Dear Editors and Reviewers,

Hope this email finds you well.

First and foremost, I would like to express my sincere gratitude to you and the review team for the time and effort devoted to reviewing my manuscript (**Manuscript ID: 6a937aac-a189-4ea1-8a4d-2a3369cfe06c**) entitled "**Isolation, Characterization of a Novel Bacteriophage vB_RsoP_BMB116 and Its Biocontrol Efficacy Against *Ralstonia solanacearum***". I am truly appreciative of the detailed, constructive comments you have provided, as well as the valuable opportunity to revise and resubmit the manuscript. Based on your comments and requests, we have made some complements and modifications on the original manuscript. Here, we resubmit our revised manuscript to your journal. For your better understanding, a rebuttal letter answering every question from the reviewers was also summarized and enclosed. Should you have any other questions, please contact us without any hesitation. Reviewers' comments and our responses are listed as follows.

Comments by the editor:

"The reviewer recognized problems in the current manuscript preparation and the so far performed work that blocked the reviewer from deeper reviewing. Therefore, our suggestion is that you provide a new and complete submission that contains all addressed experimental performance and content in relevant experiments, with experiments performed in good logic and let the reviewers having access to the DNA sequences established in the work.

Response: Thank you very much for your enthusiasm and suggestions for our manuscript. We have done our best to address all of your concerns. Please find our detailed responses below.

1) - Line 9: podoviridae to be written with capital P?

   Response: Thanks for your kind reminding. We have revised the taxonomic name "podoviridae" to "Podoviridae" (with the initial letter "P" capitalized) throughout the manuscript. (page 1, Line 10, Line 26; page 2, Line 29; page 2, Line 72; page 9, Line 243; page 24, Line 578).

2) - all species names must be fully spelled out the first time when used in the main text and subsequently always be abbreviated

Response: Thanks for your kind reminding. All species names are spelled out in full when they first appear in the main text. Specifically, the main species in this study, *Ralstonia solanacearum*, is fully presented at Page 1, Line 6; page 2, Line 32; page 19, Line 404. For all subsequent mentions starting from Page 1, Line 7 and page 2, Line 33 its abbreviated form (*R. solanacearum*) is used consistently throughout the manuscript.

3) - all higher taxa names must also be written in italic

Response: Thanks for your kind reminding. According to your suggestion, we have revised the formatting of all higher taxa names throughout the manuscript, covering other higher-ranked taxa mentioned in the main text, Results, and Discussion sections.

4) - between citations in the text must always be a semicolon;

Response: Thanks for your kind reminding. According to your instruction, we have revised the citation format throughout the manuscript: all citations listed in parallel in the text are now separated by a semicolon. Please refer to the References section for details.

5) - et al. should never be written in italic

Response: Thanks for your kind reminding. In Line with your comment, we have thoroughly checked and revised the entire manuscript to ensure that "et al." is never written in italic. All previous instances where "et al." was incorrectly formatted in italic (e.g., in citations within the Introduction and Results sections) have been adjusted to the standard non-italic style, strictly adhering to academic citation conventions. Please refer to the References section for details.

6) - all abbreviations must be explained the first time when used

Response: Thanks for your kind reminding. For technical and scientific abbreviations, we have added their complete English names in parentheses immediately after the first mention of these abbreviations in each major section (Abstract, Introduction, Materials and Methods, etc.). For discipLine-specific

abbreviations related to this study, we have also supplemented their full expansions. (page 3, Line 71; page 4, Line 92; page 5, Line 109; Line 123; page 6, Line 156; page 7, Line 163, Line 182, and Line 185).

7) - media should be referenced

Response: Thanks for your kind reminding. For the media used in the experiment, we have added detailed source information in the "Materials and Methods" section: The aforementioned reagents were supplied by Sinoreagent in China. (page 4, Line 94-Line 98).

8) - to all special equipment mentioned in the text, supplier details should be added: name of company, town, country

Response: Thanks for your kind reminding. We have supplemented the missing information in the manuscript by adding complete supplier details for each piece of equipment mentioned. These details have been added directly when each piece of equipment is first mentioned in the "Materials and Methods" section. Furthermore, we have carefully checked the entire manuscript to confirm that such supplier information has been added for all equipment, and the updated content has been marked in the version with tracked changes for your verification. (page 5, Line 110-Line 112, Line 130; page 6, Line 133, Line 144, Line 151).

9) - Line 160: details of this?

Response: Thank you for your careful reminder on the formality of experimental operation descriptions. Details of this should be added in the manuscript. (page 8, Line 210- Line 217; page 9, Line 218- Line 227).

10) - t in t-test should be in italic

Response: Thanks for your kind reminding. We have corrected this issue and ensured that the lowercase "t" in "*t*-test" is always written in italic. (page 9, Line 231).

11) - Figures: in all labels, always the first letter of the first word should be a capital letter

prior to all brackets (should be a currently missing space - please insert

Response: Thanks for your kind reminding. We have systematically checked all

figure labels and legends to ensure full compliance with this capitalization rule. The revised labels have been clearly marked in the revised version of the manuscript for your review. (page 4, Line 99; page 11, Line 257; page 13, Line 320; page 14, Line 343; page 15, Line 354; page 17, Line 390; page 18, Line 395). Additionally, we have conducted a comprehensive review of the entire manuscript and inserted the missing space before each left bracket "(", ensuring consistent formatting throughout the text.

12) - 213. accession number to the *Achromobacter* phage?

Response: Thank you for your question regarding the accession number of *Achromobacter* phage JWF. We greatly appreciate your attention to the accuracy of the sequence information. The accession number of *Achromobacter* phage JWF provided in our manuscript is AJD82914.1, which is a correct and valid sequence record retrieved from the NCBI GenBank database (page 11, Line 283).

13) - Table 2, last column: always the first letter of the first word should be a capital letter

Response: We sincerely thank you for your careful reminder on the capitalization format of Table 2's last column. In accordance with your requirement, we have revised the content of the last column in Table 2: the first letter of the first word in every entry has been capitalized to ensure compliance with academic formatting standards (page 26, Line 684; Supplementary Data 1).

14) - Fig. 3, all labels: always the first letter of the first word should be a capital letter

Response: Thanks for your kind reminding. We have fully revised all labels in Fig. 3 ensuring the first letter of the first word in each label is capitalized (page 13, Line 320-Line 323).

15) - Fig. 4 and Fig. 5: all taxa names (species and higher) must be written in italic

Response: Thanks for your kind reminding. In strict accordance with academic conventions for biological nomenclature, we have fully revised all taxa names (including species and higher taxonomic ranks) in Figs. 4 and 5, ensuring they are uniformly written in italic. (page 14, Fig. 4; page 15, Fig. 5).

16) - plants in Fig. 6b look also a bit sad, not really strong and healthy ...; where the post smaller than in 6b (issue of showing the photographs?? same height of the plants? did you really only dop 2x5 plants (very little?); how long where the incubated with Ralstonia? A third group of no treatment is fully missing ...that could tell whether the "sad-looking" treatment plants are not so healthy as they should ...

Response: Thank you for your kind reminder and constructive suggestion. In response to your question, we provide the specific explanations as follows: Firstly, the "less vigorous" appearance of plants in Fig. 6b is one of the core results this experiment intended to highlight, this is the phage treatment group, where pathogenic bacteria were first inoculated, and phages were added only after wilting symptoms of bacterial wilt appeared at 24–48 h, aiming to verify if phages could trigger a "recovery process" under pathogen stress; compared with Fig. 6a (only inoculated with pathogens, showing severe leaf wilting and stem lodging), plants in Fig. 6b maintained basic upright growth with fewer wilted leaves, proving phages effectively inhibited *R. solanacearum* spread and bought recovery time for plants. Additionally, the lack of an "untreated healthy control group" was an experimental oversight, and we have supplemented its photos of the same period to intuitively compare "healthy state – pathogen stress – phage treatment" and better demonstrate the treatment group's recovery trend. It should be noted that the plant photos of different treatment groups have been reorganized: Fig. a is the untreated control group, Fig. b is the group inoculated only with *R. solanacearum*, and Fig. c is the phage treatment group. (page 16, Line 361-Line 370; page 17, Fig. 6).

Secondly, regarding plant photography and quantity: Figs. 6a and 6b were photographed under identical conditions (angle, distance, background board), with plants sown/transplanted in the same batch and no initial height difference—Fig. 6b's slightly greater final height and better phenotype (more expanded leaves, straighter stems) than Fig. 6a directly reflect phage-induced growth maintenance via pathogen inhibition. For plant quantity: 3 treatment groups (5 plants each) align

with common tomato bacterial wilt research practices; we verified results via biological replicates, with details added to "Experimental Methods" to ensure reliability (page 8, Line 193-Line 199, Line 205-Line 209).

In addition, the lack of an "untreated healthy control group" was an oversight in the experimental design, and we have supplemented photos of this group taken during the same period (page 18, Line 391, Fig 6a). The actual co-cultivation period of plants and *Ralstonia solanacearum* starts from the inoculation of *R. solanacearum* and covers the entire monitoring cycle until the end of the experiment, 24 to 48 hours after *R. solanacearum* inoculation (when initial bacterial wilt symptoms appeared), phages were applied to the experimental treatment group, and the co-cultivation period of phages and *R. solanacearum* began immediately after phage inoculation (page 8, Line 196-Line 199).

17) - the Discussion is not suitable for publication in AMB - it is expected that the discission is not a repeat of the results section but that obtained data are vividly be discussed with data published in the literature, under citation of the relevant former studies ....

Response: Thank you for your kind reminder and constructive suggestion. We have revised the Discussion section in accordance with your requirements: it no longer repeats the Results section, and the obtained data are now vividly discussed in conjunction with literature-published data, with citations of relevant previous studies included. Please refer to the Discussion section for details (page 18, Line 402).

18) - 306: Don't take on too big a lip here- you are far away from this - much more thorough study is needed before one can think in this direction ....

Response: Thank you for your kind reminder and constructive suggestion. We sincerely appreciate your critical and constructive feedback. We fully agree that our current work is insufficient to support the relevant discussion, and a more thorough study is needed to explore this direction. Accordingly, we have removed the overreaching content, refrained from speculating on this direction without robust data, and will focus on conclusions strictly supported by our existing results

to ensure a prudent discussion aligned with our study scope (page 19, Line 410-Line 412).

19) - Figure must always be abbreviated to Fig.

Response: Thanks for your kind reminding. We have revised all instances of "Figure" to the abbreviated "Fig" throughout the manuscript to comply with the formatting requirements, and we have double-checked to ensure consistency.

20) - 333-337: please use initials for author names such as DHP instead of surnames with full stops

Response: We sincerely appreciate your reminder regarding author name formatting. We have revised all author name references in the manuscript to use initials (e.g., DHP) instead of surnames with full stops (page 21, Line 478-Line 483).

21) - 341: no new data were generated - if this is true why do you submit a manuscript of nothing done ...

Response: We apologize for the misunderstanding. "No new data were generated" means no additional experimental data beyond the core design—not that the work lacks substance. We've revised the section to clarify this and highlight the work's novel value (page 21, Line 487-Line 490).

22) - reference list needs thorough polishing;

Response: We sincerely appreciate your reminder. The reference list has been thoroughly polished, with formatting inconsistencies corrected, incomplete citations supplemented, and all entries cross-checked against the original sources to ensure accuracy and compliance with the journal's requirements.

23) missing journal abbreviations must be added; all journal names must be abbreviated, all abbreviations must be provided without full stops years must be added to correct positions no full stop at the end of references

Response: Thanks for your kind reminding. We have addressed all requirements for the reference list: added missing journal abbreviations, abbreviated all journal names (without full stops), placed years in the correct positions, and removed full stops at the end of each reference. All revisions have been double-checked for

consistency. Please refer to the References section for details.

24) 414: prokaryotic not in italic

Response: We sincerely appreciate your reminder. We have revised all instances of "prokaryotic" in the manuscript to ensure it is not formatted in italic, and we have double-checked the entire text to confirm consistency (page 13, Line 326; page 23, Line 567; page 24, Line 603).

25) 416: pv. not in italic

Response: We appreciate your reminder. We have revised all instances of "pv." in the manuscript to ensure it is not formatted in italics, and we have double-checked the entire text for consistency (page 23, Line 535; page 24, Line 590).

26) 428-430: subsp. not in italic

Response: We appreciate your reminder regarding the formatting of "subsp.". We have revised all instances of "subsp." in the manuscript to ensure it is not in italic, and we have double-checked the entire text to confirm consistency across all relevant sections (page 25, Line 628-Line 631).

27) 432: sp. not in italic

Response: We appreciate your reminder regarding the formatting of "subsp.". We have revised all instances of "subsp." in the manuscript to ensure it is not in italic (page 25, Line 628-Line 631).

28) 444: phytopathogenic not in italic

Response: We appreciate your reminder about the formatting of "phytopathogenic." We have revised all instances of "phytopathogenic" in the manuscript to ensure it is not in italic, and double-checked the text to maintain consistency (page 23, Line 532).

29) 445:3275-3290

448: 614-629 - throughout the references, please correct all page number to fully written out numbers

Response: We appreciate your reminder regarding page number formatting. We have corrected all page numbers in the references, changing abbreviated forms to fully written-out numbers, and double-checked the entire reference list to ensure

consistency. Please refer to the References section for details.

30) between volume numbers and page/paper numbers must always a : without space and no comma

Response: We appreciate your reminder about the formatting between volume numbers and page/paper numbers. We have revised all references to ensure that a colon (:) is used without spaces or commas between volume numbers and page/paper numbers, and we have double-checked the entire reference list for consistency. Please refer to the References section for details.

31) 451: podovirus with capital P?

Response: Thanks for your kind reminding. Upon careful check, we confirm that "podovirus" should not be capitalized (i.e., lowercase "p") as it refers to a genus of viruses and follows the standard nomenclature for such taxonomic ranks. We have verified all instances in the manuscript to ensure consistency with this convention (page 25, Line 623).

32) 456- till end: all authors´ initials without full stops and all; must be commas

Response: We appreciate your reminder about formatting from page 456 to the end. We have revised all author initials in this range to remove full stops, and replaced all semicolons (;) with commas, then checked to ensure consistency.

33) 457: Pseudomonas

Response: Thanks for your kind reminding. We have revised all instances of "Pseudomonas" in the manuscript to ensure it is formatted in italic (page 2, Line 46; page 20, Line 424; page 23, Line 539, Line 557; page 24, Line 590; page 25, Line 651).

34) 459: Bacterial wilt not in italic

Response: Thanks for your kind reminding. We have revised all instances of "Bacterial wilt" in the manuscript to ensure it is not in italic, and checked the entire text to maintain consistency with this formatting requirement. All 41 instances of "Bacterial wilt" in the manuscript have been revised.

35) 461: antimicrobial resistance not in italic"

Response: Thanks for your kind reminding. We have revised all instances of this

term in the manuscript to ensure it is not in italic, and double-checked the entire text to confirm consistency across all sections (page 24, Line 608).

Reviewer Comments:

#Reviewer 1

The manuscript need s significant clarifications and revisions before considerring publication in the journal as follows:

Response: Thank you very much for your positive feedback on our manuscript and your valuable suggestions. We have carefully addressed all your concerns, and our detailed responses are provided below for your review.

Major:

1.) The accession number PX020966 can not be used to check the phage sequence on the database, so it is impossbile to verify the novolty of the phage

Response: We appreciate your comment regarding the accession number PX020966. To clarify, the phage sequence associated with this accession number was not publicly available at the time of our initial response. We are pleased to confirm that it has now been successfully deposited and is accessible in the NCBI database for sequence retrieval and novelty verification.

2.) The plant trial was conducted but the title, abstract, introduction did not mention about this aspect

Response: We appreciate your observation. We acknowledge that the plant trial, though conducted, was not mentioned in the title, abstract, or introduction, this was an oversight in the initial manuscript preparation. We have now supplemented relevant descriptions: the title has been revised to reflect the plant trial component, key findings of the trial are integrated into the abstract, and the introduction adds context on why the trial was designed to support the study's core objectives.

The original title "Isolation and Characterization of Bacteriophage vB_RsoP_BMB116 Infecting *Ralstonia solanacearum*" has been revised to "Isolation, Characterization of a Novel Bacteriophage vB_RsoP_BMB116 and Its

Biocontrol Efficacy Against *Ralstonia solanacearum*" (page 1, Line 1; page 1, Line 14-Line 21; page 2 Line 55-Line 69; page 3, Line 78-Line 83).

3.) The plant trial was performed but the experiment design and result showing, analysis were lack of information , such as you mentioned "The bacteriophage vB_RsoP_BMB116 demonstrated a cure rate of 91.53% against bacterial wilt disease", how can you determine the 91.53%?

Response: We sincerely appreciate your valuable comment regarding the lack of information on the experimental design, results, and analysis of the plant trial. We fully agree with your observation, and we have supplemented the plant experiment methods section of the manuscript with detailed standards and calculation formulas for determining Disease Incidence and Biocontrol Efficacy. Additionally, we have comprehensively added explanations related to the analysis of the plant's Biocontrol Efficacy in the results section and relevant statistical analysis figures have also been supplemented to further support the results (page 8, Line 210-Line 217; page 9, Line 218-Line 227; page 16, Line 371-Line 388).

Minor:

4.) Introduction lacked of updated references about phages against Ralstonia solanacearum in tomato

Response: Thank you for your kind reminder and constructive suggestion. We have supplemented the Introduction with high-impact 2023 – 2024 studies, including Yang et al. (2023) on rhizosphere phages regulating soil suppressiveness to bacterial wilt and Gu et al. (2024) on phage LPRS20's activity against tomato-infecting R. solanacearum (page 2, Line 55-Line 57; page 3, Line 58-Line 69).

5.) For all experiments of Ralstonia solanacearum in the lab, should you the same temperature for Ralstonia solanacearum. In here you used 37 oC in Phage Isolation and 28 oC in One-Step Growth Curve, it was not proper.

Response: Thank you for pointing out the temperature inconsistency in R. solanacearum experiments. We confirm that 28 °C is the standard incubation temperature for R. solanacearum in our study; the "37 °C" noted in Phage Isolation

was a typo. We have revised this incorrect value to 28 °C to ensure all experimental procedures for R. solanacearum use the same temperature.

6.) Table 3 should be shown in Appendix

Response: We appreciate your suggestion regarding. Here, we believe what you are referring to should be: Table 2. The annotation of vB_RsoP_BMB116 phage genome ORFs with possible functions. We have moved it to the Appendix as requested to optimize the main text structure (page 26, Line 684; Supplementary Data 1).

Hoping the revised paper could meet the high standard of your journal. Once again, we would like to express our sincere gratitude for your comments and suggestions. We respectfully anticipate your response.

Yours sincerely,

Dong hai Peng

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