

Microreview of Pityriasis versicolor and *Malassezia* species

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Abstract

Recently 11 *Malassezia* species were isolated. Attention has focused on the relationship between *Malassezia* species and *Malassezia*-related disease. The causal fungus of Pityriasis versicolor is *M. globosa*. The conditions of mycelial form induction are not clear for *M. globosa*.

Key words: *Malassezia*, *Malassezia globosa*, mycelial form, nested PCR, Pityriasis versicolor

Abbreviations: AD – atopic dermatitis; CRP – confluent and reticulated papillomatosis; PV – pityriasis versicolor; SD – seborrheic dermatitis

Pityriasis versicolor

Recently, it was clarified that Pityriasis versicolor (PV) is a superficial infection of the stratum corneum caused by *Malassezia globosa* [1].

It was established that PV is an infection for the following reasons: *Malassezia* species were detected from the stratum corneum of PV, pathological change occurred by inoculating them onto human skin, and they were detected from the pathological change [2].

Clinical condition

Typical objective findings are scaly hyperpigmented (PV nigra) or hypopigmented (PV alba) lesions, usually on the upper trunk, axilla and neck. PV recurs frequently. If a pathological change area is rubbed with a surgical knife, many scales are dislodged and fall from the skin. There is slight itchiness but almost no subjective symptoms.

Epidemiology

PV varies depending on tropical or temperate climate. Although patients with PV increase in summer, it is hardly seen in winter in Tokyo. It was reported that PV patients occupied 3.1% of dermatophytosis in 1997 by the Epidemiological Investigation Committee for Human Mycoses in the Japanese Society for Medical Mycology [3]. Tan [4] reported that the most common superficial fungal infection was tinea pedis (27.3%), followed by PV (25.2%) at the National Skin Centre, Singapore.

PV is observed in the 20- to 30-year-old group, but is uncommon in children and the elderly. Jena et al. [5] reported that PV is not uncommon among children in the tropics.

Clinical examination

PV is diagnosed by its clinical appearance and the observation of many yeast cells and hyphae (so-called spaghetti and meatballs) in scales

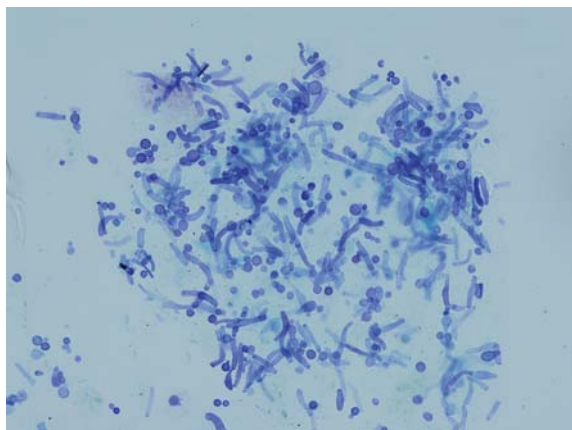


Figure 1. Direct microscopy of scales of PV patients, showing yeast cells and mycelia (methylene blue, $\times 400$).

stained with methylene blue or Parker KOH in microscopic examination (Figure 1). The range of pathological change is observed by fluorescence in Wood's lamp examination.

Differential diagnosis

Diseases resembling PV are vitiligo vulgaris, drug-induced photoleucomelanodermitis, pityriasis rosea Gibert, confluent and reticulated papillomatosis (CRP), etc. The most important point for diagnosis is to detect yeast cells and hyphae by microscopic examination. CRP resembles PV, but hyphae are rare in CRP, which is responsive to minocycline [6].

Treatment

Topical (including imidazole, allylamine and benzylamine) and oral (including itraconazole, fluconazole and ketoconazole) [7, 8] antifungal agents are effective, and no hyphae were observed in the scales of lesions after 2-week treatment. It seems that antifungal shampoo is necessary to prevent relapse.

Malassezia species

Malassezia species are lipophilic yeasts and are considered part of the normal flora of the skin,

being particularly common on the scalp, face and trunk. Sei et al. [9] reported that the number of *Malassezia* species on the face increased for 1 month after birth, and started to decrease 4 months later in parallel with the amount of sebum.

Until recently, *M. furfur*, *M. pachydermatis*, and *M. sympodialis* were the only members of the genus, but in 1996, Guého et al. [10] added four new species, *M. globosa*, *M. obtusa*, *M. restricta*, and *M. slooffiae*, based on their morphology, ultrastructure, physiology, and molecular biology. Recently, four new species were isolated, namely *M. dermatis* [11], *M. japonica* [12], *M. yamatoensis* [13], and *M. nana* [14] in Japan.

Malassezia species are associated with PV, *Malassezia* folliculitis, seborrheic dermatitis (SD), dandruff, atopic dermatitis (AD) [15], CRP [16] and acne [17]. Research attention has focused on the relationship between *Malassezia* species and skin disease.

Causative fungus of PV is *M. globosa*

Until recently, PV is a superficial infection believed to be caused by *M. furfur*. Recent studies [18, 19, 20] using morphological features and physiological tests indicate that *M. globosa* may actually be the causative agent, but the *M. globosa* rate of culture changed with reporters from 53 to 97%. On the other hand, Gupta et al. [21] reported that *M. sympodialis* was detected at 60%. We [22] also reported that *M. globosa* was isolated from 55% of lesional skin specimens in PV, while other species were below 10% according to the method devised by Guillot et al. [23]. The problems encountered in recent studies have been the changing rate of culture with reporters because culture-based methods can be biased by different growth rates and culture requirements in different species [15], and *M. restricta* may not be detected in samples in which a catalase reaction-positive *Malassezia* species is mixed [22]. In order to resolve these problems and examine the distribution of the microorganism in the skin of PV patients, we detected *Malassezia* species using a non-culture-based method consisting of nested PCR with specific primers [1]. The most frequently isolated species were *M. globosa* and *M. restricta* (both 93.9%) in 49 PV patients

(32 males, 17 females; 16–83 years old) before treatment. Other species were below 35%.

Sugita et al. [24] reported that *M. globosa* and *M. restricta* were detected in approximately 90% of AD patients. *M. globosa*, *M. restricta*, and *M. sympodialis* were detected at frequencies ranging from 44 to 61% in healthy subjects. These results suggest that *M. globosa* and *M. restricta* are common species in PV patients, similar to AD patients and healthy subjects, and there are no significant differences in the prevalence of *M. globosa* and *M. restricta* between healthy and lesional skin.

Does this result mean that both *M. globosa* and *M. restricta* are causative fungi? We observed many hyphae in scales in PV patients, but rarely in other *malassezia*-related diseases and normal skin microscopically. After PV treatment, yeast cells remained but hyphae could not be identified microscopically. It is necessary for PV to induce the pathologic mycelial form from yeasts as part of the normal flora of the skin. We tried to collect scales in which the mycelial form alone (<10 yeast cells sample⁻¹) was observed microscopically (Figure 2). In analysis of the mycelial form only, *M. globosa* was isolated (the first amplification products were cloned using a TA Cloning Kit and three positive clones from each samples were sequenced) [1].

There is no report that filaments are formed on *M. restricta* *in vitro* [10, 25]. These results mean that hyphae in PV lesions consist of *M. globosa* and yeast cells are composed of *M. globosa* and *M. restricta*. This suggests that *M. globosa* is the most causative agent of PV.

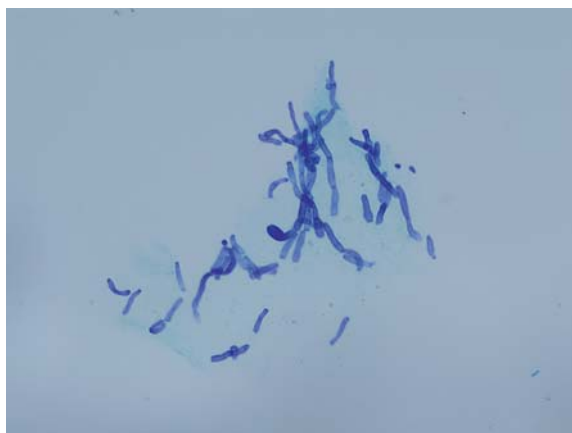


Figure 2. Direct microscopy of scales with only the mycelial form (<10 yeast cells sample⁻¹) (methylene blue, $\times 400$).

Induction of mycelial form of *Malassezia*

There have been reports [26, 27, 28] about the conditions inducing the mycelial form of *Pityrosporum ovale* and *P. orbiculare*.

We also experimented with the conditions inducing the mycelial form *in vitro* (on CHROMagar *Malassezia* Candida). Although we tried various conditions of humidity (80, 90, and 95%), sebum composition (squalene, palmitic acid, palmitoleic acid, cholesterol, cholesteryl stearate, and glyceryl monostearate), sweat (ammonia, serine, and glycine), we could not induce the mycelial form of *M. globosa*. Faergemann et al. [27] reported experimental infections could only be produced with occlusion, and microscopically short hyphae and transformation between round and oval forms were seen in both *P. orbiculare* and *P. ovale*. When *P. orbiculare* and *P. ovale* were incubated with stratum corneum epithelial cells, suspended in phosphate-buffered saline for 90 minutes at 37 °C, short filaments were produced in 11–17.5% of the yeast cells [28]. These results suggest that the stratum corneum and humidity are important in the induction of the mycelial form, but the detailed conditions are unclear. There was no report about *M. globosa*, until Tagima et al. [29] recently reported that ammonium phosphate with glycine effectively induced hyphae in *M. globosa*.

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