With reference to article #52899 titled “Microphenotyping system: A novel method for screening plant growth-promoting rhizobacteria” a revised version of the article is submitted for further consideration and processing. Article has been revised as per Editors and worthy reviewers’ suggestions. Point to point response follow below:

RESPONSE TO EDITORS COMMENTS

We are very thankful to the Editor to provide us with an opportunity to revise this article. All the sections of this article have been revised keeping in view the reviewers’ comments. In methodology, number of replicates for each experiment are now mentioned with clarity in the revised version. Different sections of the methodology have been supported with relevant references. Figures number 1-7 have been modified to increase the resolution as per Journal’s requirement. Moreover, effort has been made to remove the grammatical errors in the article.

RESPONSE TO “REVIEWER 1” COMMENTS

Basic reporting

1). Effort has been made to improve the English text or to remove grammatical errors in the revised version of this article.

2). The resolution of submitted figures was compromised during the submission process and their conversion to PDF file. Good quality figures are now provided in the revised manuscript as per Journal’s requirement.

3). Number of tables have been reduced to 4. Table No. 1 and 2 has been converted to figures. However, to elaborate the growth response of different treatments or growth parameters of mutant lines of *Arabidopsis thaliana* data is presented in the form of tables.

4). Reference to raw or supplemental data is now given in the revised article as per worthy reviewer’s suggestion.

Experimental design

1). The whole text of introduction has been formatted and research question is now well defined by adding more literature to justify the use of ‘Microphenotron’ for screening plant growth-promoting rhizobacteria (PGPR).

2). We are thankful for” reviewer 1” comments that in current study we are suggesting novel protocol based on ‘Microphenotron’ to screen bacteria for PGP traits.

3). We are thankful for reviewer’s comment that methodology is clearly stated.

Validity and findings

1). Number of replications for each experiment is now elaborated in the revised version of the article. For instance, for 'Microphenotron' for each strain or control, 8 wells or PCR tubes were used in triplicate and experiment was repeated three times. Overall, 72 wells or tubes were used for each strain to authenticate the reproducibility of the experiment. This revision had been mentioned on page 8 lines 212 to 214. Similarly, for bacteria-*Arabidopsis* pot trials, number of replicates has been mentioned in lines 230-232.

2). The trend of growth response for 'Microphenotron' and green house experiment is mentioned in the discussion section. In 'Microphenotron' screening was based on root or shoot growth response. Such parameters were not mentioned in pot trials with wild-type Col. N6000. Therefore, we did not prefer to perform correlation analysis between unrelated parameters such as root growth and rosette fresh weight or number of leaves. I hope worthy reviewer may agree with this point.

RESPONSE TO "REVIEWER 2" COMMENTS

Basic reporting

1). We are very thankful for the worthy reviewer's comments that this manuscript is generally well written. All the major sections of this article have been revised according to reviewer's suggestions. Grammatical errors have been removed and different sections have been rephrased. Especially, reviewer's concern about the replication of different experiments has been properly addressed. In first draft of the article, mean of three replicates was mentioned with reference to three different set of experiments. In tables and in text this confusion has been removed in the revised article. Figures may have lost resolution during the formation of PDF file. However, modified figures with high resolution are submitted with the revised manuscript.

Experimental design

1). For bacterial biochemical screening and for pot trials, number of replications and respective control treatments are now mentioned clearly in different sections of the methodology.

Response to general comments

1). In present study, 10 bacterial strains were short listed based on their multiple plant growth-promoting traits, especially on auxin production. Initially, we isolated number of bacterial strains that are now indicated in figure 1 and supported with reference (Raheem et al., 2018). Finally, 10 bacterial strains were selected for further experimentation for this study. Bacterial strains were

evaluated by performing different set of experiments with *Arabidopsis* including phytohormone mutant lines.

2). Reviewer's concern with reference to number of replicates is now clarified in the revised article. For 'Microphenotron' bioassay single lane of eight PCR tubes or wells was placed for each strain or control in three replication and experiment was repeated three times. Overall, 72 PCR tubes were used for single strain or B5 media (control). Similarly, for pot trials experiment was also repeated three times with overall 27 seedlings inoculated for each strains or control. Although, more replicates may be included to increase the authenticity of the experiments. But overall, currently used number of replicates may be sufficient to predict the outcome of the results. Moreover, our results indicated that strains that performed well in 'Microphenotron' bioassay also showed good results in pot trials for wild-type *A. thaliana.* Auxin production by PGPR strains in soil is not always detrimental to host plant. Nevertheless, reviewer's concern may be right for some plant pathogenic bacteria that may induce abnormal growth in plants due to increased auxin production. But for free living PGPR, auxin production is very beneficial as supported with number of references in introduction (Egamberdieva, 2012; Iqbal and Hasnain, 2013; Aslam and Ali, 2018; Raheem et al., 2018). In present study, all the strains possess beneficial traits as they were selected from several bacterial isolates as mentioned in figure 1. For 'Microphenotron' bioassay, B5 medium was used as a control in comparison with bacterial inoculations. For negative control, bacterial strain was not used as we do not have culture of bacteria that may be deficient for number of plant growth promoting traits.

3). In this article, wild-type and mutant lines of *A. thaliana* were already compared in different set of experiments in 'Microphenotron' and then in pot trials.

4). Two figures have been added in the manuscript to improve the presentation of data. Moreover, figure 7 has been converted into Box and Whisker plot to analyze the plant growth response for different growth parameters.

Response to specific comments

1). Bacterial auxin production was used as a major criterion to screen PGPR. Reviewer's concern about the detrimental effects of auxin may be true for some plant pathogenic bacteria as mentioned above. However, free living PGPR produce optimum level of auxin within the rhizosphere due to the limited availability of L-tryptophan in root exudates. In introduction section, we have quoted a few references that showed beneficial effects of microbial auxin.

2). Line 234: Bacterial strains were identified by comparing already submitted sequences in GenBank through Blast analysis. Their similarity is now elaborated in lines 249-255 in the revised manuscript.

3). Phylogenetic tree is modified to include all possible sequences (25 strains) from the same geographical location. Phylogeny is assigned according to nearest related sequence. Moreover, *Psychrobacter* and *Moraxella* are now separated and occupying different branches on the phylogenetic tree.

4). Line 245: Sentence has been modified and rephrased as per reviewer's suggestion. Correlation between bacterial auxin production and cell densities is clarifies in lines 259-261 in the revised version. Figure 2 has been modified to increase the resolution and submitted with the revised manuscript.

5). Line 268: Initially, we isolated number of bacterial strains that is mentioned in the legend of figure 1. Next, we selected only those strains that were positive for multiple plant growth promoting traits. That is why all 10 strains were positive for different traits. Negative strains were not used as we do not have bacterial isolates that are deficient for all beneficial attributes.

6). Line 277: All figures are now included in the text including the supplemental files. Figures with high resolution have been provided with the revised manuscript. As mentioned above, we selected only 10 strains with positive plant growth promoting traits from several bacterial isolates. Moreover, *E. coli* has not been used in present study as PGPR. We used two strains of *Enterobacter aerogenes* that were reported in number of studies/ in literature as PGPR.

RESPONSE TO "REVIEWER 3" COMMENTS

We are very thankful for the valuable comments of worthy reviewer to improve the text of this article. Point to point response to comments is given below.

Basic reporting

1). PGPR may show variable interaction with inoculated plants that may depend upon bacterial species or plant used in the experiment. Sometime, PGPR may show inconsistencies in results but in majority of the cases inoculated plants recorded promising growth response. In present study, we have used 'Microphenotron' to screen our potential PGPR strains for further application. Moreover, line 336 has been modified to remove the confusion for the reader.

2). Introduction has been modified as per reviewer's suggestion. The use of 'Microphenotron' has been justified in a dedicated paragraph and supported with relevant references. Results of 'Microphenotron' and pot experiments have been compared in the discussion section.

3). Line 26: Abstract and introduction has been started with the background of 'Microphenotron'. Line 26-31 have been modified in abstract and lines 56-64 have been incorporated in introduction as suggestion by the reviewer.

4). Line 29: "would allow" replaced with "allows".

5). Line 30: "were able to degrade" has been replaced with "degraded."

6). Line 58: Here we are talking about the application of 'Microphenotron' by using *Arabidopsis thaliana* as a model system. Now sentences have been modified according to reviewer's comments.

7). Line 72: Major sections of the introduction has been reorganized. Moreover, "species" has been replaced with "strains". Similarly, "have been reported to" is deleted in the revised article and Lines 72-75 have been moved at the start of the first paragraph.

8). Line 76: Fillers have been deleted as per reviewer's suggestion. Also "have been considered as" is replaced with "is".

9). Lines 89-99 have been revised and words/ phrases like "that would" and "under specified…effect" have been deleted.

10). Line 93: Correction of reference with name "Forde" has been made throughout the manuscript.

11). Lines 96-99 have been deleted.

12). Line 104: Location and mode for soil sampling is provided in the revised article in lines 117-118.

12). Line 135: "Indole" is not capitalized and converted to "indole".

13). Line 148: Name of City and State for USA locations are now given in the methodology.

14). Line 169: The name of spectrophotometer is now given in the section for auxin quantification as suggested by the worthy reviewer.

15). Word "sterilizes" replaced with "sterilized".

16). Line 210: Soil mixture ratio revised to 6:1:1.

17). Line 235 and 240 have been moved to methods.

18). Line 246: Start of results for auxin production has been modified and lines 246-248 have been deleted. Similarly, lines 269-271 have also been modified in result section.

19). Line 282: Figure 6 showed the effect of bacterial strains on the root proliferation in the inoculated plants. Line has been modified accordingly.

20). Line 322: “showed stimulatory effects on rosette growth biomass” replaced with “stimulated rosette growth”.

21). Line 328: Words changed to “controls”.

22). Line 363: Word “parameters” has been deleted.

23). For figure 5 (now figure 7), bar charts have been converted to Box and Whisker plots.

Experimental design

In all tables for pot trials, water treated plants were used as control. Indeed, majority of the bacterial strains recorded promising growth for vegetative parameters over water treated controls. For negative control, bacterial strain was not used as we do not have culture of bacteria that may be deficient for number of plant growth promoting traits.

Validity of the findings

Reviewer’s concern with reference to number of replicates is now clarified in the revised article. For ‘Microphenotron’ bioassay single lane of eight PCR tubes or wells was placed for each strain or control in three replication and experiment was repeated three times. Overall, 72 PCR tubes were used for single strain or B5 media (control). Similarly, for pot trials, experiment was also repeated three times with overall 27 seedlings inoculated for each strains or control. Although, more replicates may be included to increase the authenticity of the experiments. Overall, currently used number of replicates may be sufficient to predict the outcome of the results. Moreover, our results indicated that strains that performed well in ‘Microphenotron’ bioassay also showed good results in pot trials for wild-type *A. thaliana.*