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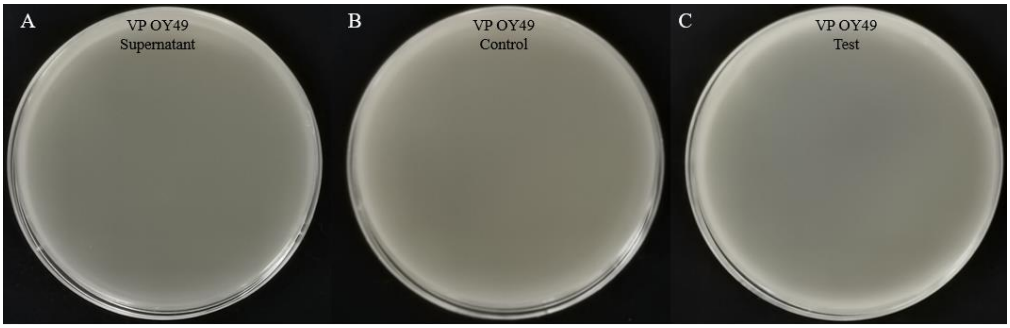
Response to reviewer

Major comments / points for correction are listed below:

Point 1: Since the authors claimed that the phage GHSM17 is lytic phage, do authors have any experiments showing that the phage does not enter lysogenic cycle? Even though the phage genome does not contain any lysogenic-related genes, it might be possible that some of them might not be predicted during the annotation. I would suggest the author to perform some wet experiments to support their claim.

Response 1: We thank the reviewer for this practical suggestion. We verified that phage GHSM17 was not a lysogenic phage by the following wet experiments:

The phage and host bacteria were mixed and cultured for 12 h. The pellets were centrifuged, washed 3 times and then inoculated into the medium, one of which was added with mitomycin C to a final concentration of 0.5 μg/mL (experimental group), and the other was added with an equal amount of PBS (control group). The mixture was incubated at 37 °C, 220 r/min overnight. The next day, two groups of cultures were centrifuged, and an appropriate amount of the supernatant was mixed with the host bacteria to make a double-layer plate for verification. If a large number of plaques appeared on the double-layer plates in experimental group and no plaques were found in the control group, it proved that the phage was lysogenic. In our experiment, no plaques appeared in both the experimental and the control groups, and it indicated that the strain was not a lysogenic phage. The data was presented as Supplementary Figure S1.



A: The supernatant of the bacterial pellet washed for the third time

B: Bacterial supernatant induced by mitomycin C

C: Bacterial supernatant added with PBS

Point 2: It seems to me that the re-growth of SC123 occurs after 4 hours; however, the cell number as measured by OD does not much increase after that. The authors then claimed that SC123 was still suppressed by the phage. I would suggest that the author should try doing the experiment at the longer period to see if the cell would re-grow or even measure the number of viable cells at the end. This will help illustrating whether the OD that was measured are from the cell debris or live cells and it will make your claim much stronger. (It would be also nice to explain why the authors included SC123 into the experiment.)

Response 2: We performed phage-inhibition experiments on *V. parahaemolyticus* over a longer period of 20 hours and observed that the bacteria can re-grow over time. The number of viable cells was measured at the end, indicating the OD measured are from the live cells. The new results description has replaced the original in the manuscript (lines 276-286).

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VP SC123 is a bacterium isolated from scallops. In the next experiment, we will verify the application of phage in the control of *Vibrio parahaemolyticus* in shellfish such as scallops and oysters, so VP SC123 was included in the scope of the experiment.

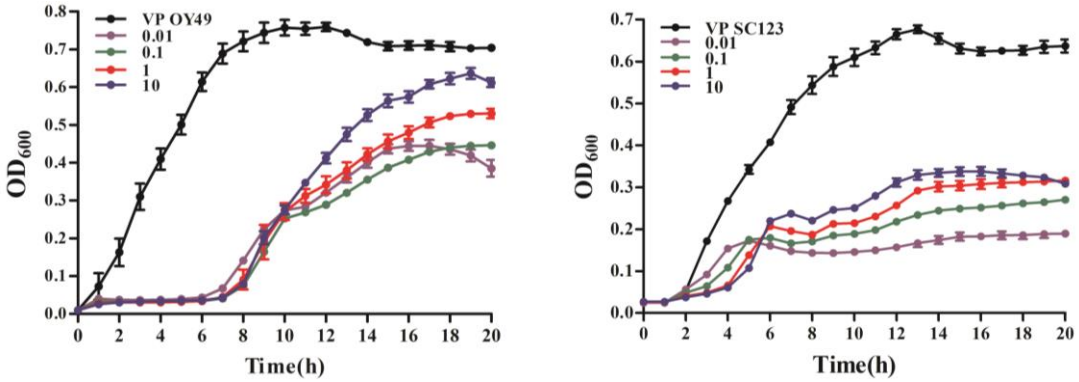


Figure 4. In *vitro* lysis of vB_VpaP_GHSM17 against (A) *V. parahaemolyticus* strain OY49 (B) *V. parahaemolyticus* strain SC123 at multiplicity of infections (MOI) 0.01, 0.1, 1 and 10 for 20 h.

Table 1 The number of viable cells of VP OY49 at 20 h.

Viable cells	0.01	0.1	1	10	VP OY49
1	1.18×10^8	1.85×10^8	2.24×10^8	1.43×10^8	5.8×10^8
2	1.58×10^8	2.16×10^8	2.33×10^8	2.30×10^8	7.7×10^8
3	1.74×10^8	2.14×10^8	2.30×10^8	3.07×10^8	6.7×10^8

Table 2 The number of viable cells of VP SC123 at 20 h.

Viable cells	0.01	0.1	1	10	VP SC123
1	1.58×10^6	2.29×10^6	9.7×10^6	8.7×10^6	1.39×10^8
2	1.74×10^6	2.76×10^6	1.07×10^7	9.3×10^6	1.19×10^8
3	1.83×10^6	2.98×10^6	9.4×10^6	9.3×10^6	1.43×10^8

Point 3: In the genome comparison, KF2 showed the highest ANI to the phage GHSM17. The author should indicate the number of their identity. This might be informative to show if the phage GHSM17 is a novel phage (For your information, based on ICTV taxonomy, genome identity more than 95% are classified as the same species).

Response 3: We thank the reviewer for this helpful suggestion. We have indicated the identity of 94.82% between phage KF2 and GHSM17 in the text, which implied that the above two phages were not the same species. The results of the genome identity between the phage GHSM17 and all phages of *Maculvirus* were shown in Supplementary Table S2, while all genome identity (Percentage Identity, P.I.) were less than 95%. The above results indicated that phage GHSM17 was a novel species belonging to the genus *Maculvirus* (lines 328-336).

Point 4: The author should be aware of indicating the number of host range since this number might be very varied as the hosts are used. I would suggest the author to use text saying

something "relatively broad host range" instead of using number.

Response 4: We agree with you. The number of host ranges in the manuscript has been indicated. The host range in the manuscript has been revised from figures to "relatively broad host range" (lines 237-238, lines 375-376).

Minor comments / points for correction are listed below:

Point 1: Do the authors have "n" number for the measurement made from the TEM image? Please indicate "n" number and add standard deviation.

Response 1: We have "n=2" for the measurement made from the TEM image and indicated "n" number and added standard deviation (lines 228).

Point 2: Italicized the scientific name

Response 2: We have carefully checked and italicized the scientific name.

Point 3: change "terile" to "sterile"

Response 3: We have changed "terile" to "sterile" (lines 93).

Point 4: Please double check the writing in "Material and Methods"

Response 4: We have carefully checked the writing in "Material and Methods" and made improvements accordingly.

Point 5: Figure 1A: Please add scale bar and add magnified image of the plaque

Response 5: We have added scale bar and add magnified image of the plaque.

Point 6: Figure 2B: the one step growth curve seems strange to me. Could you explain why the graph does not appear sigmoidal as it should when the phages are released from the host cell?

Response 6: The graph shows a relatively flat sigmoidal curve, when the phage is released from the host cell.

Point 7: Figure 3C: Please add standard deviation

Response 7: We have added standard deviation in Figure 3C.

Point 8: Line 284: please add a comma

Response 8: We have added a comma.

Point 9: No references in text to supplemental figure S2

Response 9: The supplemental table S2 has been cited in text (lines 330-332).

Point 10: Line 294-295 is not explained in the figure.

Response: We have made corresponding corrections in the article (lines 307).

Point 11: Line 311: it would be nice to illustrate the 7 clusters in the figure.

Response: The 7 clusters in the figure have been illustrated (lines 323-325).

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