Author's response to reviews

Title: Distribution of Malassezia species on the skin of patients with atopic dermatitis, psoriasias, and healthy volunteers assessed by conventional and molecular identification methods

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Author's response to reviews: see over
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**Dr Hayley Henderson**

**Senior Executive Editor**

**BioMed Central**

Dear Dr Henderson,

I would like to thank you, as well as your expert reviewers for estimating the manuscript I am a co-author, entitled: “Distribution of *Malassezia* species on the skin of patients with atopic dermatitis, psoriasis, and healthy volunteers assessed by conventional and molecular identification methods” (MS: 2006987336107279).

You will find enclosed herewith a revised version of our manuscript, which incorporates almost all the changes requested by the Reviewers. The changes are highlighted in grey in the text. The answers to the reviewer’s comments are given below and are also highlighted in grey.

We let ourselves hope that the article of ours has now reached a form allowing it to be published in the BMC Dermatology journal.

Yours sincerely,

Tomasz Jagielski

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ANSWERS TO THE REVIEWERS COMMENTS

Reviewer1:

1- The title page:
- The title should figured before the names of authors

We have now corrected this.

2- The abstract:
- Background: delete “in the causation of”

It has been deleted, as requested by the Reviewer.
- Results: should be more detailed and the statistical analysis is useless

The Results section of the Abstract has now been provided with more detailed data.
- Results: restructure “all but one subjects under the study”

We have replaced it with “17 subjects under the study”

3- Keywords:
- Delete “phenotypic methods”

It has been deleted, as requested by the Reviewer.
- “Drug susceptibility” should be at the last

It has been listed in the end, as requested by the Reviewer;

4- Background:
- Add “The” before “Identification” and delete “the “ before “Malassezia” in the

We have introduced both changes, as requested by the Reviewer.

3rd paragraph
- in the last paragraph “the bioactivity of the secondary metabolites of M. littoralis” by “ the antifungal activity of essential oil”

The suggestion above is not clearly stated. Our paper does not include the quoted phrases.

5- Methods:
- Subjects: precise the difference between the mean age and the median age

The mean is the arithmetic average of a set of numbers, or distribution. The median is described as the numeric value separating the higher half of a sample, a population, or a probability distribution, from the lower half. Both the mean and median values are used to fully characterize the study sample.
- Culture condition and yeast strains:

  - Why the yeasts were cultured for 2 weeks? only 5 to 7 days are sufficient for the growth of Malassezia species

We agree with the Reviewer that normally about one-week time is sufficient to obtain growth of Malassezia fungi. However, sometimes, to obtain a more decent fungal growth we prolonged the incubation period to 2 weeks. This was applied to all the cultures.

6- Results: there is repetition of the results

- Phenotyping identification: the term “isolates” is repeated

We have now corrected this.

- PCR-RFLP analysis: what is “ca”

The abbreviation “ca” stands for Latin “circa”, which means “approximately”

7- Discussion:

- replace “pediatric patients” by “children in the first paragraph

We have changed it, as requested by the Reviewer.

Reviewer2:

I have read with interest the article by Jagielski et al, which reports on the epidemiology and identification of Malassezia yeasts from patients with atopic dermatitis, psoriasis and controls. Furthermore an effort to assess in vitro the activity of six common antifungal agents against the isolates is performed.

We thank the Reviewer for this positive comment.

Major Compulsory Revisions

1. Abstract. Try to shorten the introduction and put in the results statistically significant observations (M sympodialis is AD) as well as a phrase on the identification comparison and MIC

We have broadened the Results section in the Abstract, as requested by the Reviewer.

2. Results. As the article states to report on the isolation rate of Malassezia species I would suggest to move the “Distribution section” first, and start it by denoting the use of molecular methods for evaluating the results. Put a simple Table for the frequencies (absolute and %) for the isolated species and state the level of significance. In the present “Distribution of Malassezia species” (Page15) the first paragraph is confusing without reporting statistically significant findings. A Table to include these findings would be more reader friendly. Remember to state the level of significance.

Data concerning frequencies for the isolated species is actually presented in the last column of Table 2. Hence we do not see the need for a table that would evidently double the one already existing. We kindly ask the Review to withdraw this request.
As for the statistical analysis of the data, it has been clearly stated that the only significant association was that isolation of *M. sympodialis* alone was more frequent among AD patients and healthy volunteers, as opposed to PS patients. Please, see the final paragraph of the Results section of the manuscript.

3. Discussion. The discussion has to focus on the key points which is the isolation of *M. sympodialis* from AD and the discrepancy of phenotypic and molecular identifications results. For example, referrel with 9 References to pityriasis versicolor and seborheic dermatitis epidemiological data is redundant, as the paper does not address this issue.

As requested by the Reviewer, epidemiological data concerning pityriasis versicolor and seborheic dermatitis were deleted from the first part of the Discussion section of the article. Isolation of *M. sympodialis* from AD patients and the discrepancy of phenotypic and molecular identification results are commented in detail in further parts of the Discussion (particularly on pages 20 and 21), which have been considered satisfactorily clear and emphasized by the other two Reviewers.

4. Table 1. Drug susceptibility pattern column is redundant as it is collectively assessed in Table 3. Furthermore, safe statistical data on MIC and disease cannot be extracted as the testing was randomly performed in few strains

Table 1 has been modified as requested by the Reviewer.

Minor Essential Revisions

None.

Discretionary Revisions

In the introduction a comment on the extensive basic research performed by Scheynious on the Malassezia sympodialis and atopic dermatitis would be helpful as the authors mainly report on this species.

We have added a short paragraph to the Introduction part of the article, as requested by the Reviewer.

**Reviewer3:**

This paper is interesting and could be a very nice contribution to the field. I see as especially important the comparison of the molecular and culture based speciation. This was interesting and well executed. It could be accepted without further experimental data, but only with significant revisions to the text.

We thank the Reviewer for these positive comments.

The authors did not adequately review the field, and hence under reported an important issue - that the data relies on culture based sampling. They should call this out more clearly and in more detail review the existing data.

The risk of potential bias arising from the use of culture-based methods has now been emphasized in the Discussion section of the article, as recommended by the Reviewer.
The following comments should all be considered as Compulsory Revisions

1. Is the question posed by the authors well defined?

The authors present a survey study. The question is relevant and important, and a necessary contribution to the field. It is not the testing of a specific hypothesis.

We thank the Reviewer for this positive comment.

2. Are the methods appropriate and well described?

The methods are reasonable, but should be better described as “culture based”. The isolation is all based on culture so it is very important to point out the potential bias introduced by the well documented poor reproducibility of Malassezia primary isolates. There are inherent biases due to the fastidious nature of Malassezia in culture which must be reviewed and accounted for.

We have now clearly addressed the issue of culture bias in the depiction of Malassezia microbiota.

3. Are the data sound?

The data are sound and well reasoned. There needs to be a clearer description of how the isolation technique influences the data and adds inherent difficulties in interpretation. The authors need to add discussion comparing non-culture based isolation techniques (for example Nat Rev Micro 9, 244-253 (2011) or J. Clin. Microbiol. vol. 40 no. 9 3350-335 2002), which indicate that Malassezia are very likely found on every human. Also, it has been previously reported and well supported that M. restricta is found on essentially all humans. The authors need to discuss their work in comparison to this previously published data.

We agree with the Reviewer that the that culture-based methods carry the risk of the underestimation of colonization rate in case of Malassezia fungi. As mentioned above, we have now highlighted this issue more clearly in the Discussion section of the article. We let ourselves to stress, however, that the aim of this study was to examine species composition of Malassezia microflora on the skin of healthy volunteers and patients with AD and PS, as well as, to compare phenotypic and molecular methods of species identification of the cultured strains.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?

Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?

Yes. The authors do an excellent job of noting the positive and negative aspects of interpreting their data on a technical level. The Conclusions and Discussion, however, could be covered with fewer words and abbreviations and this should improve the readability and clarity.

As suggested by the Reviewer, some unessential epidemiological data have been deleted from the Discussion section of the article.

6. Are limitations of the work clearly stated?

With regard to everything other than the issues associated with cultivation, yes.

We have now clearly stated the limitation associated with cultivation of the Malassezia fungi.
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?

No. The authors failed to reference a significant body of work. There are several key papers, such as Nat Rev Micro 9, 244-253 (2011), J. Clin. Microbiol. 2002 (40) 9 3350-335, PNAS 2007 (104) 47 18730-18735. They also should include previous work in the area of Malassezia resistance in the susceptibility section, such as FEMS Yeast Research 2011 (11) 1, p 80–87.

As suggested by the Reviewer we have cited some important papers on Malassezia spp., that had been missed when preparing the first draft of the manuscript, including Xu et al., Proc Natl Acad Sci U S A. 2007, 104:18730–18735; Gioti et al., mBio. 2013, 4:00572-12; Gemmer et al., J Clin Microbiol 2002; 40:3350-7, and Grice, Segre. Nat Rev Microbiol 2011; 9:244-53.

Furthermore, as requested by the Reviewer, we have incorporated in the Discussion section of the article, the paper of Kim et al., FEMS Yeast Res. 2011, 11:80-87.

I found it unnecessary for the authors to state that this “was the first time” this data has been published in Poland. See “Distribution of Malassezia species in patients with atopic dermatitis – quality assessment. El#bieta Rup, Magdalena Skóra, Pawe# Krzy#ciak, Anna B. Macura. Post Dermatol Alergol 2011; XXVIII, 3: 187–190”

We have deleted this statement, as requested by the Reviewer.