

Clarifications on Conflicting Results Published on the Amplification of the Mala s 1 Allergen Gene Sequences

To the Editor:

In the December issues of the Journal of Investigative Dermatology (JID) (Gaitanis *et al*, 2003—Online publication 26-11-2003, PMID 14675212) and Medical Mycology (Andersson *et al*, 2003—Online publication 12-03, PMID 14675212), two independent publications investigated the distribution of genes coding for *Malassezia* allergens. Although the methodology and scope of the two studies did not differ significantly, contradictory results were reported regarding the PCR amplification of sequences coding for the Mala s 1 allergen.¹

In the letter published in the JID, the authors (Gaitanis *et al*, 2003) amplified Mala s 1 allergen coding sequences only from the clinical isotype strain ATCC 42132 and from 13 reference and clinical *M. sympodialis* but not from 22 Type or clinical strains of any other *Malassezia* species. This result was obtained only after optimizing the PCR conditions.

In the Medical Mycology article, PCR products corresponding to the size of the complete coding sequence of the same gene were amplified from genomic DNA of Type strains *M. globosa* CBS 7966, *M. obtusa* CBS 7876, and *M. pachydermatis* CBS 1879 (Andersson *et al*, 2003). No mRNA was detected from any of these *Malassezia* species by RT-PCR, denoting absence of the expression of the allergen.

In order to ensure uniformity of the PCR outcome regarding amplification of Mala s 1 sequences from *Malassezia* species, our two groups collaborated exchanging raw data and unpublished observations. It was subsequently ascertained that the PCR primers used in both studies overlap different regions of the Mala s 1 complementary DNA sequence (Gene Bank Accession No. X96486; Schmidt *et al*, 1997). In the Medical Mycology study, the forward and reverse primers overlap the (22–44) and (1085–1058) regions, respectively, whereas in the JID study the corresponding primers overlap (120–140) and (860–841) regions, respectively. Therefore, it cannot be excluded that the disagreements between the published data might be because of differences in the nucleotide sequences in these different Mala s 1 regions among the various *Malassezia* species, which can in turn influence annealing of the respective primer pairs to the different DNA templates. Unfortunately, the primers designed from the Mala s 1 cDNA coding sequence (Gaitanis *et al*, 2003) could not be simultaneously aligned to the Andersson *et al* (2003) partial sequences from the PCR products.

¹Former Mal f 1.

²Velegriaki A, Gaitanis G, Frangoulis E, Legakis N, Katsambas A: First report on the distribution of the Major Allergenic Protein Mal f 1 gene sequences among the seven *Malassezia* species by PCR. Clinical and molecular aspects. Regional Meeting International Society of Dermatology "Medical Education in Dermatology", Rhodes, Greece, 2001, PO66.

Sporadic and inconsistent PCR products were amplified in earlier experiments from *M. globosa*, *M. obtusa*, and *M. pachydermatis*.² This phenomenon was resolved through PCR optimization and titration with 2-pyrrolidone (Gaitanis *et al*, 2003). Additionally, no Mala s 1 gene sequences were amplified from nine additional Type and reference *M. pachydermatis* strains that were retrospectively tested (*M. pachydermatis* CBS1880, CBS1881, CBS 1882, CBS 1883, CBS 1884, CBS 1885, CBS 6540, CBS 7044, CBS 7925). Andersson *et al* (2003) reported absence of Mala s 1 allergen transcripts from *M. globosa*, *M. obtusa*, and *M. pachydermatis*, which regarding *M. globosa*, has also been supported by earlier observations (Koyama *et al*, 2001).

In conclusion, sequences corresponding to the complete coding sequence of the Mala s 1 gene are amplified, from both DNA and cDNA, solely from *M. sympodialis* strains (Andersson *et al*, 2003) when increased stringency and optimum PCR conditions are attained (Gaitanis *et al*, 2003). In one of the studies (Andersson *et al*, 2003), the Mala s 1 sequence could also be PCR amplified from the DNA of *M. globosa*, *M. obtusa*, and *M. pachydermatis*. These PCR products, however, were only partially sequenced and whether their full-nucleotide sequence also shows 100% sequence similarities to Mala s 1 has to be determined.

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