**From:** em.jove.145d9.5d0ee5.a0fac39d@editorialmanager.com <em.jove.145d9.5d0ee5.a0fac39d@editorialmanager.com> **On Behalf Of** Vineeta Bajaj  
**Sent:** Monday, August 06, 2018 1:51 PM  
**To:** Deborah Neher <Deborah.Neher@uvm.edu>  
**Subject:** Revisions required for your JoVE submission JoVE58767 - [EMID:9f2e9c61d2374625]

CC: [tweicht@uvm.edu](mailto:tweicht@uvm.edu)  
  
Dear Dr. Neher,  
  
Your manuscript, JoVE58767 Plate competition assay as quick preliminary assessment of disease suppression, has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.  
  
After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually. Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 pixels x 1080 pixels or 300 dpi.  
  
Your revision is due by **Aug 20, 2018**.  
  
To submit a revision, go to the [JoVE submission site](http://www.editorialmanager.com/jove) and log in as an author. You will find your submission under the heading "Submission Needing Revision".  
  
Best,  
  
Vineeta Bajaj, Ph.D.  
Review Editor  
[JoVE](http://www.jove.com/)  
617.674.1888  
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**Editorial comments:**  
Changes to be made by the Author(s) regarding the written manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.   
2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

Requested on 18 August 2018.  
3. Keywords: Please provide at least 6 keywords or phrases.

Added 3 words: root diseases, soilborne fungal pathogens, bioassay   
4. Please expand the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

Done  
5. Long Abstract: Please do not include references here. Removed the reference  
6. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

Updated; not sure if days is d, so left it as days or weeks  
7. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.

Updated  
8. 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: American Type Culture Collection, etc. The only one that I noticed was Erlenmeyer which is now changed to conical flask.

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

10. Please revise the protocol to contain only action items that direct the reader to do something (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.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Removed ‘should be’ from 4.1.1

Added laminar hood or bleached counter top to the Notes

11. Please add more details 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. 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. Some examples:  
1.1.2: Please describe how to isolate from root lesions.  
1.1.2.1: How to excise lesions? Added 1.1.2.1 through 1.1.2.3  
1.1.2.2: What container is used? Added 1.1.2.4  
1.2.1: Please describe how to establish a daughter culture. Added a note  
12. Please include single-line spaces between all paragraphs, headings, steps, etc. Done  
13. Please remove the weblink and use a superscripted numbered reference instead. I removed the web links in the references  
14. For in-text references, the corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text (before punctuation but after closed parenthesis). The references should be numbered in order of appearance. Done.  
15. References: Please do not abbreviate journal titles. Wrote them out  
  
**Reviewers' comments:**  
  
  
  
**Reviewer #1:**  
Manuscript Summary:  
The manuscript details a methodology of a plate-based assay to identify plant disease suppression capacity of compost.  
  
Major Concerns:  
The authors have done a decent attempt of highlighting the steps but the manuscript needs refinement. The intro paragraph is very weak and does not really provide a hook to keep the readers reading. The discussion section also reads weak. The authors should really highlight the strength of the protocol rather than ending on a negative note. The methods section also needs work.  
being introduced like multiple indicators, etc.  
Line 2: Disease suppression in what/where? Plant roots  
Line 34-38: Please choose a better word than tool. Perhaps "medium" or "bioproduct". I also don't necessarily agree that it allows manipulation of soil microbial communities? I would suggesting rephrasing this entire paragraph. Reworked the paragraph  
Feedstocks used to prepare compost? Also what process are these? Do you want to convey biological processes? Please provide clarity. I can only draw vague understanding of the message here. Changed feedstocks to raw materials (ingredients)  
LIne 35: Feedstocks used to prepare compost? Also what process are these? Do you want to convey biological processes? Please provide clarity. I can only draw vague understanding of the message here. Changed ‘process’ to ‘method’  
Line 36: Fungi and bacteria in compost? is this plant disease? No, beneficial microbes

Line 37: What do you mean by antagonism? Please elaborate. Leaves the readers wondering what kind of antagonism it detects. Even straightaway highlighting which process is being detected here in this manuscript will also help. I would take some time to craft this paragraph. It is the opening paragraph but desires much to be understood. The message is not clear, and the paragraph reads choppy at its best. Reworked the paragraph.  
Line 40: Provide examples of such time consuming assays. There are no more examples  
Line 42-43: What does finer degrees of suppression mean? Finer than what? Bioassays or excavation of roots  
Line 45: Potential of what? suppression  
Line 47-48: Why should the readers care about R. solani with respect to the bigger picture of the manuscript? This is the first instance that R. solani has been introduced in this manuscript, but I haven't understood why. Added some text for explanation  
Line 62: What kind of infested soil is this? Is this easily available/safe to use? Those naturally containing R. solani  
Line 63: Provide composition of water agar.(water plus agar)  
LIne 64: Provide composition of corn meal agar. What percent? Are the isolates kept in dark? added  
Line66: Provide composition and what percent added  
Line 73: What is meant by each compost? How many are there? clarified to a single compost  
Line 77: After 24 hours? changed  
Line 94: How many wells per plate? And are the wells+R solani placed centrally in the plate or dispersed throughout the plate? A petri dish only has one well  
Line 95: Perhaps elaborate this for readers who are not familiar with this measurement technique?   
Line 101: Sentence seems to have error. Recheck for grammar. Thanks, changed.  
Line 108: days of challenge? Or incubation? Is this a common term in plant pathogenesis? Challenge relates to the definition in the prior section  
Line 112: Avoid using such words in scientific literature. Significantly is appropriate. Also, background information of the representative results should be provided. Changed to significantly.  
Line 113: Again, avoid such phrases….how much is almost all?? Changed  
Line 114: How can your method serve as a negative control? Please edit/rephrase. Explained that it was the autoclaved plate A of the 2 plate pair  
Line 118-119: How did you test this? Your methods don't highlight this. You tested one type of compost, unless you tested more. If you did, this should be highlighted in the methods. Added text to explain that a. variety of composts were tested  
Line 118: Avoid words like most/more/almost all….this is an unscientific way of discussing results. Ok, removed them  
Line 120: check grammar. Done  
Line 135: Why isn't there error bars in control? Were replicates not performed for controls? Too small to see  
Line 139: ??? Check for grammar. Reads incorrect. Good catch, thanks.  
Line 150: This is a new concept being introduced in the discussion. Is filtering an established protocol? If so, it should be highlighted in the introduction. Added some text so it is less abrubt.  
Line 154-156: References? Is this something you did or are you citing others' results?? Yes, we did this, but I also added references.  
Line 158: Figure 2 is place above in line 116…..Why should it be placed here again? Good catch, the second mention is removed.  
Line 170: Table 1 seems to be placed at line 127, although it would make more sense to place it here. Removed the Table per Reviewer 2  
LIne 173-174: "takes care to avoid contamination". Rephrase sentence, incorrect grammar; Done  
Line 177-178: Was expecting the discussion to end on a positive note. Since you just highlighted the limitation, that is what sticks in mind. Is there a way you can highlight the benefits of your method and discuss some potential applications of it, rather than ending it in a negative note and one random sentence about the future application. The future application sentence reads odd as well, since the future application is not particularly relevant to your method's future application. But I can see how using the plate assay would allow you to access the inhibition zone and attempt community identification. I would recommend highlighting applications like this to end on a robust note. It should make readers want to adopt this protocol for their work. Added a positive ending.  
Line 182: Why is Lynn Fang not a co-author on a method that she/he tested and published???? Tom developed the method for Lynn to use and Deb ran statistics, created figures, and wrote manuscripts.  
  
Minor Concerns:  
Grammatical errors.  
  
  
**Reviewer #2:**  
The paper titled "Plate competition assay as quick preliminary assessment of disease suppression" by Deborah A Neher and Thomas R Weicht submitted to JoVE, reports a methodological study addressing the development of a rapid and affordable test to assess the suppressive capability of compost towards the pathogen Rhizoctonia solani.  
  
The idea of developing a rapid and affordable assay is very interesting. Moreover, the authors report a correct materials & methods section to reproduce the experiment. The results are interesting and support the basic idea of the authors. My only concerns is about the way of data and method presentation. The Introduction is too short and only some of the topic are properly introduced. So, the reader when arrive to the results become confused for the appearance of concepts and details that were not previously introduced. Moreover, the quality of several figure must be improved.  
  
Below I summarize a list and my comments that may improve future version of this manuscript:  
1. Line 34. Compost allow manipulation of soil microbial communities to modify soil functioning, including disease suppression. Thanks for the specific suggestion  
2. Line 36. "Beneficial fungi and bacteria into compost…" Completed  
3. Line 41. "from week to months" instead of "2-4 weeks" Completed  
4. Lines 46-48. I think that a short sentence that explain the reasons for the selection of R. solani is required. A short paragraph was added  
5. Lines 49-50. A couple of sentence describing the experiment could be very useful here. The experiment, in the present version, is described only in the caption of Figure 3. So, is quite difficult to follow the rationale of the experiment. I think that the authors can make a synthetic description of the factors included in the experiment: i.e. decomposition time and feedstock types. A couple of sentences were added  
6. Lines 120-121. Please, check English. Good catch, thanks.  
7. Lines 124-127, Table 1. I do not understand the logic of this table. What is the nematode index? Why reporting some enzymatic activities only here? A short background is necessary to explain their significance. In fact, presented in this way the table create only confusion to the reader. This section must be properly introduced explaining the importance of identify the suppressiveness of compost not only with bioassay but also with microbiological, chemical and/or biochemical parameters. Removed the table.  
8. Figure 1. This figure must be improved. A structure with some arrow showing autoclaved and autoclaved sample can be useful. The quality of the cartoon is low and can be misleading. For instance, the R. solani culture seems liquid instead is solid in water agar or PDA. I think that the use of pictures would be better than the proposed cartoon. For instance, a panel with pictures of water agar, water agar + compost and these two materials inoculated with R. solani can be very useful. In case of the plate with R. solani, please report a plate with not complete growth but where the mycelium cover only 50-75% of the plate. This picture will be more explicative. Remade the figure  
9. Figure 2. Why standard deviation is not present for autoclaved bar? Too small to see

10. Figure 3. Y axe label is wrong because growth cannot be negative. The correct info are reported in the caption of figure 3. Please, change the label and make this consistent with the caption. Revised the legend to explain the negative values  
  
  
**Reviewer #3:**  
Manuscript Summary:  
Based on a master thesis and a published journal article by the authors, the protocol described a plate assay with comparison of mean radius of mycellium to detect disease suppressive ability of compost against R solani, a soilborne fungus pathogen. The protocol is well written. The procedures are clear, and results are well described and supportive to conclusion.  
  
Major Concerns:  
Line 45. please provide more details about the method by Alfano, then explicitly explain what the current method differs from the one by Alfano. Display the advantages if any. More explicitly stated the two differences and why those changes were made  
Lines 140-142. To improve the readability and clarity, the batch of compost terminology need to be explained and elaborated. Attempted to use simpler terms

Minor Concerns:  
Line 73. 2.1 rephrase the sentence; Done  
Line 78. 2.3. what is a liquid cycle? please explain or be explicit. Rephrased as a slow exhaust setting  
Line 120, delete is. Done  
Lines 140-142. this is confusing. separate the stats results from those abbreviations. Moved the statistics to the graphs and rearranged the legend.  
Figure 2. what is the error bar for Autoclaved? Too small to see  
  
**Reviewer #4:**   
Manuscript Summary:  
This is a useful assay for studying disease suppression potential of mixed microbial communities in compost.  
  
Major Concerns:  
The introduction is clear and the methods are fairly good as well, but then in the results you talk a lot about different assays (ie. ecoenzymes) that you never explained and never end up showing how the results in your plate assay relate to disease suppression potential in plants (the gold standard for estimating compost disease inhibition potential). I would ask that you delete table 1 (you can still mention those other assays in the discussion if you want, but don't dwell on them), and instead replace it with a direct comparison of plate assay results juxtaposed against plant disease assay results. Some photos of what disease results in the plant assay look like, as well as what plate assay growth inhibition looks like would also be useful.

Deleted Table 1 but let the explanation in the text.   
  
Minor Concerns:  
You missed a few details that might be important. For example, what genotypes of radish should one use as bait? What composts did you test and where did you obtain them? What could one use as positive controls to know the technique is properly being conducted? Did you get your R. solani from ATCC or did you isolate it yourself? Why is maintaining moist, warm conditions a limitation? Please do a better job of explaining the drawbacks of plant bioassays (maybe just stick to your original explanation that they take too long).

Added the Latin name for radish, and clarified that the authors isolated their own Rhizoctonia solani.   
  
You need to prove or better reference why it would not be reliable as a standalone assay. Please reference: Shehata, Hanan R., et al. "Relevance of in vitro agar based screens to characterize the anti-fungal activities of bacterial endophyte communities." BMC microbiology 16.1 (2016): 8. That paper shows that plate competition assays are reliable as standalone assays, so why shouldn't your assay be as well? Please elaborate. Added some explanation in the text to provide more clarification.