

Response Letter

Reviewer 2:

Comments and Suggestions for Authors: *Bacillus velezensis* FZB42 is the model strain for Gram-positive plant-growth-promoting and biocontrol rhizobacteria, which has been the subject of more than 100 published articles in the last 20 years. The current manuscript is devoted to further previously unknown aspects of this bacterium. The results obtained are very interesting. The authors determined, among others, the role of *sprT* in biofilm formation, motility, 'endospore development', and antibiotic production of *B. velezensis* FZB42. Manuscript should be published in IJMS after making some additions.

Response: Thanks a lot for the constructive comments, to which we have responded one by one as shown below.

Comments 1: Line 50 sporulation - it should be explained what process exactly is involved and what does it matter whether they are produced in greater or lesser numbers (e.g. depending on *sprT*). Most bacteria do not produce spores like fungi, which can be referred to as sporulation. In *Bacillus*, these are probably endospores, which have a different character than typical spores in fungi.

Response 1: Yes, sporulation is a strategy *Bacillus* uses to survive harsh conditions, which results in the production of endospores that are highly resistant to heat. In this article, we have discussed some differences between bacterial and fungal spores. As the mechanism involved in the sporulation process is rather complex and the present article does not primarily focus on spores, we have not elaborated on the specific process of sporulation.

Comments 2: Line 49-50 The production of these antibiotics in *B. velezensis* FZB42 is well connected with other phenotypes such as biofilm formation, motility, and sporulation – relevant literature should be cited.

Response 2: We have added a reference on line 50.

Comments 3: Line 140, Line 142 it should be *P. sojiae*.

Response 3: The error has been corrected.

Comments 4: Line 167 it should be *Bacillus spp.* (spp. not italic, and one dot).

Response 4: The error has been corrected.

Comments 5: Line 214 Chapter 4.1 It is necessary to provide the origin of the *Staphylococcus aureus* and *Phytophthora sojae* strains. How were they identified?

Response 5: *Staphylococcus aureus* 9144 was obtained from the Guangdong Microbial Culture Collection Center (GDMCC). *Phytophthora sojae* was obtained from Institute of Forest Protection of Nanjing Forestry University They are identified based on colony morphology, with *Staphylococcus aureus* 9144 appearing as a pale yellow color, *phytophthora sojae* has obvious mycelia.

Comments 6: Figure 2C requires further detailed explanation.

Response 6: Thanks for this suggestion. Information on the Figure 2C has been added in the 2.2 part.

Comments 7: Figure 4A requires further explanation (where is *S. aureus* and where is *P. sojae*) so that there is no need to guess

Response 7: Thank you for this suggestion. The location information for *S. aureus* and where is *P. sojae* has been labeled in Figure 4A.

Comments 8: Line 142-143 Antagonism radius – it should be defined in Materials & Methods.

Response 8: Revised as suggested(4.8).

Comments 9: Chapter 4.4 The methodology for biofilm formation is not complete. Further procedure should be provided. The same applies to chapters 4.6 and 4.7. There are also no details regarding inhibition zone measurements (Chapter 4.8). Much of the data that should be included in the methodology is found in Results.

Response 9: Revised as suggested(4.4, 4.6, 4.7, and 4.8). We filled in the experimental details.

Comments 10: Line 252 Sporulation test - the methodology must be completed, it is as if there was no completion of how to proceed to determine the sporulation.

Response 10: Many thanks for these comments. In the Sporulation assay, we took the bacterial suspension of FZB42 and *sprT* knockout strains in the late growth phase (24h), and subjected them to heat treatment at 85°C. Generally, bacteria would be killed and lose their ability to grow under such high temperature. However, *Bacillus* can produce spores and has a high-temperature tolerance characteristic (we have added this information in introduction). Therefore, it still retains the ability to grow. Here, we used the dilution method with concentration gradients, followed by spot plating, to measure the Sporulation ability of FZB42 and *sprT* knockout strains at different concentrations.

Comments 11: Line 347 the authors should be corrected in accordance with the requirements of the Editorial Office.

Response 11: Revised as suggested.