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.

EVALUATION

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

The study is a continuation of the research previously conducted by the group that concerned isolation and antimicrobial properties evaluation of lipopeptides produced by Bacillus subtilis RLID 12.1. The current data obtained using single Candida glabrata strain and two lipopeptides AF4 and AF5 confirmed by means of fluorescent staining the membrane destabilizing effect of the compounds. CFU counts and propidium iodine (PI) staining indicated that CF4 at 16mg/L reduced C. glabrata viability by over 99% in both methods, while CF5 provided 70% PI positive cells and 99% CFU reduction. Confocal laser scanning microscope observations using PI and FUN-1 stains further confirmed the finding.

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

The data provided are limited to only one strain of target C. glabrata (ATCC 2001) and no information is provided on statistical analysis. The lipopeptides of B. subtilis RLID 12.1 including AF4 and AF5 were characterized in previous works of the group in detail (Evaluation of antifungal efficacy of three new cyclic lipopeptides of the class bacillomycin from Bacillus subtilis RLID 12.1. Antimicrobial Agents and Chemotherapy, 62(1)published in 2018 as an example). Though the current manuscript has a brief report format and the amount of data presented is sufficient, the authors should refer to the previous results that described C. glabrata ATCC 2001 susceptibility including MICs.

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**

The type of statistical analysis of data should be provided.

Check List

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

No.

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

Yes.

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

No.

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

No.

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

Yes.

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

Yes.

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

Yes.

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.)

Not Applicable.

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

Not Applicable.

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):

The main suggestions are included in questionnaire 2 nad 3. The number of repetitions and replicates and how the data were statistically estimated should be included. Minor remarks: Two times of treatment with AF4 and AF5 were used against C. glabrata, please state the incubation time for results in Fig.3 and Fig.4. In the

Keywords section line 43 the word "cytometry" is missing. In the Introduction part please correct lines 55–57, the sentence is not complete. The reference in line 80–81 refers not only to Bacillus culture conditions but also to chromatography purification procedure, it should be shifted within the paragraph. Lines 119–120 the sentence is not grammatically correct. Please consider if more current reports on Candida threat could be used in Introduction.

QUALITY ASSESSMENT

Q 15	Originality	██ ██ ☐ ☐ ☐
Q 16	Rigor	██ ██ ██ ☐ ☐
Q 17	Significance to the field	██ ██ ██ ☐ ☐
Q 18	Interest to a general audience	██ ██ ██ ☐ ☐
Q 19	Quality of the writing	██ ██ ██ ☐ ☐
Q 20	Overall quality of the study	██ ██ ██ ☐ ☐