Jundishapur Journal of Microbiology
Volume 17 · Issue 8 · e150118

OPEN PEER REVIEW

Application of Prebiotics and Probiotics to Improve Oral and Dental Health by Inhibiting *Streptococcus mutans*

Ronak Bakhtiari, Zahra Sheybani, Malihe Aminzadeh, Mohammad Mehdi Soltan Dallal

SUBMITTED	REVISED	ACCEPTED	DOI
24 Jun 2024	12 Aug 2024	14 Aug 2024	10.5812/jjm-150118

About this report

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1 Original submission

3 comments · 13 Jul 2024 – 28 Jul 2024

Referee 1 Reviewer

MINOR REVISION 13 Jul 2024

The manuscript is novel and has a lot of valuable information. I would like to express my appreciation to the authors.
1. In the background, line 43, Streptococcus mutans is described as “a facultative anaerobic”; this should be written in the correct form.
2. In the background, lines 53 and 59, the names of all bacteria should be written in full the first time they appear, for example: Lactobacillus reuteri, not L. reuteri. After the first time, you can write L. reuteri.
3. In line 70, “chicory root” was written twice, and one occurrence should be removed.
4. In the material and methods, lines 99 to 106, Agar overlay interference assay, the cited references do not match the contents of the paragraph. The methods described match this paper more closely: Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S. Indigenous Probiotic Lactobacillus Isolates Presenting Antibiotic like Activity against Human Pathogenic Bacteria. Biomedicines. 2017 Jun 16;5(2):31. doi: 10.3390/biomedicines5020031. PMID: 28621711; PMCID: PMC5489817.
5. In line 162, “106 CFU”, the number 6 should be written as a superscript.
6. In table 2, it seems that the value for M18 is 11.21±0.3.
7. In the results, figure 2 shows that the effect of M18 is greater than that of K12; however, in the text, K12 has a greater effect. Please check it.
8. In figure 3, the effect of Inulin is greater; however, in the results text, the effect of Nisin was greater.

Seyed Hossein Shahcheraghi Reviewer

MAJOR REVISION 27 Jul 2024

Dear Editor
This work is favorable after the following revisions are made:
1- The bacterial name should be written in full at its first mention and abbreviated thereafter, for example, S. salivarius.
2- In the title, it is better to use “Application of” instead of “use of”.
3- In the discussion, the results have been mentioned again. It is essential to delete many of them, present them in abbreviated form, and then compare them.

Associate Editor

MINOR REVISION 28 Jul 2024

According to the reviewers' comments, the manuscript needs Minor revision.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Letter to Reviewers

Dear Reviewers,

Thank you for your detailed comments, corrections were carefully made to the text.

Reviewer 1

The manuscript is novel and has a lot of valuable information. I would like to appreciate the authors.

1. In the background, line 43. Streptococcus mutans is a facultative anaerobic and should be written in correct form.

Reply: Correction was made (Line 39).

2. In the background, line 53 and 59, for first time, the name of all bacteria should be written completely like: Lactobacillus reuteri not L. reuteri. After first time, you can write L. reuteri

Reply: Changes were made (Lines 39–41, 54–56 and 61–63).

3. In line 70 chicory root, it was written twice and one of them should be removed.

Reply: Done.

4. In the material and methods, line 99 to 106, Agar overlay interference assay, the written references are not match with the contents of paragraph. The written methods is more match with this paper: Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S. Indigenous Probiotic Lactobacillus Isolates Presenting Antibiotic like Activity against Human Pathogenic Bacteria. Biomedicines. 2017 Jun 16;5(2):31. doi: 10.3390/biomedicines5020031. PMID: 28621711; PMCID: PMC5489817.

Reply: Reference was replaced (Line 375).

5. In line 162, bacterial 10^6 CFU, the number 6 should be written in superscript.

Reply: Done (Line 173).

6. In table 2, it seems the number about M18 is 11.21±0.3

Reply: Correction was made.

7. In the results, figure 2 shows the effect of M18 is greater than K12, however in the text, K12 has more effect. Please check it.

Reply: In Figure 2, the correct title of the Y-axis is "*S. mutans* growth" and not "growth inhibition" as modified in the figure.

8. In figure 3, the effect of Inulin is greater however in the result text the effect of Nisin was greater.

Reply: In Figure 3, the correct title of the Y-axis is "*S. mutans* growth" and not "growth inhibition" as modified in the figure.

Reviewer 2

This manuscript evaluated the effect of prebiotics and probiotics on improving oral and dental health by inhibiting Streptococcus mutans. However, the manuscript is not suitable for publication as there are fundamental flaws in the study design and execution.

1.The author did not introduce inulin and nisin. Are these chemical compounds or drugs already on the market?

Reply: Inulin and nisin are introduced in the background (lines 71-74). Both compounds are already commercially available. The following references provide further evidence of their market availability:

- Roberfroid MB. Introducing inulin-type fructans. British journal of nutrition. 2005 Apr;93(S1):S13–25.
- Santos JCP, Sousa RCS, Otoni CG, Moraes ARF, Souza VGL, Medeiros EAA, et al. Nisin and other antimicrobial peptides: Production, mechanisms of action, and application in active food packaging. Innov Food Sci Emerg Technol. 2018;48:179–194.

2.Why did the author choose inulin and nisin as prebiotics for this study? What advantages do they have compared to other prebiotics such as galactooligosaccharides and fructooligosaccharides?

Reply: Nisin, one of the most important type I bacteriocins, is produced *by Lactococcus lactis* strains during the logarithmic phase of growth, with production ceasing in the stationary phase. It is the only bacteriocin approved for food applications and is widely used in the food industry. Additionally, nisin is a rare example of the broad-spectrum bacteriocins effective against several Gram-positive microorganisms. Unlike prebiotics such as GOS and FOS, which primarily enhance gut health, nisin actively inhibits the growth of harmful bacteria, providing a direct antimicrobial benefit. This characteristics makes nisin particularly advantageous in extending the shelf life of vulnerable food products by preventing spoilage and pathogen growth.

Inulin, known for its benefits and compatibility in the food industry, is frequently added to processed food products and supplements. Inulin's ability to act as a prebiotic, dietary fiber and functional food ingredient (fat replacer and texture modifier) gives it a multifunctional role in food products. In addition to its prebiotic effects, inulin offers multiple health benefits such as improved mineral absorption, enhanced satiety and better blood sugar regulation, making it advantageous for various aspects of health.

Therefore, these two compounds have been discussed in this study.

3. There are millions of chemicals found in nature with potential antibacterial activity, but not all can be consumed by humans. Chemicals or any drug adjuvant must go through extensive toxicity screening to be considered safe for human consumption. There is no mention of any preliminary toxicity study conducted on inulin and nisin.

Reply: Since inulin is present in various plants, including chicory root, leek, onion, garlic and asparagus and its regulatory effects on gut microflora have been established, its consumption is suggested as non-toxic for humans. Additionally, nisin has been approved by the US Food and Drug Administration (FDA). This information with relevant references is discussed and highlighted in the Background and Discussion sections of the manuscript.

4. Where were these two compounds (nisin and inulin) purchased?

Reply: Prebiotics of nisin and inulin (Sigma-Aldrich, Germany) were used by preparing serial dilutions with physiological serum.

5. Where did the microbial strains come from? Many important details have been omitted in the methods section.

Reply: Additional information was added (Lines 81–82 and 88–91).

6. In the microdilution assay section, S. mutans was used at 100 CFU per well. What do you mean by this? Is it soluble in water or not?

Reply: *S. mutans* was diluted in BHI broth culture media, the number of bacteria was set at 1000 CFU/ml using spectrophotometer as 100 µl contained 100 CFU.

7. In the combination studies, the growth rate must be calculated using a specific formula. This method should be described in more detail in the methods section.

Reply: It was added to Page 3 (Lines 97–102).

8. Why did the author not use statistical tests? It seems that the authors have only presented raw data without providing robust statistical analysis.

Reply: We appreciate your valuable feedback regarding use of statistical tests in this study. This was included in Page 6 (Lines 175–181).

9. How are the statistical results discussed in the discussion section if there is no mention of statistical analysis in the methods and results sections?

Reply: That is right—statistical results should clearly be explained in Methods and Results sections before they are discussed in Discussion section. This was carried out in Results section (Lines 193–194, 198–200 and 203–204).

Reviewer 3

Dear Editor

This work is favorable after following revisions:

1. Bacteria name in first time is complete and other times should be for example *S. salivarius*.

Reply: Corrections were made.

2. In title is better Application of instead use of

Reply: Done.

3. In discussion, your results have been mentioned again. It is essential that you delete many them and state them as abbreviated and then compare them.

Reply: Discussion was improved (Lines 302–308).

2 Revision 1

0 comments

No public comments in this round.