

Detection of Mala f and Mala s allergen sequences within the genus *Malassezia*

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Malassezia species are opportunistic yeasts that are involved in the pathogenesis of a number of skin diseases including atopic eczema/dermatitis syndrome. Previously, we cloned six allergens from *Malassezia sympodialis* isolate ATCC 42132; these allergens are designated Mala s 1, and Mala s 5–Mala s 9. Three additional allergens, Mala f 2–Mala f 4, have been isolated from *M. furfur* by other investigators. The objective of the present study was to investigate the presence of these Mala sequences in seven *Malassezia* species. Genomic DNA amplification by PCR and sequencing showed that *M. globosa*, *M. obtusa* and *M. sympodialis* contain DNA sequences corresponding to all the allergens except Mala f 2 and Mala f 3. *M. pachydermatis* contains Mala s 1, Mala f 4, and Mala s 5–Mala s 8. *M. restricta* and *M. slooffiae* possessed Mala f 4 and Mala s 6. *M. furfur* was seen to possess Mala f 2–Mala f 4 as well as Mala s 5–Mala s 7. Our data from reverse-transcriptase PCR showed a more species-specific pattern of amplification. *M. furfur* evidenced expression of Mala f 2–Mala f 4. *M. globosa* and *M. obtusa* appeared to express only Mala s 6. *M. pachydermatis* expressed Mala f 4, Mala s 6, and Mala s 8, while *M. restricta* and *M. slooffiae* expressed Mala f 4 and Mala s 6. *M. sympodialis* expressed all the allergens except Mala f 2 and Mala f 3. Different *Malassezia* species appear to contain both common and species-specific allergen sequences.

Keywords AEDS, atopic eczema/dermatitis syndrome, *Malassezia*, yeast

Introduction

The genus *Malassezia*, previously called *Pityrosporum* by some authors, is comprised of yeasts that belong to the normal cutaneous microflora. *Malassezia* was revised relatively recently to include seven species, namely, *M. furfur*, *M. globosa*, *M. obtusa*, *M. pachydermatis*, *M. restricta*, *M. slooffiae*, and *M. sympodialis* [1–3]. Under certain conditions, these yeasts can become pathogenic [4]. Numerous studies have documented the importance of *Malassezia* species in pityriasis (tinea) versicolor, *Malassezia* (*Pityrosporum*) folliculitis, seborrhoeic dermatitis, and atopic dermatitis (AD) [5–8].

Atopic dermatitis is a chronically relapsing inflammatory skin disease. It is associated with itching that increases the inflammation and results in secondary skin lesions. This in turn increases the susceptibility of the skin to micro-organisms and environmental allergens [9]. Recently, the nomenclature task force of the European Academy of Allergology and Clinical Immunology (EAACI) proposed the term atopic eczema/dermatitis syndrome (AEDS), as a term more suitable than AD for the correct representation of this ‘disease’ as an accumulation of several diseases with certain clinical characteristics [10]. Approximately 80% of AEDS patients have elevated serum IgE levels with immediate-type skin reactions to environmental allergens [11], a finding that is in agreement with the idea that allergens are an important factor in AEDS. Furthermore, the discovery of IgE on epidermal Langerhans cells supports the idea that allergens are important in the pathogenesis of AEDS and that their

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effect is exerted by activation of IgE-mediated pathways [12,13].

Studies have indicated that between 40 and 65% of AEDS patients have IgE antibodies, positive skin prick tests (SPT), or positive atopy patch test (APT) reactions to extract from *Malassezia* [14–19]. Extract from *M. sympodialis* induces a significantly stronger T-cell response in AEDS subjects than in healthy individuals and this response strongly tends to be associated with production of T helper (Th)2-like cytokines [14,20,21]. These data suggest that *Malassezia* plays a role in maintaining IgE-mediated skin inflammation in AEDS.

Nine allergens have so far been cloned from two strains of *Malassezia* [22–26]. Six of these allergens were cloned from strain ATCC 42132 (American Type Culture Collection, Manassas, VA, USA) and have been expressed as recombinant proteins in our laboratory [22,24,25]. ATCC 42132, which is used in the commercially available ImmunoCap 'm70' test for measuring allergen-specific serum IgE (Pharmacia, Diagnostics, Uppsala, Sweden), was previously designated *P. orbiculare* and later *M. furfur*, but has recently been reclassified as *M. sympodialis* [[27]; J. Faergemann, personal communication]. Therefore, in accordance with the allergen nomenclature of the World Health Organisation [28], the Mal f 1, and Mal f 5 to Mal f 9 allergens, were renamed as Mal s 1, and Mal s 5 to Mal s 9. The other three allergens, Mal f 2, Mal f 3 and Mal f 4, were cloned from the *M. furfur* strain TIMM 2782 (Teikyo University Institute for Medical Mycology, Tokyo, Japan) which is identical to strain CBS 1878 (Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands) [[23,26]; H. Yasueda, personal communication]. Interestingly, Mal s 1, and Mal s 7 to Mal s 9 are unique proteins without significant sequence similarity to other known proteins [22,24,25]. In response to characterization of an increasing number of allergens from different sources, the International Union of Immunological Societies Allergen Nomenclature Sub-Committee (<http://www.allergen.org/List.htm>) has redesignated the *Malassezia* allergens as bearing the nomenclature Mala, since Mal stands for apple, also an allergen. This new nomenclature will be used here.

The potential contribution of each individual *Malassezia* species to AEDS remains to be clarified, as does the extent to which various *Malassezia* species share allergens. The aim of the present study was therefore to investigate the presence of the known Mala f and Mala s allergen sequences in the different species of *Malassezia*.

Materials and methods

Yeast strains and culture conditions

M. sympodialis isolate ATCC 42132 and *M. slooffiae* isolate CBS 7956 were obtained from the culture collections indicated. *M. furfur* isolate CBS 7019, *M. globosa* isolate CBS 7966, *M. obtusa* isolate CBS 7876, *M. pachydermatis* isolate CBS 1879, *M. restricta* isolate CBS 7877, and *M. sympodialis* isolate CBS 7222 were kindly supplied by G. Midgley (Department of Medical Mycology, St John's, Institute of Dermatology, King's College London, UK). The strains were grown on modified Dixon agar [1] for 2 days at 32°C.

DNA/RNA extractions and cDNA synthesis

Poly(A)⁺ RNA was isolated using a commercially available kit (PolyA Tract System 1000; Promega, Madison, WI, USA) according to the manufacturer's instructions. The isolated RNA was converted to cDNA using the ProSTAR™ First-strand RT-PCR kit (Stratagene, Cedar Creek, TX, USA). DNA was extracted from the cells by using a phenol/chloroform/isoamyl alcohol procedure [29].

PCR-restriction endonuclease analysis (PCR-REA)

This assay was performed as previously described [30]. Briefly, a region of the large subunit (LSU) of the ribosomal RNA (rRNA) gene was amplified by PCR using the LSU primers shown in Table 1. PCR reactions were carried out in a final volume of 10 or 20 µl containing 0.2 mmol/l dNTP (Amersham Pharmacia Biotech, Piscataway, NJ, USA), 0.2 pmol of each primer, either 50 × Taq DNA Titanium polymerase (Clontech, Palo Alto, CA, USA) or 1.25–2.50 U AmpliTaq Gold (PerkinElmer, Norwalk, CT, USA) with their respective 10 × PCR reaction buffer, and finally 1 µl template (cDNA or DNA). PCR amplifications were performed in either an Eppendorf Mastercycler gradient (Eppendorf–Netheler–Hinz, Hamburg, Germany) or a PE DNA Thermocycler 480 (PerkinElmer) with the following conditions: an initial denaturation at 95°C for 3 min (for Titanium DNA polymerase) or 10 min (for AmpliTaq Gold polymerase), followed by 30 cycles of amplification (denaturation at 94°C for 45 s, annealing at 60°C for 1 min and elongation at 72°C for 1 min) and a final elongation step at 72°C for 10 min. The PCR products were separately restricted with three restriction endonucleases, *Ban*I, *Hae*II and *Msp*I (New England BioLabs, Inc., Beverly, Mass., USA). The digested fragments were analysed by electrophoresis in 4% agarose gel (agarose 1000;

Table 1 Oligonucleotides used in PCR amplifications and sizes and sequence accession numbers of the products they generate

Primer	Sequence (5'–3')	Product size (bp)	EMBL accession number [Reference no.]
Mala s 1 forward	ATGCGATACAGCACCGTCTTGGCAGC	1061	X96486
Reverse	CGCTCTCTGACTTAAAAGTTCAGGATGC		[25]
Mala f 2 forward	TCCTACTGCTACTGCCAAGG	558	AB011804
Reverse	GACCTTGGTGAGGATCGTGT		[26]
Mala f 3 forward	CGTTTGCATACGTGCCGTAC	410	AB011805
Reverse	AGTACTCGACCTTGAGGTCG		[26]
Mala f 4 forward	TCATTGGTGGCCACTCGGGT	375	AF084828
Reverse	AGGCGTCGAGCAGCTTCTGC		[23]
Mala s 5 forward	TCTGCTACTACCGTTCTCAG	546	AJ011955
Reverse	GAGCTGCGCCAGCACCGCGTC		[22]
Mala s 6 forward	ATGTCTAACGTTTTCTTCGACATTACC	535	AJ011956
Reverse	GCACACACCGCACTTGGCGA		[22]
Mala s 7 forward	ATGAATAATGAAAAGTACTGTTTCAGTC	458	AJ011957
Reverse	GGCGTTCTTCTTGAGATTGAGC		[22,24]
Mala s 8 forward	ATGGCCCCCTCTAGCATGGATCGC	530	AJ011958
Reverse	GCACTATTGGCCAGGGATGAC		[22,24]
Mala s 9 forward	ATGAGCAACGTTATCAAGAAG	1029	AJ011959
Reverse	CTGAGAGAGAGGCAGGCCAC		[22,24]
LSU forward	AGCGGAGGAAAAGAACT	574	AF063214-5, AF064024-8
Reverse	GCGCGAAGGTGTCCGAAG		[30]

Gibco-BRL, Life Technologies, Täby, Sweden) with ethidium bromide in $1 \times$ Tris-borate-EDTA (TBE) buffer.

PCR amplifications

PCR amplifications were performed as described above, using gene-specific primers (Table 1) designed in our earlier studies [22,24,25]. These primers amplify the complete coding sequences of Mala s 1 and of Mala s 5 to Mala s 9. An exception to this was the 5' primer for Mala s 9, which was designed from the recently obtained 5' end sequence of the Mala s 9 cDNA (accession number AJ 011959) currently updated in the EMBL sequence database. The sequences of the primers for Mala f 2, Mala f 3 and Mala f 4, partially amplifying each gene, were designed according to the published sequences [23,26]. The oligonucleotides were synthesised by Medprobe (Oslo, Norway) or DNA Technology (Aarhus, Denmark). PCR products were electrophoresed in 1% agarose gels containing ethidium bromide in $1 \times$ TBE buffer.

DNA sequencing

After purification of the PCR products from 1% agarose gel using a QIAGEN gel extraction kit (Qia-gen, Hilden, Germany), they were directly subjected to nucleotide sequencing using either forward or reverse primers shown in Table 1. Sequencing was performed on an ABI prism™ 377 sequencer (PerkinElmer) using the ABI Prism™ BigDye Terminator Cycle Sequencing

Ready Reaction Kit v2.0 (PerkinElmer). Pairwise alignments between partially amplified sequences, ranging from 113 to 503 bp, and the known Mala f and Mala s sequences were performed using Lasergene software by DNASTAR (Madison, WI, USA). Of the 44 sequences that were aligned, 37 were longer than 270 bp and seven ranged between 113 and 170 bp. The software calculated similarity index as the number of matching residues divided by the sum of all residues and gap characters multiplied by 100 to give a similarity as percentage.

Results

PCR-REA

To confirm the identity of each *Malassezia* species, PCR-REA was performed. Part of the LSU rRNA gene was amplified from the DNA of each *Malassezia* strain. A single specific band, 580 bp in size, was generated with all *Malassezia* isolates as exemplified for *M. sympodialis* in Fig. 1A. Subsequently, the PCR products were cleaved separately with *Ban*I, *Hae*II and *Msp*I. A specific and unique restriction pattern was seen for each of the *Malassezia* species (data not shown) in accordance with previously published data [30]. The PCR-REA pattern of the two *M. sympodialis* strains ATCC 42132 and CBS 7222 was found to be identical, confirming the correctness of identifications originally made using biochemical testing [27; J. Faergemann, personal communication].

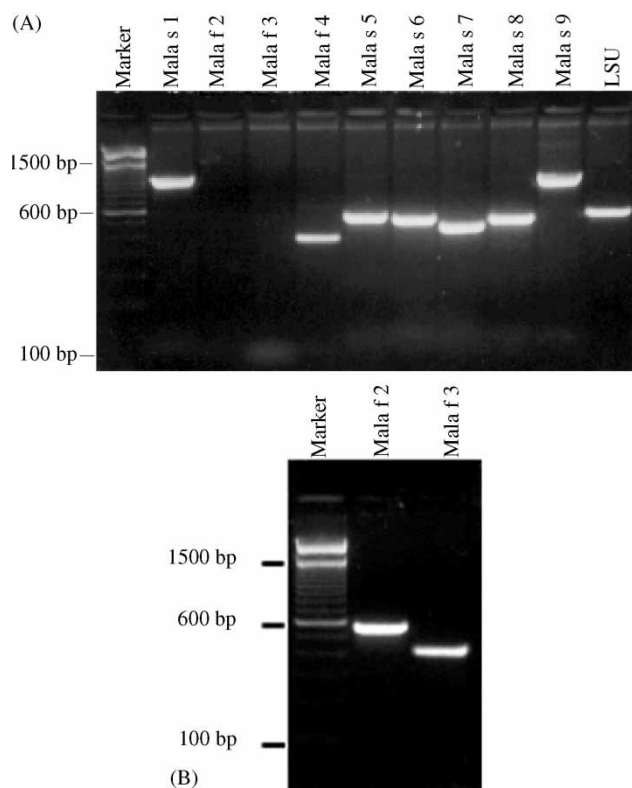


Fig. 1 PCR amplification patterns of the Mala f, Mala s and LSU genes obtained using DNA from *Malassezia sympodialis* (ATCC 42132) (A) and *M. furfur* (CBS 7019) (B). A 100-bp DNA ladder (Gibco-BRL, Life Technologies) was used as marker.

Amplification of allergen DNA by PCR

Using specific primers for the allergen coding sequences (Table 1), together with DNA as template, the PCR amplification data showed that from *M. globosa*, *M. obtusa* and *M. sympodialis*, sequences corresponding to all allergens except Mala f 2 and Mala f 3 could be specifically amplified (Table 2). On the other hand, *M. pachydermatis* contained Mala s 1, Mala f 4, and Mala s 5 to Mala s 8, while *M. restricta* and *M. slooffiae* contained Mala f 4 and Mala s 6. Mala f 2 and Mala f 3 were amplified only in *M. furfur* (Fig. 1B); this species also yielded amplifications of Mala f 4 and of Mala s 5 to Mala s 7 (Table 2). The amplification pattern of the *M. sympodialis* DNA is shown in Fig. 1A. All the primer pairs used in amplification gave rise to bands of appropriate size in each of the species for which the corresponding allergen sequence was amplified.

DNA sequencing

In order to confirm that the PCR fragments obtained corresponded to the Mala f and Mala s allergen

sequences, the amplified products from the genomic PCR were partially sequenced. In similarity index calculations, values ranging from 71 to 100% were obtained in comparisons between sequences of the PCR products and of the known Mala allergens (Table 2).

Amplification of allergen mRNA by RT-PCR

In RT-PCR (Table 2), all the allergens except Mala f 2 and Mala f 3 were specifically amplified from the cDNA of the two *M. sympodialis* strains. However, when cDNA from *M. globosa* or *M. obtusa* was used as the PCR template, only Mala s 6 primers gave rise to an amplified product. The primers of Mala f 4 and Mala s 6 were able to amplify cDNA fragments of expected sizes in *M. restricta*, *M. slooffiae* and *M. pachydermatis*. *M. pachydermatis* cDNA also yielded a PCR product of correct size when Mala s 8 primers were used. Amplification of Mala f 2 and Mala f 3 could be seen only in *M. furfur*, which also contained Mala f 4.

Discussion

Our results show that the different *Malassezia* species contain both common and species-specific Mala f and Mala s allergen sequences. Only parts of the PCR products were sequenced and compared with the known sequences of Mala f and Mala s allergens, which means that we are dealing with only putative allergens. Whether these sequences resembling Mala f and Mala s bind IgE as the confirmed allergens do remains to be determined. However, in a study using extracts from the seven *Malassezia* species and inhibition immunoblotting, it was shown that in addition to species-specific allergens, the different *Malassezia* species contain a high proportion of mutually similar IgE-binding components [31]. This finding appears to support the idea that the Mala f- and Mala s-like sequences obtained from heterologous *Malassezia* species in our study may also code for IgE-binding proteins.

That our data show amplification of Mala f 2 and Mala f 3 only in *M. furfur*, for example, might cause concern that our failure to amplify the DNA of some of the allergen types in some species was merely due to minor interspecific sequence differences in the regions binding the primers. This concern appears unwarranted, however, since at least five allergens were specifically amplified in the majority of the different *Malassezia* species.

Table 2 Data from genomic PCR (DNA) and reverse transcriptase PCR (RNA), showing the amplification pattern of Mala f and Mala s allergens in the different *Malassezia* species

Strain	Mala s 1	Mala f 2	Mala f 3	Mala f 4	Mala s 5	Mala s 6	Mala s 7	Mala s 8	Mala s 9
<i>M. furfur</i> (CBS 7019)	DNA	100*	100	100	99	80	99	—	—
<i>M. globosa</i> (CBS 7966)	RNA	+	+	+	—	—	—	—	—
<i>M. obtusa</i> (CBS 7876)	DNA	—	—	72	100	84	100	100	99
<i>M. pachydermatis</i> (CBS 1879)	RNA	—	—	—	—	—	—	—	—
<i>M. restricta</i> (CBS 7877)	DNA	—	—	—	92	92	100	100	100
<i>M. slooffiae</i> (CBS 7956)	RNA	—	—	—	—	—	—	—	—
<i>M. sympodialis</i> (CBS 7222)	DNA	—	—	71	100	88	99	100	—
<i>M. sympodialis</i> (ATCC 42132)	RNA	—	—	+	—	+	—	+	—
	DNA	—	—	72	—	90	—	—	—
	RNA	—	—	83	—	87	—	—	—
	DNA	—	—	+	—	+	—	—	—
	RNA	—	—	79	100	100	100	100	100
	DNA	—	—	+	+	+	+	+	+
	RNA	—	—	79	100	100	100	100	100
	DNA	—	—	+	+	+	+	+	+
	RNA	—	—	+	+	+	+	+	+

*The numbers represent percent similarities between the known Mal f/Mal s allergen DNA sequences and the corresponding, partially amplified sequences obtained from the other *Malassezia* species tested. +, product amplified; —, product not amplified.

It is interesting that the pattern of allergens amplified in RT-PCR was more species-specific than that seen in genomic DNA amplifications. One could speculate that the absence of some allergen mRNA types in different species was an artefact of the conditions in which the yeasts were cultured or of the time allowed for growth. It should be noted, however, that our use of 2-day-old cultures grown at 32°C was based on our previous observation that such cultures of ATCC 42132 express high levels of IgE-binding proteins, whereas cultures incubated more than 4 days lost most proteins in the range between 10 and 100 kDa [32]. In our PCR studies, sequences corresponding to Mala f 4 and Mala s 6 were consistently amplified from genomic DNA of all the *Malassezia* species tested. This is not particularly surprising, in that these two allergens have strong sequence similarities with proteins from other organisms. Mala f 4 resembles mitochondrial malate dehydrogenase, while Mala s 6 is similar to cyclophilin. These correspondences suggest that these allergens represent evolutionarily conserved elements [22,23].

As mentioned above, we found that *M. globosa*, *M. obtusa* and *M. sympodialis* had DNA for all the *Malassezia* allergens examined except Mala f 2 and Mala f 3. On the RNA level, parallel results were obtained only with *M. sympodialis*. Differences among species in expressed allergen component might be reflected in studies revealing differences in patient responses to various species and their known allergens. Koyama *et al.* found specific IgE-antibodies reacting against *M. globosa* and *M. sympodialis* extracts in 83 and 74%, respectively, of 54 AEDS patients [33]. The corresponding data for the other investigated *Malassezia* species were as follows: 65% of the patients had IgE antibodies directed against *M. furfur*, 56% against *M. restricta* and 50% against *M. slooffiae* [33]. We found that out of 156 AEDS patients, 74 (47%) had a positive ImmunoCAP reaction to extract from *M. sympodialis* (ATCC 42132), and 86 (55%) to at least one of six recombinant (r) Mala s allergens, with the highest sensitivity being found in response to rMala s 9 [34]. Mala s 1, Mala s 6, Mala s 8 and Mala s 9 all fulfil the necessary criteria to be designated major allergens, as each recombinant allergen was recognized by more than 50% of the sera that were ImmunoCAP-positive to whole extract of *M. sympodialis* [34]. However, it is important to mention that we have recently discovered that the sequence earlier reported for Mala s 9 [24], coding for a 14 kDa protein used in the above-cited study [34], was only a partial 3' sequence; therefore the IgE reactivity towards the complete Mala s 9 sequence (1029 bp encoding a protein with 342 amino acid residues and a calculated mass of 36.7 kDa) needs to be

investigated. When we studied IgE reactivity [as measured by ImmunoCAP, SPT and APT] shown against *M. sympodialis* extract as well as reactivity shown against the individual allergens rMala s 1, rMala s 5, and rMala s 6 or mixtures of those allergens, we found that two-thirds of 132 AEDS patients analysed showed a positive reaction to at least one of the challenge preparations in at least one of the tests [35]. Taken together, these data suggest an important role of the *M. sympodialis* allergens in AEDS.

Since the establishment of the new *Malassezia* classification, several studies have reported different and sometimes even contradictory results in regard to detection of these species on the skin of healthy individuals and on that of persons with dermatological diseases [36–42]. Recently, still another *Malassezia* species was isolated from AEDS patients and named *M. dermatis* [43]. This complexity evidences a need to standardise techniques for a more reliable identification of these *Malassezia* species and for more accurate assessment of their association with different diseases. Today, *Malassezia* extracts are used for detecting *Malassezia*-specific IgE reactivity among AEDS patients. Discovery and analysis of common and specific IgE-binding components in these yeasts and production of recombinant allergens may contribute to the development of a superior source of test allergens. Such an optimised allergen mixture may make it easier to develop reliable diagnostic tools [44], resulting in a better understanding of the mechanisms involved in allergic reactions and an improvement in our knowledge of relationships between allergen structure and function [45]. Our findings indicate the importance of optimising the allergen source used in the diagnosis of AEDS patients with IgE reactivity to these yeasts.

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References

- Guého E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie van Leeuwenhoek* 1996; **69**: 337–355.
- Guillot J, Guého E, Lesourd M, Midgley G, Chévrier G, Dupont B. Identification of *Malassezia* species, a practical approach. *J Mycol Méd* 1996; **6**: 103–110.
- Gupta AK, Kohli Y, Summerbell RC. Molecular differentiation of seven *Malassezia* species. *J Clin Microbiol* 2000; **38**: 1869–1875.
- Guého E, Boekhout T, Ashbee HR, Guillot J, Van Belkum A, Faergemann J. The role of *Malassezia* species in the ecology of human skin and as pathogens. *Med Mycol* 1998; **36**: 220–229.
- Faergemann J. *Pityrosporum* species as a cause of allergy and infection. *Allergy* 1999; **54**: 413–419.
- Hazen KC. New and emerging yeast pathogens. *Clin Microbiol Rev* 1995; **8**: 462–478.
- Marcon MJ, Powell DA. Human infections due to *Malassezia* spp. *Clin Microbiol Rev* 1992; **5**: 101–119.
- Scheynius A, Johansson C, Buentke E, Zargari A, Tengvall-Linder M. Atopic Eczema/Dermatitis Syndrome and *Malassezia*. *Int Arch Allergy Immunol* 2002; **127**: 161–169.
- Wahlgren CF. Pathophysiology of itching in urticaria and atopic dermatitis. *Allergy* 1992; **47**: 65–75.
- Johansson SGO, OB Hourihane J, Bousquet J et al. A revised nomenclature for allergy. *Allergy* 2001; **56**: 813–824.
- Öhman S, Johansson SGO. Allergen-specific IgE in atopic dermatitis. *Acta Derm Venereol* 1974; **54**: 283–90.
- Bruynzeel-Koomen CAFM, Van Wichen DF, Toonstra J, Berrens L, Bruynzeel PLB. The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch Dermatol Res* 1986; **278**: 199–205.
- Ring J, Abeck D, Neuber K. Atopic eczema: role of micro-organisms on the skin surface. *Allergy* 1992; **47**: 265–269.
- Johansson C, Eshaghi H, Tengvall-Linder M, Jakobsson E, Scheynius A. Positive atopy patch test reaction to *Malassezia furfur* in atopic dermatitis correlates with a Th2 like PBMC response. *J Invest Dermatol* 2002; **118**: 1044–1051.
- Kieffer M, Bergbrant IM, Faergemann J, et al. Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrhoeic dermatitis. *J Am Acad Dermatol* 1990; **22**: 739–742.
- Nissen D, Petersen LJ, Esch R, et al. IgE-sensitization to cellular and culture filtrates of fungal extracts with atopic dermatitis. *Ann Allergy Asthma Immunol* 1998; **81**: 247–255.
- Nordvall SL, Johansson S. IgE antibodies to *Pityrosporum orbiculare* in children with atopic diseases. *Acta Paediatr Scand* 1990; **79**: 343–348.
- Rokugo M, Tagami H, Usuba Y, Tomita Y. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. *Arch Dermatol* 1990; **126**: 627–632.
- Tengvall-Linder M, Johansson C, Scheynius A, Wahlgren CF. Positive atopy patch test reactions to *Pityrosporum orbiculare* in atopic dermatitis patients. *Clin Exp Allergy* 2000; **30**: 122–131.
- Tengvall-Linder M, Johansson C, Zargari A, et al. Detection of *Pityrosporum orbiculare* reactive T cells from skin and blood in atopic dermatitis and characterization of their cytokine profiles. *Clin Exp Allergy* 1996; **26**: 1286–1297.
- Tengvall-Linder M, Johansson C, Bengtsson Å, et al. *Pityrosporum orbiculare*-reactive T-cell lines in atopic dermatitis patients and healthy individuals. *Scand J Immunol* 1998; **47**: 152–158.
- Lindborg M, Magnusson CGM, Zargari A, et al. Selective cloning of allergens from the skin colonizing yeast *Malassezia*

- furfur* by phage surface display technology *J Invest Dermatol* 1998; **113**: 156–161.
- 23 Onishi Y, Kuroda M, Yasueda H, *et al.* Two-dimensional electrophoresis of *Malassezia* allergens for atopic dermatitis and isolation of Mal f 4 homologs with mitochondrial malate dehydrogenase. *Eur J Biochem* 1999; **261**: 148–154.
- 24 Rasool O, Zargari A, Almquist *et al.* Cloning, characterization and expression of complete coding sequences of three IgE binding *Malassezia furfur* allergens, Mal f 7, Mal f 8 and Mal f 9. *Eur J Biochem* 2000; **267**: 4355–4361.
- 25 Schmidt M, Zargari A, Holt P, *et al.* The complete cDNA sequence and expression of the first major allergenic protein of *Malassezia furfur*, Mal f1. *Eur J Biochem* 1997; **246**: 181–185.
- 26 Yasueda H, Hashida-Okado T, Saito A, *et al.* Identification and cloning of two novel allergens from the lipophilic yeast, *Malassezia furfur*. *Biochem Biophys Res Commun* 1998; **248**: 240–244.
- 27 Mayser P, Gross A. IgE antibodies to *Malassezia furfur*, *M. sympodialis* and *Pityrosporum orbiculare* in patients with atopic dermatitis, seborrhoeic eczema or pityriasis versicolor, and identification of respective allergens. *Acta Derm Venereol* 2000; **80**: 357–361.
- 28 WHO/IUS Allergy Nomenclature Subcommittee World Health Organization. Allergen nomenclature. *Clin Exp Allergy* 1995; **25**: 27–37.
- 29 Hoffman CS. Preparation of yeast DNA, RNA, and proteins. In: Ausubel FA, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds). *Current Protocols in Molecular Biology*, vol. 2. New York: Wiley & Sons, 1997: 13.11.2–13.11.3.
- 30 Guillot J, Deville M, Berthelemy M, Provost F, Guého E. A single PCR-restriction endonuclease analysis for rapid identification of *Malassezia* species. *Lett Appl Microbiol* 2000; **31**: 400–403.
- 31 Zargari A, Midgley G, Bäck O, Johansson SGO, Scheynius A. IgE-reactivity to seven *Malassezia* species. *Allergy* 2003; **58**: 306–311.
- 32 Zargari A, Doekes G, Van Dijk AG, *et al.* Influence of culture period on the allergenic composition of *Pityrosporum orbiculare* extracts. *Clin Exp Allergy* 1995; **25**: 1235–1245.
- 33 Koyama T, Kanbe T, Ishiguro A, *et al.* Antigenic components of *Malassezia* species for immunoglobulin E antibodies in sera of patients with atopic dermatitis. *J Dermatol Science* 2001; **26**: 201–208.
- 34 Zargari A, Eshaghi H, Bäck O, Johansson SGO, Scheynius A. Serum IgE reactivity to *Malassezia furfur* extract and recombinant *M. furfur* allergens in patients with atopic dermatitis. *Acta Derm Venereol* 2001; **81**: 418–422.
- 35 Johansson C, Sandström MH, Bartosik J, *et al.* Atopy patch test reactions to *Malassezia* allergens differentiate subgroups of atopic dermatitis patients. *Br J Dermatol* 2003; **148**: 479–488.
- 36 Aspiroz C, Moreno LA, Rezusta A, Rubio C. Differentiation of three biotypes of *Malassezia* species on normal human skin: correspondence with *M. globosa*, *M. sympodialis* and *M. restricta*. *Mycopathologia* 1999; **145**: 69–74.
- 37 Arzumanyan VG. The yeast *Malassezia* on the skin of healthy individuals and patients with atopic dermatitis. *Vestn Ross Akad Nauk* 2001; **2**: 29–31.
- 38 Gupta AK, Kohli Y, Summerbell RC, Faergemann J. Quantitative culture of *Malassezia* species from different body sites of individuals with or without dermatoses. *Med Mycol* 2001; **39**: 243–251.
- 39 Midgley G. The lipophilic yeasts: state of the art and prospects. *Med Mycol* 2000; **38**(Suppl. 1): 9–16.
- 40 Nakabayashi A, Sei Y, Guillot J. Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol* 2000; **38**: 337–341.
- 41 Sandström MH, Bartosik J, Bäck O, *et al.* The prevalence of *Malassezia* yeasts in patients with atopic dermatitis, seborrhoeic dermatitis and healthy controls. *Acta Soc Med Suecanae* 2001; **110**: 169.
- 42 Sugita T, Suto H, Unno T, *et al.* Molecular analysis of *Malassezia* microflora on the skin of atopic dermatitis patients and healthy subjects. *J Clin Microbiol* 2001; **39**: 3486–3490.
- 43 Sugita T, Takashima M, Shinoda T, *et al.* New yeast species, *Malassezia dermatis*, isolated from patients with atopic dermatitis. *J Clin Microbiol* 2002; **40**: 1363–1367.
- 44 Hiller R, Laffer S, Harwanegg C, *et al.* Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002; **16**: 414–416.
- 45 Bufe A. The biological functions of allergens: Relevant for the induction of allergic diseases? *Int Arch Allergy Immunol* 1998; **117**: 215–219.