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Review Article

Phenotypic and Genotypic Characterization of *Malassezia* Species Isolated from *Malassezia* Associated Skin Diseases: review article

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Abstract

Lipophilic yeasts called *Malassezia* are naturally occurring in the skin flora of 75%–98% of healthy adults. They live in areas of the skin that are highly rich with sebaceous glands. The genus *Malassezia* consists of 22 species. They are also the cause of some skin-related diseases, such as: Pityriasis versicolor (PV), seborrheic dermatitis (SD), atopic dermatitis (AD), dandruff and complicated systemic fungal infections, particularly in infants and kids with impaired immune system. Since *Malassezia* species are dimorphic fungi, their transformation from the yeast to the mould phase allows them to invade the stratum corneum and break through corneocytes, which results in pityriasis versicolor. It is a chronic condition characterized by skin pigmentation that is difficult to cure and has high chance of relapse and recurrence. Seborrheic dermatitis (SD) is a common inflammatory dermatosis that can affect children, adolescents and adults of various ethnicities and races. Since *Malassezia* species have similar morphological and biochemical characteristics, pityriasis versicolor cannot usually be accurately and quickly diagnosed with the phenotypic approaches currently in use. Consequently, a number of genetic typing methods have been effectively applied, resulting in the identification and classification of new *Malassezia* species. Molecular techniques such as DNA sequence analysis, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and pulsed field gel electrophoresis (PFGE) are used in genotypic identification. This review aims for phenotypic and genotypic identification of *Malassezia* and *Malassezia* species. Also aims for antifungal susceptibility testing for *Malassezia* species by disc diffusion method.

Keywords: *Malassezia*, lipophilic, Pityriasis versicolor, Phenotypic, Genotypic.

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Introduction:

For more than a century, lipophilic organisms known as *Malassezia* have been identified as both agents of specific skin disorders and typical components of the human cutaneous flora. Furthermore, they have been linked to opportunistic systemic infections since the 1980s. ⁽¹⁾

The genus *Malassezia* consists of 22 species, namely *M. globosa*, *M. sympodialis*, *M. furfur*, *M. restricta*, *M. slooffiae*, *M. obtusa*, *M. dermatis*, *M. japonica*, and *M. yamatoensis* are associated with normal human flora but can also cause skin lesions, while *M. pachydermatis*, *M. nana*, *M. equina*, *M. caprae*, and *M. cuniculi* are associated with animals. There have been few studies that have found that *M. pachydermatis* can be transmitted from pets to man. All species are lipid dependent, but only *M. pachydermatis* is lipid independent. ⁽²⁾

Malassezia arunalokei, *Malassezia brasiliensis* from parrots, *Malassezia equi* from horse skin, *Malassezia muris* from mouse skin, *Malassezia ochoterenai*, *Malassezia psittaci* from parrots, *Malassezia tropica* from humans, and *Malassezia vespertilionis* from vesper bats were among the eight species that were later identified; most of them were found in animals. ⁽³⁾

Epidemiology:

More than 90% of all skin fungi are members of the genus *Malassezia*, which is the most prevalent fungus on mammalian skin. The pathogenic potential of the *Malassezia* yeast lies in their ability to interact with the host immune system and either directly or through chemical mediators penetrates the stratum corneum. *Malassezia* is thought to affect around 140 million people globally each year and is linked to a number of skin conditions, some of which are severe and chronic. Less frequently, members of the genus cause invasive disease in premature infants and other immunocompromised and debilitated individuals, and the majority of infections are endogenous in origin. The species are likely distributed on seborrheic skin areas because they

lack fatty acid synthase genes, which explain the site of *Malassezia* folliculitis, pityriasis versicolor, seborrheic dermatitis, and atopic dermatitis. ⁽⁴⁾

Pathophysiology of *Malassezia* on Human Skin:

Using culture-independent techniques, it was determined that the most prevalent skin eukaryotes, accounting for 50% to 80% of the total skin mycobiome, were *Malassezia* yeasts. It was discovered that bacteria were more prevalent on human skin than fungus. *Malassezia* species predominated at all sampled body sites except the foot. ⁽⁵⁾

Malassezia begins colonizing a baby's skin as soon as they are born and keeps growing until they are between six and twelve months old. After that, the rate of colonization remains low until just before puberty, when sebaceous gland activation improves the environment and *Malassezia* populations stabilize. Recent metagenomic data indicates that skin colonization changes with puberty and age. It has been proposed that a greater colonization of adult *Malassezia* has a protective effect on the skin by preventing the colonization of more pathogenic species, including dermatophytes and other more prevalent pathogenic species. ⁽⁶⁾

The complex interactions of *Malassezia* with the skin, an organ that has been under intense selection pressure throughout evolution, have left the pathophysiology of skin illnesses caused by or worsened by this commensal poorly understood. Without causing disease, *Malassezia* yeasts develop and grow in healthy skin by utilizing vital nutrients. When this process is disturbed, *Malassezia* yeasts adapt by changing the expression of energy-gaining enzymes such as lipases and phospholipases and simultaneously producing a class of bioactive indoles that function via the aryl-hydrocarbon receptor (AhR), which is expressed on nearly all types of cells in the epidermis. ⁽⁷⁾ Figure(1)

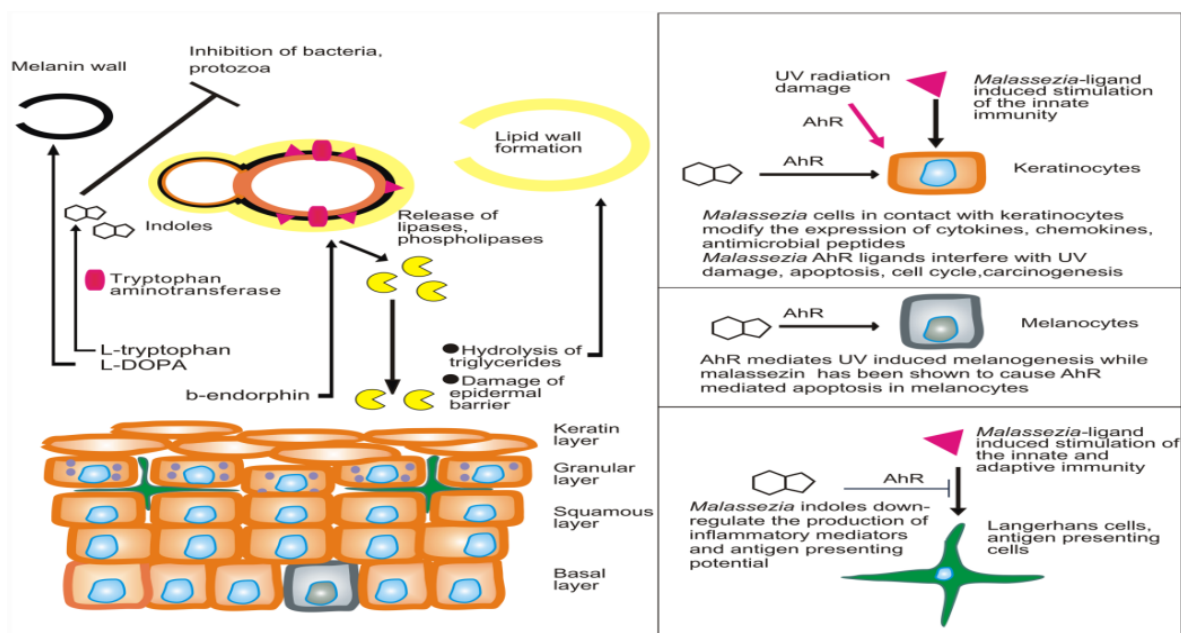


Figure (1): Model showing the interactions of *Malassezia* yeasts with the skin ⁽⁷⁾

In addition to absorbing nutrients, *Malassezia* yeasts also absorb amino acids required for the production of melanin and AhR indolic ligands and sebum lipids that form the outer layer of *Malassezia* yeast. Under the influence of β -endorphin, they simultaneously alter the production of lipases and phospholipases. The innate and adaptive immune systems recognize cellular components as (proteins, enzymes, glyceroglycolipids, and mannosyl fatty acids) and change how they work. Indirubin and indolo carbazole (ICZ) are examples of AhR ligands that may restrict immune stimulation, alter the epidermal cell function, inhibit AhR-induced UV damage and melanogenesis, and most likely suppress antagonist microorganisms. ⁽⁸⁾

Virulence factors of *Malassezia* species:

Malassezia yeast is mostly known for its lipolytic activity, unique cell wall construction, hyphae formation, and the recently discovered tryptophan-dependent pigment synthesis. These characteristics have significant implications for infections as the relatively high lipid content in the cell wall of *Malassezia* fungi appears to protect the yeasts from phagocytosis and down regulate the inflammatory immune response. Phospholipase production has been observed in *Malassezia furfur*, but the involvement of this enzyme in the development of human skin disease is not well understood. *Malassezia* yeasts are able to produce reactive oxygen species (ROS) which are supposed to play a role in the pathogenesis of

seborrheic dermatitis and the pigmented variant of pityriasis versicolor, since hyphae are widely distributed in scales removed from lesions, it is believed that the hyphal stage contributes to the pathophysiology of pityriasis versicolor (PV) and melanin or melanin-like pigments are thought to be responsible for multiple events, including host immune system evasion and resistance to antifungal drugs. ⁽⁹⁾

Predisposing factors to *Malassezia* –associated skin diseases:

Hyperhidrosis, systemic corticosteroid usage, HIV infection, and other host factors, as well as exogenous factors such as; tropical climates and higher temperature of summer months, contribute to the development of *Malassezia* –associated skin diseases. ⁽¹⁰⁾

***Malassezia*-Associated Skin Diseases:**

Pityriasis versicolor is a chronic superficial fungal illness characterized by round to oval, hyper- or hypopigmented skin lesions. It is most usually observed on the neck, trunk, and upper arms, which are considered seborrheic areas. The infection is caused by *Malassezia* yeasts. The lesions are usually asymptomatic, however occasionally they can be itchy. Young adults and adolescents, when sebaceous gland activity is usually at its highest, are the age groups in which PV most frequently occurs. ⁽¹¹⁾

Seborrheic dermatitis (SD) is a chronic scaly inflammatory skin disease with frequent relapses.

It mainly affects regions of the body that are highly rich in sebaceous glands, such as the face, scalp, chest, and upper trunk, where scaly erythematous patches are present. These are best found behind the ears, on the chest above the sternum, in the nasolabial folds, on the scalp, and in the eyebrows. Men are more likely than women to have the disease, which affects 1-3 % of the overall population. People with immunosuppression have a higher prevalence of SD. ⁽⁹⁾

Dandruff is an extremely common scalp condition. Typical symptoms of dandruff include Scaling and flaking, it is thought that dandruff is a mild form of SD or a separate condition that can deteriorate into actual SD. The main difference between SD and dandruff is that the dandruff does not appear to be associated with inflammation. ⁽¹²⁾

The chronic disease known as atopic dermatitis (AD), also known as dermatitis with pruritus, is characterized by cycles of remission and regression. The main feature of AD is hypersensitivity to dry skin, which may be brought on by IgE antibodies and a variety of environmental factors. Patients with AD have reduced cutaneous surface barrier function, so their skin can be easily stimulated by normal cutaneous microorganisms. ⁽¹³⁾

Malassezia folliculitis (MF) is characterized by itchy papules and pustules that mostly affect the trunk and upper arms. The development of folliculitis is primarily caused by follicular obstruction, with *Malassezia* proliferation and overgrowth occurring as a subsequent event. Patients with MF often have dilated follicles with an abundant number of round or oval yeast cells in their biopsy results. Also temperature and humidity may have an impact on how the disease develops. ⁽¹⁴⁾

Laboratory Diagnosis of Malassezia:

It is important to do microscopic examination following treatment with KOH (10%) to identify the characteristic appearance of spaghetti and meatballs. Following staining with methylene blue, lactophenol cotton blue, and gram staining, the presence of rounded or oval budding yeast cells of *Malassezia*, with or without hyphae, is examined under a light microscope. Culture is usually done on Sabour dextrose agar (SDA) supplied with olive oil, modified Leeming-Notman agar, and modified Dixon's medium (MDM). Phenotypical species identification is usually done by examining colonial and microscopic morphologies such as;

colony color, shape and texture, as well as cell size, shape and bud pattern in stained smears and characterizing the activity of the enzymes catalase, urease and β -glucosidase. The tween assimilation test is performed using tween in different concentrations (20, 40, 60, and 80). Genotypic identification of *Malassezia* can be done by amplification of 26SrDNA region by conventional PCR. Many molecular biology techniques are used to overcome the limitations of culture-based methods which sometimes make it difficult to identify every species of *Malassezia*. These techniques include nested polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), real-time PCR, pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA analysis (RAPD), and sequencing analysis. ⁽⁹⁾

Treatment of Malassezia skin infections:

Malassezia species-induced infections and skin disorders are usually treated with antifungal therapy and anti-inflammatory drugs are often added if there are concomitant inflammatory skin lesions. Different patterns of responsiveness to antifungal drugs have been observed among different types of *Malassezia*. Therefore, in some cases, identification of the species of *Malassezia* may be crucial to choose the most effective antifungal drug. ⁽¹⁵⁾ Azoles, polyenes (Amphotericin B, AmB), and echinocandins are the three main types of antifungals used to treat *Malassezia* fungal infections. Commonly used to treat infections or skin conditions in humans and animals brought on by *Malassezia* fungi. Patients with PV and SD frequently receive appropriate topical treatment for *Malassezia*-related human skin disorders; however, maintenance therapy is usually advised to prevent relapse. ⁽¹⁶⁾

Topical ketoconazole (KTZ) shampoo (twice weekly) or miconazole cream (twice daily) can be used to treat PV and SD. In particular, SD was usually treated with keratolytic medications or topical corticosteroids. But because *Malassezia* is thought to be the cause of SD, the majority of the available treatments are topical antifungal drugs, either by itself or in combination with corticosteroids. For sever PV lesions and in situations where topical treatment is ineffective, systemic treatment with Fluconazole FLZ (300 mg/week for 2-3 weeks) or Itraconazole ITZ (200 mg/day for 5 or 7 days up to 3 weeks) may be used. Although the effects of these two medications seem to be

comparable, FLZ is usually advised for PV and MF and ITZ for SD.⁽¹⁷⁾

Mechanism of antifungal drug resistance:

Fungi can weaken or avoid the effect of the antifungal drugs through a series of methods, so as to achieve the purpose of drug resistance by modifying the drug targeting molecules by changing the structure of drug targeting proteins and lowering drug sensitivity to make drugs ineffective, decreasing the drug concentration by lowering the permeability of cells to reduce the amount of drug entering them, target enzyme overexpression, which causes the antifungal drug to be overwhelmed⁽¹⁸⁾. Modifications of metabolic pathways: One method that antifungal drugs work against fungi is by preventing the formation of essential compounds at important phases of fungal metabolism. For example, azole drugs prevent 14 α -methyl-3, 6-diol from being converted to ergosterol⁽¹⁹⁾ and The formation of biofilms which are complex three-dimensional structures and composed of a collection of core microbial cells, (which may be a single species or a mixture of species), attached to host tissues or antifungal surfaces and buried in an extracellular polysaccharide substance. In addition to their ability to protect fungi from harmful medications, fungal biofilms also greatly reduce their susceptibility to most antifungal drugs and can lead to the development of high-level resistance.⁽²⁰⁾

Progress in the development of new anti-drug-resistant fungal drugs:

There have only been two new antifungal medications approved since the start of the twenty-first century Caspofungin and Isavuconazole so there is an increasing need to discover new medications with a wider range of benefits due to the high incidence of fungal infections, the high mortality rate from invasive fungal infections, and the limitations of currently utilized antifungal agents⁽²¹⁾.

Developing a drug with all of these properties will be a hard task. In contrast to the development of novel antimicrobial agents, the development of antifungal agents has been prohibited by the fact that fungal pathogens are very closely related to humans, use the same eukaryotic mechanisms and therefore have few pathogen-specific targets. In order to reduce the high mortality rate of invasive mycosis and combat resistance to existing

therapies, new antifungal agents such as natural compounds, semisynthetic compounds, synthetic compounds, nanoparticles, peptides, or new antifungal therapies that need to work through new mechanisms need to be developed. However, with the development of a number of promising drugs, the future looks bright.⁽²¹⁾

Conclusion:

In conclusion, *M. furfur*, *M. globosa* and *M. pachydermatis* represent the most common *Malassezia* species causing *Malassezia* skin infections in our locality. Culture is very beneficial, but it is time consuming and it requires considerable experience. PCR-RFLP is the most ideal and rapid method for differentiation of *Malassezia* species but it is financially expensive. Antifungal susceptibility testing revealed that *M.furfur*, *M. globosa* and *M.pachydermatis* are resistant to fluocytosine.

Recommendatio

To determine *Malassezia*'s role in diseases and to choose the best antifungal treatment, it is necessary to correctly identify the species of *Malassezia* causing the disease. Antifungal medications should be used appropriately following antifungal sensitivity testing to prevent resistance. Due to its strong resistance pattern, fluocytosine should not be used to treat skin infections caused by *Malassezia*.

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