

IDENTIFICATION AND DIFFERENTIATION OF MALASSEZIA SPECIES IN PITYRIASIS VERSICOLOR BY PCR-RFLP METHOD

Thesis Submitted for partial fulfillment of Master Degree
in Dermatology, Venereology and Andrology

Presented by:
Nargz Hussein Abdullah Al-Azawi

M. B.Ch.B.

Supervised By:
Prof.Dr. Adel Imam M.D.

Head of Department & Professor of Dermatology , Venereology and Andrology

Faculty of Medicine

Ain Shams University

Dr. Ranya Lotfi M.D.

Assistant Professor of Dermatology , Venereology and Andrology

Faculty of Medicine

Ain Shams University

Dr. Wael Saudi M.D.

Lecturer of Dermatology , Venereology and Andrology

Faculty of Medicine

Misr University for Science and Technology

Faculty of Medicine

Ain Shams University

2013

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَقُلْ
رَبِّ زِدْنِي عِلْمًا

وَقُلْ رَبِّ زِدْنِي عِلْمًا

Acknowledgement

First, and above all I would like to kneel thanking our God for the unlimited help he granted me to conduct this work.

I would like to express my deepest gratitude, thanks and appreciation to **Prof. Dr. Adel Imam** M.D., Professor & Head of Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University, for his encouragement, advice, valuable guidance and support throughout this study.

I would like to express my thanks and gratefulness to **Dr. Ranya Lotfi** M.D., Assistant Professor of Dermatology, Venereology and Andrology Faculty of Medicine, Ain Shams University, for her assistance, advice and valuable guidance to accomplish this study.

I am profoundly grateful to **Dr. Wael Saudi** M.D., Lecturer of Dermatology, Venereology and Andrology, Faculty of Medicine, MISR University for Science and Technology, for his great support and his co-operation to complete and finish this work.

I wish to extend my thanks to all members of the department of dermatology, mycology and molecular units staff MISR university hospital for their great support and scientific guidance, with special thanks to: **Dr. Mohamad Mostafa**, Associated professor of Biotechnology, MISR University and **Dr. Marwa Saad**, Lecturer of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University.

Last but not least, I would like to express my sincere thanks to all my family, who helped me, supported me, encouraged me, and prayed to me.

List of Contents

Title	Page No.
Introduction	1
The Aim of the Work	3
Review of Literature	4
Malassezia Yeast	4
Historical Review and Nomenclature	5
Taxonomic Classification.....	7
Epidemiology	8
Biological Characteristics of <i>Malassezia</i> Organism	9
Structure	9
Reproduction.....	10
Biochemistry	12
Dermatological Diseases Caused By <i>Malassezia</i>	12
Pityriasis Versicolor	12
Definition	12
Incidence	13
Etiology	15
Pathogenesis.....	15
Clinical Picture.....	19
Diagnosis.....	21
Mycological Examination.....	21
Culture.....	24
Skin Biopsy and Histopathology	26
Blood Examination	28
Identification of <i>Malassezia</i> Species	28

Conventional identification of <i>Malassezia</i> Species	28
Molecular Identification of <i>Malassezia</i> Species	32
Polymerase Chain Reaction (PCR)	33
PCR used in <i>Malassezia</i> Species Identification	36
Other Dermatological diseases caused by <i>Malassezia</i>	38
Dandruff	38
Seborrheic Dermatitis (SD)	39
Malassezia Folliculitis	41
Atopic Dermatitis (AD)	42
Psoriasis	43
Neonatal Cephalic Pustulosis	44
Acne	45
Onychomycoses	45
Confluent and Reticulate Papillomatosis (CRP)	47
<i>Malassezia</i> in Systemic Infections	48
<i>Malassezia</i> Species Infections in Children and Adults	48
<i>Malassezia</i> Species Infections in Infants	49
HIV Infection and <i>Malassezia</i>	50
Patients & Method	51
Results	56
Discussion	66
Summary	71
Recomondations	73
References	74
Arabic Summary	

LIST OF FIGURES

<i>Figure No.</i>	<i>Title</i>	<i>Page No.</i>
Figure (1):	Microscopic appearance of <i>Malassezia</i>	9
Figure (2):	Reproduction of <i>Malassezia</i>	11
Figure (3):	Direct examination of PV with potassium hydroxid	22
Figure (4):	Direct examination of PV with potassium hydroxide plus ink.....	23
Figure (5):	Photograph of colonies of <i>Malassezia furfur</i> on GYP-S agar.	25
Figure (6):	Culture of M. furfur on Dixon's agar	25
Figure (7):	Histopathology of PV.....	27
Figure(8):	Primer	33
Figure(9):	The different steps in PCR	35
Figure (10):	Eppendorf tube in vortex device	56
Figure (11):	Gel- electrophoresis device.....	56
Figure(12):	Direct examination of PV scales.....	58
Figure (13):	sex distribution in PV.....	58
Figure (14):	Body site distribution in PV.....	59
Figure (15):	single band specific for <i>Malassezia</i> as detected by gel electrophoresis.....	60
Figure (16):	PCR-RFLP restriction fragment patterns of 26S rDNA digested with <i>HhaI</i> restriction enzyme.....	61

Figure (17):	Relation of <i>Malassezia</i> species in PV cases to age.....	62
Figure (18):	Relation of <i>Malassezia</i> species in PV cases to sex.....	63
Figure (20):	Relation of <i>Malassezia</i> species in PV cases to clinical form of lesion.....	65

LIST OF TABLES

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (1):	Morphological characteristics of the <i>Malassezia</i> species.....	29
Table (2):	Biochemical Characteristics of the <i>Malassezia</i> species.....	31
Table (3):	Description of dermagraphism of cases	57
Table (4):	Description of isolated <i>Malassezia</i> species and PCR results	60
Table (5):	Relation of <i>Malassezia</i> species in PV cases to age and sex	62
Table (6):	Relation of <i>Malassezia</i> species in PV cases to body site and clinical Form	64

Abbreviations

PV.....Pityriasis versicolor

M*Malassezia*

PCRPolymerase chain reaction

SD.....Seborrheic Dermatitis

AD.....Atopic Dermatitis

PS.....Psoriasis

CRP.....Confluent and Reticulate Papillomatosis

ICUs.....Intensive care units.

GYP-S.....Glucose-Yeast-Pepton-Supplement

RFLPRestriction fragment length polymorphism

INTRODUCTION AND AIM OF THE WORK



Introduction

Pityriasis versicolor, is a chronic superficial fungal infection that appears as flat, slightly scaly discolored patches on the upper trunk, neck, and upper arms.

The word “versicolor” implies that this rash can have several different colors, and indeed the eruption may be lighter or darker than a person’s normal skin or even have a reddish appearance (**Deepak et al., 2005**). PV is one of the most common dermatomycosis, and is especially prevalent in regions with a warm humid climate, where up to 40% of the population may be affected, Pityriasis versicolor is the prototypical skin disease etiologically connected to *Malassezia* species (**Gaitanis et al., 2012**).

Members of the genus *Malassezia* are lipophilic and/or lipid-dependent, unipolar budding yeasts, characterized by a thick cell wall. These yeasts are often common commensals of the skin of humans and other animals (**Cafarchia et al., 2011**).

Malassezia yeasts, since first reported in 1889, are known to be implicated in various diseases, including pityriasis versicolor, seborrheic dermatitis, and *Malassezia* folliculitis. Recently, there have been a growing number of reports which show the implication of *Malassezia* yeasts in atopic dermatitis, confluent and reticulated papillomatosis (Gougerot-Carteaud), and *Malassezia* onychomycosis. Furthermore the pathogenicity of *Malassezia* yeasts comes into the surface as systemic infections with *Malassezia* are identified in premature neonates and immunodeficient adult patients undergoing lipid replacement therapy via vein catheter (**Jang et al., 2009**).

The use of molecular methods has revolutionized the study of disease due to *Malassezia* species because the organisms are fastidious and difficult to identify, the introduction of a new taxonomy in 1996 led to a series of revealing

studies of the etiology of diseases linked to *Malassezia* species (**Hay and Jones, 2010**). *Malassezia* species can be identified through their morphological features and biochemical characterization (**Guillot et al., 1996**).

However, these phenotypic methods are usually time consuming, lack sufficient discriminatory power, and are unable to unambiguously differentiate newly identified species. Although various DNA-based molecular methods have been described to overcome this problem (**Theelen et al., 2001**). A simple, reliable, and cost effective method is still needed for differentiation of *Malassezia* species, therefor, the application of PCR-based technique using restriction enzyme digestion used for discrimination between *Malassezia* species (**Mirhendia et al., 2005**).

Aim of the work

To isolate and identify different species of *Malassezia* in pityriasis versicolor patients by using the rapid and accurate molecular biology method (PCR- RFLP) which overcome the limits of morphological and biochemical methods.

REVIEW OF LITERATURE



Malassezia Yeast

The genus *Malassezia* comprises a group of superficial dimorphic fungi occurring as normal skin flora on the human body, however, they can also cause infection or are associated with certain skin diseases. Rarely, they can become invasive, to cause opportunistic systemic infection in the presence of certain predisposing factors (**Inamadar and Palit, 2003**).

However, the complexity of the interaction of a unicellular eukaryotic organism (*Malassezia*) with a tissue of a multicellular organism (skin) makes understanding the interactions and development of disease a complex process (**Gaitanis et al., 2012**).

Their habitat is primarily the skin of mammals and birds, with the highest counts being on the areas rich in sebaceous gland such as head and upper trunk, the organisms become part of the normal skin flora by three to six months of age. The frequency and density of colonization in healthy individuals is related to the age and to the activity of the sebaceous glands in the area studied. It has been demonstrated that *Malassezia* yeasts inhabit various body sites including scalp, forehead, shoulder, abdomen, lower axilla, groin and forearm, due to increased density of sebaceous glands at these sites (**Smolinski, 2005**).

During the past two decades, this group of fungi has gained on increasing importance. The nomenclature has been changed, newer species have been identified and associations of the organism with different disease entities have been described (**Midgley et al., 1998**).