OCCURRENCE OF *MALASSEZIA* SPP. ON HEALTHY HUMAN SKIN

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ABSTRACT

The genus *Malassezia* currently includes seventeen species that have been isolated from healthy and diseased human and other animal skin. *Malassezia* are implicated in a range of cutaneous diseases in humans: pityriasis versicolor, atopic or seborrheic dermatitis, dandruff, folliculitis and psoriasis. The outbreak of the disease depends on the interaction between the host immune system and *Malassezia* species. *Malassezia* stimulates both the cellular and humoral immune response in humans. Although *Malassezia* species have been associated with various dermatological diseases in people, the detailed pathological role of *Malassezia* remains obscured. *Malassezia* yeasts require lipids for their growth and therefore to a greater extent they colonize the sites with more sebaceous glands. The ecosystem on skin is complex and its balance depends on several factors. The aim of this study was to determine the presence of *Malassezia* yeasts in clinically normal skin of 42 healthy, randomly selected individuals of different ages. In the group of people examined, up to 30 persons (71.4 %) represented by chil-

dren, adults and the elderly were positive to *Malassezia* yeasts. It has been shown that the back is an area with a higher incidence (66.7 %) of observed yeast compared to the head (40.5 %).

Key words: healthy skin; *Malassezia*; man; occurrence; yeasts

INTRODUCTION

The lipophilic yeasts *Malassezia* are usually members of the normal skin flora and mucosa in warm-blooded humans and other animals. At the same time *Malassezia* can act as an opportunistic organism that under certain conditions can become pathogenic and they may cause various diseases of skin or fungaemia [9]. The pathophysiology of *Malassezia*-caused or *Malassezia*-exacerbated skin conditions is largely unknown [26]. A range of skin microenvironmental factors, such as the bacterial microbiota, pH, salts, immune responses, biochemistry, and physiology, may play a role in the adherence and growth of *Malasse-*

zia species, favouring distinct genotypes depending on the geographical area and/or the skin sites involved [8].

Malassezia yeasts are associated with many dermatological disorders of the human skin, such as: atopic dermatitis, dandruff, folliculitis, pityriasis versicolor (PV) or seborrheic dermatitis and intravascular catheter-acquired infections [15]. They have participated in different skin disorders in animals, especially otitis externa and dermatitis [6]. In animals, only one species —*M. pachydermatis*, is important. All *Malassezia* species are lipid-dependent, except for *M. pachydermatis* that only one which is lipophilic. The genus *Malassezia* currently includes 17 species: *M. globosa*, *M. restricta*, *M. slooffiae*, *M. obtusa*, *M. furfur*, *M. sympodialis*, *M. japonica*, *M. yamatoensis*, *M. dermatis*, *M. pachydermatis*, *M. caprae*, *M. equina*, *M. nana*, *M. cuniculi*, *M. vespertilionis*, *M. brasiliensis* and *M. psittaci*. These can be divided into three groups according to the hosting organism: *Malassezia* occurring only in animals (*M. vespertilionis*, *M. brasiliensis*, *M. psittaci*, *M. caprae*, *M. equina*, *M. cuniculi* and *M. nana*), *Malassezia* occurring primarily in humans (*M. dermatis*, *M. japonica*, *M. obtusa*, *M. restricta*, *M. yamatoensis*) and *Malassezia* occurring both in humans and other animals (*M. furfur*, *M. globosa*, *M. slooffiae*, *M. sympodialis* and *M. pachydermatis*) [3, 4, 14].

In humans, *Malassezia* yeasts inhabit sebum-rich areas of the skin, including the trunk and the head region (face and scalp). The skin ecosystem is complex and its balance depends on several factors. Sebaceous gland activity in children is small, but increases with age according to androgen production.

The aim of this study was to determine the presence of *Malassezia* yeasts from clinically normal skin of healthy randomly selected human individuals of different ages.

MATERIALS AND METHODS

The survey was carried out on 42 clinical healthy persons with no skin diseases and without any known underlying disease. Six children (4 boys and 2 girls) aged 4 to 11 years old and 36 adults (9 men and 27 women) aged 22 to 62 years were examined. From each person, two samples originating from different anatomical sites of the body that is the hair area of head and back (interscapular region) were collected by a standard swab method. A sterile cotton swab (Fungi-Quick, Dispolab) soaked with sterile saline

was used to rub against the skin surface, with continuous rotation of the swab over at least 15 seconds, and immediately applied evenly onto three agar media. The samples were inoculated on specific media for the culturing of *Malassezia*: Sabouraud dextrose agar with chloramphenicol

Table 1. Sabouraud dextrose agar with chloramphenicol—composition

Substance	Quantity per 1 litre of medium
Enzymatic digest of casein	5 g
Enzymatic digest of animal tissue	5 g
Dextrose monohydrate	40 g
Chloramphenicol	0.05 g
Agar	15 g

Table 2. Modified Leeming & Notman agar (MLNA)—composition

Substance	Quantity per 1 litre of medium
Mycological peptone	10 g
Glucose	10 g
Yeast extract	2 g
Bovine bile	8 g
Glycerol	10 ml
Glycerol monostearate	0.5 g
Tween 60	5 ml
Olive oil	20 ml
Agar	15 g

Table 3. Modified Candida-Chrom agar—composition

Substance	Quantity per 1 litre of medium
HiCrome Candida differential agar	42.72 g
Mycological peptone	15 g
Yeast extract	4 g
Dipotassium phosphate	1 g
Chloramphenicol	0.5 g
Chromogenic mixture	7.22 g
Tween 40	10 ml
Agar	15 g

(Table 1), Modified Leeming and Notman agar medium (Table 2) and Modified Candida-Chrom agar with Tween 40 (Table 3). The yeasts were incubated on growth media at 32°C and were monitored every day for two weeks. The identification of yeasts was based both on macroscopic appearance of colonies and microscopic cell morphology. Each sample was stained by Gram staining and examined by a microscope for the presence of typical *Malassezia* yeast cells (Fig. 1). The identification of *Malassezia* genus was confirmed by their morphological and physiological properties according to K a n e k o et al. [11].

RESULTS

Thirty of the 42 persons scored positive for *Malassezia* spp. (71.4 %) from which 28 persons had a confirmed occurrence of *Malassezia* spp. on their back. Seventeen of the people had *Malassezia* yeasts on their head. Synchronously, *Malassezia* was confirmed in 13 samples from both the head and back (Table 4). A total of 45 positive samples (53.6 %) were obtained. No sample was identified as *M. pachydermatis*, since all isolated yeast species were lipid-dependent.

Out of the 6 children, 2 children had *Malassezia* present at both areas mentioned, while in another 2 children it

was diagnosed only on their back. Of the 9 men, *Malassezia* has been confirmed in 4 persons both on the back and the head. In 7 cases, *Malassezia* yeasts were identified on the back and in 4 cases on the head. There were 22 women in the group, 15 of whom had *Malassezia* on the back and 9 on the head. Concurrently, 7 women had *Malassezia* on both body areas.

Table 4. Results of human samples

Number of individuals with confirmed <i>Malassezia</i> spp.	30 (71.4 %)
Number of <i>Malassezia</i> isolates from the back	28 (66.7 %)
Number of <i>Malassezia</i> isolates from the head	17 (40.5 %)
Number of <i>Malassezia</i> isolates from both sites	13 (31.0 %)
Total	42

DISCUSSION

Most of the available articles have focused on the incidence of *Malassezia* in humans in connection with skin diseases such as pityriasis versicolor, psoriasis, atopic and seborrheic dermatitis, dandruff and folliculitis. *Pityriasis versicolor* is the only cutaneous disease etiologically connected to *Malassezia* yeasts. In other dermatoses, such as

Fig. 1. Isolated *Malassezia* cells

Malassezia folliculitis, seborrheic dermatitis, atopic dermatitis, and psoriasis, these yeasts have been suggested to play pathogenic roles either as direct agents of infection or as trigger factors because there is no evidence that the organisms invade the skin [20]. Pityriasis versicolor is a superficial fungal infection of the skin caused by *Malassezia* species that induces a characteristic rash of well demarcated, thin, scaly plaques that can be hypopigmented, hyperpigmented, or erythematous [10].

Seborrheic dermatitis is a chronic relapsing inflammatory skin disorder clinically characterized by scaling and poorly defined erythematous patches. Although the cause of seborrheic dermatitis has yet to be understood, *Malassezia* yeasts, hormones (androgens), sebum levels and immune responses are known to play important roles in its development. Additional factors including drugs, winter temperatures and stress may exacerbate seborrheic dermatitis [2].

The pathophysiology of atopic dermatitis is complex and multifactorial, involving elements of barrier dysfunction, alterations in cell mediated immune responses, IgE mediated hypersensitivity, and environmental factors. One of several factors that complicate atopic dermatitis is *Malassezia* [7].

Malassezia folliculitis is a form of folliculitis caused by yeast infections. It is characterized by dome-shaped papules, pustules, nodules, and cysts in severe cases [21]. Formation of dandruff is a common but complex event which has been associated with numerous causal factors. An imbalance between physiological factors (pH, water content, or sebum secretion) can disturb the physiological equilibrium of the scalp that can lead to dandruff formation. However, the severity of the condition is strongly related to *Malassezia* yeasts [16]. Psoriasis is a chronic inflammatory skin disease, whose pathogenesis involves dysregulated interplay among immune cells, keratinocytes and environmental triggers, including microbiota, such as *Malassezia* spp., *Streptococcus* spp., *Micrococcus* spp., *Staphylococcus* spp.) [22].

Although *Malassezia* species is associated with various dermatological diseases, the exact pathological role of *Malassezia* yeasts remains obscure. *Malassezia* yeasts are commonly found on the skin of healthy people and do not usually cause any health problems. However, in some interplay of conditions (immune deficiency, genetic predisposition, systemic diseases, skin diseases, etc.), it can cause unpleas-

ant skin disorders. A y h a n et al. [1] and N a g a t a et al. [17] describe the gradual settlement of baby skin by *Malassezia* immediately after birth. L e e et al. [12] found that the distribution of *Malassezia* on the body depends on the amount and type of lipids present on the skin. Even in adolescents during puberty, but generally also in adults, the incidence of *Malassezia* depends on the activity of the sebaceous glands.

In women the highest incidence of yeast in the population was recorded from the chest, upper back and forehead, while men yielded more yeasts on the lower back and thigh areas compared to women [13]. Some authors prefer that *Malassezia* microbiota of healthy skin is gender-dependent and changes with age. We assume that assessing the incidence of *Malassezia* yeasts in certain groups (age, gender) is of little importance, even if it has a certain informative value. The *Malassezia* occurrences should be assessed in a particular individual. In our group of people, we have found that *Malassezia* is fairly common on the skin of healthy people, whether children or adults. *M. pachydermatis* was not recorded from any of our samples either from the trunk or from the healthy scalp skin. This species is better adapted to animals, although it can be involved in some systemic human infections. The presence of this species on human skin is rare and transient, occurring possibly by transmission from pets or environmental sources [19].

In one group of people examined, we detected only lipid-dependent *Malassezia* yeasts, both in children and adults. Of the 42 subjects, up to 30 were positive for *Malassezia* (71.4 %), with the most common being detected on the back in up to 28 people (66.7 %). Of these, in 13 samples (31 %) *Malassezia* yeasts were present on both body areas concurrently. Other authors also consider these body areas as typical for the occurrence of *Malassezia* yeasts and this is explained by the higher number of sebaceous glands. Of the lipid-dependent species, *M. restricta* is most common on the head and *M. sympodialis* with *M. globosa* on the torso [5, 12, 18, 23, 24, 25].

CONCLUSIONS

Malassezia species have been associated with a variety of dermatological disorders. Certain environmental, genetic, and immunological factors can predispose to this pathogenic influence and contribute to the development

of the disease. Ten *Malassezia* species have been isolated in humans, namely *M. furfur*, *M. sympodialis*, *M. slooffiae*, *M. pachydermatis*, *M. globosa*, *M. obtusa*, *M. restricta*, *M. dermatis*, *M. japonica*, and *M. yamatoensis*. The most common site of occurrence is the scalp and the trunk. Up to 71.4 % of the person were positive for *Malassezia* yeasts and their highest incidence was confirmed on the back area (66.7 %). The results show that the *Malassezia* spp. are part of the normal skin microflora in healthy people and they usually do not cause any clinical problems.

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