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June 18, 2020
Dr. Vidhya Iyer
JoVE

Re: 62812

Dear Dr. Vidhya Iyer

Thank you for your May 31 email regarding our manuscript entitled “Bacteriophage effectiveness for biocontrol of foodborne pathogens evaluated via high-throughput settings”. ‘Clean’ versions of our revised manuscript including main body and Tables of Materials are uploaded with this letter.

Reviewer’s comments are copied below, with the authors’ responses inserted between.

The authors thank the reviewers for their constructive comments, and look forward to publishing future work in the JoVE Journal.

Thank you very much!

With kind regards,

Sincerely,

Dongyan Niu, PhD

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Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.
   Thank you. We have asked Dr. John Kastelic, a professional Editor, to proofread the manuscript.
2. Please provide an email address for each author.
   Thank you. Added.

3. Please increase the word count of your abstract to be 150-300 words.

Thank you. Added new content in the abstract, meet the word requirement now.

4. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s), but before punctuation.

Thank you. Corrected as suggested.

5. Please revise the following lines to avoid overlap with previously published work: 25-27, 32-33, 61-65, 113-118, 119-126, 134-139, 155-160, 198-203, 205-211, 219-225, 227-228, 230-232.

Thank you. Reworded.

6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: BioTek, Winooski, VT, USA; GLIMMIX; SAS (Version 9.4, 1999);

Removed BioTek, Winooski, VT, USA ; But we kept GLIMMIX; MIXED and SAS, as they are standard name of program model and seems no alternative name can be replaced.

7. Please revise the text, especially in the protocol, to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Thank you. Revised.

8. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

Thank you. Revised as suggested.

9. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add

references to published material specifying how to perform the protocol action. Please add more specific details (e.g., button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Thank you. More steps and details added.

10. After including a one line space between each protocol step, highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. This will clarify what needs to be filmed.

Thank you. highlighted in yellow.

11. In the text and figures, ensure that you have a space between the numbers and units (except for %). Write µL not uL.

Thank you. corrected as suggested.

12. Ensure that you cite figures and tables in order in the text—Table 1 is cited after Table 2.

Thank you. The tables should be in the right order now

13. Please include a table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the Materials Table alphabetically by the name of the material.

Thank you. Added material table.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The present study evaluated a proposed method for the development of phage cocktails, considering factors such as the phage genera and various combinations, multiplicity of infection, temperature, and time.

Major Concerns:

While the approach and documentation of a systematic protocol is of importance, and the authors were throughout with the analysis and results, the use of overnight culture is particularly problematic (lines 194-196), as usually bacterial cultures at log phase are used in order to maximize the number of bacteria that are viable for phage infection. How would results change if log phase cultures were used instead.

Thank you. We explained why using overnight culture instead of log culture in line 299. We enumerated diluted overnight culture each time when doing the assay. So the bacterial number for phage infection

are consistent (~10^4-10^5 CFU/mL) across different trials. We are not sure how results would change if using log culture, but can test it in the future.

In addition, the protocol itself is not completely novel. The title does not reflect what was done in the sense of the bacterial strains tested. Since only E. coli O157 was used, the title should reflect this, as well as the abstract. More info of E. coli O157 as a foodborne pathogen may be needed to benefit readers.

The protocol was also successfully executed in phage-killing kinetics against Salmonella in our published work. We added the discussion in line 287.

Minor Concerns:

Line 56: The authors may have forgotten to add the date in the Bourdin reference.

Thank you. The reference was added now.

Line 73: Is incubation with shaking needed at this stage?

No shaking is required. We added “statically”.

Line 76: Explain the importance of adding MgSO4

Thank you. We explained use of MgSO4in the discussion, Line 295-296.

Line 85: Why were phage stocks diluted in mTSB and not a buffer (e.g., PBS)?

Thank you. We need to make E. coli grow as well.

Lines 134 and as needed throughout the text: Add the word "phage" before describing the designations T4, T5, T1, etc. so it reads "phage T5" instead of just "T5"

Thank you. Added as suggested.

Line 149: Strain 3081?

Yes, we added strain.

Reviewer #2:

Manuscript Summary:

The manuscript is pretty straight forward and describes a strategy for optimal use of phage cocktails for biocontrol. Such descriptive protocols are useful for not only in this setting but also in other scenarios such as clinical applications in human phage therapy. The authors describe a systematic approach of using phage and bacterial dilutions and mixes for assessing the efficacy of the individual and cocktails. Some additional questions that could be addressed are: do suboptimal phage concentrations (low MOIs) induce resistance?

Yes, it may be, but the current protocol was not able to monitor the anti-phage resistance. We discussed the limitation in lines 306.

What is the ideal number of phages in a cocktail to obtain maximum benefit of biocontrol?

It depends the bacteria target and how much to kill and environmental condition. Usually MOI (phage to bacteria ratio) > 10 are required.

These experiments are done buffer system. What happens with these cocktails in actual biocontrol settings? These questions are not to be addresses here for additional experiments. But perhaps, discussed for the benefit of the readers.

We discussed in the lines 307-309.

Major Concerns:

None

Minor Concerns:

None

Reviewer #3:

Manuscript Summary:

In the manuscript "Bacteriophage effectiveness for biocontrol of foodborne pathogens evaluated via high-throughput settings", the authors provided a method for screening high-efficiency phages. The procedure was well described and easy to follow.

Minor Concerns:

1. Please check the references carefully and format them according to the JoVE.
   Thank you, References corrected.
2. Lines 187-188, please make sure whether the abbreviation month "mo" is acceptable by JoVE.
   We changed to months

Lines 199, "37C" should be "37°C". Please go through the manuscript for such minor errors.

Thank you. Corrected.

3. I am confused about the tables. "Panels A–G were assigned to each phage treatment", why some panels have more than one treatment? Some panels have more treatments than others? What kind of statistical analysis was used? The first row was not assigned properly. The E. coli strains also should be mentioned in the legends.

Number of panels were originated from statistical analysis of each phage treated bacteria culture.

As sensitivity of strain to phage was not uniform, the different assignment of panels reflects this variance. The statical analysis was described as the following

5. Data analyses

1. *Repeat at least 2 independent experiments as described above. Compile results from all the independent trials. Calculate the average and standard deviation of the OD_{600} from each phage-treated and -free culture.*
2. *For each bacterial strain and temperature, square-root transform the OD values at 600 nm and analyze using an appropriate statistical model. For SAS software, select the MIXED model and least-squares to differentiate means ($P < 0.05$). For each strain, assign panels A−G to each phage treatment of which overall anti-O157 efficacy across time and MOIs differed ($P < 0.05$).*
3. *Define superior phage efficacy based on OD_{600} value ≤ 0.01 that is corresponding to no detectable bacterial growth (limit of detection:300 CFU/mL). Analyze effects of incubation temperature, time, MOIs, E. coli O157 strains and phage types on phage efficacy using an appropriate statistical model. For SAS software, use GLIMMIX with random measures. Calculate Odds Ratios to compare superior efficacy for different environmental and biological factors of interests.*

Corrected others.
