

## Peer Review Report

# Review Report on Membrane Permeabilization of *Candida glabrata* Induced by Two Promising Natural Lipopeptides Co-Produced by *Bacillus subtilis*

Brief Research Report, Acta Biochim. Pol.

Reviewer: Adam Junka

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### EVALUATION

#### **Q 1** Please summarize the main findings of the study.

The authors isolated two peptides with antifungal activity against a single strain of *Candida glabrata* and assessed the level of this activity using three relevant techniques.

#### **Q 2** Please highlight the limitations and strengths.

Strengths:

The *C. glabrata* has become an increasing clinical problem due to its persistence and drug resistance. Therefore, the search for new antifungal agents is urgently needed. Moreover, the authors investigated two peptides of bacterial origin that meet the criteria of biomimetics, a highly esteemed field of science.

Limitations:

The English language used requires significant correction. The descriptions of the methods lack scientific rigor. The results also lack scientific rigor.

#### **Q 3** Please comment on the methods, results and data interpretation. If there are any objective errors, or if the conclusions are not supported, you should detail your concerns

Methods:

The description of the extraction/purification process is insufficient for replicating the experiments. The absence of SYTO-9, especially when PI is used, is puzzling. Quantitative culturing should be performed in triplicate at the very least, and ideally in six repetitions, if the data were to be presented as the authors have done.

Results:

Mostly presented in the proper manner. The exceptions are the results from quantitative culturing and the histogram from Figure 3 (details provided in Q14).

Data Interpretation:

There are overly brief statements combined with instances of jumping to conclusions.

### Check List

#### **Q 4** Is the English language of sufficient quality?

No.

#### **Q 5** Is the quality of the figures and tables satisfactory?

Yes.

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**Q 6** Does the reference list cover the relevant literature adequately and in an unbiased manner?

Yes.

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**Q 7** Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)

No.

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**Q 8** Are the methods sufficiently documented to allow replication studies?

No.

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**Q 9** Are the results presented correctly and interpreted in light of previous knowledge?

No.

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**Q 10** Do the discussion and conclusion address the research questions or hypothesis posed in the introduction?

No.

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**Q 11** Are the data underlying the study available in either the article, supplement, or deposited in a repository? (Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data are required to be deposited in public repositories prior to publication.)

No.

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**Q 12** Does the study adhere to ethical standards including ethics committee approval and consent procedure?

Not Applicable.

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**Q 13** Have standard biosecurity and institutional safety procedures been adhered to?

Not Applicable.

**Q 14** Please provide your detailed review report to the editor and authors (including any comments on the Q4 Check List):

Dear Authors,

You have done an excellent job selecting *C. glabrata* and antifungal, bacterial-derived peptides as the focus of your study. Nevertheless, I can not accept it in the present form. I believe that addressing my remarks will clarify specific issues and enhance the quality of your manuscript.

Here are my comments:

a) Please improve the language of the manuscript. In its current form, it is not acceptable. The language issues obscure the significance of your data and make the introduction difficult to follow. There are stylistic and typographical errors throughout the whole manuscript.

b) Abstract:

- introduce the fact that you conducted quantitative culturing. It represents the highest and most direct form of scientific evidence showcasing the fungicidal activity of the two peptides.
- How did you determine that these peptides were antifungal at that stage? If this knowledge stems from your previous research, it is not mentioned in your manuscript. This needs correction in abstract and throughout the manuscript.

c) Contribution to the field: Please avoid reiterating the abstract. You have aptly highlighted the importance of your research (e.g., drug resistance). Now, it would be beneficial to discuss potential applications of the peptides you described.

Introduction:

L50 - There's no need to provide an abbreviation in brackets.

L52: Please include a reference from this decade if you're discussing the "past two and a half decades." The most recent reference in this context is from 2014 ;-)

L54-56: This is a prime example of why the manuscript should be reviewed by a native English speaker. I won't list other similar sentences, but they are evident.

L67: Kindly add a statement like: "The aim (or goal, purpose, etc.) of this study was to..."

Materials and Methods:

L78-87: Why "in brief"? Please provide a precise reference. In its current form, other researchers might find it challenging to reproduce this experiment. How did you determine that AF4 and AF5 were antifungal at this stage? Were other fractions also tested?

L88-93: Specify the medium. RPMI?

L95-105: Provide a precise reference for the fluorimetry method. Why did you use only PI and not PI + SYTO-9? They are typically used in combination for such experiments.

L106-111: If I understand correctly, you performed quantitative culturing before microscopy and cytometry. Thus, the description of QC should precede the latter techniques. Notably, why did you opt for duplicate culturing instead of 3 or 6 repeats? Averaging percentages and standard deviations from two results is problematic, even if you achieved high repeatability. Please elaborate on the culturing process to clarify if these were independent or technical repeats.

P133: Please justify the exposure time of *Candida* to the peptides. For potential systemic use, durations of 24 hours or longer are acceptable. For topical applications, typically 15 minutes or less is used. Why 5 hours?

P157: I'm confused about the "green fluorescence." You used PI, which results in red fluorescence.

P158: You mention "...and probably cell death." Given that you performed quantitative culturing, what prevents a more definitive statement on this matter?

Figure 3: The histogram has asterisks indicating the application of a statistical test. However, no statistical method is described in the manuscript.

Dear Authors, I kindly request that you revise the manuscript in line with my comments.

#### QUALITY ASSESSMENT

<b>Q 15</b> → Originality	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Q 16</b> → Rigor	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Q 17</b> → Significance to the field	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<b>Q 18</b> → Interest to a general audience	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Q 19</b> → Quality of the writing	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Q 20</b> → Overall quality of the study	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>