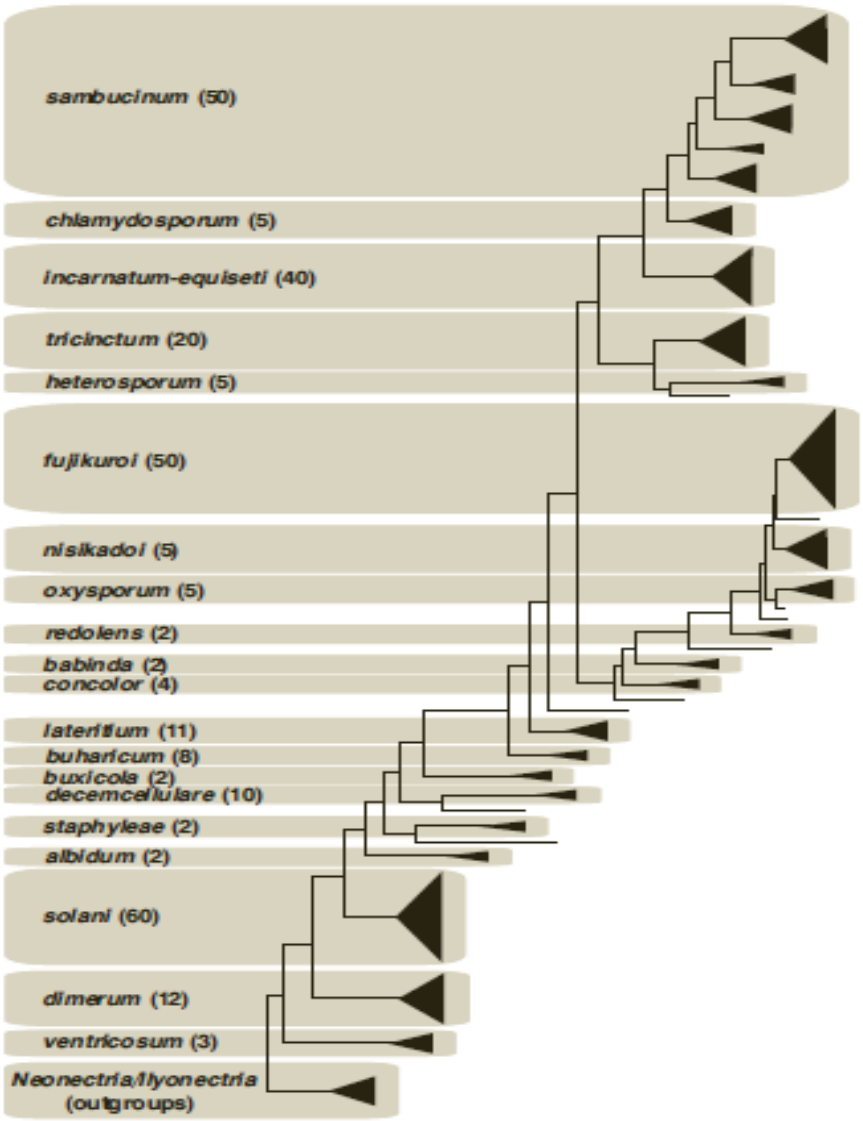

Figure 1.1. Diagrammatic representation of *Fusarium* phylogeny inferred from a combined *RPB1* + *RPB2* gene dataset (Adopted from O'Donnell K, Ward TJ, Robert VA, Crous PW, Geiser DM, Kang S. DNA sequence-based identification of *Fusarium*: current status and future directions. *Phytoparasitica.* 2015;43(5):583-595).

1.2 Clinical significance

For over 200 years of *Fusarium* has been found, numerous extensive studies have done in which *Fusarium* recognized as a pathogen that causes a broad range of plant diseases.

Some *Fusarium* species have emerged to cause a broad spectrum of opportunistic infections in human over the past decades.[4,26,27] In human, invasive and disseminated infections likely occur in severely immunocompromised patient[4], and generally manifest as fever not responding to antimicrobial therapy.[28] Superficial infections such as keratitis and onychomycosis are frequently manifested in immunocompetent persons and often associated with previous trauma.[29,30] Invasive infection was also reported in an immunocompetent patient, as *Fusarium* was responsible for brain abscess in 33-year-old Hispanic woman, which successfully treated with surgical aspiration and antifungal treatment. [31]

In the severely immunocompromised patient, *Fusarium* species has recently emerged as the second most common pathogenic mould after *Aspergillus* in high-risk patients with haematological malignancy, and in recipients of solid organ,[26,32] and allogeneic bone marrow or stem cells transplant [26]. Invasive Fusariosis is characterized by poor prognosis in neutropenic patients [33] and highly resistance to antifungal agents and therapy [34-36]. Most species exhibit high minimal inhibitory concentrations (MICs) towards currently used antifungals, especially azoles group[34].

1.3 Laboratory identification of *Fusarium*

1.3.1 Conventional identification methods

Identification of fungi to the species or at least to the genus level is important in order to direct appropriate treatment. Phenotypic identification is fundamental for the identification of *Fusarium.* Morphological examination with lactophenol cotton blue (LPCB) rapid stain still the standard for identification in many laboratories. Many *Fusarium* species look similar in culture and direct microscopic examination on

morphological characteristics, have been shown to represent species complexes instead of single species.[3] These conventional methods include the description of colonies appearance, in term of texture, colour and pigment; and microscopic description of conidiogenous cells and conidia. Further genotypic characterization of *Fusarium* to species level is usually not done routinely in clinical laboratories.

Phenotypically, *Fusarium* colonies are fast-growing, pale or bright coloured (depending on the species) with or without a cottony aerial mycelium. *Fusarium* species grow readily on many media, such as Potato dextrose agar (PDA) and Sabouroud dextrose agar (SDA) without cycloheximide which is inhibitory. *Fusarium* colonies may appear as white, yellow, orange, lavender, pink, salmon, grey or purple shades[37] and may appear to be quite mucoid.[38] (Figure 1.4 and figure 1.5). On Lactophenol direct or slide culture examination, *Fusarium* typically produces both macro- and microconidia from slender phialides as shown in figure 1.2 and 1.3. The production of both fusoid macroconidia and microconidia are the characteristics of the genus *Fusarium*. Microconidia are one or two-celled, hyaline, smaller than macroconidia which are multicelled in pyriform, fusiform to ovoid, straight or curve shape. Chlamydospores may be present or absent.[37]

Besides the similarity of phenotypically among *Fusarium* species, morphological identification is often difficult due to variability between isolates in term of shape and size of microconidia and colony colour. This is because not all features required always well developed on subculture isolate, such as the absence of macroconidia.[37]

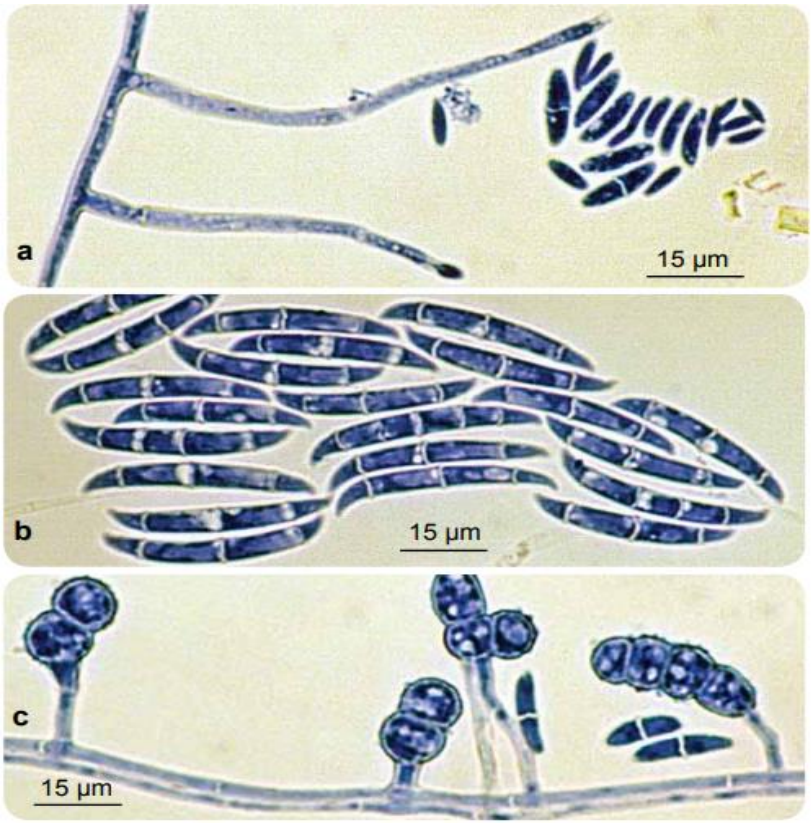

Figure 1.2. Microscopic differences between *Fusarium solani* and *Fusarium oxysporum.* Picture of microscopic direct visualization of *Fusarium solani* complex. (a) microconidia on long phialides, (b) macroconidia, (c) chlamydospores.

(Pictures are adopted from

https://mycology.adelaide.edu.au/descriptions/hyphomycetes/fusarium/*)*

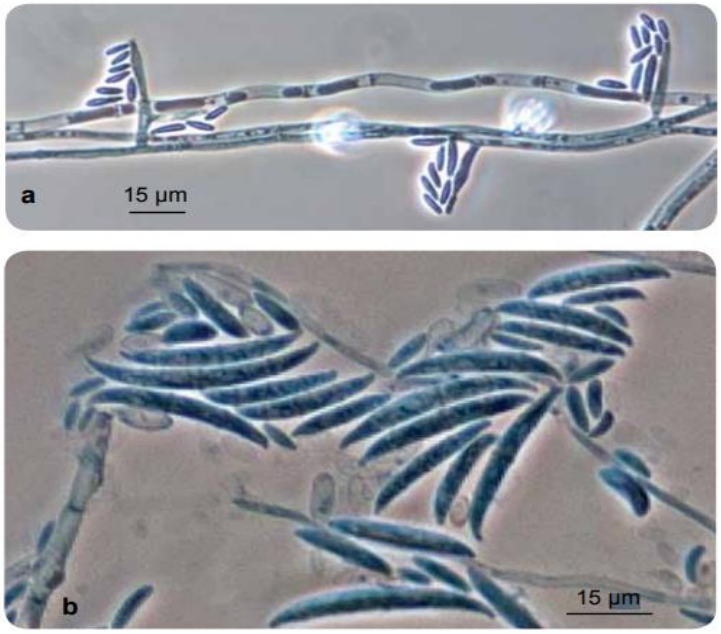

Figure 1.3. Microscopic differences between *Fusarium solani* and *Fusarium oxysporum. Fusarium oxysporum* complex. (a) miccroconidia on short phialides and (b) macroconidia.

(Pictures are adopted from

https://mycology.adelaide.edu.au/descriptions/hyphomycetes/fusarium/*)*

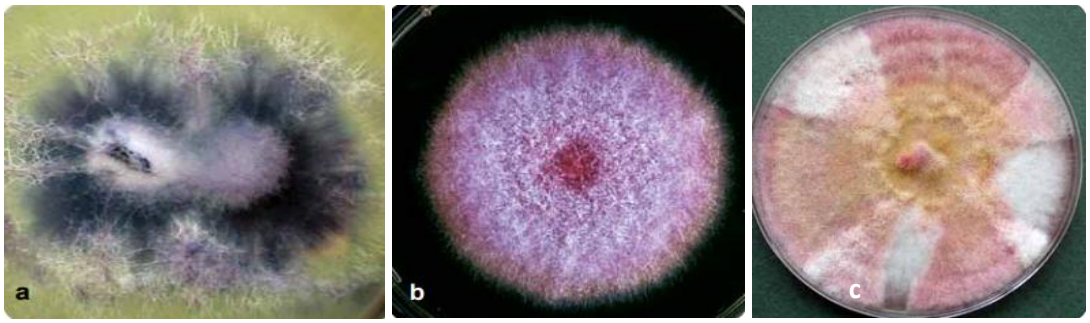

Figure 1.4. Picture of various colonies *Fusarium* species complex

(a) *Fusarium oxysporum* complex showing purple pigmentation

(b) *Fusarium fujikuroi* complex showing pink pigmentation

(c) *Fusarium chlamydosporum* complex, showing pink to ochraceous to brownish surface

(Pictures are adopted from

https://mycology.adelaide.edu.au/descriptions/hyphomycetes/fusarium/*)*

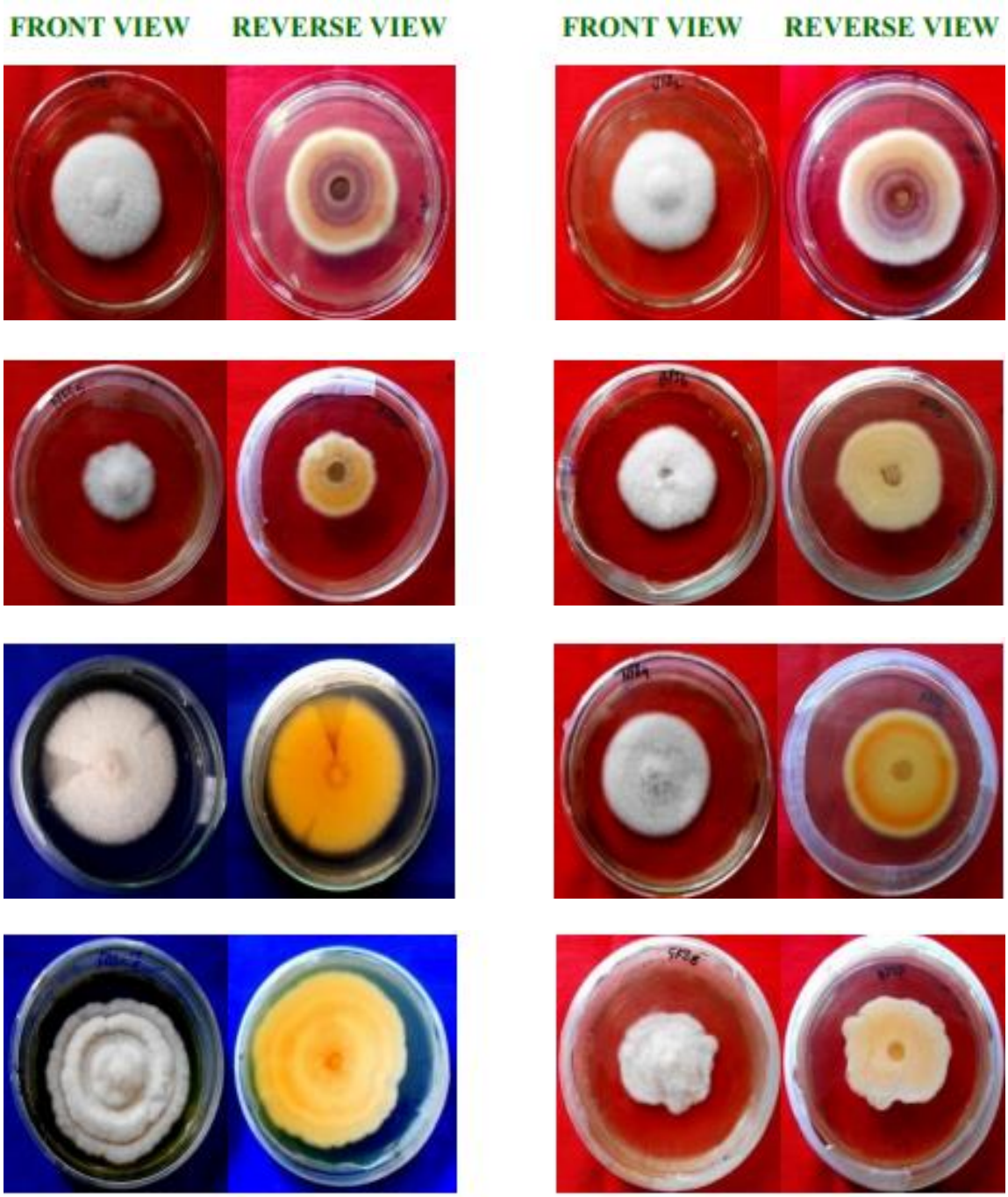

Figure 1.5. Morphological characteristics of different *Fusarium solani* species on Potato Dextrose Agar (PDA).

(*Adopted from* http://doi.org/10.20546/ijcmas.2017.611.225, ISSN: 2319-7706 Volume 6 Number 11 (2017) pp. 1889-1901)

1.3.2 Molecular methods

As phenotypic identification has become more complicated in recent years as the phylogenetic taxonomy revealed cryptic species within morphologically indistinguishable isolates[39]. Thus, combinations of phenotypic and molecular approach are more accurate in *Fusarium* identification. The amplification of DNA sequences through the polymerase chain reaction (PCR) has wide application in the diagnosis and fungi detection[40].

1.3.2a Fungal DNA sequencing

To date, only three marker loci tested meet the three important criteria for phylogenetic species recognition which are: 1) applicable across the phylogenetic breadth of *Fusarium* (Figure 1.1), 2) Informative or at near the species-level, and 3) orthologous across the genus.[24] Internal transcribed spacer (ITS) and D1/D2 sequences, have been used mainly to identify pathogenic fungi, but are too conserved to resolve species limits of the most *Fusarium*. Identifications based on morphology and/or ITS and D1/D2 sequences should be reported as species complexes. For accurate species identification, sequencing of *TEF*, *RPB1* and/or *RPB2* is required.[41]

A study by Thomas *et al.* in 2018, which did a comparison on type of gene and database to choose in clinical practice for molecular identification of species complexes, five identification scheme were evaluated: ITS/GenBank; ITS/*Fusarium* MLST; TEF/*Fusarium* MLST; RPB1/*Fusarium* MLST; RPB2/*Fusarium* MLST. Their study observed identification performance of ITS, *RPB1* and *RPB2* genes depending on the species complex to be identified. For example, *RPB2* analysis with *Fusarium* MLST was indicative for FSSC, but less performance on FOSC and *Fusarium fujikuroi* species

complex (FFSC). *TEF-1α* was the most versatile gene, in which its performance was less variable, and also depending on the species complexes identified.[42]

1.3.2b Translation elongation factor 1-alpha (*TEF-1α*) Sequencing

The *TEF-1α* gene has been widely used by researchers for molecular identification as it has high phylogenetic utility because (i) it is highly informative at the species level in *Fusarium*; (ii) non-orthologous copies of the gene have been detected in the genus; and (iii) availability of universal primers that work across the phylogenetic breadth of the genus.[43] *TEF-1α* encodes an essential part of the protein translation machinery in eukaryotic cells. These primers (EF1 – forward primer; and EF2 – reverse primer) amplify an approximately 700 bp region of *TEF-1α,* with flanking introns that total over half of the amplicon's length, in all known fusaria (Figure 1.6)[43]. This gene amplification shows a high level of polymorphism among closely related species. For these reasons, *TEF-1α* has become the marker of choice as a single locus-identification tool in *Fusarium.* Amplification sequence from *TEF-1α* then will be compared against FUSARIUM-ID sequence database using BLAST to identify the closest matches of *Fusarium* species.

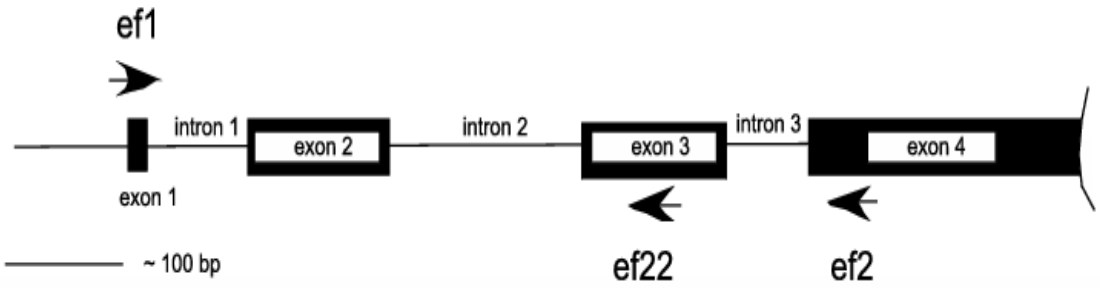

Figure 1.6. Schematic representation of the *TEF-1α* gene region in *Fusarium* used in FUSARIUM-ID, with primer locations (Adopted from Geiser DM, del Mar Jiménez-

Gasco M, Kang S, et al. FUSARIUM-ID v. 1.0: a DNA sequence database for identifying Fusarium. *European Journal of Plant Pathology.* 2004;110(5-6):473-479).

1.4 Treatment

Prompt and appropriate therapy of localised disease is vital to prevent progression to disseminated infection, which includes surgical debridement, and probably systemic antifungal therapy.[26] The optimal treatment for disseminated fusariosis remains unclear.[30] Most *Fusarium* species show resistance to multiple antifungal agents[4], however resistant patterns vary among *Fusarium* species. Voriconazole, itraconazole and polyenes (amphotericin B and its lipid formulations) have been associated with some success from few studies which conducted in the 1990s.[44,45] Recent study of Molecular Characterization and antifungal susceptibility of clinical *Fusarium* species from Brazil has shown that amphotericin B had relatively high activity with MICs value ranging from 0.5 to 32 mcg/ml. One of the clinical *Fusarium* isolates exhibit higher MICs values of 8 and 32mcg/ml. All isolates had shown high MICs to itraconazole, and other azoles were showed to be less effective against *Fusarium solani* species complex (FSSC); in which FSSC is the most common pathogen affecting humans.[35]

1.5 Rationale of the study

Regardless of the growing attention being focused on all fungal infections, little is known about the current epidemiology of human *Fusarium* infections, especially in Malaysia. Despite the knowledge that it has been emerged as major opportunistic in immunosuppressed patients and can cause superficial infection in an immunocompetent individual, data on its course and evaluation is still scarce. In addition, specific identification of *Fusarium* species is necessary as few species have been shown associated with severe clinical presentation.

This study aims at determining the specific identification of *Fusarium* species using the molecular method. In Malaysia, few studies have conducted on *Fusarium* at the species level among major plant pathogen. However, there is a lack of data on *Fusarium* infection at the species level in human infection. The clinical epidemiology of *Fusarium* infection and the clinical significance of isolates grown from various clinical specimens will be studied. The clinical manifestation and risk factors of being infected by *Fusarium* species will be identified.

This study will benefit and evoke clinicians so that they will be more alert to the prevalence of *Fusarium* infection, especially in immunocompromised patients with high-risk association. In a few data gathered regarding human *Fusarium* infection in Malaysia, there are only few studies which done which in 1999, 9.2% of *Fusarium* species isolated from onychomycosis caused by mold[46] and in 2008, 4.7% *Fusarium* species have been identified as fungal pathogen isolated from keratitis in HUSM.[47] The third study in 2012, *Fusarium* species accounted for 46.34% (19/41), which is the most commonly fungal isolated for fungal keratitis in 5 years retrospective review in HUSM.[48] A study by Tzar *et al.* on Dermatomycoses in Kuala Lumpur, Malaysia in

2014, the prevalence of *Fusarium* infection infects the nails and skin was 3.5%.[49] The first phylogenetic analysis of *Fusarium* focused on *Fusarium solani* species complex done by School of Biological Science as a plant pathogen.[50] Up to date, there is no molecular study has published on *Fusarium* at the species level which causes human infection in Malaysia. Early diagnosis of *Fusarium* infection is important as appropriate antifungal will be initiated, and poor clinical outcome will be prevented.

1.6 Study objective

1.6.1 General objectives

To study the proportion *Fusarium* species, the clinical manifestations of their infections, the risk factors and outcomes for *Fusarium* isolation

1.6.2 Specific objectives

1. To determine the molecular identification of *Fusarium* species isolated from clinical samples
2. To determine the proportion of *Fusarium* species isolated from clinical samples
3. To describe the clinical manifestations and outcomes of *Fusarium* infections
4. To determine the risk factors of *Fusarium* infections

REFERENCES

1. Nelson PE, Dignani MC, Anaissie EJ. Taxonomy, biology, and clinical aspects of *Fusarium* species. *Clinical Microbiology Reviews.* 1994;7(4):479-504.
2. Austen B, McCarthy H, Wilkins B, Smith A, Duncombe A. Fatal disseminated *Fusarium* infection in acute lymphoblastic leukaemia in complete remission. *Journal of Clinical Pathology.* 2001;54(6):488-490.
3. Van Diepeningen AD, Brankovics B, Iltes J, Van der Lee TA, Waalwijk C. Diagnosis of *Fusarium* infections: approaches to identification by the clinical mycology laboratory. *Current Fungal Infection Reports.* 2015;9(3):135-143.
4. Nucci M, Anaissie E. *Fusarium* infections in immunocompromised patients. *Clinical Microbiology Reviews.* 2007;20(4):695-704.
5. Lamprecht S, Tewoldemedhin Y, Botha W, Calitz F. *Fusarium graminearum* species complex associated with maize crowns and roots in the KwaZulu-Natal province of South Africa. *Plant disease.* 2011;95(9):1153-1158.
6. Nucci M, Anaissie E. Cutaneous infection by *Fusarium* species in healthy and immunocompromised hosts: implications for diagnosis and management. *Clinical Infectious Diseases.* 2002;35(8):909-920.
7. Anaissie EJ, Kuchar RT, Rex JH, et al. Fusariosis associated with pathogenic *Fusarium* species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infections. *Clinical Infectious Diseases.* 2001;33(11):1871-1878.
8. Al-Hatmi AM, Bonifaz A, de Hoog GS, et al. Keratitis by *Fusarium temperatum*, a novel opportunist. *BMC Infectious Diseases.* 2014;14(1):588.

9. Salah H, Al-Hatmi AM, Theelen B, et al. Phylogenetic diversity of human pathogenic *Fusarium* and emergence of uncommon virulent species. *Journal of Infection.* 2015;71(6):658-666.

10. Van Diepeningen AD, Al-Hatmi AM, Brankovics B, de Hoog GS. Taxonomy and clinical spectra of *Fusarium* species: where do we stand in 2014? *Current Clinical Microbiology Reports.* 2014;1(1-2):10-18.

11. Shi W, Tan Y, Wang S, et al. Mycotoxigenic potentials of *Fusarium* species in various culture matrices revealed by mycotoxin profiling. *toxins.* 2017;9(1):6.

12. Link H. Observationes in Ordines plantarum naturales, Dissertatio 1 ma (Berlin Ges. NatKde 3: 1–42). *Berlin, Germany*. 1809.

13. Wollenweber H, Reinking O. *Die Fusarien*, ihre Beschreibung. *Schadwirkung und Bekämpfung.* 1935:1-355.

14. Raillo A. Fungi of the genus *Fuzarium.* 1950.

15. Booth C. The genus *Fusarium*. The genus *Fusarium.* 1971.

16. Gerlach W, Nirenberg H. The genus *Fusarium*--a pictorial atlas. *Mitteilungen aus der Biologischen Bundesanstalt fur Land-und Forstwirtschaft Berlin-Dahlem.* 1982(209).

17. Toussoun TA, Nelson PE. A pictorial guide to the identification of *Fusarium* species according to the taxonomic system of Snyder and Hansen. *A pictorial guide to the identification of Fusarium species according to the taxonomic system of Snyder and Hansen.* 1968.

18. Nelson PE, Toussoun TA, Marasas W. *Fusarium* species: an illustrated manual for identification. 1983.

19. Babadoost M. *Fusarium*: Historical and Continued Importance. *Fusarium: Plant Diseases, Pathogen Diversity, Genetic Diversity, Resistance and Molecular Markers.* 2018:13.

20. Leslie JF, Summerell BA. *The Fusarium laboratory manual.* John Wiley & Sons; 2008.

21. Geiser D, O'Donnell K. Defining the genus *Fusarium* in a scientifically robust way that best preserves longstanding use. Paper presented at: Meeting Abstract 2013.

22. Gräfenhan T, Schroers H-J, Nirenberg H, Seifert K. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora, Acremonium, Fusarium, Stilbella,* and *Volutella*. *Studies in Mycology.* 2011;68:79-113.

23. Taylor JW, Jacobson DJ, Kroken S, et al. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology.* 2000;31(1):21-32.

24. O'Donnell K, Ward TJ, Robert VA, Crous PW, Geiser DM, Kang S. DNA sequence-based identification of *Fusarium:* current status and future directions. *Phytoparasitica.* 2015;43(5):583-595.

25. Al-Hatmi AM, Hagen F, Menken SB, Meis JF, De Hoog GS. Global molecular epidemiology and genetic diversity of *Fusarium*, a significant emerging group of human opportunists from 1958 to 2015. *Emerging Microbes & Infections.* 2016;5(1):1-11.

26. Boutati EI, Anaissie EJ. *Fusarium*, a significant emerging pathogen in patients with hematologic malignancy: ten years' experience at a cancer center and implications for management. *Blood.* 1997;90(3):999-1008.

27. Pushker N, Chra M, Bajaj M, et al. Necrotizing periorbital *Fusarium* infection—an emerging pathogen in immunocompetent individuals. *Journal of Infection.* 2002;44(4):236-239.

28. Nucci M, Anaissie EJ, Queiroz-Telles F, et al. Outcome predictors of 84 patients with hematologic malignancies and *Fusarium* infection. *Cancer.* 2003;98(2):315-319.

29. Ninet B, Jan I, Bontems O, et al. Molecular identification of *Fusarium* species in onychomycoses. *Dermatology.* 2005;210(1):21-25.

30. Dignani M, Anaissie E. Human fusariosis. *Clinical Microbiology and Infection.* 2004;10:67-75.

31. Peterson A, Pham MH, Lee B, et al. Intracranial *Fusarium* fungal abscess in an immunocompetent patient: case report and review of the literature. *Journal of Neurological Surgery Reports.* 2014;75(02):e241-e245.

32. Sampathkumar P, Paya CV. *Fusarium* infection after solid-organ transplantation. *Clinical Infectious Diseases.* 2001;32(8):1237-1240.

33. Walsh TJ, Gamaletsou MN. Treatment of fungal disease in the setting of neutropenia. *ASH Education Program Book.* 2013;2013(1):423-427.

34. Al-Hatmi AM, van Diepeningen AD, Curfs-Breuker I, de Hoog GS, Meis JF. Specific antifungal susceptibility profiles of opportunists in the *Fusarium fujikuroi* complex. *Journal of Antimicrobial Chemotherapy.* 2014;70(4):1068-1071.

35. Herkert PF, Al-Hatmi A, de Oliveira Salvador GL, et al. Molecular characterization and antifungal susceptibility of clinical *Fusarium* species from Brazil. *Frontiers in Microbiology.* 2019;10:737.

36. Guevara-Suarez M, Cano-Lira JF, De García MCC, et al. Genotyping of *Fusarium* isolates from onychomycoses in Colombia: detection of two new species within the *Fusarium solani* species complex and in vitro antifungal susceptibility testing. *Mycopathologia.* 2016;181(3-4):165-174.

37. Kidd S, Halliday CL, Alexiou H, Ellis DH. *Descriptions of Medical Fungi.* David Ellis; 2016.

38. Vyzantiadis T-AA, Johnson EM, Kibbler CC. From the patient to the clinical mycology laboratory: how can we optimise microscopy and culture methods for mould identification? *Journal of Clinical Pathology.* 2012;65(6):475-483.

39. Zhang N, O'Donnell K, Sutton DA, et al. Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. *Journal of Clinical Microbiology.* 2006;44(6):2186-2190.

40. Louie M, Louie L, Simor AE. The role of DNA amplification technology in the diagnosis of infectious diseases. *Cmaj.* 2000;163(3):301-309.

41. O'Donnell K, Rooney AP, Proctor RH, et al. Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. *Fungal Genetics and Biology.* 2013;52:20-31.

42. Thomas B, Audonneau NC, Machouart M, Debourgogne A. Molecular identification of *Fusarium* species complexes: Which gene and which database to choose in clinical practice? *Journal de Mycologie Medicale.* 2019;29(1):56-58.

43. Geiser DM, del Mar Jiménez-Gasco M, Kang S, et al. FUSARIUM-ID v. 1.0: a DNA sequence database for identifying *Fusarium*. *European Journal of Plant Pathology.* 2004;110(5-6):473-479.

44. Goldblum D, Frueh BE, Zimmerli S, Böhnke M. Treatment of postkeratitis *Fusarium* endophthalmitis with amphotericin B lipid complex. *Cornea.* 2000;19(6):853-856.

45. Wolff MA, Ramphal R. Use of amphotericin B lipid complex for treatment of disseminated cutaneous *Fusarium* infection in a neutropenic patient. *Clinical Infectious Diseases.* 1995;20(6):1568-1568.

46. Ng K, Saw T, Madasamy M, Soo Hoo T. Onychomycosis in Malaysia. *Mycopathologia.* 1999;147(1):29-32.

47. Norina T, Raihan S, Bakiah S, Ezanee M, Liza-Sharmini A, Wan Hazzabah W. Microbial keratitis: aetiological diagnosis and clinical features in patients admitted to Hospital Universiti Sains Malaysia. *Singapore Med J.* 2008;49(1):67-71.

48. Mohd-Tahir F, Norhayati A, Siti-Raihan I, Ibrahim M. A 5-year retrospective review of fungal keratitis at Hospital Universiti Sains Malaysia. *Interdisciplinary Perspectives on Infectious Diseases.* 2012;2012.

49. Tzar M, Zetti Z, Ramliza R, Sharifah A, Leelavathi M. Dermatomycoses in Kuala Lumpur, Malaysia. *Sains Malaysiana.* 2014;43(11):1737-1742.

50. Chehri K, Salleh B, Zakaria L. Morphological and phylogenetic analysis of *Fusarium solani* species complex in Malaysia. *Microbial Ecology.* 2015;69(3):457-471.

CHAPTER 2:

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