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REVIEW ARTICLE

Progress in *Malassezia* Research in Korea

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Yeasts of the genus *Malassezia* are part of the normal flora of human skin. However, they are also associated with various skin diseases. Since the introduction of *Malassezia* to the Korean Dermatologic Society two decades ago, remarkable progress has been made in our knowledge of this genus. In this paper, we review recent developments in *Malassezia* research, including taxonomy and methods for species identification, recent genome analyses, *Malassezia* species distribution in healthy conditions and in specific skin diseases, trials investigating the mechanisms underlying *Malassezia*-related diseases, as well as therapeutic options. This review will enhance our understanding of *Malassezia* yeasts and related skin diseases in Korea. **(Ann Dermatol 27(6) 647~657, 2015)**

-Keywords-
Atopic dermatitis, Korea, *Malassezia*, *Malassezia* folliculitis, Pityriasis versicolor, Seborrheic dermatitis

INTRODUCTION

Malassezia yeasts are lipophilic fungi that are part of the normal flora of the human skin. They are typically recovered from 75% to 98% of healthy adults[1,2]. However, these yeasts are also known to be associated with various diseases, including pityriasis versicolor (PV), seborrheic dermatitis, *Malassezia* folliculitis, and more recently, atopic dermatitis, which demonstrates their powerful allergenicity[3].

Malassezia belongs to the kingdom Fungi, phylum Deuteromycota, class Blastomycetes, order Cryptococcales, and the family Cryptococcaceae[4]. The type species of the genus, *Malassezia furfur* (Robin) Babillon, was first described in 1889[4]. Early taxonomic descriptions of *Malassezia* yeasts were limited by failure to culture these fungi, and thus, they were based solely on micromorphological descriptions in skin samples. Microbiological culture only became possible after the lipophilic nature of this fungus was uncovered[5]. Historically, *Malassezia* species were thought of as two separate organisms, a yeast (*Pityrosporum*) and a mycelial (*Malassezia*) fungus[6]. This dimorphism was finally resolved by Dorn and Roehnert[7] and Porro et al.[8], and since 1986, *M. furfur* is the name that has been formally accepted for both growth phases[9]. Using the medium validated by Leeming and Notman[10], Cunningham et al.[11] classified *M. furfur* isolates into three different cultural groups, which correspond to the three serological groups A, B, and C. Prior to 1996, the genus *Malassezia* comprised three taxa, *M. furfur* (serotype A, B, C), *M. pachydermatis*, and *M. sympodialis*[11-13]. In 1996, Guého et al.[12] isolated four new species, *M. globosa*, *M. obtusa*, *M. restricta*, and *M. slooffiae* on the basis of morphology, ultrastructure, physiology, and molecular biology (Fig. 1~3). Between 2002 and 2004, four new species, *M. dermatis*[14], *M. japonica*[15], *M. yamatoensis*[16], and *M. nana*[17], were identified by Japanese scientists using molecular analyses, and since then, three more species, *M. caprae*[18], *M. equina*[18], and *M. cuniculi*[19], have been identified in animals (Fig. 4). Thus, the genus *Malassezia* currently comprises 14 species, and new species continue to be discovered.

In Korea, various attempts have been made to identify members of this species. In 1996, Ahn and Ashbee[20] introduced *Malassezia* to the Korean Dermatologic Society and compared the antifungal activities of azoles against *M. furfur* serovars A, B, and C by determining their minimal inhibitory concentrations using Leeming and Notman

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Type species: *Malassezia furfur* (Robin) Baillon 1889

On glucose/peptone agar
- Growth ---- *M. pachydermatis*
- No growth
  - Catalase reaction negative ---- *M. restricta*
  - Catalase reaction positive
    - On glucose/peptone agar with 0.5% Tween 60
      - No growth
        - Long cylindrical cells; growth at 37°C ---- *M. obtusa*
        - Spherical cells; usually no growth at 37°C ---- *M. globosa*
      - Growth
        - On glucose/peptone agar with 0.1% Tween 80
          - No growth ---- *M. slooffiae*
          - Growth
            - Sympodial budding cells ---- *M. sympodialis*
            - Bud on a broad base, filament formation ---- *M. furfur*

Fig. 1. Key to species of the genus *Malassezia*. Guého et al. (Antonie Van Leeuwenhoek 1996;69:337-355)[12] and Ahn (Korean J Med Mycol 1998;3:81-88)[2].

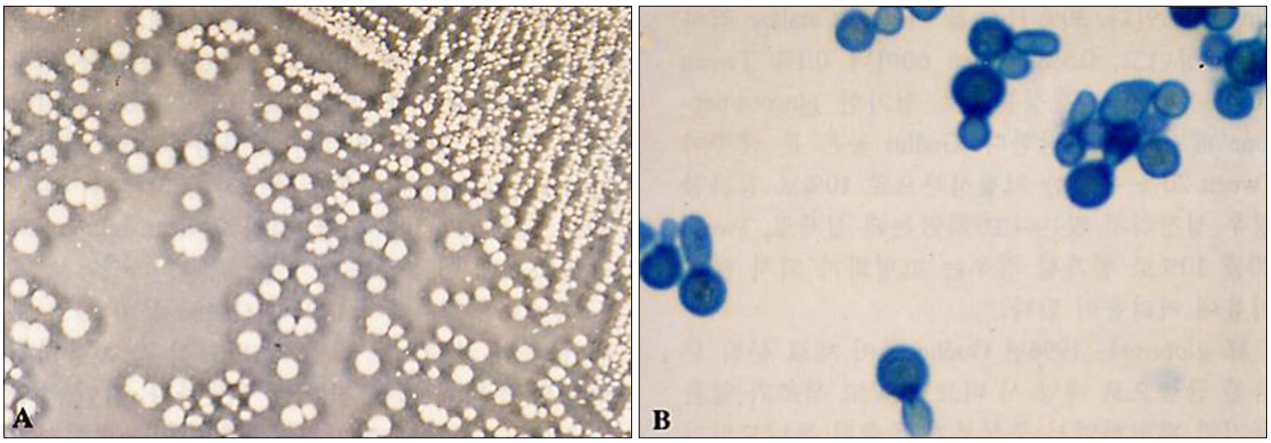

Fig. 2. *Malassezia globosa*. (A) Medium-sized, lighter in color, friable and crenated flat colonies with a pointed button center (Leeming and Notman medium, 34°C, 14 days). (B) Spherical, circular cells with buds on a narrow base (Parker Quink-KOH stain, ×1,000). Data from the article of Ahn (Korean J Med Mycol 1998;3:81-88)[2].

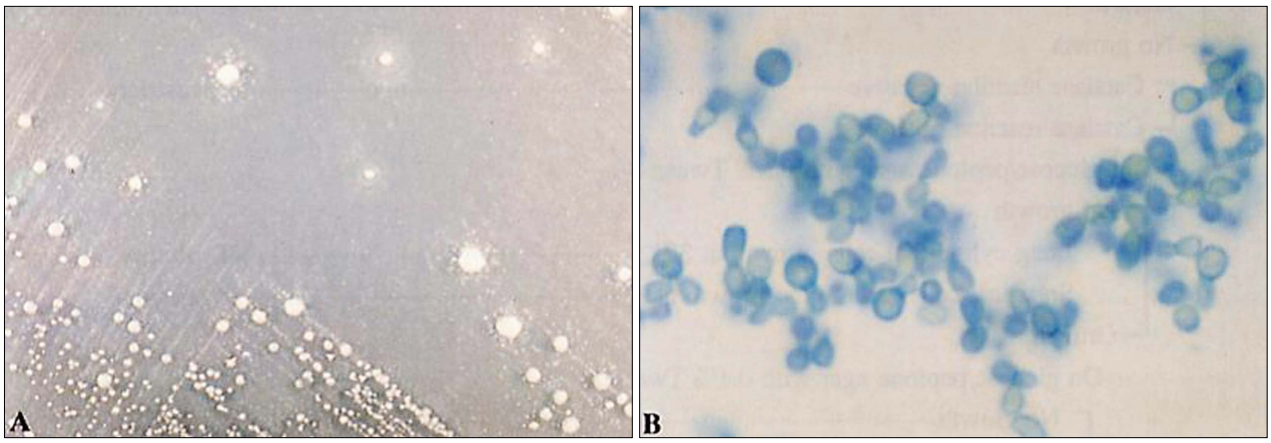

Fig. 3. *Malassezia restrica*. (A) Small-sized, circular, umbonate, entire, dull colonies (Leeming and Notman medium, 34°C, 14 days). (B) Small, spherical or oval cells with buds on a relatively narrow base (Parker Quink-KOH stain, ×1,000). Data from the article of Ahn (Korean J Med Mycol 1998;3:81-88)[2].

medium.

MAIN SUBJECTS

Malassezia-associated skin diseases

Malassezia yeasts have been implicated in various skin diseases, including PV, seborrheic dermatitis, *Malassezia* folliculitis, atopic dermatitis, and psoriasis (Fig. 5)[2]. An association between *Malassezia* yeasts and skin diseases was first reported by Eichstedt[21] in 1846, and was based on the presence of yeasts and filaments in material from the infected scales of patients with PV. In Korea, correla-

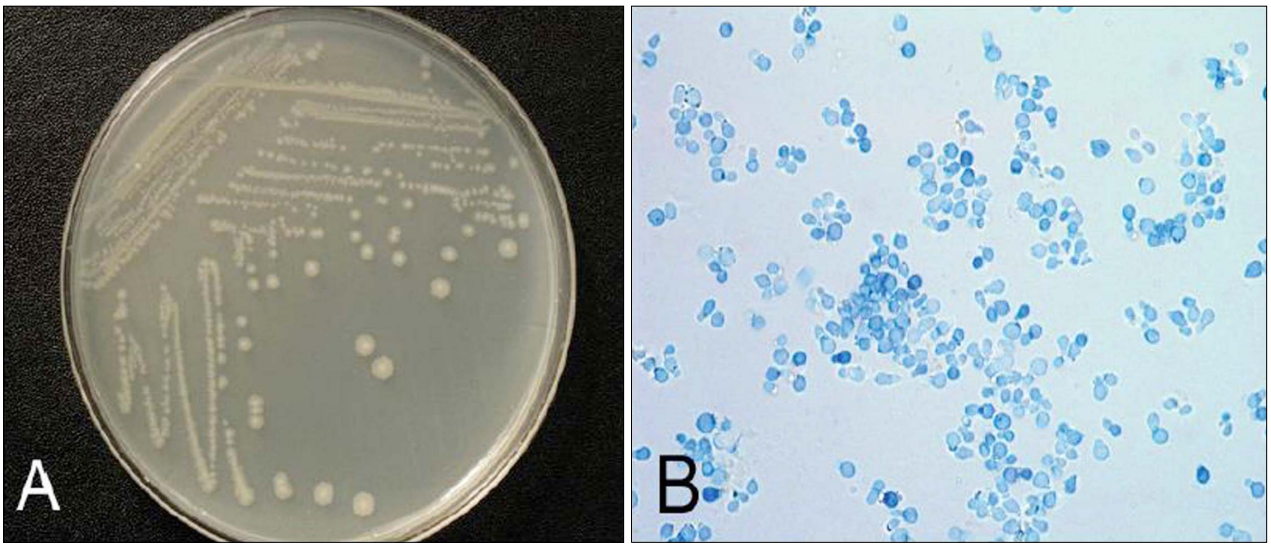

Fig. 4. *Malassezia dermatis.* (A) Large-sized, circular, smooth colonies (Leeming and Notman medium, 34°C, 14 days). (B) Spherical, oval, or ellipsoidal vegetative cells with monopolar budding (Parker Quink-KOH stain, ×1,000). Data from the article of Lim et al. (Korean J Dermatol 2007;45:1020-1030)[52].

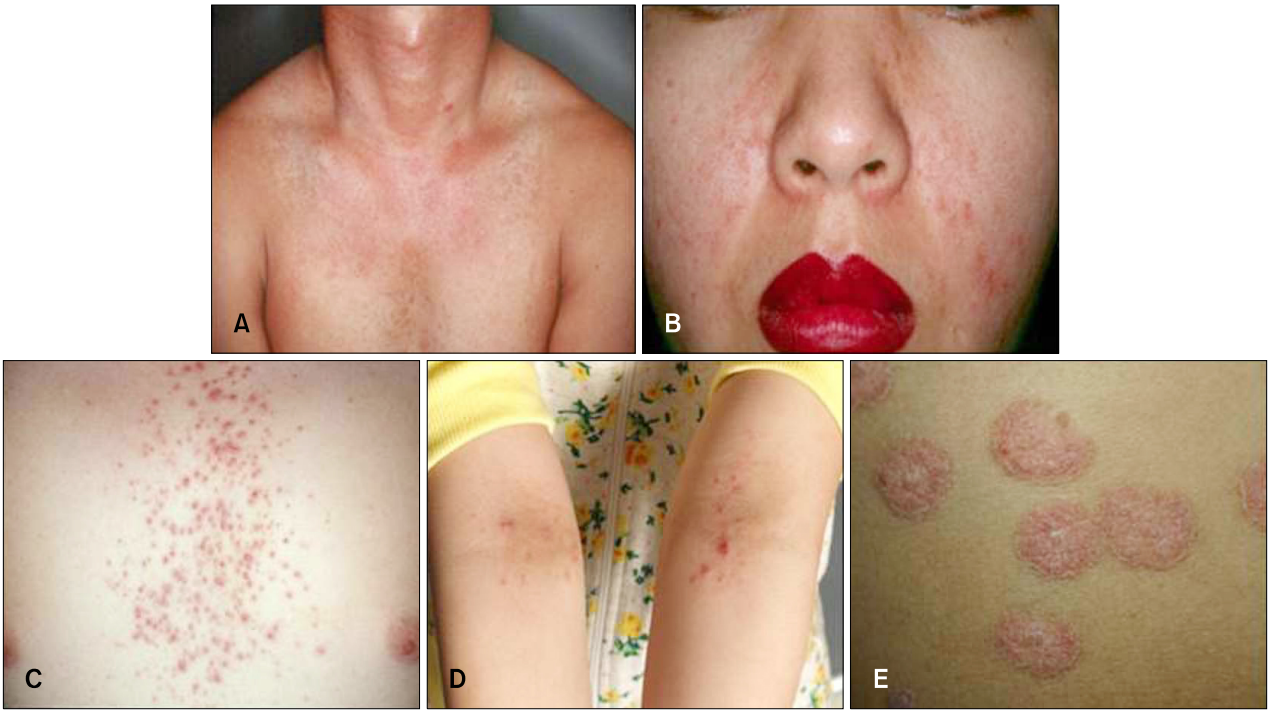

Fig. 5. Skin diseases associated with *Malassezia* species. (A) Pityriasis versicolor, (B) seborrheic dermatitis, (C) *Malassezia* folliculitis, (D) atopic dermatitis, and (E) psoriasis.

tions between various skin diseases and *Malassezia* species have been reported since 1997. After Guého et al.[12] classified *Malassezia* into seven species in 1996, Ahn[22] cultured the yeast from PV lesions to comply with the newly revised taxonomy, and reported that *M. globosa* was the most common causative agent of PV in Korea. Studies on the incidence of *Malassezia* species in acneiform eruptions[23], steroid acne[23], atopic dermatitis[24], and seborrheic dermatitis[24] were reported in 1998.

In 1999, Kang and Kim[25] investigated the frequency of *Malassezia* yeasts in the comedones of patients clinically diagnosed with acne vulgaris and found that 25% of these patients had *Malassezia* folliculitis; however, no species-specific relationship could be identified. Kim et al.[26] performed a skin prick test and measured total immunoglobulin E (IgE) and specific IgE antibodies to *M. furfur* in patients with head and neck dermatitis (HND). Of the 80 patients with HND, 45% were positive for *M. furfur* in the skin prick test, and 68% had specific IgE antibodies to *M. furfur*. The patients who showed a positive response to anti-*M. furfur*-specific IgE antibodies had more severe clinical symptoms and had higher total IgE levels. In conjunction with other environmental factors, such as sweat, heat, dryness, sun exposure, and stress, *M. furfur* was shown to aggravate HND.

Malassezia on human skin

Colonization of neonates and infants with *Malassezia* yeasts is a controversial topic. Oh et al.[27] found that 60.5% (121/200) of neonates and infants under 12 weeks carried *Malassezia* yeasts in at least one of five examined sites: scalp, forehead, cheek, earwax, and back. Ahn et al.[28] confirmed the efficacy of a one-week regimen of itraconazole by studying 20 patients with PV, and found that *M. globosa* infection was closely related to PV.

With the aim of obtaining baseline data, several studies have investigated the distribution of *Malassezia* species on normal human skin according to body region using swab and scrub-wash techniques[29-31]. The incidence of *Malassezia* yeasts was 78.4% on the scalp, 86.5% on the forehead, 100% on the chest, and 97.3% on the back[31]. In normal subjects, *M. restricta* was found predominantly on the forehead and scalp, whereas *M. globosa* was predominantly present on the chest and back[31].

In 2001, Lee et al.[32] found that *M. restricta* was also the most frequently recovered species from the face of patients with seborrheic dermatitis. A 2002 study aimed at determining the clinical features of acne associated with *Malassezia* as well as the efficacy of antifungal treatments, showed that *Malassezia*-associated acne was characterized by polymorphous eruptions composed of open and closed comedones, inflammatory papules, and pustules, accompanied by seborrhea and seborrheic dermatitis. *Malassezia*-associated acne was also found to be aggravated during the summer, because of the use of systemic corticosteroids, and during menstruation. A one-week regimen of systemic itraconazole (200 mg/d) improved the lesions[33].

In 2003, Lee et al.[34] found that the efficacy and safety of 1.5% ciclopirox olamine (a hydroxypyridone derivative) shampoo was comparable to that of 2% ketoconazole shampoo (a highly effective antifungal treatment) in patients with mild-to-moderate dandruff. Moreover, in 2003, Jang et al.[35] found that *M. restricta* and *M. globosa* are the most common causative species for *Malassezia* folliculitis of the face and trunk, respectively.

Subsequently, in 2004, Choe et al.[36] performed a study on the distribution by age group and body site of *Malassezia* yeasts on normal human skin. Subjects between 21 and 30 years old showed the highest positive culture rate (88%), and the chest (91%) and thigh (60%) had respectively the highest and lowest positive culture rates of all evaluated body parts. *M. globosa* was most frequently recovered from the chest, whereas *M. restricta* was most frequently recovered from the forehead.

Jang et al.[37] conducted a study on earwax in 2005, and showed that *M. restricta* is the most commonly isolated species.

Most of the aforementioned studies were qualitative. However, in 2006, Lee et al.[38] conducted a quantitative study on the distribution of *Malassezia* species in a healthy Korean population. In this study, the qualitative distribution of *Malassezia* species varied according to anatomic site: *M. restricta* was most commonly found on the scalp and forehead, whereas *M. globosa* was the predominant species on the chest. Quantitative analysis showed that, although the values varied among age groups, the yeast count per unit area of skin (cm^2) was higher for the chest and scalp and lower for the upper arm and thigh. By age, the *Malassezia* yeast count on the scalp, forehead, and chest was the highest in the 11 to 20 and 21 to 30 years age groups.

In 2009, Moon et al.[39] compared *Malassezia* species from atopic dermatitis head and neck lesions in children and adults and showed that *M. globosa* was the predominant species in children, whereas *M. furfur* was the predominant species in adults. Both *Pityrosporum ovale*-specific IgE and clinical severity grade were higher in the adults, and they were significantly correlated.

In 2014, Song et al.[40] performed a retrospective study of patients with folliculitis who were previously diagnosed with *Malassezia* folliculitis or non-*Malassezia* folliculitis and compared their clinical features. Among the 80 cases

of non-*Malassezia* folliculitis, yeasts were retrospectively found in 16 patients by serial tissue sectioning and diastase periodic acid Schiff staining. Therefore, the diagnosis of these cases was changed to *Malassezia* folliculitis. *Malassezia* folliculitis presented with trunk involvement and male predilection.

Molecular techniques for the isolation of *Malassezia*

In order to overcome the limitations of conventional approaches based on morphology, microstructure analysis,

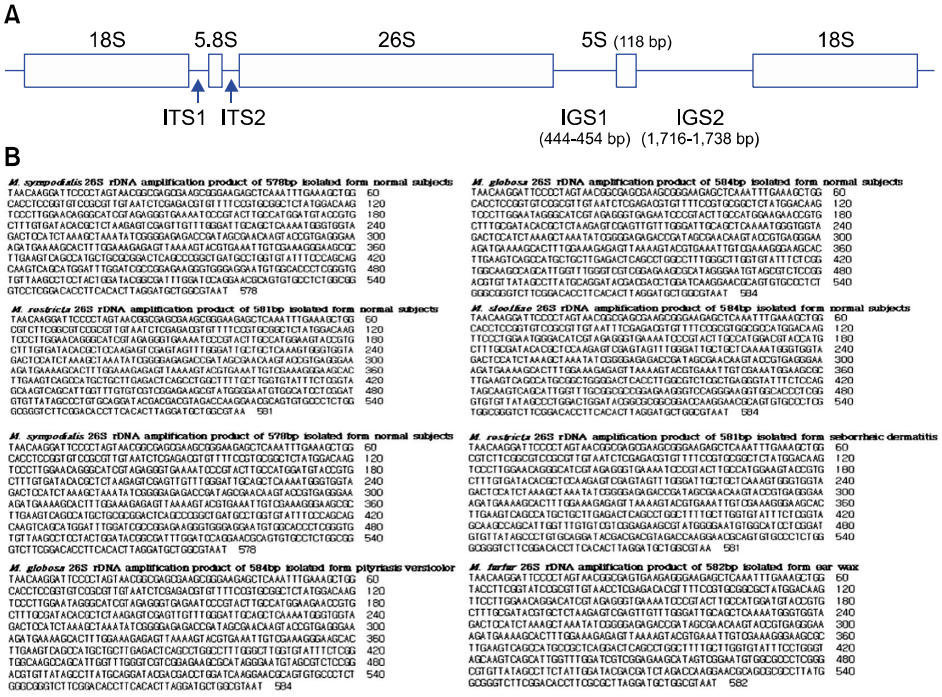

Fig. 6. (A) Schematic representation of the rRNA gene in the type strain (CBS 7966) of *Malassezia globosa*: the 26S rDNA, internal transcribed spacer (ITS1) region, and intergenic spacer 1 (IGS1) region sequences are used for species identification and strain typing. Data from the article of Sugita et al. (J Clin Microbiol 2003;41:3022-3027)[48]. (B) Sequences of the amplified 26S rDNA products from clinical isolates of *Malassezia*. Data from the article of Lee et al. (Korean J Med Mycol 2006;11:141-153)[51].

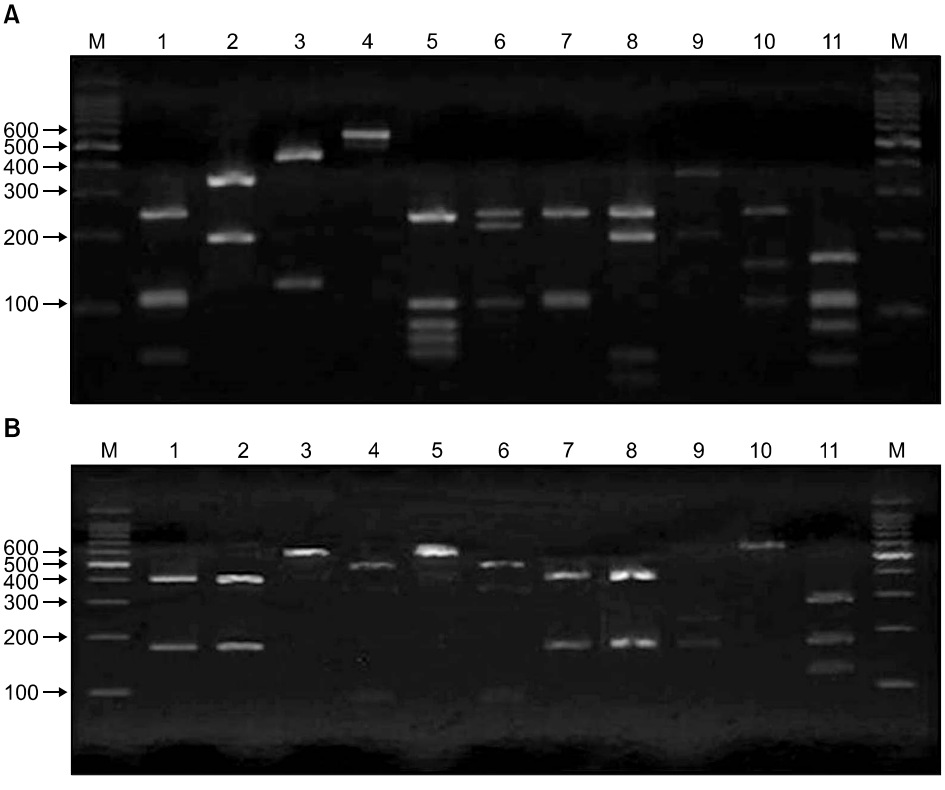

Fig. 7. Polymerase chain reaction (PCR) and restriction fragment length polymorphism patterns of the 26S rDNA PCR products digested with (A) *Hha1* and (B) *BstF51* from *Malassezia* standard strains. Lane M, 100-bp DNA ladder; Lane 1, *Malassezia furfur*; Lane 2, *M. sympodialis*; Lane 3, *M. globosa*; Lane 4, *M. restricta*; Lane 5, *M. slooffiae*; Lane 6, *M. pachydermatis*; Lane 7, *M. japonica*; Lane 8, *M. nana*; Lane 9, *M. dermatis*; Lane 10, *M. obtusa*; and Lane 11, *M. yamatoensis*. Data from the article of Lee et al. (Korean J Med Mycol 2006;11:141-153)[51].

and physiology for the delineation of closely related *Malassezia* species, molecular methods have been developed for the reliable isolation of *Malassezia*[41-50]. For the last 15 years, the molecular studies on *Malassezia* species in Asia have been performed mainly in Japan[14-17,46-50]. Japanese scientists have designed non-culture-based methods, including the direct application of OpSite transparent dressings[47]. These methods avoid culture in Leeming and Notman agarose gels, as DNA is extracted directly from the dressings, followed by gene sequencing and nested polymerase chain reaction (PCR) for species identification and strain typing. The preferred targets for strain identification are the D1/D2 region of the 26S ribosomal DNA (rDNA), the internal transcribed spacer (ITS) regions, and the intergenic spacer 1 region of the ribosomal RNA gene (Fig. 6A). Quantitative analyses of *Malassezia* species have also been performed using real-time (RT) PCR[49,50].

In Korea, Lee et al.[51] were the first to use 26S rDNA PCR and restriction fragment length polymorphism (PCR-RFLP) to identify *Malassezia* species in 2006 (Fig. 6B, 7). In 2007, new experimental molecular methods were introduced in Korea to identify and classify *Malassezia* species. Lim et al.[52] successfully isolated *M. dermatis* from healthy subjects and patients with seborrheic dermatitis by using 26S rDNA PCR-RFLP and 26S rDNA and ITS1 sequencing for the first time in Korea (Fig. 8). Kim et al.[53] implemented a colony PCR method that eliminated the need for DNA extraction, as a fast and simple way to amplify *Malassezia* target DNA, and assessed its clinical utility. In this method, *Malassezia* colonies were lysed in a microwave and used as a template for PCR instead of purified genomic DNA, and this colony PCR method was compared to two DNA extraction methods (the boiling and glass bead methods). Song et al.[54] employed pyrosequencing to identify *Malassezia* yeasts, but the result showed limitations (Fig. 9). Lim et al.[55] performed a nested PCR to differentiate various *Malassezia* species obtained from clinical strains and skin scales in 2008 (Fig. 10, 11). The detection rate for the nested PCR method was 96% for clinical strains and 87%

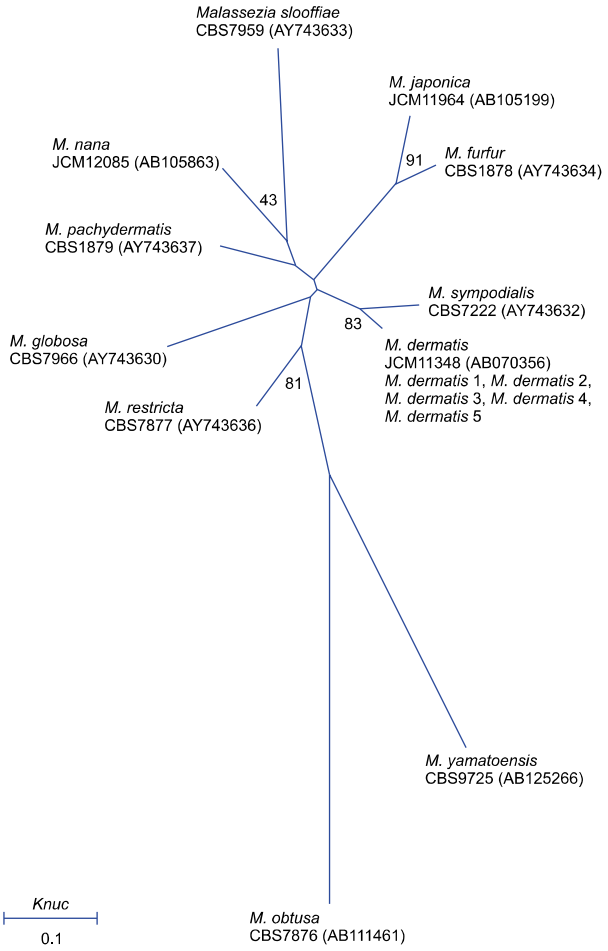

Fig. 8. A molecular phylogenetic tree constructed by the neighbor-joining method using the internal transcribed spacer 1 sequences of members of the genus *Malassezia*. Data from the article of Lim et al. (Korean J Dermatol 2007;45:1020-1030)[52].

Fig. 9. Sequence alignment of the rRNA internal transcribed spacer 2 variable regions (box) of 11 *Malassezia* standard strains for pyrosequencing. Lane 1, *Malassezia dermatis*; Lane 2, *M. furfur*; Lane 3, *M. globosa*; Lane 4, *M. japonica*; Lane 5, *M. nana*; Lane 6, *M. obtusa*; Lane 7, *M. pachydermatis*; Lane 8, *M. restricta*; Lane 9, *M. slooffiae*; Lane 10, *M. sympodialis*; and Lane 11, *M. yamatoensis*. Data from the article of Song et al. (Korean J Med Mycol 2007;12:189-197)[54].

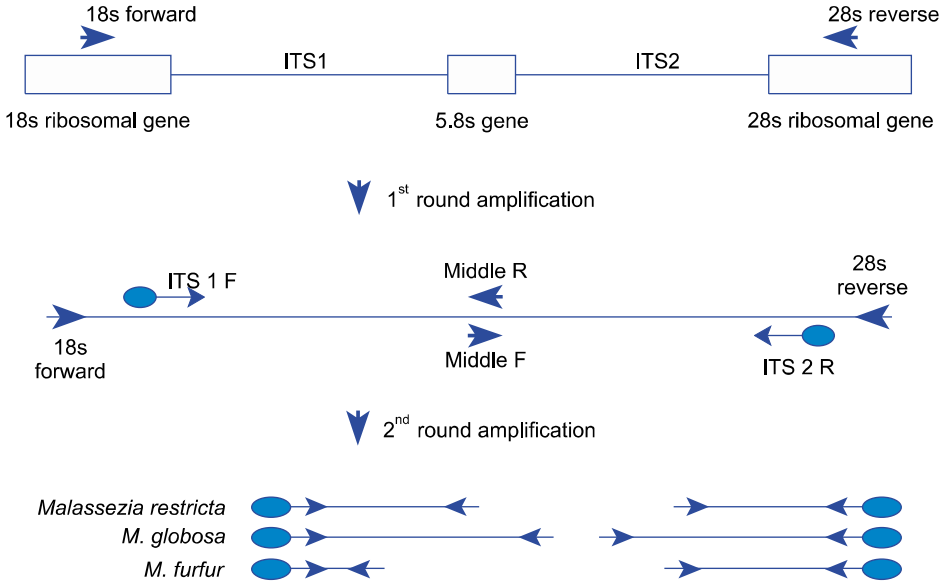

Fig. 10. Nested polymerase chain reaction: structure of the internal transcribed spacer (ITS) gene region and location of primer sites. Data from the articles of Lim et al. (Korean J Dermatol 2008;46:446-452)[55].

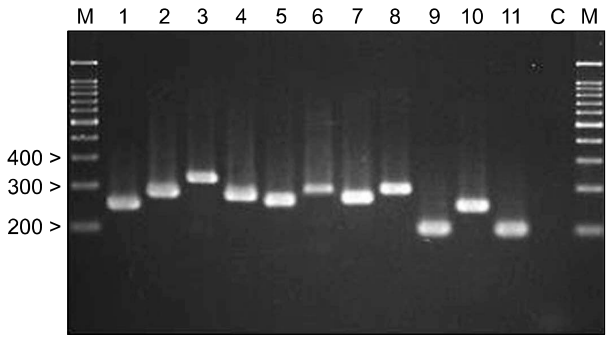

Fig. 11. Nested polymerase chain reaction products from standard *Malassezia* species. Lane M, molecular marker; Lane 1, *Malassezia* dermatis; Lane 2, *M. furfur*; Lane 3, *M. globosa*; Lane 4, *M. japonica*; Lane 5, *M. nana*; 6, *M. obtusa*; Lane 7, *M. pachydermatis*; Lane 8, *M. restricta*; Lane 9, *M. slooffiae*; Lane 10, *M. sympodialis*; Lane 11, *M. yamatoensis*; and Lane C, negative control. Data from the article of Lim et al. (Korean J Dermatol 2008;46:446-452)[55].

for skin scales. *M. globosa*, *M. sympodialis*, and *M. restricta* were the most common causative agents in patients with PV, *Malassezia* folliculitis, and seborrheic dermatitis, respectively. Lee et al.[56] isolated 19 strains of *M. dermatis* from healthy human skin in Korea by using 26S rDNA PCR-RFLP.

Using the same technique, in 2009, Jang et al.[57] attempted the qualitative isolation of *Malassezia* yeasts from 80 healthy males and 80 healthy females in different age groups, from different body areas. They isolated *M. dermatis* and found that *M. restricta*, *M. globosa*, and *M. sympodialis* were the most commonly identified species. In addition, in 2009, Oh et al.[58] compared PCR-RFLP and nested PCR for the isolation of *Malassezia* species and showed that PCR-RFLP is the preferred method for differentiation, although nested PCR is advantageous with respect to time and simplicity (Fig. 12). In 2009, there was also a special trial to study the effects of detergents on the morphology and immunomodulatory activity of *M. furfur*. In this study, Kim et al.[59] found that detergent altered the surface of *M. furfur* and that detergent-treated *M. furfur* induced higher levels of TNF-α expression in monocytes than untreated *M. furfur*. They concluded that the detergents in shampoos or soaps affect the lipid layers of the *Malassezia* cell walls, which can induce or aggravate some inflammatory conditions.

The disorders associated with *Malassezia* yeasts have also been studied extensively in Korea. In 2010, Yim et al.[60] demonstrated evidence of a relationship between *M. sympodialis* and atopic dermatitis by showing that it is the dominant species in patients with this disorder. However, in 2010, Oh et al.[61] showed that there was no statistically significant difference in the distribution of *Malassezia* species between patients with seborrheic dermatitis and healthy controls. Kim et al.[62] developed and evaluated the accuracy of a multiplex PCR kit using ITS1 specific primers with six *Malassezia* standard strains.

In 2011, Song et al.[63] detected *Malassezia* yeasts in acne patients using 26S rDNA PCR-RFLP. The authors showed that the growth rate of *Malassezia* was clearly lower in patients with acne (50%) than in controls (70.6%) and that *M. restricta* was the dominant species in patients with acne, whereas *M. globosa* was the most common species found in healthy controls. Using the same method, Lee et al.[64] investigated the distribution of *Malassezia* species on

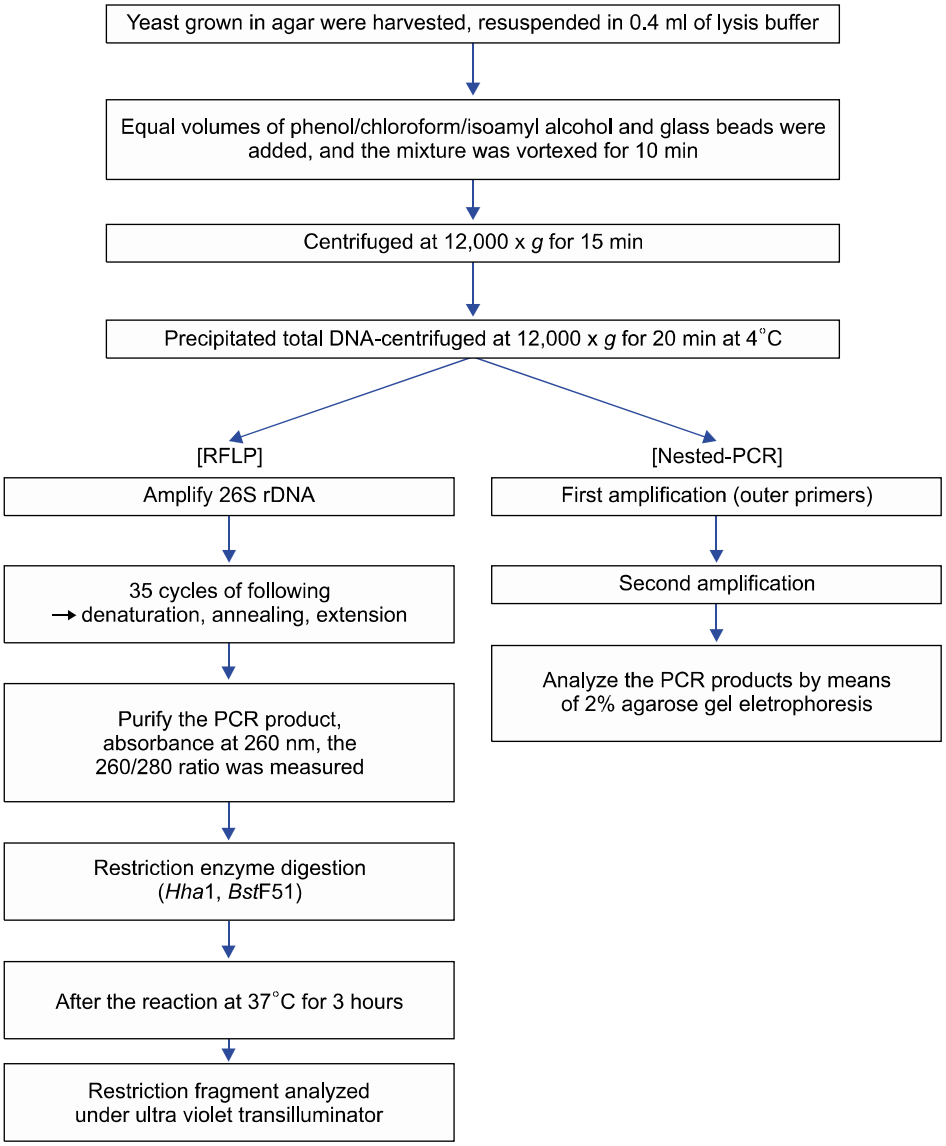

Fig. 12. Flowchart of the nested polymerase chain reaction (PCR) and restriction fragment length polymorphism (PCR-RFLP) methods. Data from the article of Oh et al. (Ann Dermatol 2009;21:352-357)[58].

the scalp of patients with seborrheic dermatitis. The most common species in these patients was *M. restricta*, whereas *M. globosa* was most common in healthy controls, which suggests that *M. restricta* is the most important *Malassezia* species in Korean patients with seborrheic dermatitis.

In 2012, Lim et al.[65] compared the yields of *Malassezia* DNA isolated by the conventional culture-based technique or by a non-culture-based technique via OpSite adhesive tape developed by Sugita et al.[47]. They found that the culture rate of the non-culture-based technique was better than that of the culture-based technique (100% vs. 57.8%); the only disadvantage of the non-culture-based technique was the need for repeated rounds of PCR because of the low amounts of extracted DNA.

A recent genome analysis of *M. globosa* revealed that the absence of a gene encoding fatty acid synthase might be compensated by genes encoding lipases and phospholipases, which showed higher expression levels on the human scalp[66]. Based on this finding, in 2013, Lee et al.[67] analyzed the *in vivo* expression of the lipases and phospholipases of *M. restricta*, the most frequently isolated *Malassezia* species from the scalp of patients with seborrheic dermatitis in Korea, by two-step nested RT-PCR. Another study in 2013 examined the use of pyrosequencing for the identification of *Malassezia* species[68]. Using different primers targeting ITS1 and ITS2, designed in a previous study by Song et al.[54], *Malassezia* species were successfully isolated by pyrosequencing, and this method was more rapid and accurate than 26S rDNA PCR-RFLP.

Biophysical properties of *Malassezia*-associated skin diseases

In 2011, Lee et al.[69] measured the changes in transepidermal water loss (TEWL), stratum corneum hydration, and skin pH in PV lesions of 11 patients. They found significantly higher TEWL and reduced hydration in lesional skin in comparison to in the adjacent non-lesional skin; there was no change in skin pH. The authors concluded that infection with *Malassezia* species alters the biophysical properties of skin, especially the function of the stratum corneum as a barrier to water loss.

Park et al.[70] investigated the skin characteristics of patients with PV using MPA5® (Courage and Khazaka, Köln, Germany). Both hyperpigmented and hypopigmented PV lesions showed higher humidity, sebum levels, and TEWL than healthy controls, indicating that higher humidity and sebum levels provide a better environment for *Malassezia* yeasts in the skin, leading to disruption of the skin barrier, which causes a further increase in TEWL.

Therapeutic approaches for *Malassezia*-associated skin diseases

Regarding alternative therapies for *Malassezia*-associated skin diseases, Lee et al.[71] conducted a pilot study on the efficacy of methyl 5-amino-levulinic acid photodynamic therapy for recalcitrant *Malassezia* folliculitis, and showed that it is an effective treatment option. In 2012, Wi et al.[72] examined the antifungal effect of light-emitting diode (LED) irradiation on *M. furfur*, *M. sympodialis*, and *M. globosa*. They found that an LED that emitted light at wavelengths of 380 ± 2 nm and 392.5 ± 1 nm had an antifungal effect on *Malassezia* species, and they observed an increase in both intracellular and extracellular reactive oxygen species following LED irradiation at 392.5 ± 1 nm. In addition, Kim et al.[73] reported the efficacy of a shampoo with a new formula that contains natural ingredients, including an extract of *Rosa centifolia* petals and epigallocatechin gallate, which is known to exert anti-inflammatory and antifungal effects on scalp seborrheic dermatitis. In a randomized double-blind controlled study, the shampoo had an efficacy comparable to that of 1% zinc pyrithione shampoo and of 2% ketoconazole shampoo; thus, it could be used as an alternative treatment for seborrheic dermatitis.

CONCLUSION

Many common skin diseases, such as seborrheic dermatitis, atopic dermatitis, and *Malassezia* folliculitis, are associated with *Malassezia* yeasts. Although these yeasts are part of the normal flora of human skin, under certain conditions, they can induce or aggravate skin diseases. Many studies have tried to elucidate the mechanism and role of *Malassezia* in disease by comparing the distribution of *Malassezia* in specific diseases to that in healthy controls. These efforts have been supported by the development of highly accurate molecular methods for the identification of each species. In addition, several studies have described the clinical manifestation of related diseases and have investigated various therapeutic options. Recent genomic analyses of *Malassezia* species have accelerated the elucidation of the mechanisms underlying these skin diseases. More prolific studies are underway in Korea, in sync with the global trend.

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