Comments to the Authors

Manuscript: Addressing the challenges of symbiont-mediated RNAi in aphids

General comments:

In this study, Elston et al., test a new approach, symbiont-mediated RNAi (smRNAi), for its use in aphids. The authors engineered a native aphid symbiont *Serratia symbiotica* to produce dsRNAs that target different aphid genes. However, using this method, the authors could not generate consistent results, therefore concluding that smRNAi was not a reliable method for aphid gene knockdown.

As agreeing to the authors' discussion on varies ways to optimize this method, I think they have reached their "unreliable" conclusion too early. I think this smRNAi is a promising RNAi method with high potential in terms of testing gene functions, as well as applying in the field for aphid control. I have a few general comments and suggestions for the authors, and hopefully will help them to improve their assays:

First of all, there are many missing information/gaps in the successfulness of smRNAi itself: For examples, what are the abundance of engineered *Serratia* in infected aphids? Can the author achieve a more or less consistent abundance of engineered *Serratia* in infected aphids? Where those *Serratia* localized and are they always infect the same location after infection? The authors have tested the presence of dsRNAs in the infected aphids, using whole aphids (includes the engineered *Serratia*), this does not prove the transfer of dsRNA from *Serratia* to the result of aphid body. Is there any evidence of this release? The mechanism of this release, active or passive, is also crucial in terms of RNAi efficiency. If there is evidence for this release, how far those dsRNA can reach in the aphid body from the location of *Serratia*? I think before they confidently conclude, those questions about this technique need to be answered.

Second, I have some suggestions for the authors in regard their aphid bioassays:

For all their aphid bioassays, the author only used two trails with 45-60 aphids or even fewer. The population is too small to generate a conclusive result. If I were the author, I would take each of their trail as a single data point, for each experiment, I would set up 3-5 of those trails in each treatment as replicates, and then repeat this experiment for at least three times. I know it is a lot of work, but it is better way to see the trends in effects.

For selecting of targets, the authors based on the results of previous studies and chose the targets that generate the most prominent effects in others hand, but another crucial factor in selecting target genes is depended on the delivery method for RNAi agents. For example, C002 might not be the best target to test using smRNAi. C002 is a salivary effector, and based on the location of *Serratia* after infection, the dsRNA may not be effectively transfer to the tissues that expressed C002, e.g. salary gland. Maybe genes specific to gut are better targets, as in some of the trails the author even saw effects on knocking down the gut specific Nuc1.

For detecting gene expression in qPCR, the authors used whole aphid tissue. This could bias their results in multiple ways. One is that if the target gene is tissue-specific, the qPCR may not detect the significant changes in that specific tissue by using the whole body. Two

is that when gene expression changed by RNAi in one tissue, their expression maybe complemented by other tissue thereby veil the RNAi effects. To improve this, if it is known, I would suggest the author to dissect the specific tissues such as gut/salivary gland, for example C002, to measure the gene expression level.

All that said, I think if the authors could figure out those questions to optimize the smRNAi, and improve their bioassays to make their observation more conclusive. I would expect to see the smRNAi as a significant novel method in aphid. However, obviously it will require much more work to optimize. At this stage, the overall writing and data presentation are clear, and the discussions covered most of the concerns about this method. The manuscript could be a good start for people to discuss about the challenges in current available methods in aphid RNAi. But it will be unfortunate if the authors stop trying on optimizing this smRNAi method.