OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Jundishapur Journal of Microbiology
Article DOI: 10.5812/jjm.112449
Published in: Jundishapur Journal of Microbiology: 13(11); e112449

Peer Review Report for "Antagonistic Effects of *Lactobacillus plantarum* on *Candida albicans* in ME-180 Cervical Carcinoma Cell Culture"

Author(s): Young Ki Lee, Young Sam Yuk, Ga-Yeon Kim

Review Timeline:

Submit Date: 26 Dec 2020
Revised Date: 20 Feb 2021
Accepted Date: 25 Feb 2021

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0)

10 Jan 2021

Dear AE,
Unfortunately, the submitted text is not understandable. Conclusions are not extracted from the article data. I have divided the conclusion into several separate sentences, which may be understandable. In my opinion, these sentences are contradictory. In the Method, no experiment was performed to measure hydrogen peroxide and bacteriocin secretion of L. plantarum , but it is discussed in the conclusion.
Conclusion
1. Therefore, an antagonistic effect of L. plantarum against C. albicans has been observed in ME-180 cell cultures inoculated first with L. plantarum, and the effect increased as the cell concentration of L. plantarum increased.
2. However, almost no antagonistic effect was detected in ME-180 cell cultures simultaneously inoculated with both L. plantarum and C. albicans, as well as in those inoculated with C. albicans first, regardless of the L. plantarum cell concentration.
3. These findings may indicate that L. plantarum does not directly antagonize C. albicans by inhibiting its hydrogen peroxide and bacteriocin secretion but rather inhibits the early stage of biofilm development, which subsequently inhibits the growth and mucosal adhesion of C. albicans. Further studies on the
safety, side effects, and metabolism of L. plantarum and its lack of infectivity to animals

are required before applying L. plantarum isolated from kimchi as a treatment for vaginitis.
Minor comment
- Background: Candidiasis is caused by each Candida species “Candidiasis is a fungal infection caused by Candida albicans and affects the mucosa and internal organs in humans”
Thanks.

Donya Nikaein: Reviewer | **Revision** (0) 28 Jan 2021

Dear AE,
I reviewed the manuscript, however, I think this research has some fundamental problems especially in the materials and methods part. what were the reference authors used to evaluate the effect of Candida and/or Lactobacillus on ME-180 cells?
- there are many methods to evaluate necrosis and apoptosis, I don't think microscopy is alone suitable. there was no statistical analysis as well. how many replicates were used?
- and the conclusion part was somewhat not clear because L. palntarum is a known probiotic and as far as I know it is GRS, so why should its safety be examined?
- according to these and other comments which you could find in the attached file, I am afraid, this article is not suitable for publication in this journal.
regards

REFEREE: Reviewer | **Revision** (0) 30 Jan 2021

Dear AE,
Comments for the authors:
1- Write a little bit about biofilm and probiotics first and then explain your work.
2-use very new references in both introduction and discussion sections like refs 11-12.
3- write a sentence for the public about the prevention and the role of probiotics.
Thanks.

Saeid Mahdavi Omran: Associate Editor | **Revision** (0) 1 Feb 2021

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author. According to the comments, the document needs major revision.
Kind regards,

REFEREE: EIC | **Revision** (0) 1 Feb 2021

Dear Authors,
Thank you for submitting your manuscript to JJM. I have completed the review of your manuscript and a summary is appended below (Pleases find attached files). The reviewers recommend the reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments. Please answer point by point to reviewer's comments on a separate sheet under WORD format and write your

modifications in the revised version (WORD format) in colored or highlighted letters. Your answers to reviewers' queries are not only for the Editorial Board but for the readers and should be included, when appropriate, in the manuscript for improvement and a better understanding.

When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made a point by point, and provide suitable rebuttals for any comments not addressed. In addition, JJM INSTRUCTION attached to the mail, Pleases find it.

Note:

Please add ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf. Pleases follow the below link for the process. https://sites.kowsarpub.com/jjm/knowledgebase/category/tree.html#orcid.html

Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Date: Feb. 18, 2021

Manuscript ID: 112449

Dr. Ali Zarei Mahmoudabadi

Editor-in-Chief

Jundishapur Journal of Microbiology

Dear editor,

Please find enclosed our revised manuscript, entitled **“Antagonistic effects of *Lactobacillus plantarum* On *Candida albicans* in ME-180 cervical carcinoma cell culture”**, by Yuk et al., which we would like you to consider for publication in the *Jundishapur Journal of Microbiology*.

We are grateful to the reviewers for the critical comments and useful suggestions, which have helped us improve the quality of our paper considerably. We have incorporated the suggestions in the revised version of our paper.

The comments provided by each reviewer have been addressed in the point-by-point responses. Along with our response to each comment or request for revision, the page and line numbers in the manuscript where the changes have been made have been indicated.

We are grateful to you for considering our paper for publication. We look forward to hearing from you soon.

Sincerely yours,

Department of Public Health, Graduate School, Dankook University, 119 Dandae-ro, Dongnan-Gu, Cheonan-Si, Chungnam 31116, South Korea.

Response to comments provided by reviewers (112449)

We are grateful to the reviewers for the critical comments and careful suggestions, which have helped us improve our paper considerably. As indicated in the following responses, we have incorporated the suggestions in the revised version of our paper.

Reviewer 1

Unfortunately, the submitted text is not understandable. Conclusions are not extracted from the article data. I have divided the conclusion into several separate sentences, which may be understandable. In my opinion, these sentences are contradictory. In the Method, no experiment was performed to measure hydrogen peroxide and bacteriocin secretion of L. plantarum, but it is discussed in the conclusion.

Conclusion

1. Therefore, an antagonistic effect of L. plantarum against C. albicans has observed in ME-180 cell cultures inoculated first with L. plantarum, and the effect increased as the cell concentration of L. plantarum increased.

2. However, almost no antagonistic effect was detected in ME-180 cell cultures simultaneously inoculated with both L. plantarum and C. albicans, as well as in those inoculated with C. albicans first, regardless of the L. plantarum cell concentration.

3. These findings may indicate that L. plantarum does not directly antagonize C. albicans by inhibiting its hydrogen peroxide and bacteriocin secretion but rather inhibits the early stage of

biofilm development, which subsequently inhibits the growth and mucosal adhesion of C. albicans. Further studies on the safety, side effects, and metabolism of L. plantarum and its lack of infectivity to animals are required before applying L. plantarum isolated from kimchi as a treatment for vaginitis.

Minor comment

Background: Candidiasis is caused by each Candida species “Candidiasis is a fungal infection caused by Candida albicans and affects the mucosa and internal organs in humans”

Methods.

Response: We are grateful for the careful evaluation of our paper. In earlier studies conducted by us (11,12), the inhibitory effects of probiotic strains were directly examined. In this study, instead of evaluating the inhibitory effects of *L. plantarum* on ME-180 cells, the effects of the inhibition of biofilm development at an early stage or the inhibition of *C. albicans* growth and mucosal adhesion were primarily assessed. Therefore, only H_2O_2 production was assessed (unpublished), and bacteriocin production was not.

In the H_2O_2 production experiment, the TMB Plus medium was used to check the presence or absence of H_2O_2. Horseradish peroxidase present in the medium oxidizes TMB in the presence of H_2O_2, which induces blue coloration in the medium. Of the 140 probiotic strains isolated from the kimchi samples, 43 (30.7%) produced H_2O_2, of which *L. plantarum* produced H_2O_2 in 25 weeks (47.2%) of 53 weeks. This information has been added to the main manuscript (Page 3, Lines 12–15).

Reviewer 2

Dear Editor in Chief and authors;

Q1) I reviewed the manuscript, however I think this research has some fundamental problems

specially in materials and methods part. what was the reference authors used to evaluate the effect of Candida and/or Lactobacillus on ME-180 cells.

there are many methods to evaluate necrosis and apoptosis, I don't think microscopy is alone suitable. there was no statistical analysis as well. how many replicates were used?

Response: We are grateful for the careful evaluation of our paper. The findings related to this study have been published earlier in two journals (11, 12). To evaluate the mutually antagonistic effects of *Candida* and the probiotic strain in ME-180 cervical cancer cell culture, we examined the treated cultures using a microscope. In other words, the cytopathic effects, such as necrosis or transformation, were observed. Is not shown; however, most images displayed the results clearly. Of course, as correctly pointed out by the reviewer, the use of statistical analysis or cell number calculation may be more relevant. However, instead of focusing on the differences in cell number, we performed microscopic observation to confirm the effects and changes induced by the probiotic strain in the cancer cells. Only a single *L. plantarum* isolate, which was considered to exert the most potent inhibitory effect against *C. albicans*, was selected from the 140 probiotic strains isolated in the study conducted earlier. We only confirmed the cytopathic effects, and detailed experiments will be added later.

Q2) and the conclusion part was somewhat not clear, because *L. palntarum* is a known probiotic and as far as I know it is GRS, so why should its safety be examined?

according to these and other comments which you could find in the attached file, I am afraid, this article is not suitable for publication in this journal. regards

Response: Most probiotic strains are known to exhibit stability in cell cultures. However, according to a guideline published by the Ministry of Food and Drug Safety (MFDS) in Korea, strains of only ten species can be considered safe. Additionally, probiotic strains also

express certain pathogenic genes, and therefore, the chances of complications caused by these strains remain. Accordingly, we have added this information to prevent lack of clarity regarding this approach.

Reviewer 3

Q1) Write little bit about biofilm and probiotics first and then explain your work.

Response: The related content has been added below. (Page 2, Line 23–Page 3, Lines 1-6)

> Meanwhile, the biofilm, in which microorganisms aggregate, exhibits self-protection, and the microorganisms communicate with each other via antibiotic resistance and exhibit social behavior through horizontal gene transfer and quorum sensing mechanisms.
> Probiotic strains secrete antagonists such as surfactants, bacteriocins, exopolysaccharides, organic acids, lactic acid, fatty acids, enzymes, and hydrogen peroxide, and reduce the biofilm biomass by changing the pH and initiating nutrient competition, which prevents biofilm formation by pathogens.

Q2) Use very new references in both introduction and discussion sections like refs 11-12.

Response: The related content has been added. (Page 3, Lines 6–15)

> In a previous study, 140 probiotic strains were isolated from 35 types of Korean kimchi using 16S rRNA sequencing. Among them, *Lactobacillus plantarum* strains exhibiting antimicrobial activity and broad antibiotic resistance were selected (11). The growth of the microorganisms exhibiting highest pathogenicity, including *C. albicans*, was found to be almost completely inhibited in the mixed culture containing the probiotic strains and six pathogenic microorganisms (12).
> In the hydrogen peroxide production test (unpublished), 43 of 140 probiotic strains (30.7%) isolated from kimchi produced H_2O_2, among which 25 of 53 *L. plantarum* strains (47.2%) produced H_2O_2. This was attributed to direct inhibition via lactic acid, hydrogen peroxide, and bacteriocin production and low pH induced by probiotics (12).

Q3) write a sentence for public about prevention and role of probiotics.

Response: The related content has been added. (Page 2, Lines 19–22)

Most probiotics are a part of the human mucosal microbiota, and are effective in preventing and treating atopy, eczema, dermatitis, and diarrhea as well as in treating inflammatory enteritis. Probiotics also play an important role in maintaining the vaginal environment in healthy women.

List of changed sentences

No	Location	Revised manuscript	Original manuscript
1	Page 1, Lines 1	Antagonistic effects of Lactobacillus plantarum On Candida albicans in ME-180 cervical carcinoma cell culture	Antagonistic effects of Lactobacillus plantarum against Candida albicans in ME-180 cervical carcinoma cell culture
2	Page 2, Lines 7	Cervical cancer, Vaginitis.	cervical carcinoma, Candida vaginitis
3	Page 1, Lines 4–6	*Candida albicans* is an yeast species that colonizes the vaginal and oral mucosa of healthy women. However, it exhibits pathogenicity when the balance between yeast and mucous membranes and host defense mechanisms is disrupted.	*Candida albicans* is an important yeast species, which forms colonies on the vaginal and oral mucosa of healthy women and can become pathogenic when the balance between the yeast's pathogenicity and the defense mechanism of the host is disrupted
4	Page 1, Lines 9–10	To develop an auxiliary treatment for vaginitis, we evaluated the inhibitory effects of a probiotic bacterial strain isolated from kimchi on C. albicans.	In order to develop a treatment for vaginitis from kimchi, this study was conducted to investigate the inhibitory effects of a probiotic bacterium against Candida albicans, which causes Candida vaginitis.
5	Page 2, Lines 11–15	Candidiasis is a fungal infection caused by *Candida* spp. that affects the mucosa and internal organs in humans (1). *Candida albicans* is an important yeast species that colonizes the vaginal and oral mucosa of apparently healthy	Candidiasis is a fungal infection caused by *Candida albicans* and affects the mucosa and internal organs in humans (1). *Candida albicans* is an important yeast species, which forms colonies on the vaginal and oral mucosa of

		women. However, it can become pathogenic if and when the balance between yeast and mucous membranes and host defense mechanisms is disrupted (2-4).	healthy women and can become pathogenic when the balance between the yeast's pathogenicity and the defense mechanism of the host is disrupted (2-4).
6	Page 2, Line 19–Page 3, Line 6	Most probiotics are a part of the human mucosal microbiota, and are effective in preventing and treating atopy, eczema, dermatitis, and diarrhea as well as in treating inflammatory enteritis. Probiotics also play an important role in maintaining the vaginal environment in healthy women. Meanwhile, the biofilm, in which microorganisms aggregate, exhibits self-protection, and the microorganisms communicate with each other via antibiotic resistance and exhibit social behavior through horizontal gene transfer and quorum sensing mechanisms. Probiotic strains secrete antagonists such as surfactants, bacteriocins, exopolysaccharides, organic acids, lactic acid, fatty acids, enzymes, and hydrogen peroxide, and reduce the biofilm biomass by changing the pH and initiating nutrient competition, which prevents biofilm formation by pathogens (6-10).	Most probiotics naturally occur as part of the human mucosal microbiota and belong to the genera *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, and *Streptococcus* (6). Probiotic bacteria attach to the mucosa, secrete acids, bacteriocins, and hydrogen peroxide, act as surfactants, inhibit pathogens via coaggregation, and affect the immune system (7-10).
7	Page 3, Line 6–15	In a previous study, 140 probiotic strains were isolated from 35 types of Korean kimchi using 16S rRNA sequencing. Among them, *Lactobacillus plantarum* strains exhibiting antimicrobial activity and broad antibiotic resistance were selected (11). The growth of the microorganisms exhibiting highest pathogenicity, including *C. albicans*, was found to be almost completely inhibited in the mixed culture	In a previous study, 140 probiotic strains were isolated from commercial kimchi in Korea; these bacteria were phylogenetically identified based on their 16S ribosomal RNA gene sequences and examined for their resistance to 18 antibiotics (11). Examination of the inhibitory effects of the probiotics using ME-180 cervical carcinoma cells revealed that the

		containing the probiotic strains and six pathogenic microorganisms (12). In the hydrogen peroxide production test (unpublished), 43 of 140 probiotic strains (30.7%) isolated from kimchi produced H2O2, among which 25 of 53 *L. plantarum* strains (47.2%) produced H2O2. This was attributed to direct inhibition via lactic acid, hydrogen peroxide, and bacteriocin production and low pH induced by probiotics (12).	growth of *C. albicans* was inhibited in ME-180 cultures initially inoculated with relatively high concentrations of *Lactobacillus plantarum* cells, regardless of the concentration of *C. albicans* (12).
8	Page 4, Line 5	*L. plantarum* as an auxiliary treatment agent for vaginitis.	*L. plantarum* as a treatment for candidiasis.
9	Page 5, Lines 2–3	ME-180 cells were inoculated with both strains simultaneously, following which the cells were cultured for 5 h at 37 °C in a 5% CO_2 incubator.	Next, ME-180 cells were simultaneously inoculated with the two strains at 0.5 mL/well each, followed by cell culture at 37 °C in a 5% CO_2 incubator for 5 h.
10	Page 8, Line 15–21	The inhibitory effect of *L. plantarum* on *C. albicans* in the ME-180 cell culture was not direct (e.g., via hydrogen peroxide, bacteriocin, lactic acid, and organic acids). Rather, the effect was presumed to be mediated by a reduction in the growth and mucosal adhesion ability of *C. albicans* by inhibition at the early stages of biofilm development. *L. plantarum*, which exerted strong inhibitory effects on *C. albicans*, can be used as an auxiliary treatment agent for female vaginitis after safety studies have been conducted for assessing its metabolic activities and it has been confirmed to be non-infectious in animals.	
11	Page 9, Lines 5–	These findings indicate that *L. plantarum* may not have	These findings may indicate that *L. plantarum* does not directly

	12	antagonized *C. albicans* directly by inhibiting its hydrogen peroxide, bacteriocin, lactic acid, and low pH. Rather, it may have inhibited biofilm development at an early stage, which subsequently inhibited the growth and mucosal adhesion ability of *C. albicans* in the ME-180 cell culture. Further studies should be conducted on the safety, side effects, and metabolism of *L. plantarum*, and it should be confirmed to be non-infectious in animals before the *L. plantarum* isolate derived from kimchi can be used as an auxiliary treatment agent for vaginitis.	antagonize *C. albicans* by inhibiting its hydrogen peroxide and bacteriocin secretion but rather inhibits the early stage of biofilm development, which subsequently inhibits the growth and mucosal adhesion of *C. albicans*. Further studies on the safety, side effects, and metabolism of *L. plantarum* and its lack of infectivity to animals are required before applying *L. plantarum* isolated from kimchi as a treatment for vaginitis.
12	Page 11, Lines 20–22	9. Choi AR, Patra JK, Kim WJ, Kang SS. Antagonistic Activities and Probiotic Potential of Lactic Acid Bacteria Derived From a Plant-Based Fermented Food. Front Microbiol. 2018 Aug;9:1963. doi:10.3389/fmicb.2018.01963 . PMID: 30197633.	9. Leão MVP, Tavares TAA, Gonçalves ESCR, Dos Santos SSF, Junqueira JC, de Oliveira LD, et al. Lactobacillus rhamnosus intake can prevent the development of Candidiasis. *Clin Oral Investig*. 2018;**22**(7):2511-8. doi:10.1007/s00784-018-2347-8. [PubMed:29372447].

116

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: EIC | **Revision (1)** 25 Feb 2021

Dear authors
According to the associated editor report, your manuscript is accepted for publishing in JJM and send to the publisher for the next process.
Note:
Please add the ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf. For more details:
https://sites.kowsarpub.com/jjm/knowledgebase/category/tree.html#orcid.html
Thanks.