J. Mycopathol, Res, 49(1) : 123—126 (2011)

Form conversion of *Malassezia furfur* and its pathogenic significance

ASIS MANNA,[1] JHUMU MANNA,[2] SWAGATA GANGULY[3], AND PRASANTA KUMAR MAITI[4]
Department of Microbiology[1], Department of Physiology, Sabang SK Mahavidyalay, Paschim Medinipur[2] Calcutta National Medical College,[3] Calcutta School of Tropical Medicine, IPGMER, Kolkata.[4]

Received : 27.11.2009 Accepted : 30.10.2010 Published : 25.04.2011

Presence of short hyphal forms with yeasts in scraping materials of Pityriasis Versicolor is diagnostic, though in routine isolation media only their yeast forms were grown. Only yeast form was also demonstrable from non-lesional skin. So yeast to hyphal form conversion of any *Malassezia* isolate may discriminate pathogen from commensal. With this aim we attempted to develop such form conversion test for *Malassezia furfur.*

We collected castoff skin scales from human volunteers and added to 2% Agar, Sabouraud Dextrose Agar and modified Dixon Agar separately at 1% concentration. Each medium was solidified as thick film on sterile slide surface. Maximum hyphal conversion was noted on - slide culture with skin scales in agar, a few in MDA or SDA. No form conversion was noted in medium wilhout human scales.

The test may establish clinical relevance and pathogenic potentialitv of this lipophilic fungus.

Key words: *Malassezia furfur,* form conversion, yeast, hypha

INTRODUCTION

The genus *Malassezia* comprised of basidiomycetous, lipophilic yeasts that multiplies by percurrent, monopolar budding with a broad base. They can be easily cultivated on simple media, but a lipid source proved essential for growth of most species. Despite its two morphological forms, it is not regarded as a dimorphic fungus in the true sense as both the morphological structures are simultaneously found *in vivo* irrespective of the temperature variations (Chander, 2002),

These fungi are found in abundance in the hypopigmented patches of Pityriasis Versicolor as well as from normal skin. However, materials obtained from the lesions seem to possess predominance of hyphal forms in contrast to materials from non-affected skin. A possibility is there that the hyphal form being the pathogenic one outnumbered the yeast form in the lesions. A constellation of factors may be responsible for the conversion of forms. This report highlights the preliminary findings of a study investigating such possibilities.

The aims of the study are to investigate the effects of various growth environments including incorporated skin scales in different media in order to establish relationship between the yeast and mycelial forms of *Malassezia* species.

MATERIALS AND METHODS

Malassezia species were isolated from the scrapings of clinically obvious lesions inoculated in Sabouraud's dextrose agar (SDA) with olive oil overlay and modified Dixon's agar (MDA). Species identification was made with the help of an array of physiological characteristics and biochemical properties.

Major components of sweat were postulated to bring some impact on the pattern of growth of these fungi. Hence they were challenged against pH from 4.6 to 7.6 (at the intervals of 0.2) in addition to gradients of concentration (from 0.5% to 2.5% at the interval of 0.5%) of sodium chloride (Merck) and urea (HiMedia).

To explore the effects of human stratum corneum cells on the growth as well as form conversion (from yeast to mycelial one) of *Malassezia* fungi, a modification of Dalmau culture method was exploited (Milne, 1996). Cast off skin scales from human volunteers were pooled and cleaned by suspending in normal saline followed by repeated centrifugation. The pellet thus formed was dried by placing it in an incubator at 37°C overnight. These scales were then added at the concentration of 1% to sterile media (2% agar, SDA and MDA separately) and were sterilized in an autoclave at 121°C for 15 minutes.

The media were distributed on the surface of sterile glass slides into a thin film and kept in sterile Petri dishes with bent glass rod platform on a filter paper. Pure culture of *Malassezia* species from clinical isolates (identified by proper physiological tests to be *Malassezia furfur*) was taken and even, solidified medium was then aseptically streaked in a linear fashion by those cultures with the help of a flamed straight inoculation wire. A cover slip was dipped in alcohol, sterilized by flaming and cooled. It was placed on the inoculated medium over the slide. The filter papers on the bottom of the Petri dishes were then soaked with sterile distilled water. Similar slide cultures were made on 2% agar, SDA and MDA (without skin scales). Identical batches of the same were placed over slide carriers in sterile Petri dishes and incubated at 25°C, 30°C and 37°C. The slides were examined everyday under low followed by high magnifications of a microscope for formation and nature of growth.

After thorough scanning of the slide, when any type of growth was encountered, the cover slip was removed and a portion of the growth within media was examined by lacto-phenol cotton blue mount for morphological confirmation of purity of culture of *Malassezia*. sp.

Repeat tests were done with five isolates of the same species of the yeast.

RESULTS AND DISCUSSION

Colonies of *Malassezia* on SDA with overlaid olive oil incubated in a humidified environment at a temperature of 25-37°C for 5-7 days showed cream to yellow colored slightly raised colonies, 3-6 mm in diameter with irregular edges when viewed through a magnifying glass. The colonies possessed a characteristic brittle texture — when preparation of a suspension was attempted on a glass slide or over the surface of an agar medium, the cells got stuck together to form small clumps. Lacto-phenol cotton blue mount of those colonies yielded 2-5 μm yeasts with lateral budding. Mycelial forms were not detected in this medium.

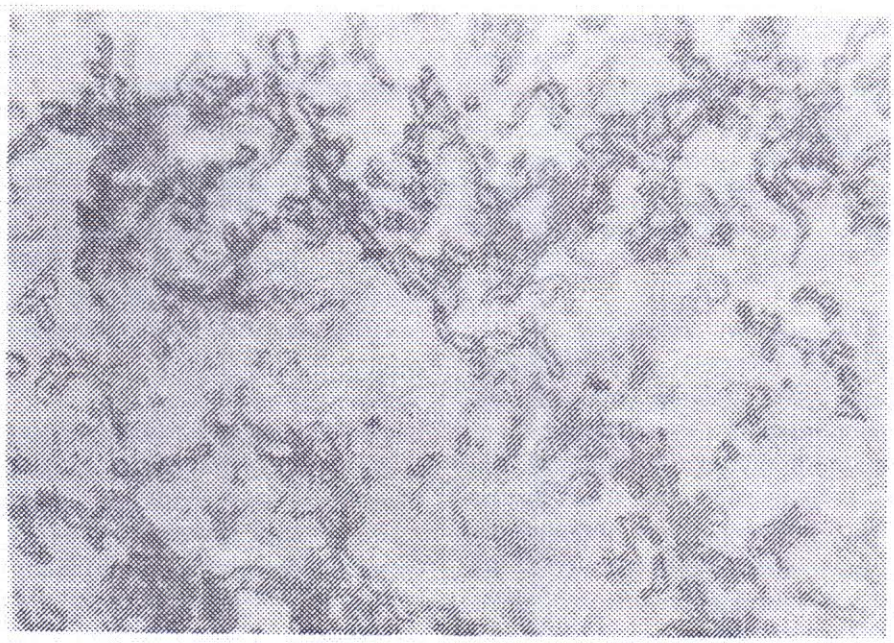

Fig 1. Growth of *M. furfur* on slide culture with skin scales, showing budding yeasts and short hyphae (400x).

The yeasts had grown over media having pH ranging from 4.6 to 7.6 but growth was inhibited at pH 4.4 and below. Sodium chloride at the concentration of 2.5% was also found to be inhibitory. *Malassezia* colonies were found to form on SDA plates containing 0.5 to 2.5% of urea.

The slide cultures in SDA, MDA and MDB (with or without skin scales) illustrated development of ovoid or ellipsoid yeast cells with double contour, 2-5 μm in size, within 24 hrs of incubation. Nevertheless, the yeast cells were abundant just around the skin scales even in 2% agar, although no such growth was observed in agar without skin scales, On prolonged incubation of the same (for 3 to 7 days), some of the yeast cells (specially those at the margins of the clumps of the fungi) showed formation of 10-30 μm long cylindrical, stout, sinuous, often septate tubular structures resembling pseudomycelium (Fig.1.). Similar results were obtained in slides kept at 25°C and 30°C, but at 37°C the relative proportion of pseudomycelium formation appeared to be higher.

The numbers of the genus *Malassezia* were isolated from normal human skin in addition to some other clinically discernable dermatological as well as systemic disorders. To enumerate, the association of cutaneous maladies included Pityriasis Versicolor (Midgley *et al.*,1998), Seborrhoeic Dermatitis. Onychomycosis, Atopic Dermatitis (Plewig *et al.*,1999; Nakabayashi *et al.*, 2000) in children and Malassezia Folliculitis (Martin *et al.*, 1999). Besides, it had been implicated in the etiology of neonatal pustulosis and blepharitis or dacryocystitis after keratoplasty. These fungi were also opportunistic to cause deep seated infections especially in the immunodeficient or immunocompromized settings. Several cases of catheter related fungemia were reported in patients of bone marrow transplantation and very low birth weight infants having parenteral nutrition through indwelling catheters (Nguyen *et al.*,2001). Disseminated infections might also be seen in neutropenic children or neonates.

With the exponential increase in the number of AIDS cases globally, the role of these opportunistic fungi assumed greater importance. Severe forms of Malassezia Folliculitis and Seborrhoeic Dermatitis were clinically recognized hallmarks of AIDS (Nakabayashi *et al.*,2000).

Patients who had undergone organ transplantation were given long continued immunosuppressive medications to counteract rejection. They were at an increased risk of systemic invasion by the supposedly innocuous fungi.

These fungi had shown ready growth on simple media containing glucose and an organic nitrogen source with overlay of sterilized oil. Many others were used like simple glucose or peptone agar, either with 4% sugar, 1% peptone and an acid pH (SDA) or with 2% sugar, 1% peptone and a neutral pH (Emmon's modification of SDA) pouring oil on it. The choice of oil seemed a varied one from widely utilized olive oil to wheat germ oil to milk fat (also ghee and butter) to glycerol and oleic acid and lanoline (Panja, 1927). The primary culture medium used, therefore, was largely incompatible with some physiological studies. Antibacterials such as gentamicin (0.0025%) and/or chloramphenicol (0.005%) might be added to reduce contamination, and the addition of cycloheximide at 0.04% would inhibit the growth of non-dermatophyte moulds Malt extract agar 3-4% was also frequently used for primary culture.

For obvious reasons, search towards a lipid-incorporated medium was on the way. Dixon's agar, first described by van Abbe in 1964, utilized ox bile and Tween 40 (polyoxyethylenesorbitan monopalmitate) to assimilate glycerol and oleic acid within the medium itself. Liquid form (Dixon's broth) was also effective. Later, modification of the original recipe became popular for speedier and substantial growth of *Malassezia* species with easily discernable colony characters. Quantitation of colonies was also feasible on this media. Leeming and Notman medium (with whole fat cow's milk) and GYP-S agar [containing glucose-yeast extract peptone agar, Tween 80 (polyoxyethylenesorbitan monooleate), glycerol monostearate and olive oil] were reported to be successful (Midgley *et al.*, 1998). Recently, a medium containing coconut milk was claimed to be at least as effective as, if not better than, MDA towards culture of Malassezia (Maiti *et al.*, 2008).

Occurrence of filamentous form of *Pityrosporum ovale* grown on human stratum corneum *in vitro* was described by Faergemann *et al.*(1996). Dead cells shed off human skin might put forth comparable consequences without the requirement for organization of cell culture systems. As chlamydospore test demonstrated form conversion of *Candida albicans*, a

modification of the same was employed in this study as 'slide culture'.

It may be inferred from our findings that presence of skin scales might be recognized as an important promoting factor for the growth of *Malassezia* fungi. The switching of yeast cells to hyphal ones might be possible *in vitro* by creating the natural environment in an artificial medium. Sweat components, namely pH, salt and urea had no significant role on conversion of forms.

However, these findings were only preliminary ones. Towards substantiating the actual role of cultural aspects on form conversion of these fungi, some supplementary clarifications were desired, as (i) whether the method would substantially differentiate pathogens from the commensal forms; (ii) whether the method would be universally applicable to all the species of *Malassezia* genus; (iii) whether the tests were reproducible using reference strains of the yeasts, if available; (iv) towards clenching the pathogenic role of mycelial form of *Malassezia*, some more information needed to be available, namely; (a) whether mere presence of or attainment of a 'critical number' by the specific form would have to be required; and (b) whether application of that specific form on the skin of healthy animal models and human volunteers would yield expected results.

REFERENCES

Chander J. 2002. In: *Textbook of Medical Mycology*, 2nd ed. Mehta Publishers. New Delhi-I 10028: India. 69-78, 376-383. and 391-392pp.

Milne. LJR, 1996. *Fungi*, in: *Mackie & McCartney Practical Medical Microbiology*. 1989 14th ed: International student edn. Churchill Livingstone, New York. 695-717pp.

Midgley, G.; Gu'eho. E. and Gutllot, J. 1998. Diseases caused by *Malassezia* species In:Topley and Wilson's *Microbiology and Microbial infections*. Vol. 4, 9th ed. Arnold, London. 201-211pp.

Plewig, G. and Jansen, T. 1999. Seborrheic dermatitis. In Fitzpatrick's *Dermatology in General Medicine*. Vol. 1, 5th ed. International edn. Mc Graw-Hill, Health Professions Division, New York. 1482-1489pp.

Nakabayashi, A.; Sei, Y. and Guillot, J. 2000 Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol* **38**: 337-341.

Martin, AG. and Kobayashi, GS. 1999. Yeast Infections: Candidiasis, Pityriasis (Tinea) versicolor. In: Fitzpatrick's *Dermatology in General Medicine*. Vol. 2, 5th ed. International edn. Mc Graw-Hill, Health Professions Division, New York. 2368-2371pp.

Nguyen,T. Lund, CH. and Durand, DJ.2001 Thrombolytic therapy for adhesion of percutaneous central venous catheters to vein intima associated with *Malassezia furfur* infection. *J Perinatal.* **21**: 331-333.

Panja, G. 1927 The *Malassezia* of the skin: their cultivation, morphology and species. *Trans Far Eastern Assoc Trop Med.* **1**: 422-456.

Maiti, P K. Bose, M. Ganguly. S. Ghosh, S. and Manna, A. 2008 Coconut based in-house developed medium for fungal growth. *J Mycopathol Res.* **46**(2): 215-211.

Faergemann, J. and Bratel, AT. 1996 The *in vitro* effect of fluconazole on the filamentous form ot *Pityrosporum ovale. Acta Derm Venereol.* **76**(6): 444-446.