Review of the manuscript #52339

The multi metal-resistant bacterium *Cupriavidus metallidurans* CH34 affects growth and metal mobilization in *Arabidopsis thaliana* plants exposed to copper

Overview

In this manuscript, the authors studied the role of multi metal resistant bacterium *Cupriavidus metallidurans* CH34 on the growth and metal accumulation of the model plant *Arabidopsis thaliana* in the presence and absence of Cu. Generally, the topic of this research is interesting and worth publishing. However, the manuscript needs some major improvement before publishing. Certainly, discussion section is the weakest part of the manuscript and needs re-working. At the moment, results are given without much of the context and their implications remain elusive to me. It almost feels like authors themselves are not sure what their findings mean.

BASIC REPORTING:

- The objective of the manuscript is rather clear.
- English require verification (check by a native speaker)
- Intro & background should be further developed, at the moment too general.
- Literature well referenced & relevant, however more literature review for the discussion section is necessary.
- Structure conforms to PeerJ standards: Results and discussion should be separately following the journal's requirements.
- Figures are definitely too small and need some improvement. Table are missing important information (see my specific comments).
- Raw data supplied, however, metadata not provided (description of columns' names is needed)

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful.
- It is not clearly stated how the research fills an identified knowledge gap.
- Investigation performed to a rather low but sufficient technical standard.
- Some methods described with insufficient detail & information to replicate (see my specific comments).

VALIDITY OF THE FINDINGS

- Moderate impact and novelty.
- Inconclusive results.
- All underlying data have been provided, however not always presented in a clear way.
- Data are robust, statistically sound, & controlled.
- Conclusions are not well stated, rather vague and general. Not enough linked to original results

General comments:

- Authors should highlight the importance, novelty, and significance of this work.
- All the experiments were conducted in growth media and hydroponic cultures. Did the authors consider using more environmentally relevant matrix such as soil? That could significantly change the uptake and translocation of metals and certainly, it would give a better idea about the potential use of CH34 strain in bioremediations. It should be mentioned in the text…
- The plant root exudes (PRE) analysis is barely mentioned in the discussion and results are not presented in any form (figure/table). Whether authors further elaborate on this result or completely remove it. At the moment I don't feel it contributes much to the discussion and interpretation of results.
- Generally, many sentences are written in a clumsy way. I strongly recommend giving the text for a revision to a native speaker.

Specific comments

Abstract:

1. Line 21. …. Such as…Please give examples of these detrimental effects… When does it become detrimental? Is it only at high Cu concentration? How is it expressed? By plan disease or death? Please give more background.
2. Lines 27-29. This is a very general statement and it likely was expected before even starting the experiment. I would recommend being more specific. When the effect was positive and when was it negative? Give a brief result sentence...
3. Line 30-31. Again, this is too general. What were these significant changes in metal accumulation? Please be more specific. The result is the most important part of your work! Based on the abstract and these few results sentences reader will continue reading your manuscript or stop right here.

Introduction:

4. Line 42. Please introduce abbreviation of the element (Cu)
5. Lines 43-48. It's a very long sentence. Perhaps it's better to split it.
6. Lines 54-60. Again, a very long and complex sentence. I suggest to divide it.
7. Line 63. I would say PRE-microbes interaction "might" affect trace metals bioavailability in soil, as not all of them do.
8. To provide a better background I would suggest adding a short paragraph regarding Cu in the environment (particularly in agricultural soils). Specifically, what are average concentrations, where the Cu contamination comes from, does it accumulate in the soil or it is easily leached, how soil microbiota respond to high Cu concentration etc.

Materials and Methods

9. Line 99. Why 95C? Are authors sure that the bacteria culture was inactivated at this temperature? Usual sterilization protocol takes 30 min at a minimum temperature of 121C.

10. Line 97. Please provide a formula that was used to calculate CFU. I couldn't find it either in the manuscript or in the excel sheets.
11. Line 109. Please specify all concentrations used in the experiment.
12. Line 112. Why only 21 days of growth?
13. Line 118. Why in the rhizosphere and plant colonization only 50 uM Cu was used while in In vitro plant-bacteria co-cultivation assays from 0 to 70 uM Cu was applied?
14. Line 159. I must say I'm a bit confused about concentrations of Cu used in different experiments. Why in Bacterial gene expression tests in plant-bacterium co-cultivation assays 0 and 25 uM was used while in previous it was 50 or from 0 to 75? Any specific reason?
15. Line 162. Why 30, 60, and 180 min time interval was used? This feels a bit short considering that bacteria require some adaptation period.
16. Lines 178-180. Please provide instrument information.
17. Lines 180-181. Please provide manufacturer and software information.
18. Lines 184-186. Please specify what ae these genes and what the primers are meant to target. Are they all related to copper resistance?
19. Line 218. decreasing it by 20.0 and 86% compared to uninoculated control, right? Please specify.
20. Line 220. It is surprising that for the inoculated plants FW and DW decreased when the rosette area and root growth increased at lower Cu concentrations. Any explanations for that?

Results and Discussion:

21. Lines 223-224. Why this data is not shown? As I already mentioned in point #9, I have doubts that microorganisms were fully inactivated at the temperature of 95C. Is it possible that some activity was still recorded? Could you present this data in supplementary materials?
22. Lines 242-244. Please refer here to a figure/table where the data regarding PRE composition can be found.
23. Lines 245-247. The authors state that "Cupriavidus strains are in general unable to use sugars as carbon and energy source, however, they usually encode a significant group of aromatic compounds degrading genes". But was sugar degradation/consumption observed in this experiment? The fact that the bacterium carries a given gene doesn't mean it was expressed. Also, what authors mean by "ecologically relevant advantages"?
24. Line 239. Table 2 is not discussed enough. How authors explain such high standard deviation in the CFU of rhizosphere (both with and without Cu).
25. Line 256. Sometimes authors use Arabidopsis and sometimes *A. thaliana*, and another time *Arabidopsis thaliana*, please stay consistent and stick to one form.
26. Line 256-261. It is extremely long and confusing sentence. I suggest to re-write it and split into 2 o 3 sentences.
27. Lines 262-264. Before discussing all analyzed elements, I would recommend to write a short paragraph why these elements are important and how they can affect the plant growth. Please provide some background… Also, this sentence is not very clear: "Mo and Zn levels decreased and increased only in roots, respectively". So, Mo and Zn decreased in rosettes and both increased in roots? Besides it increased/decreased compared to what? Please reformulate it and be more specific.
28. Table 3. I have two major criticisms about this table. First of all, what is the unit of given values? Second of all, is it calculated by g or mg of dry or fresh weight? This is important

information that is missing. The tables as well as figures need to be self-explanatory, so reader by looking at them without even reading the text can understand the content.

29. Table 4. I'm not sure if I fully understand. The percentage of increase of given metal is calculated based on non-inoculated plants vs. inoculated? This need to be explained in caption of the table.
30. Lines 260-268. I think it would help reader to have the values of the metals given in the brackets directly in the text. I find it annoying to scroll down each time to look at table 3 and 4.
31. Lines 275-278. Similarly, as above, it would be much easier to have TFs values given in the text rather than just in the table.
32. Line 280-289. The discussion about remediation/phytoremediation is insufficient. I would recommend to explore this topic a bit farther, to look more into the literature, refer to different plant/bacteria strains etc. This is an important aspect and I believe it deserves a bit more attention.
33. Fig.2. Did authors consider bar plots? With only 3 timepoints it might be more appropriate. I would recommend to make the graphs bigger, there is still a lot of space. Please make a value bigger. Also, to make a legend a bit clearer and save time of the reader to look it up in materials and methods, I suggest adding information what plant and control treatments are. E.g. Plant (A. thaliana + CH34, no Cu); Control (CH34 + Cu). Or just simply write it in the caption of the Figure. I think there is no need for 5 same legends, just make one but well visible and clear. Also, I'm wondering why copC gene is so much more expressed in control compared to other treatments. Any explanation to that?
34. Line 309. Authors refer to Figure 2A, but figure 2 doesn't have A,B, C, D panels labeled. Please correct that.
35. Line 322. Sometimes it's written Fe and sometimes spelled out as iron. Please stay consistent
36. Line 325. I think authors should re-consider naming of each treatment, so it would be easier to follow. Plant, that's no inoculation and no Cu? I'm confused…
37. Fig. 3 is ridiculously small, why? Same comments as Fig. 2… Consider bar plots, label panels, make axis tittle and scales bigger, make one legend - clear and common for all panels (write what each treatment represents)
38. Line 326. Non-planted conditions mean control? It is really needed to make the naming clear.
39. Lines 291-239. I feel all this section needs some improvement. Just saying that one gen was expressed and other wasn't, or one was after 30 and another after 60 min doesn't bring much to the discussion. What are implications of these findings? What does it mean for the bacteria but also for the plant? What can be concluded out of these observations. The way it is right now it doesn't bring any value to the manuscript. Make it more like a discussion.

Conclusions

40. Conclusion section also needs some more work. At the moment it is very vague. The authors should put a bit more effort into summarizing the main finding. Saying that CH34 has beneficial or detrimental effects on the plant isn't sufficient. Be more specific about your results and what are implications of your finding, how it can be used and why is it important…