Review 2

This manuscript is a review of current advances in gene editing tools available for species of Streptomyces. In particular, the content focuses most heavily on CRISPR-related editing systems. The manuscript is well organized, easy to read, and highlights the key developments that, at least in my opinion, are needed to bring the reader up to date in the field. The topic is significant and of great interest to a large community of researchers working on Actinomycetes and in particular, the natural products produced by these bacteria.

Reply: Thanks for your accurate summary of our manuscript!

General comments:

Apparently there are two figures and a table that accompany this review, but those were not available to me and have not been reviewed here. I think the figures would be very helpful in delineating some of the key differences between CRISPR systems in use for Streptomyces.

Reply: Actually, two figures and a table were included in the submitted manuscript. In Figure 1, we briefly illustrated the development course of genome editing technologies in Streptomyces; In Figure 2, we gave a brief illustration of the CRISPR/Cas system-based technologies for genome editing in Streptomyces, which is helpful in delineating the key differences between CRISPR systems used in Streptomyces.

The authors refer to a toolbox and to the different plasmid constructs as useful tools for the field. In addition to a listing in the Table, as indicated by the text, it would be extremely helpful to the community to have information about how and where the different tools can be obtained, i.e. are the available by request from individual laboratories, available through plasmid repositories (as is the case for the pCrispomyces), etc.

Reply: We have added these information according to your suggestion! Please see them in Table 1. Thanks!

A recently published article would be good to include in this review, because it incorporates some new plasmid constructions. The article reference is: Culp et al., Nature Biotechnology, Vol 37: 1149-1154, 2019.

Reply: We have included this article in the revised manuscript. Please see the description from Line 185-193. Thanks!

Other comments:

1- Lines 70-78. Label the four steps 1,2,3,4 – the current text doesn’t demarcate the steps well.

Reply: We have revised them according to your comment!
2- line 85. “independent used” needs to be corrected
   Reply: We have revised it. Thanks!

3- line 98. ‘are often difficult to occur’ grammar needs correction
   Reply: We have replaced “occur” by “be obtained”. Thanks!

4- lines 145-146. It is unclear from the text what is meant by “deletions of double genes.”
   I think it would help to be more explicit – deletion of two genes or BGCs within a target
   genome, or something like that.
   Reply: We have revised it to “...simultaneous deletion of two genes (actII-orf4 and
   redD) and two BGCs (21.3 and 31.6 kb)”. Thanks!

5- line 198. The ‘genome of Streptomycetes’ What species do you mean or do you mean
   genomes in general?
   Reply: To avoid confusion, we rephrased it to “The genomes of Streptomyces strains
   contain....”.

   Reply: We have revised it. Thanks!

7- lines 313-315. Describe the reasons why Cas9 is toxic above, where it is first
   mentioned (lines 229-231.
   Reply: We have described the reason for the toxicity of Cas9. (Lines 184-186) Thanks!