VILNIUS UNIVERSITY

Auksė ZINKEVIČIENĖ

YEAST IN ATOPIC DERMATITIS ETIOLOGY

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This dissertation was prepared during the period of 2006-2012 at the Department of Microbiology and Biotechnology, Faculty of Natural Sciences of Vilnius University.

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The dissertation will be defended at the public session of the Council of Biological Science at 2 p.m. on October 26, 2012 in the prof. Jonas Dagys's auditorium at the Faculty of Natural Sciences, Vilnius University.

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VILNIAUS UNIVERSITETAS

Auksė ZINKEVIČIENĖ

MIELĖS ATOPINIO DERMATITO ETIOLOGIJOJE

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INTRODUCTION

Allergy is defined as an overreaction of the immune system to otherwise harmless, nontoxic environmental substances – allergens. The inflammatory response can be triggered by hereditary and/or environmental factors. The genetic background is an important factor in the onset of an allergy e.g. it can cause impairment of the barrier integrity of epidermal layer. Epidermal barrier dysfunction is a prerequisite for the penetration of high-molecular weight allergens from the environment. The prevalence of allergic diseases has been increased in industrialized countries during these later decades. This phenomenon cannot be explained by genetic factors only. The risk of allergy can also be effected by environmental factors, such as urban living environment, exposure to pollutants and improved hygiene. It has been estimated that environment and genetic variation each account for about 50 % of the risk of allergic diseases.

Atopic dermatitis (AD) is a chronic relapsing form of skin inflammation manifested by dry skin and IgE-mediated sensitization to environmental allergens. In recent years, the incidence of AD has increased. As the prevalence of other allergic diseases, the observed increase in the prevalence of AD cannot be explained by one or a few dominant risk factors. One of the possible explanation for this phenomenon could be the most widely accepted theories, the so-called “hygiene hypothesis”, which states that increasing use of antibiotics and decreasing exposure to microbial antigens during early childhood is associated with increasing severity and prevalence of atopic diseases. AD patients are easily infected by microorganisms due to the defect skin barrier. A major difference from other allergenic agents is that in AD, microorganisms may colonize the human body and aggravate AD as a result of another allergic reaction.

The importance of fungi as allergens has been increasing considerably over the last decade. The yeast *Malassezia* is considered to be an important factor in the course of AD clinical history. Sensitization to the yeast can be detected in approximately 30–50 % cases of AD patients. To date, the genes for 13 allergens from two different *Malassezia* species have been cloned. There are some reports that specific IgE response towards *Candida albicans* is associated with saprophytic *C. albicans* carriage, as *C. albicans*

inhibits delayed skin response and elevates IgE response. Two C. albicans allergens are listed in the official list of allergens of the International Union of Immunological Societies Allergen Nomenclature Subcommittee (www.allergen.org).

Many fungal proteins responsible for allergenic reactions have been described, and many of them are proved to be serologically cross-reactive with each other. Apart from sensitizations to species-specific antigens, a number of polyvalent sensitizations have been found that probably are caused by cross-reactivity between homologous proteins and the polysaccharides common among different fungal species. More than a half of the cross-reactive fungal allergens have homologous IgE-reactive proteins in non-fungal species.

Microbiological samples to control yeast microflora are rarely taken in AD. The majority of studies involving yeasts and AD patients focus mainly on the members of the genus *Malassezia*.

The aim of the dissertation work was: to isolate and identify all the yeast species found on skin affected by atopic dermatitis, to evaluate their influence to the synthesis of IgE antibodies, and to assess the possible cross-reactivity between different yeast species.

The following **tasks** have been formulated to achieve this aim:

1. To isolate and identify all the yeast species found on skin affected by atopic dermatitis.
2. To identify atypical yeast species.
3. To compare the differences in cutaneous yeast flora by age and body parts of atopic dermatitis patients.
4. To evaluate the presence of specific IgE antibodies to isolated yeast species in the sera of atopic dermatitis patients.
5. To assess the possible cross-reactivity of IgE antibodies between isolated yeast species.
6. To assess the possible cross-reactivity of IgE antibodies between isolated yeast species and other allergens.

Scientific novelty

- For the first time in Lithuania evaluation of the cutaneous yeast flora of atopic dermatitis patients was performed.
- For the first time in Lithuania evaluation of *Malassezia* yeast distribution on atopic dermatitis affected skin was performed.
- For the first time it was shown that the sera of atopic dermatitis patients have specific IgE antibodies to *Candida famata*, and *Candida pelliculosa*.
- For the first time it was shown that the sera of atopic dermatitis patients have specific IgE antibodies to cross-reactive *Candida famata*, *Candida pelliculosa*, *Candida guilliermondii*, and *Rhodotorula rubra* antigens.
- For the first time it was shown that *Candida pelliculosa* and house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* might share some allergenic epitopes.

Volume and structure of the dissertation. The dissertation is written in the Lithuanian language. It consists of the Introduction, Material and methods, Results, Discussion, Conclusions, and the List of references (223 reference sources). There are 8 tables and 18 figures in the dissertation. In total, the dissertation consists of 106 pages.

MATERIAL AND METHODS

Subjects. In total 241 samples were obtained in the Centre of Diagnosis and Treatment of Allergic Diseases in Vilnius, Lithuania from the patients with clinical diagnosis of atopic dermatitis exacerbation. Samples were divided into groups by patients' age and sampled body parts (areas mostly affected by AD) (Table 1).

Table 1. Division of samples into groups.

Groups	Total of samples	Males/females	Average age
Group I (samples were collected from patients aged 2-16 years with skin lesions on hands or legs)	69	35/34	5.8±4.3
Group II (samples were collected from patients aged 2-16 years with skin lesions on face, neck or trunk)	63	30/33	4.9±3.9
Group III (samples were collected from patients aged 16-65 years with skin lesions on hands or legs)	58	23/35	33.9±10.7
Group IV (samples were collected from patients aged16-65 years with skin lesions on face, neck or trunk)	51	19/32	36.7±11.2

40 samples were taken from clinically healthy individuals and used as controls. The patients had not been taking antifungal agents or topical corticosteroids for at least four weeks. The study was approved by the Lithuanian Bioethics Committee. Written consent was obtained from each patient.
Additionally, sera from 34 AD patients were obtained, including 15 males and 19 females, aged 16 to 65 years (average age 36.7 ± 11.7 years). The results of the Immulite® 2000 3GAllergy™ assay (Siemens Healthcare Diagnostics Products Ltd., UK) revealed that 19 patients were positive (> 0.7 kU/l) for D1 (*Dermatophagoides pteronyssinus*) and D2 (*D. farinae*) and that 15 were patients were negative (< 0.35 kU/l) for D1 and D2. 14 serum samples were taken from clinically healthy individuals and used as controls: 10 males and 4 females, aged 16 to 65 years (average age 33.6 ± 7.8 years).

Collection and cultivation of the samples. The samples were collected from a 5-cm^2 area of affected body parts (scalp, face and trunk; also legs and hands) by swabbing. Samples were transported to the laboratory within 1-2 hours of collection. Specimens were then cultured qualitatively on modified Leeming and Notman agar (10 g glucose, 10 g peptone, 8 g bile salts, 2 g yeast extract, 0.5 g glycerol monostearate, 10 ml glycerol, 5 ml Tween 60, 20 ml olive oil, 15 g agar, 50 mg chloramphenicol and 50 mg cycloheximide per liter) and glucose, peptone, yeast extract (GPY) agar (40 g glucose, 5

g peptone, 5 g yeast extract, 20 g agar and 50 mg chloramphenicol per liter). Incubation was performed at 32 ^{o}C for 2 weeks.

Physiological identification of the yeasts. The DBB test was performed as described in Boekhout et al. 2010. The identification of the DBB test negative yeast strains was performed with the help of assimilation test api ID32C (bioMerieux sa, France). The DBB test positive yeasts were investigated by further presuming that they belonged to genus *Malassezia.* Tween 20, 40, 60 and 80 assimilation tests were performed as described in Gueho et al. 1996 and Guillot et al. 1996. Selective growth with cremophor EL, the presence of β-glucosidase as revealed by the splitting of esculin (Boekhout et al. 2010), the ability to produce pigments on p-medium (Mayser et al. 1998, 2004), and temperature requirements were also tested. The ability to growth on Sabouraud agar (SGA; 20 g glucose, 10 g peptone, 15 g agar per liter) was verified by multiple (four) transfers on SGA with intervals of five days incubations at 32^{o}C.

Molecular analysis of *Malassezia*. Colonies grown on mLNA agar at 32^{o}C for five days were collected by scraping them from the surface of the agar plate. DNA was extracted by modified glass beads protocol of Vogelstein and Gillespie (DNA Extraction Kit, Fermentas, Lithuania). The samples were dissolved in sterile distilled water and used for PCR amplification. Primer pair for D1/D2 26S rDNA amplification was based on the sequences reported in Fell et al. 2000. Forward primer F63: 5'-GCA TAT CAA TAA GCG GAG GAA AAG-3'; reverse primer LR3: 5'-GGT CCG TGT TTC AAG ACG-3'. Primer pair for internal transcribed spacer (ITS) regions of the rRNA gene was based on the sequences reported in Sugita et al. 1999. Forward primer pITS-F: 5'-GTC GTA ACA AGG TTA ACC TGC GG-3'; reverse primer pITS-R: 5'-TCC TCC GCT TAT TGA TAT GC-3'. Amplification reactions were performed in an Eppendorf PCR system using the following cycling parameters: 95^{o}C for two min.; followed by 29 cycles of 95^{o}C for one min., 50^{o}C for two min., and 72^{o}C for three min.; followed by the final extension at 72^{o}C for seven min. To confirm the absence of false positive reactions the PCR products were inserted into the plasmid vector pTZ57R/T (InsTAclone^{TM}PCR Cloning Kit, *Fermentas*, Lithuania) according to the manufacturer's instructions. Plasmids were extracted from the overnight culture of *Escherichia coli* using the lysis by alkali method, dissolved in sterile distilled water and used for PCR amplification. The PCR products were purified. The protocol BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Nieuwerkerk aan de IJsel, The Netherlands) was used for sequencing with genetic analyzer 3130xl (Applied Biosystems). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 program (Tamura et al. 2007) by the neighbour-joining method (Kimura two-parameter model, transition to transversion rate of 2.0).

Preparation of yeast extracts. The yeast extracts were prepared from the following cultures: *R. rubra* 345, *C. pelliculosa* 18, *C. famata* 94 and *C. guilliermondii* 65. All strains were cultured in glucose, peptone, yeast extract (GPY) broth (40 g glucose, 5 g peptone, 5 g yeast extract per liter) at 35^{o}C with shaking for four days. Yeast cells were disrupted using a BBx24 Bullet Blender® (Next Advance, Inc., USA) according to the manufacturer's protocol for *Candida albicans* homogenization in the Bullet Blender. After the extraction of yeast cells, homogenate was separated from debris and beads by

centrifugation at 800 g for 5 minutes. The protein concentration of the extracts was determined by spectrophotometry at 280 nm and adjusted to 250 µg/ml in PBS. The extracts were stored at -20°C.

Specific IgE enzyme linked immunosorbent assay (ELISA). Yeast protein extracts were adapted to a protein concentration of 25 µg/ml with PBS (pH 7.2). The ELISA plates (Greiner Bio-one, Germany) were coated with 100 µl of each extract and incubated at 4°C overnight. The content of the wells was aspirated, and the free surface was blocked with 150 µl of 1% bovine serum albumin (BSA) in PBS for one hour at room temperature. The test sera were diluted (1:10) in PBS buffer containing 0.1% Tween 20 (PBS-T), then distributed at 50 µl/well, and incubated overnight at 4°C. The bound antibodies were detected using biotin-labeled mouse anti-human IgE antibodies (BD Biosciences, NJ USA) and streptavidin-horseradish peroxidase (Thermo Fisher Scientific Inc, IL USA). Bound conjugate was detected calorimetrically using o-phenylenediamine / H_2O_2 substrate (100 µl per well). The reaction was stopped with 2M H_2SO_4, and absorbance was measured with a Synergy 2 microtiter plate reader (BioTek, USA) at 490 nm. The plates were washed three times with PBS-T after each step. Several negative controls were included to estimate non-specific binding. PBS buffer was used for the first control instead of the test serum. The second negative control was performed for each tested serum separately: the uncoated wells were blocked and incubated with each serum, biotin labeled mouse anti-human IgE antibodies, and streptavidin–horseradish peroxidase conjugate. Nonspecific binding was always lower in the second negative control than in the first one. Thus, we used the first control to calculate the results. Blank wells were coated with PBS buffer (instead of the yeast extract), and incubated with PBS buffer (instead of the test serum). **A**ssays with test sera and negative controls were performed in duplicate. The results were calculated as follows: (average OD_{490} of the two wells with test serum – OD of the blank wells) – (average OD_{490} of the two wells with PBS instead of test serum – OD of the blank wells).

Competitive ELISA assay. ELISA competition tests were carried out to evaluate possible cross-antigenicity between the four yeast species. The inhibiting fluid phase antigens comprised 75 µg/ml of protein extracts in PBS from the four yeast species. The serum samples from three patients (reacting against all four yeast species) were pooled and mixed (1:10) with each yeast extract. The pooled sera mixed with PBS buffer instead of the yeast extract were used to estimate the maximal binding. The mixtures were preincubated overnight at 4°C and transferred into the wells of ELISA plate coated with yeast extracts (solid phase antigens) as described above. The plates were incubated overnight at 4°C. Plates were further incubated with biotin-labeled mouse anti-human IgE antibodies and streptavidin-horseradish peroxidase as described above.
PBS buffer was included as a blank control instead of the sera-yeast extract mixture.
The percentage of inhibition was calculated as follows:
Inhibition % = (A/B) ×100; where: A=OD_{490} with inhibiting fluid phase antigens – blank OD_{490}; B=OD_{490} without inhibiting fluid phase antigens – blank OD_{490}

Immulite® inhibition assay. In order to assess possible cross-reactivity between the four yeast species and house dust mites, two mite-positive sera were pooled, then mixed with each yeast extract, and transferred into the wells of an ELISA plate coated with the same

yeast extract (100 µl of 25 µg/ml yeast extract was added into the microtiter wells and incubated overnight at 4°C) with the aim of adsorbing the yeast-specific antibodies (binding of antibodies to the solid phase antigen) or blocking the yeast-specific epitopes of the remaining antibodies (binding of antibodies to the antigen in the liquid phase). A pooled sera sample preincubated with PBS (instead of the yeast extract) served as a control. The sera with adsorbed yeast-specific antibodies (or blocked yeast-binding epitopes) were analyzed using Immulite® 2000 3GAllergy™ for IgE antibody to D1 and D2 according to the manufacturer's instructions.
Additionally, ten D1 and D2 positive sera were selected. Microtiter wells were coated with 100µl of 25 µg/ml *C. pelliculosa* extract. The selected serum samples were added, and the wells were incubated overnight at 4°C. Serum samples preincubated with PBS (instead of the yeast extract) served as controls. The IgE antibody value against D1 and D2 was determined using Immulite® 2000 3GAllergy™.
The inhibition percentage was calculated as (A/B) ×100; where A= value of serum preincubated with yeast extract; B= value of serum preincubated with PBS.

Flow cytometry. In the flow cytometry analysis, 5 µl of yeast cells suspension in PBS (10^7 cells/ml) was mixed with 100 µl of test sera diluted 1:10 in staining solution (100 ml PBS, 2 ml bovine serum, 0.1 g sodium azide), and incubated for 24 hours at 4 °C. Yeast cells were then mixed with 20 µl of biotin labeled mouse anti-human IgE antibodies (BD Biosciences, USA) diluted 1:100 in staining solution, and the mixture was incubated for 30 min. in ice. Yeast cells were resuspended in streptavidin-fluorochrome conjugate (Anti-IgE Pe Cy5), incubated for 30 min. in ice, and mixed with 200 µl of staining solution. The fluorescence intensity was measured with flow cytometer FACS Calibur (BD Biosciences, USA).

Data analysis. All statistical analysis was performed using standard statistical software SPSS version 15.0, and STATISTICA 6. The Student's *t*-test and paired *t*-test were used for statistical analysis. The Mann-Whitney *U* test was used to investigate possible differences between the two groups in ELISA. The Spearmans rank order correlation test was used for correlation analysis in Immulite® inhibition assay. A P-value < 0.05 was considered as statistically significant.

RESULTS

1. Taxonomic characteristics of atypical *Malassezia* strains

The genus *Malassezia* belongs to anamorphic basidiomycetous yeasts (Ahearn, Simmons 1998). At present, fourteen *Malassezia* species are reported: *M. furfur*, *M. globosa*, *M. restricta*, *M. sympodialis*, *M. slooffiae*, *M. obtuse*, *M. pachydermatis* (Gueho et al. 1996), *M. dermatis* (Sugita et al. 2002), *M. japonica* (Sugita et al. 2003), *M. yamatoensis* (Sugita et al. 2004), *M. nana* (Hirai et al. 2004), *M. equine*, *M. caprae* (Cabanes et al. 2007), and *M. cuniculi* (Cabanes et al. 2011). All *Malassezia* species are recognized as lipid-dependent. Even *M. pachydermatis*, designated as non-lipid-dependent, requires growth media enriched with peptone (*i.e.* Sabouraud medium), which contains short chain fatty acids (Boekhout et al. 2010). The main physiological characteristics of the known *Malassezia* species and our atypical isolates are summarized in the Table 2.

We isolated three non-lipid-dependent strains (M47, M54 and M235) of *M. furfur* from patients with diagnosed atopic dermatitis. Although these atypical isolates were in many ways similar to *M. furfur*, biochemical identification was not possible because of their ability to grow on Sabouraud glucose agar without lipid supplementation. The growth on SGA was different from the growth on mLNA (fig. 1 and 2): the colonies were very small (~0.3 mm in diameter to compare with 2-3 mm colonies developing on mLNA), but the growth was seen on the second day of cultivation.

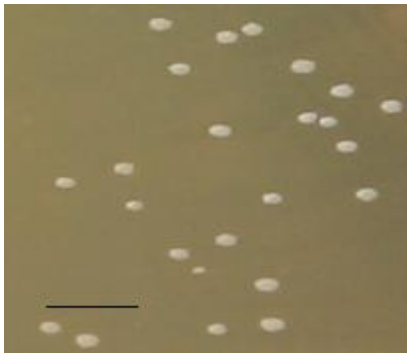

Figure 1. Appearance of the colonies of atypical strain of M. furfur (M47) on SGA after incubation at 32^{o}C for five days. Bar 1.5 mm.

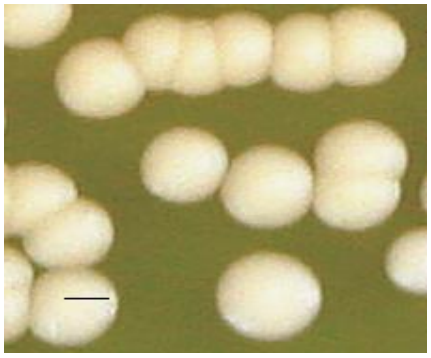

Figure 2. Appearance of the colonies of atypical strain of M. furfur (M47) on mLNA after incubation at 32^{o}C for five days. Bar 1.5 mm.

Table 2. Main characteristics of *Malassezia* species.

Species	Growth on SGA	Tween 20	Tween 40	Tween 60	Tween 80	Cremophor EL	Catalase	ß-Glucosidase	Pigment production	Growth at 37 °C	Growth at 40 °C
M. caprae[a]	–	+	w	w	w	–	+	+	?	–	–
M. cuniculi[b]	–	–	–	–	–	–	+	+	?	+	+
M. dermatis[c]	–	+	+	+	+	w, +	–	–	?	+	+
M. equina[a]	–	–	w	w	w	–	+	–	?	w	–
M. furfur[d]	–	+, –	+, –	+, –	+, –	+, –	+, –	w	+	+	+
M. globosa[d]	–	–	–	–	–	–	+	–	–	–, w	–
M. japonica[e]	–	–	w	+	–	–	+	+	?	+	–
M. nana[f]	–	+	+	+	w	–	+	–	?	+	w
M. obtusa[d]	–	–	–	–	–	–	+	+	–	w	–
M. pachyder.[d]	+, w	+	+	+	+	+	+, w	+	w	+	+
M. restricta[d]	–	–	–	–	–	–	–	–	–	w	–
M. slooffiae[d]	–	+, w, –	+	+	–, w	–	+	–	–	+	+
M. sympod.[d]	–	–, w	+	+	+	–, w	+	+	–	+	+
M. yamatoen.[g]	–	+	+	+	+	?	+	?	?	+	–
M.47,M.54,M.235	+	+	+	+	+	+	+	w	+	+	+
M.8	–	+	+	+	+	–	+	–	–	+	–

+: positive; –: negative; w: weak; ?: no data.

[a] Cabañes et al. 2007; [b] Cabanes et al. 2011; [c] Sugita et al. 2002; [d] Guého et al. 1996; [e] Sugita et al. 2003; [f] Hirai et al. 2004; [g] Sugita et. Al. 2004.

In order to check the viability of yeasts and their ability to survive without lipid supplementation for longer periods of time, four repeatedly transfers from SGA on SGA with the intervals of five days were performed.

The fourth atypical *Malassezia* strain (M8) was in many ways similar to *M. nana*, but this species is associated with animals, and has never been isolated from human skin.

2. Molecular phylogenetic analysis of atypical *Malassezia* strains

The atypical yeast isolates (M47, M54 and M235) obtained from the AD patients showed nearly identical D1/D2 and ITS-5.8S-ITS2 sequences indicating that they are conspecific strains. Similarities between M47, M54 and M235 isolates and the reference *M. furfur* strain CBS 1878^{T} in their D1/D2 regions were 99.3 %, 98.9 %, and 99.5 %, respectively (Fig. 3).

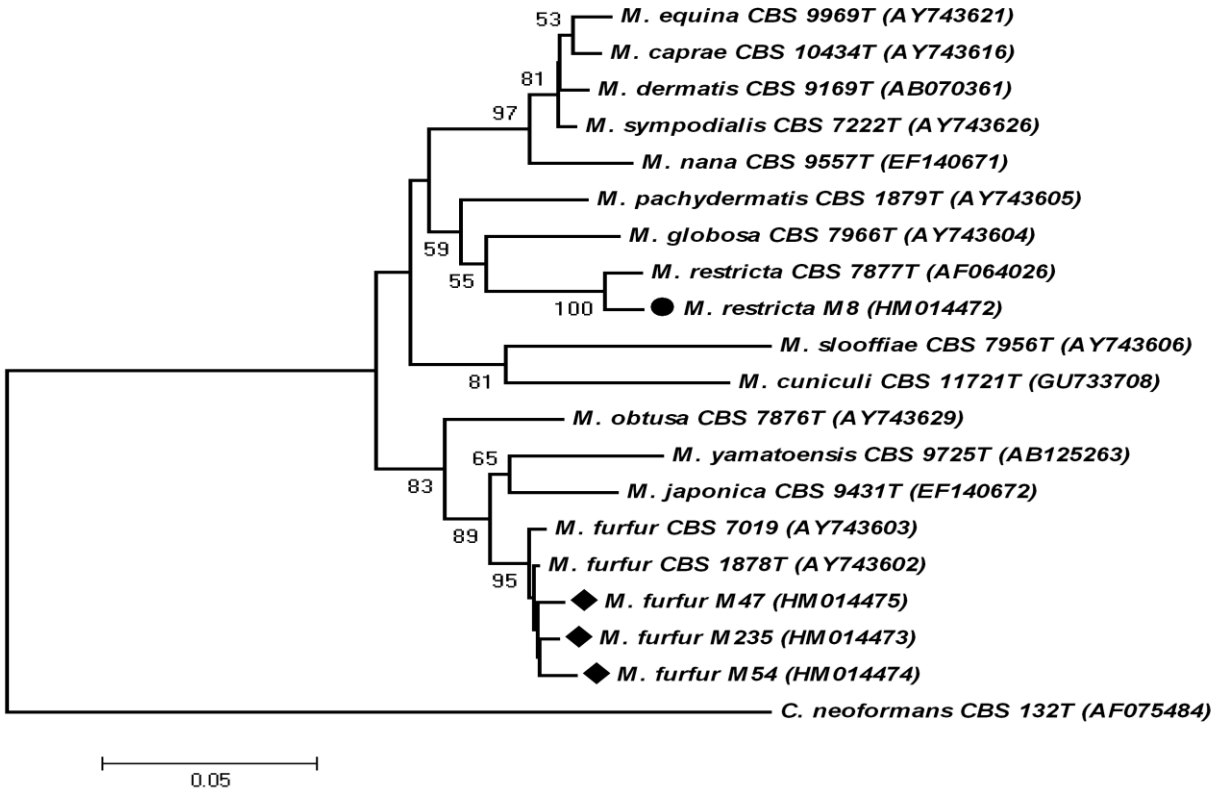

Figure 3. Phylogenetic tree constructed using the D1/D2 26S rRNA sequences of atypical isolates of *Malassezia* and other members of the genus *Malassezia.* Outgroup: *Cryptococcus neoformans* CBS 132T. GenBank accession numbers are indicated in parentheses. The evolutionary history was inferred using the Neighbor-Joining method.

Similarities between M47, M54 and M235 isolates and the *M. furfur* CBS 1878^{T} strain in their ITS-5.8S regions were 97.9 %, 98.2 %, and 98.5 %, respectively (Fig. 4).

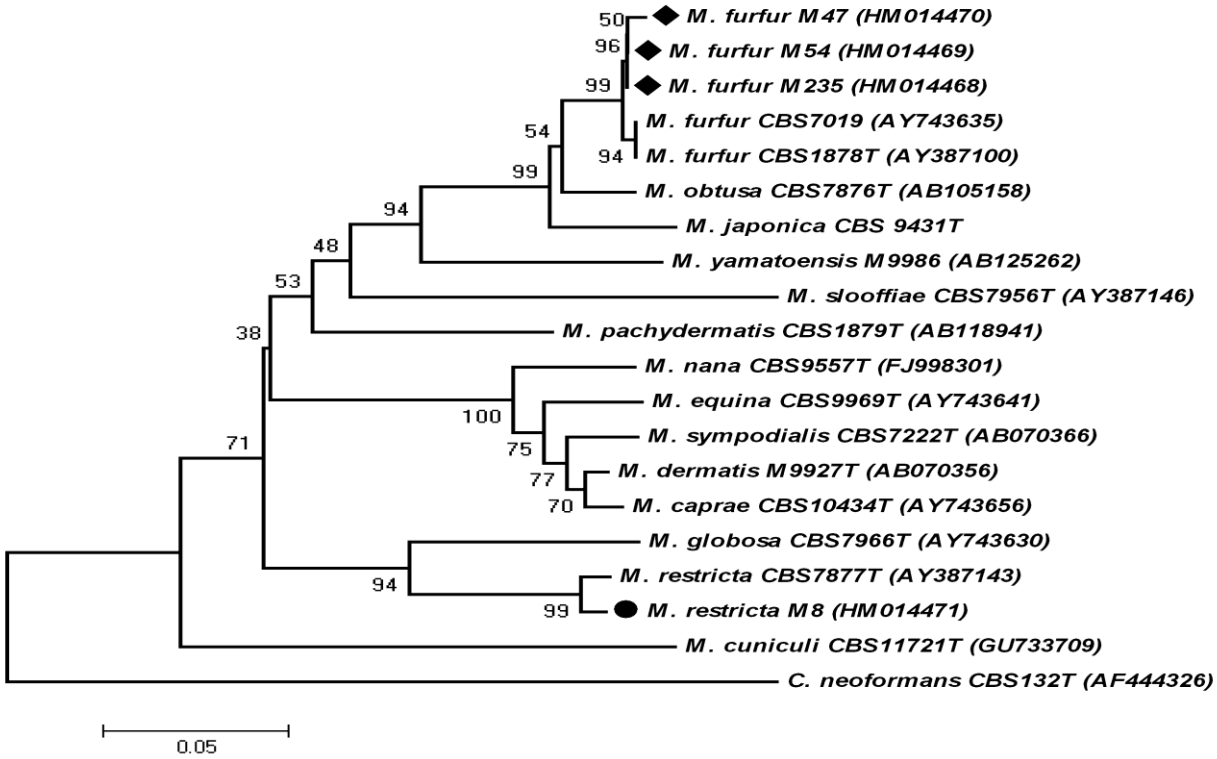

Figure 4. Phylogenetic tree constructed using the ITS-5.8S rRNA gene sequences of atypical isolates of *Malassezia* and other members of the genus *Malassezia.* Outgroup: *Cryptococcus neoformans* CBS 132T. GenBank accession numbers are indicated in parentheses. The evolutionary history was inferred using the Neighbor-Joining method.

Phylogenetic analysis of the sequences obtained from these atypical strains indicated that they did not exceed the variation generally observed to occur between species (Scorzetti et al. 2002). These isolates were identified as non-lipid-dependent variants of *M. furfur*.

Similarities between the atypical yeast isolate M8 and the reference *M. restricta* strain CBS 7877^{T} in their D1/D2 regions were 97.8 % (Fig. 3). Similarities between the atypical yeast isolate M8 and the reference *M. restricta* strain CBS 7877^{T} in their ITS-5.8S regions were 73 % (Fig. 4). Phylogenetic analysis of the sequences obtained from the atypical strain M8 indicated that this isolate could be a member of a new yeast species.

M. furfur atypical strain M47 is maintained in the American Type Culture Collection (ATCC), Manassas, Virginia, USA as strain ATCC MYA-4874.

M. restricta atypical strain M8 has been deposited in the Japan Collection of Microorganisms (JCM), Saitama, Japan.

3. Evaluation of the yeast species distribution by age and affected body parts of AD patients

In total, 89 (36.9 %) of samples were positive. Isolated yeast belonged to the following three genera: *Candida, Malassezia* and *Rhodotorula*. The most commonly isolated genus detected in 66 specimens (27.4 %) was *Candida*. Only 16 samples (6.6 %) yielded *Malassezia* in culture. *Rhodotorula rubra* was detected in seven cases (2.9 %). Species most frequently isolated from both AD groups were *C. famata, C. pelliculosa, C. parapsilosis*, and *M. furfur*. The predominant species in both age groups was *C. famata*, which was obtained in the 26.5 % of positive samples in the children's group, and in 25.5 % of positive samples in the adults' group. Other yeast species were detected across the range of 0–17.6 % in the children's specimens, and 0–23.6 % in the adults' specimens.

Cutaneous colonization with yeast was 2-fold higher in the adults' group compared to the rates in the children's group, and this difference was statistically significant ($P < 0.0001$) (Fig. 5).

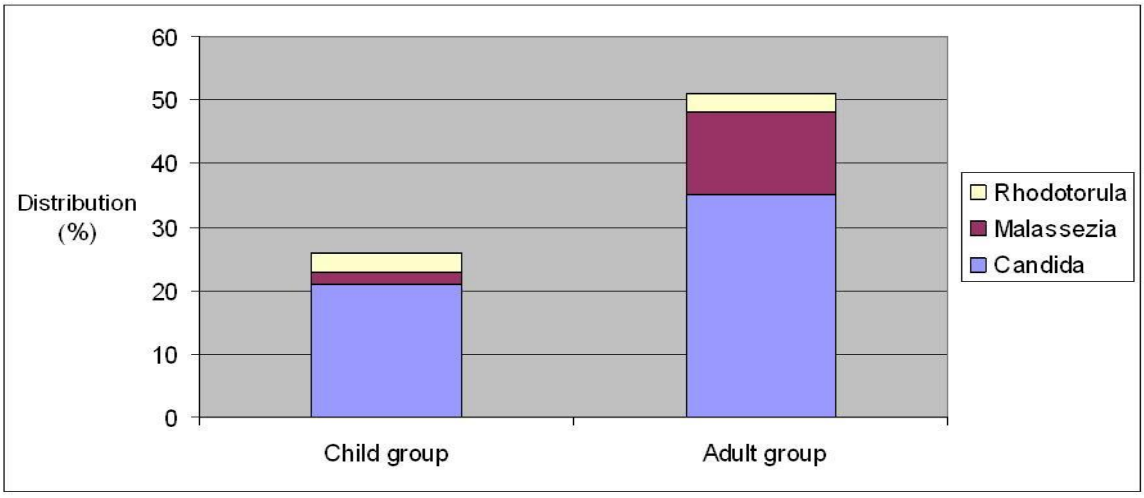

Figure 5. Cutaneous fungal microbiota of AD skin and distribution of yeast species among different age groups.

The distribution of the isolated *Malassezia* and *Candida* species was highly dependent on the body part sampled: *Malassezia* mostly appeared in face, neck, and trunk regions (P = 0.0047), and *Candida* generally colonized hands and legs (P = 0.0029) (Fig. 6).

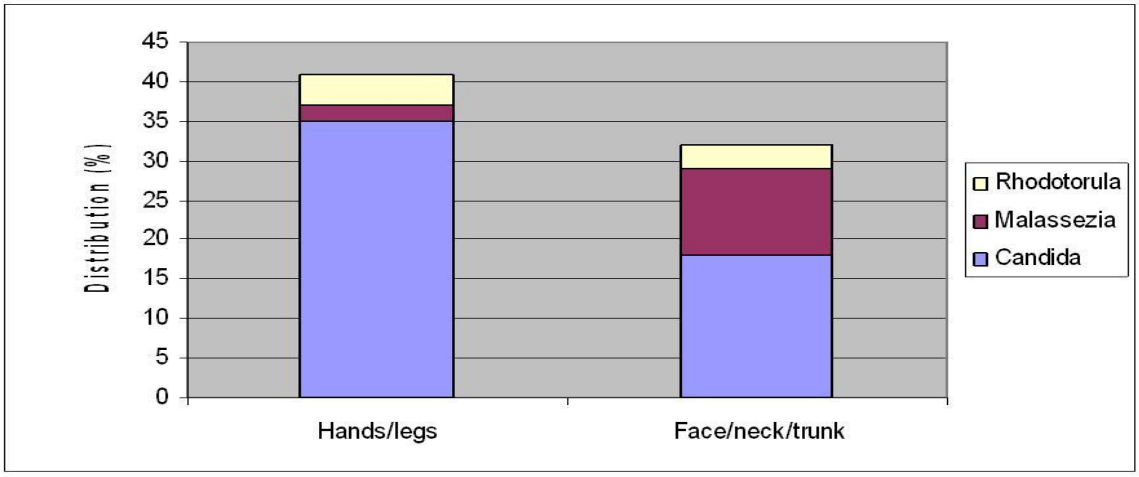

Figure 6. Cutaneous distribution of *Candida*, *Malassezia* and *Rhodotorula* species in lesional skin depending on affected body parts.

In the children's group, *Malassezia* species were in the minority (2 samples) and *Candida* species were in the majority (28 samples). Differences in the distribution of yeasts on the AD-affected skin were observed between the subgroups I (samples obtained from hands/legs) and subgroups II (samples obtained from face/neck/trunk). The highest prevalence of yeasts was found on legs and hands (67.6 % of the positive samples) with *Candida sp* as predominant.

Being found in 38 samples, *Candida* species were also dominant in the adults' group. *Malassezia* yeasts were found in 14 samples and *R. rubra* in 3 samples, respectively. In the adults' group, distribution of yeasts was almost equal: legs and hands region prevalence reached 50 %, and face, neck, trunk area was colonized by yeasts in 51 % of the cases. In other words, yeasts were isolated from the AD-affected skin in one-half of the samples in adult patients. Relevant differences were observed with regard to isolated yeast genera: *Candida* species were predominant on hands and legs (65.8 %) and *Malassezia* species were mostly found on face and trunk (85.7 %).

No yeast was observed growing in control group of healthy individuals.

4. *Candida* and *R. rubra* specific IgE antibodies in the sera of patients with AD

Specific IgE antibodies to different yeast species were tested in the sera of patients with AD. The control group comprised the sera from clinically healthy individuals. We observed that the sera from patients allergic to house dust mite reacted with the yeast extracts.

The results of ELISA showed that all D1/D2 positive sera contained IgE antibodies to the yeast species tested. Most of these sera reacted with more than two yeast species: 47 % of the sera had specific IgE antibodies to three yeast species, and 42 % of the sera reacted with all four yeast species.

The weakest response and minor differences in IgE binding between D1/D2 positive and D1/D2 negative sera were observed for *C. famata*. No specific IgE antibodies were found in the control group (Fig. 7).

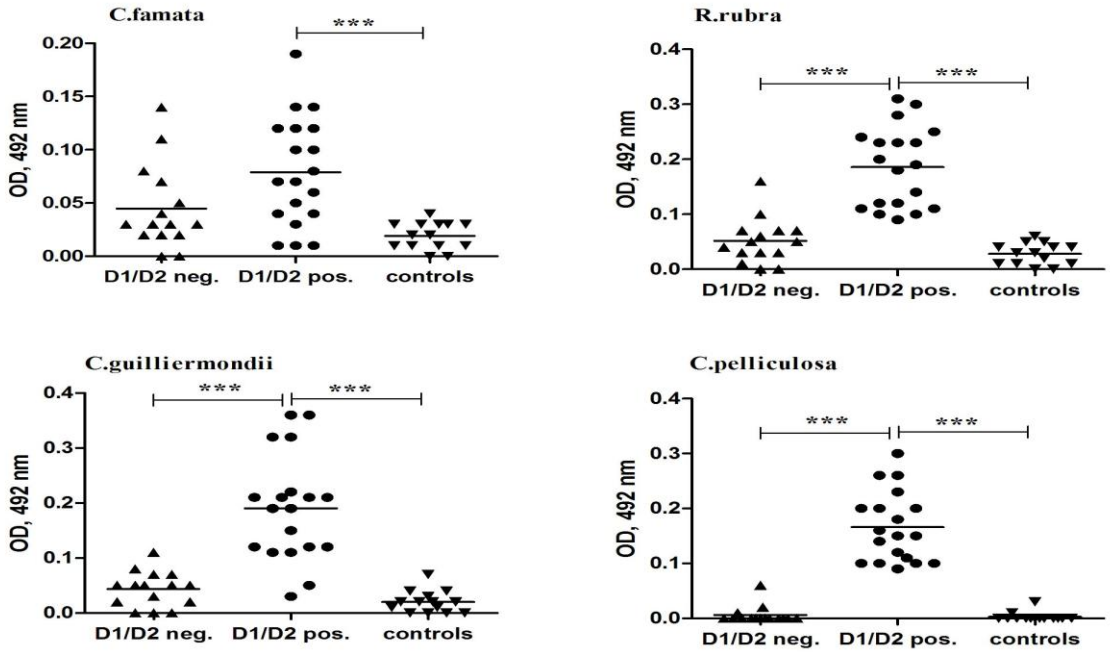

Figure 7. Specific IgE antibodies against the four yeast species in the sera of AD patients determined using an ELISA method. D1/D2 negative group contained the sera of AD patients with no specific IgE antibodies to house dust mites *D. pteronyssinus* and *D. farinae* (< 0.35 kU/L), whereas D1/D2 positive group contained the sera of AD patients with specific IgE to house dust mites (> 0.7 kU/L). Controls group contains the sera of clinically healthy individuals. ***Differences between the two groups P < 0.001 (Mann-Whitney *U* test)

We found significant differences between house dust mite-positive and control sera: $P < 0.0001$ for *R. rubra, C. pelliculosa, C. guilliermondii*, and $P < 0.0003$ for *C. famata*. Differences were also evident between D1/D2 positive and D1/D2 negative sera: $P < 0.0001$ for *R. rubra, C. pelliculosa*, and *C. guilliermondii*.

We also investigated whether the serum specific IgE antibodies to the yeast extract could recognize yeast surface antigens; for this purpose, the flow cytometry was used. The tested antibodies did not bind to the yeast surface, indicating that they recognized some intracellular antigens. This observation is consistent with the results of other studies, indicating that most identified fungal allergens are intracellular proteins (Simon-Nobbe et al. 2008).

5. Cross-reactivity between the three *Candida* species and *R. rubra* specific IgE antibodies

Table 3 shows the inhibition of IgE binding to the solid phase antigens with the fluid phase antigens prepared from the extracts of different yeast species. The fluid phase antigens from *C. guilliermondii* inhibited IgE binding to solid phase *C. famata* and *C. pelliculosa* antigens; *C. pelliculosa* markedly inhibited *R. rubra*, and *R. rubra* antigens mildly inhibited *C. pelliculosa*. A reduction of 40 % and 51 % in specific IgE reactivity of homologous inhibition were obtained, respectively, for *C. guilliermondii* and *R. rubra*.

Table 3. Cross-inhibition (%) of IgE binding to solid phase antigens after preincubation with fluid phase antigens

Solid phase antigen	Fluid phase antigen			
	C. guilliermondii	*C. famata*	*C. pelliculosa*	*R .rubra*
C. guilliermondii	40±11.3	167.5±13	130±8	100±1
C. famata	**55±4**	152±12	129±5	120.5±2
C. pelliculosa	**40±7**	106.5±9	96.5±12	**63±9**
R. rubra	104.5±4	123.5±5	**22±18**	51±21

Data represent average ± standard deviation obtained from the two experiments with pooled sera of three AD patients.

6. Cross-reactivity between IgE specific antibodies to *C. pelliculosa* protein extract and house dust mites allergens D1 and D2

Of four yeast extracts tested, only *C. pelliculosa* extract showed inhibition of IgE binding to the allergens D1 (63.4 %) and D2 (71 %). The other three yeast species showed no inhibition of specific IgE binding (Fig. 9).

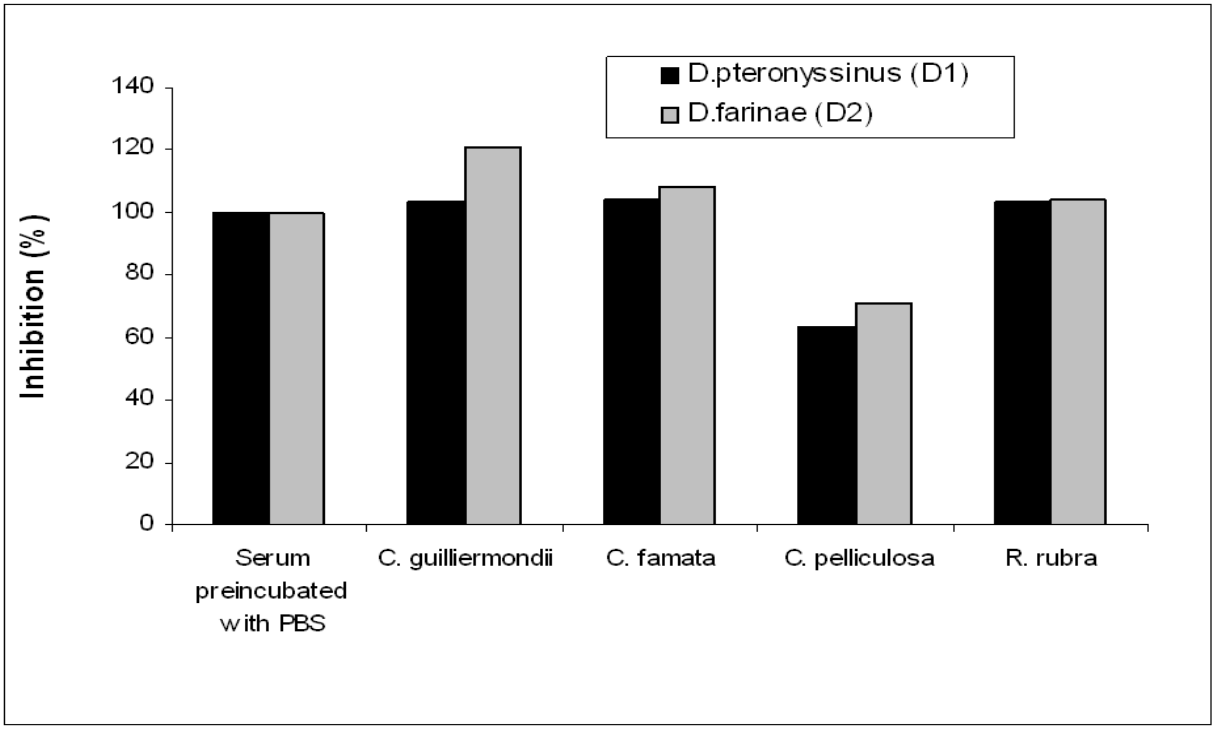

Figure 9. Inhibition of IgE binding to house dust mites *D. pteronyssinus* (D1) and *D. farinae* (D2) allergens by using four yeast species protein extracts determined using Immulite® inhibition assay.

The Immulite® inhibition assay was performed to study cross-reactivity between the *C. pelliculosa* extract and the allergens of the house dust mites. Eight sera from ten D1/D2 positive patients showed inhibition of IgE binding to D1 and D2 allergens after preincubation with *C. pelliculosa* protein extract (Fig. 10). The mean inhibition of specific IgE binding was 73.0 % ± 23.8 % (average ± standard deviation) for D1 and 82.4 % ± 11.3 % for D2. In all cases, the *C. pelliculosa* extract inhibited IgE binding to both or neither of the D1 and D2 allergens. The inhibition value against the D1 antigen showed a significant correlation with the inhibition value against D2 (r = 0.669, P = 0.03; nonparametric Sperman correlation).

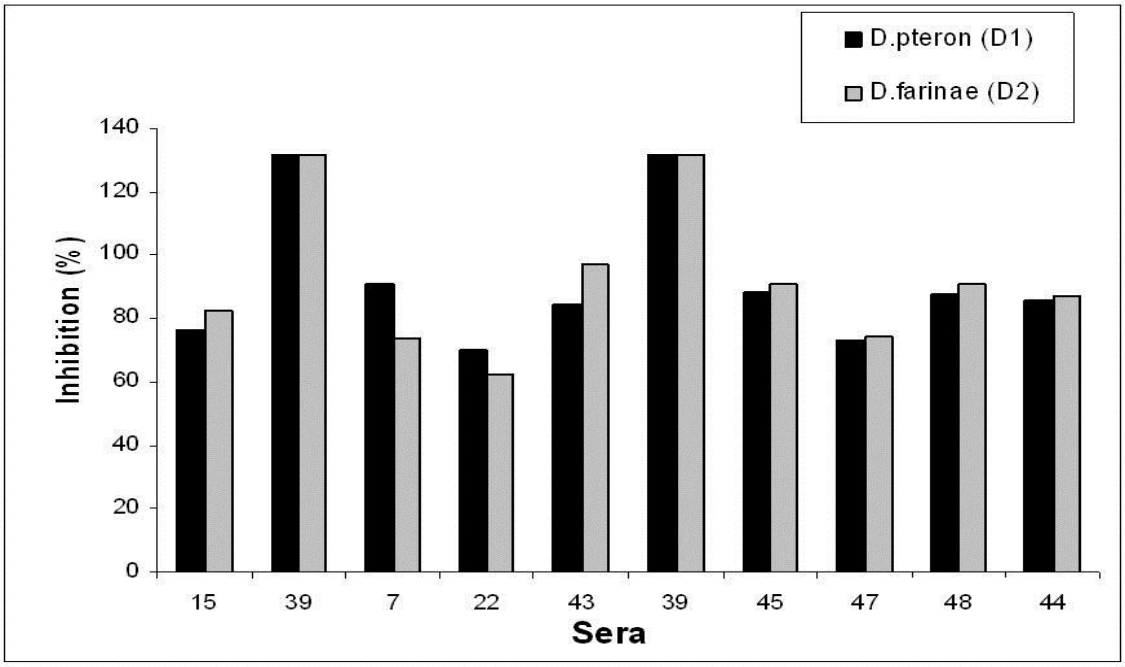

Figure 10. Inhibition (%) of IgE binding to house dust mites allergens D1 and D2 from *C. pelliculosa* determined using the Immulite® inhibition assay.

DISCUSSION

We isolated three non-lipid-dependent strains (M47, M54 and M235) of *M. furfur* from the patients with diagnosed atopic dermatitis. All *Malassezia* species are recognized as lipid-dependent. Even *M. pachydermatis*, designated as non-lipid-dependent, requires growth media enriched with peptone (*i.e.* Sabouraud medium), containing short chain fatty acids (Batra et al., 2005). New physiological features which characterize atypical *Malassezia* strains mainly are mainly associated with variations in Tween assimilation pattern - such isolates still require lipids for growth. This feature seems to be related to the distribution of these yeasts on sebum rich areas of the body (DeAngelis et al., 2005) and to virulence factors such as the *Malassezia* lipases and phospholipases (some authors claim that these enzymes are involved in pathogenicity mechanisms) (Brunke, Hube, 2006; Cafarchia, Otranto, 2004; Pini, Faggi, 2011). In addition to that, some isolates of *M. pachydermatis* have been shown to be lipid-dependent (Bond, Anthony, 1995; Cafarchia et al., 2007; Duarte et al., 2002). This species usually occurs on animals, but could appear on the skin of dog owners as commensal yeast (Morris, 2005). Some authors suggested that these yeasts may be in a state of adaptation to a specific host, associated with an increasing dependency on exogenous lipid supplementation (Cafarchia et al., 2007).

M. furfur has an essential requirement for olive oil or oleic acid for growth on Sabouraud agar. But it is one of the most robust lipid-dependent species, as any lipid supplement is sufficient for its growth (Batra et al., 2005). *M. furfur* is known from various hosts and body sites. The species has also been detected on dogs, horses, cows, bats and even on a hospital floor (Cafarchia et al., 2007; Crespo et al., 2000, 2002; Gandra et al., 2008; Tanaka et al., 2001). *M. furfur* has been described as showing high genotypic variability and these specific genotypes could be related to geographic origin of the isolates, skin disease origin, age groups, body sites from which they came, or the host (human or animal associated subtypes) (Castella et al., 2005; Gaitanis et al., 2009; Gandra et al., 2006; Takahata et al., 2007). In Lithuania, cutaneous colonization with *Malassezia* species is very low compared to other studies from different countries (Yim et al., 2010; Lugauskas et al., 2002; Nakabayashi et al., 2000; Saghazadeh et al., 2010). The cutaneous microenvironment is generally considered to be important for *Malassezia*

populations. Skin diseases associated with *Malassezia* species seems to be more common in the tropics, suggesting that increased cutaneous temperature and humidity favours yeast growth (Aspiroz et al., 2002; Dutta et al., 2002; Gupta et al., 2004; Khosravi et al., 2008; Midgley, 2000; Ramadan et al., 2012).

There is a hypothesis that phenotypic and genotypic variability in some yeast species can act as a determining factor in the infection strategy used by the microorganism, which is associated with switching of various metabolic pathways in order to accommodate to the availability of nutrients in some circumstances (Lan et al., 2002; Staib et al., 2001). This theory was revealed for *Candida spp*. but, in our opinion, it is could also be applicable for *Malassezia spp*. These two genera are phylogenetically distant human pathogenic and commensal fungus, but whole-genome shotgun sequencing revealed that *M. globosa* and *C. albicans* secrete a similar set of extracellular hydrolases. It was suggested that the prevalence of similar gene families in these two yeast species may point to similar roles in colonization and virulence (Staib et al., 1999; Xu et al., 2007). It is known that *Malassezia spp*. fatty acids incorporates directly into cellular lipids without further metabolism and some authors suggest that the lipids present on the skin surface may affect *Malassezia* resulting in either immunosuppressive (or commensal) or immunostimulatory (or pathogen) phenotype (Ashbee, Evans, 2002).

The conclusion that *M. globosa* genome contains a mating locus indicates that the fungus may be capable of sexual development, although mating has never been observed in *Malassezia* species. The possibility of a sexual cycle might be important in the distribution of virulence-related genes among populations (Xu et al., 2007). Genes at the mating locus are highly conserved, even in asexual fungi. The presence of a coding sequence does not mean that the gene is correctly transcribed and translated (Butler et al., 2010). Nevertheless, the results of one research have suggested that genetic exchange occurs within and between the various genetic groups of *M. pachydermatis* (Midreuil et al., 1999). A similar conclusion was proposed for *M. furfur* also (Guillot et al., 1997; Mittag, 1994). Increasing number of *Malassezia* isolates, which were identified as a lipid-dependent *M. pachydermatis* or pleomorphic variants of *M. furfur*, led us to the assumption of genetic material exchange between these two species (Cafarchia et al., 2007, 2011).

Two of the most extensively used DNA regions for the basidiomycetous yeast typing comprise the D1/D2 domains of the 26S rRNA gene, and the ITS-5.8S regions. It was demonstrated that conspecific strains show less than 1 % substitution in 26S rDNA region (Fell et al., 2000; Scorzetti et al., 2002), and that strains of a single biological species have less than 3 % dissimilarity in the ITS1 and ITS2 regions (Cabanes et al., 2005; Makimura et al., 2000; Scorzetti et al., 2002; Sugita et al., 1999). It was demonstrated, that the sequence difference in ITS between some mating species exceeded 8 % (Hamamoto et al., 2002). In the present study we isolated atypical *M. restricta* strain M8. The rate of similarity between the atypical yeast isolate M8 and the reference *M. restricta* strain CBS 7877^{T} in their ITS-5.8S regions was 73 %. Phylogenetic analysis of sequences from atypical strain M8 indicated that this isolate could be a member of a new yeast species.

Most epidemiological studies involve only molecular-based identifications of *Malassezia* species (Paulino et al., 2006; Sugita et al., 2006; Tajima et al., 2008; Zhang et al., 2011). Observation of *Malassezia* strains with atypical physiological characteristics demonstrate the biology of the species to be essential criterion in yeast systematics. A sequence, per se, does not describe a species (Scorzetti et al., 2002). There is a possibility, that species with atypical physiological features could be detected in more cases if using traditional, culturing methods and molecular techniques for identification.

Colonization with *Malassezia* is the most common yeast infection in AD. The majority of studies involving yeasts and AD patients focus mainly on the members of the genus *Malassezia*. *Malassezia* has been isolated in 46–100 % of patients with atopic dermatitis, and in 75–80 % of healthy adults worldwide. *M. sympodialis, M. globosa, M. furfur* and *M. restricta* were the most commonly isolated *Malassezia* species (Casagrande et al., 2006; Yim et al., 2010; Nakabayashi et al., 2000; Rincon et al., 2005; Sugita et al., 2006; Zhang et al., 2011). In the present study, we have shown that both particular yeast species and their carriage rate are associated with the age of patients and with the part of the body on which they were found. There can also be a link observed between the body part affected by atopic dermatitis and the prevalent yeast genera: if *Malassezia* mostly colonize lipid-rich areas; *Candida* usually appears on hands and legs.

The data obtained in the present study are similar to those from previous studies (Nakabayashi et al., 2000; Scheynius et al., 2002; Akaza et al., 2010).

In the children's group, yeast colonization rate was significantly lower than in the adults' group. This observation supports the suggestion that the bacterial infection (*Staphylococcus aureus*) dominates among children with AD. It also supports the notion that the cases of colonization with yeasts (rather rare) in children may be associated with a frequent use of antibiotics, because *Candida* numbers increase significantly following antibacterial therapy (Baker, 2006; Bunikowski et al., 1999, 2000).

Malassezia colonization rates in the children's group were very low (1.5 %); these data are consistent with the results of the study of Cunningham et al., 1992 , which indicated that carriage of *Malassezia* increases around puberty, and is correlated with the increase in sebaceous gland activity seen at this time.

In the adults' group, the rate of yeast colonization was rather high (51 %). In this group, the predominant species were *C. famata* and *C. pelliculosa*, and they affected mainly hands and legs. These two yeasts species, together with *R. rubra*, could be assumed to be transient microorganisms; however, the rather high incidence rate does not foreclose the possible importance of these microorganisms in clinical evaluation of AD. Interestingly, the mycological colonization rate in adult patients with AD is similar in all studies independently of isolated fungal species and geographical distribution, with an approximately frequency of 50 % (Yim et al., 2010; Saghazadeh et al., 2010).

No yeast growth was observed in the control group. With regard to non-*Malassezia* fungi, this finding is not surprising given that healthy skin forms a physical barrier with many defensive strategies, and that transient or commensal yeasts from the environment are incapable of surviving on an intact skin for long periods of time. *Malassezia* yeasts are members of the normal human cutaneous flora and are found on the skin of healthy adults (Akaza et al., 2010; Ashbee, Evans, 2002). In the present study, we observed no *Malassezia* growth in the control group and, in general, colonization with these yeasts was very low as compared with other studies. These differences are probably related to geographical location as has been suggested by several authors (Faergemann, 2002; Gupta et al., 2004).

An interesting point to note is that *Malassezia* and *Candida* were not found together in any of the study samples. *Malassezia* and *Candida* share the same ecological niche;

therefore, it could be expected that in some specimens a culture of both genera is possible. One of the possible explanations of this phenomenon may be related to different growth rates: fastidious, slow-growing *Malassezia* can be overgrown by *Candida*, which has no special requirements for growth media. Additionally, the ability to secret a substance lethal to other species (so-called killer phenomenon) has been reported to be widespread in yeasts (Golubev, 1998). In the present study, some *Candida* species demonstrated killer activity against *M. furfur* (data not shown), which can also explain the predominance of *Candida* over *Malassezia*.

Fungal allergy is not as well researched as are other allergies, but the number of the identified fungal allergens is increasing. Many AD affected persons show sensitivity to fungal allergens; such individuals commonly react to many fungal species, and sensitization to a single species only is quite rare. It has been demonstrated that repeated cutaneous application of a hapten can induce a shift from Th1 (lymphocyte dependent delayed type hypersensitivity) to Th2 (immediate hypersensitivity with IgE production) (Kitagaki et al., 1995). These findings indicate that individuals with an AD history tend to show enhanced immediate hypersensitivity to cutaneous microflora, which seems to be related to antigenic variability and cross-reactivity between species. Skin barrier defects play a primary role in the pathogenesis of AD. A defective function of permeability barrier enables penetration of environmental allergens from grass pollens, house dust mites, and microorganisms into the skin thus initiating immunological reactions. Our investigated yeasts are commensal microorganisms which could be present in soil, food, or elsewhere in the environment. If these yeasts come in contact with primarily sensitized subject, an aggravation of the allergic skin symptoms may occur due to cross-reactive epitopes. In consideration of these data we hypothesise that cutaneous colonization by yeast species may be involved in aggravation of AD.

In the present study, we have investigated the presence of specific IgE antibodies to three *Candida* species and *R. rubra* in the sera of AD patients. We found that specific IgE antibodies to the yeast species investigated are often present in the sera of the patients who also have specific IgE antibodies to house dust mite allergens. Additionally, testing of specific IgE antibodies to *C. pelliculosa* extract showed cross-inhibition with house dust mite allergens. House dust mite is recognized as the most important risk factor in indoor allergies**,** with *D. pteronyssinus* and *D. farinae* as the most abundant species in

house dust throughout the world (Takai et al., 2005). It has been shown that the most important allergenic proteins in these mites are highly homologous and react with 80 % of sera from mite-sensitive patients. Mite allergens do exhibit variable degrees of cross-reactivity between different mite and other invertebrate species (Sidenius et al., 2001; Thomas et al., 2004). More than half of all cross-reactive fungal allergens have homologous IgE-reactive proteins in nonfungal species (Simon-Nobbe et al., 2008). Considering this we hypothesized that the yeast species investigated in our research may be involved in exacerbation of AD as a result of cross-reactivity with house dust mites. Our findings support the results of other investigations, demonstrating that moderate-to severe AD is strongly associated with sensitization to house dust mites and fungi (Scalabrin et al., 1999).

No correlation was found between specific IgE values with the yeast species analyzed and total IgE values in the sera of AD patients. Strong correlations between total IgE and specific IgE antibody levels to yeast, particularly *C. albicans* and the genus *Malassezia*, were reported (Scalabrin et al., 1999). *Malassezia* is a part of the normal human cutaneous commensal flora; however, under certain conditions it is an important triggering factor for AD. Although numerous *Candida* allergens have been described, their potential role in AD remains open to debate. The frequency of specific IgE to *Candida* antigens varies from study to study. A possible explanation could be that *Candida* has weak allergenic properties and probably acts more as an aggravating factor as a result of cross-reactivity with other allergens. In some atopic patients, increased immediate-type reactions with specific IgE synthesis and diminished delayed skin reactions are observed. The depressed delayed immune response leads to the growth of saprophytic *C. albicans*. Continuous exposure to saprophytic yeast could induce synthesis of specific IgE (Savolainen et al., 1990).

Most of the sera tested tended to react with all four yeast species or with none. This finding is consistent with the results of previous studies indicating that sensitizations to multiple fungal species are common among sensitized subjects (Savolainen et al., 1998; Simon-Nobbe et al., 2008). We hypothesized that this multiple binding is resulting from common epitopes. Our inhibition study presented herein demonstrated partial cross-reactivity between IgE class antibodies and the yeasts analyzed. Our data show that different *Candida* species and *R. rubra* have species-specific and shared antigens. It has

been suggested that, if a subject develops IgE reactivity to a defined allergenic component (panallergen), the patient's serum could react with cross-reactive components in various unrelated species (Mari, 2001). Although this hypothesis was originally put forward for plant allergens, we believe it can be applied to fungi.

We used flow cytometry to determine whether serum specific IgE antibodies to the yeast extract could recognize yeast surface antigens. The antibodies tested did not bind to the yeast surface, indicating that they recognized some intracellular antigens. This observation is consistent with the results of other studies, indicating that most identified fungal allergens are intracellular proteins (Simon-Nobbe et al., 2008).

Over the last decade, a number of fungal allergens have been identified, purified, and characterized. The molecular cloning of fungal genes encoding relevant allergens has made detailed studies on cross-reactivity possible. Both unique and shared allergens were reported in phylogenetically related or unrelated fungal species. A large number of recombinant fungal allergens have been demonstrated to have similarities to one or several other fungal enzymes (Simon-Nobbe et al., 2008). Fungal allergens have been recently characterized by specifying whether or not the respective cross-reactive allergen may be found within one fungal phyla, all fungal phyla, or even non-fungal species (Simon-Nobbe et al., 2008).

In conclusion, the results of our study suggest that specific IgE antibodies to saprophytic yeasts are often present in the sera of the patients who also have specific IgE antibodies to house dust mite. Competitive ELISA tests revealed that these yeasts have species-specific and shared antigens with partially overlapping epitopes. Specific IgE antibodies to a *C. pelliculosa* extract showed cross-inhibition with house dust mites, indicating that C. pelliculosa and house dust mites might share some allergenic epitopes. We suggest that attention should be given to a cutaneous colonization by saprophytic yeast since the immune response to the allergens could further exacerbate allergic inflammation due to cross-reactive epitopes.

CONCLUSIONS

1. In 36.9 % of the cases of atopic dermatitis, the affected skin was colonized with yeast belonging to three genera: *Candida, Malassezia* and *Rhodotorula*.
2. Systematic and phylogenetic analysis of sequences from atypical *Malassezia restricta* strain M8 indicated that this isolate could be a member of a new yeast species.
3. Three atypical *Malassezia* isolates M47, M54 and M235 were identified as non-lipid-dependent variants of *Malassezia furfur*.
4. In atopic dermatitis, cutaneous colonization with yeast is two-fold higher in adults than in children ($P < 0.0001$).
5. The sera of atopic dermatitis patients have specific IgE antibodies to cross-reactive intracellular yeast antigens.
6. *Candida pelliculosa* and house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* might share some allergenic epitopes.

PUBLICATIONS

1. **Zinkevičienė A.**, Vaičiulionienė N., Baranauskienė I., Kvedarienė V., Ėmužytė R., Čitavičius D. Cutaneous yeast microflora in patients with atopic dermatitis, Cent. Eur. J. Med. 2011; 6, 713-719.

2. **Zinkevičienė A.**, Norkūnas V., Čitavičius D. Atypical non-lipid-dependent strains of *Malassezia furfur*, Cent. Eur. J. Biol. 2012; 7, 241-249.

3. **Zinkevičienė A.**, Girkontaitė I., Čitavičius D. Specific immunoglobulin E antibodies to saprophytic yeasts in sera of atopic patients allergic to house dust mites, J. Investig. Allergol. Clin. Immunol. – accepted.

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SANTRAUKA

Alergija apibūdinama kaip imuninių mechanizmų sukelta padidėjusio jautrumo reakcija į nekenksmingas, netoksiškas, nesidauginančias organizme egzogeninės kilmės medžiagas – alergenus. Paskutiniais dešimtmečiais labai padidėjo sergamumas alerginėmis ligomis. Literatūros duomenimis, padažnėję alergijų atvejai susiję su padidėjusiu alergenų kiekiu aplinkoje, užterštumu ir pasikeitusiais higienos įpročiais. Manoma, kad riziką išsivystyti alerginiam susirgimui vienodai įtakoja tiek aplinkos, tiek genetiniai veiksniai. Kaip ir kitų alerginių ligų atveju, padidėjusio sergamumo atopiniu dermatitu (AD) negalima paaiškinti vienu ar keliais dominuojančiais veiksniais. Yra žinoma, kad AD pažeista oda dažniau infekuojama mikroorganizmais. Mikroorganizmai nuo kitų alergizuoti galinčių medžiagų skiriasi tuo, kad jie geba kolonizuoti pažeistą odą ir, indukuodami alergines reakcijas, sunkinti AD eigą.

Per pastarąjį dešimtmetį išaugo susidomėjimas mikroskopiniais grybais kaip alergizuoti gebančiomis medžiagomis. Literatūros duomenimis, *Malassezia* genties mielės yra svarbus veiksnys AD patogenezei, kadangi 30–80 % vyresnių kaip 14 metų AD sergančių asmenų kraujo serume aptinkama prieš šias mieles nukreiptų IgE antikūnų. Nustatyta, kad dauguma mikroskopiniuose grybuose esančių alergizuojančių baltymų turi savo homologus kitų rūšių ar genčių grybuose. Daugelis šių mikroorganizmų išskiria medžiagas, kurios gali pačios sukelti alerginę reakciją arba stimuliuoti imuninę sistemą dėl kryžminių reakcijų, vykstančių su kitais alergenais. Kryžminėmis reakcijomis tarp šių homologinių baltymų aiškinamas pastebėjimas, kad AD sergančio individo kraujo serume aptinkama prieš kelias grybų rūšis nukreiptų IgE antikūnų. Literatūroje surinkti duomenys apie ne *Malassezia* genčiai priklausančių mielių rūšių paplitimą ant AD pažeistos odos yra skurdūs. Atsižvelgiant į aplinkoje esančių saprofitinių mielių įvairovę ir jų konservatyvių baltymų gausą, svarbu nustatyti, kurie šių tranzitinių mikroorganizmų geba kolonizuoti AD pažeistą odą ir sukelti specifinių IgE antikūnų sintezę.

Disertacinio darbo metu buvo nustatyta, kad 36,9 % atvejų atopinio dermatito pažeista oda yra kolonizuojama *Candida*, *Malassezia* ir *Rhodotorula* genties mielėmis.

Darbo metu išskirtas netipinėmis fiziologinėmis savybėmis pasižymintis *Malassezia restricta* kamienas M8 gali būti naujos rūšies atstovas. Išskirti netipinėmis fiziologinėmis savybėmis pasižymintys *Malassezia* genties kamienai M47, M54 ir M235 identifikuoti kaip nuo išorinio lipidų šaltinio nepriklausantys *Malassezia furfur*.

Įrodyta, kad mielės suaugusių asmenų atopinio dermatito pažeistą odą kolonizuoja du kartus dažniau negu vaikų ($P < 0,0001$).

Darbo metu įrodyta, kad atopiniu dermatitu sergančių asmenų kraujo serume aptinkama prieš kryžmiškai reaguojančius mielių viduląstelinius antigenus nukreiptų specifinių IgE antikūnų. Taip pat nustatyta, kad *Candida pelliculosa* ir namų dulkių erkių *Dermatophagoides pteronyssinus* ir *Dermatophagoides farinae* alergenai gali turėti panašius epitopus.

Mūsų darbo rezultatai patikimai rodo, kad atopinio dermatito pažeistą odą kolonizuojančios komensalinės mielės *Candida famata*, *Candida pelliculosa*, *Candida guilliermondii* ir *Rhodotorula rubra* gali turėti įtakos atopinio dermatito eigai, sukeldamos prieš jas nukreiptų specifinių IgE antikūnų sintezę. Todėl būtina sekti mielių mikrofloros paplitimą atopinio dermatito pažeistoje odoje ir atitinkamai skirti prevencines priemones.

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