

In the manuscript titled '*Sulfolobus* Spindle-Shaped Virus 1 Growth Kinetics', the author describes a series of experiments aimed at determining the adsorption kinetics of *Sulfolobus* Spindle-Shaped Virus 1 (SSV1), in order to understand the very first step of the viral cycle of SSV1: the binding of the viral particles to *Sulfolobus solfataricus*. The determination of the adsorption kinetics would allow the design of experiments to address the growth kinetics of this virus, and get a better understanding of how this virus operates in nature.

The experiments described in the manuscript are based in measuring viral titers at different times post infection. The approach followed is valid for this purpose; unfortunately, the results did not allow the author to draw conclusions about the adsorption of the virus.

The main concerns of this reviewer regarding the manuscript are:

- The fact that cell debris appeared with uninfected cultures (Table 2), as well as the necessity to show diluted preparations in Figure 1B –where there shouldn't be any virus– suggest that there is a contamination that could be altering the results of the experiments.
- The main results are shown in Figure 2, where a time-based curve of the viral titer upon infection can be observed, and this curve remains uninterpreted in the text. There is a decline in virus titer that would be expected upon virus adsorption, but there is no explanation for the increases in viral titer between points 0 and 0.5 and between 1.5 and 2.
- The errors shown in Table 1 for trials 1 and 2 are greater than the values themselves. The author should find a way to measure virus titer in triplicate without having such a dispersion, like choosing a single dilution from which the virus can be titered.
- The negative control shown in Table 2 should have equal or similar virus titers, unless the viruses are degraded with time, but then, why there is no virus titer at time 0.5?
- For the assays to give an idea of the adsorption kinetics, they need to rule out the possibility that the following steps of the viral cycle may occur, thus, giving some information about the replication times for the virus, if known, would be useful to the reader.

In addition, there are some minor corrections that need to be revised:

- There is an overall inconsistency in the references' format in the main text and in the references list at the end of the manuscript.

Abstract:

- I suggest writing "viral particles" instead of "virus-like particles" to avoid misinterpretations about the infectivity of these entities.

- *Fuselloviridae* is a latin term that should go in italics.

Introduction:

- In the first paragraph, I suggest writing "Prokaryotic-infecting viruses" instead of "prokaryotic viruses", since the terms "prokaryotic" and "eukaryotic" are limited to cellular organisms.
- At the beginning of the second sentence in the first paragraph, "They" should allude to viruses in general, and not to prokaryotic-infecting viruses, thus, "they" should be substituted by "viruses".
- In the second sentence of the second paragraph, a comma (,) can be introduced after Archaea to ease the reading of this phrase.
- In the third paragraph, there is a typo in "hyperthmophilic". Please change to "hyperthermophilic".

Methods:

- In the first paragraph, the term S441, used in the next section, should be introduced.
- In the second paragraph ("SSV1 production and stably-infected S441 cells"), the centrifuge force should be written as "6,000 X g", instead of "6,000 g".
- In the fifth sentence of the second paragraph ("SSV1 production and stably-infected S441 cells"), I suggest to introduce a comma (,) between "equal volume" and "and viral titer".
- In the last sentence of the second paragraph ("SSV1 production and stably-infected S441 cells"), I suggest writing "plaque assays done with cell-free supernants" instead of " plaque assays done on cell-free supernants". The latter could lead to misinterpretations, since the plaque assays are done on cells.
- At the end of the last paragraph of Methods ("Plaque Assays"), the temperature at which the soft-layers are incubated for 48 hours should be indicated.
- In the virus titer formula at the end of the Methods section, "PFU observed" should be substituted by "plaques observed" (the PFUs are not observable), and it should read "Dilution factor" instead of just "Dilution".

Results and Discussion

- As the second sentence of the paragraph describes the method mentioned in the first sentence, an introductory word such as "briefly, ..." can be introduced for clarification.

- In the fourth sentence of the first paragraph "The infected SSV1 cultures and an uninfected control...", the "an" can be substituted by "the", since the uninfected control has been introduced in the previous sentence.
- The seventh sentence of the first paragraph "In stably-infected cultures several hours the titer significantly decreased over similar time ranges" should be revised and eventually rewritten in a more clear way.
- The eleventh sentence of the first paragraph says: "Similar results were obtained for infected cultures, in which dramatic decrease in titer were observed after 30 minutes (Figure 1A)", but this can be observed in Figure 2 and not in Figure 1A.
- In the twelfth paragraph on the first paragraph, the author says that "ideally, it was expected that for infected cultures viral titer would increase after incubation for longer than 1 hour". According to my understanding, the adsorption of the virus would produce a decrease in viral titer in the supernatant (those virus particles would be pelleted together with the cells) and not an increase, as the author indicates. This point should be clarified.

Figures and tables

- The numbering format of the Tables and Figures is inconsistent.
- In Figure 1A, the dilutions of the plates should be the same to ease the comparison between them.
- In Figure 1B, dilutions should not be made, since no plaques should be detected in an uninfected sample.
- Rewrite Figure 1 caption to "Plaque assay results for infected S441 with SSV1 immediately after mixing with SSV1 (10^{-1} dilution), after 1 hour of incubation (10^{-2} dilution), and after 2 hours of incubation (10^{-1} dilution), respectively".
- The scale in Figure 2 should be revised: there is an enormous jump from 0.00E+00 to 2.00E+04, and negative values don't make sense when talking about virus titer. My suggestion is to use a logarithmic scale from 0 to $1\text{E}+06$ and to make time point 0 match the Y-axis.
- In the Figure 2 caption, the points are referred as circles, but they are more like points or diamonds.
- In the last sentence of Figure 2 caption, the nature of the two independent cultures should be clarified: does it refer to the cultures from the kinetics or from the plaque assays?
- It is not clear whether Table 2 and Figure 2 are redundant or not, because the titers don't quite match. If they are redundant, one of them should go.
- If the viral titer was measured in triplicate, as mentioned in the caption, errors should be shown.

- In caption of Table 2, I would suggest to change "plaque assay morphology [...] determined" to "plate aspect [...] observed".

Conclusion

- Comma after "Clearly".
- There is no need to use acronyms like "OSGC" for the first time at the very end of a manuscript, since this is not going to be used again. I would suggest changing it to "one-step growth curve".