Review of: "Host-associated rhizobia fitness: Dependence on nitrogen, density, community complexity, and legume genotype"

Xia Li[1]

1 Huazhong Agricultural University

Potential competing interests: The author(s) declared that no potential competing interests exist.

Legumes-rhzobia interaction and symbiotic nitrogen fixation have drawn much attention. However, the majority of the researches focus on single host and/or single rhizobial strain. Since host-rhizobia interaction occurs naturally and the external environment must have an impact on the interaction, however, the related researches are quite limited. In this work, the authors evaluated the effects of multiple factors including host genotype, nitrogen availability, rhizobial density, and community complexity on 68 rhizobia strains selection in the *Ensifer meliloti-Medicago truncatula* mutualism. They found that host genotype and rhizobial density have greater effects on strain fitness and nodule phenotypes, but the effect of inoculation density and inoculum density on plant nodule phenotypes was host-specific. In addition, they found that the relative strain rankings were stable across increasing community complexity. These results identified key factors influencing rhizobia-legume symbiosis success in agro-ecological conditions, and highlight the importance of multi-level approach in enhancing rhizobia-legumes interaction and nitrogen fixation efficiency.

Major comments

1. From their results, it is apparent that host genotype has a strong effect on infection rate and nodule development, which reflects in nodule number and size. A17 nodules harbored less diverse strain communities, but I do not see the evidence for that the these strains are more beneficial. What is the definition of “beneficial”? beneficial for host or for strain? Normally, these many but small nodules are not good for host, instead, good for rhizobia. Unless, the authors show that the nitrogen fixation efficiency and fixed nitrogen of A17 nodules per plant are higher than R108. Also, to conclude this, more genotypes should be evaluated.
2. The authors concluded that nitrogen has a weak effect on strain composition, diversity and benefit. Based on what they described, they either used N-free fertilizer or 3 mM KNO3 fertilizer once a week when inoculated the seedlings with rhizobia. I think that the levels of nitrogen are not enough. I suggest that at least zero, low, normal, and high levels of nitrogen were compared to make conclusion. It will be even better if they can compare the effects of short- or long-term N treatments.
3. Similarly, they only compared two densities of rhizobia on the changes of community composition, strain diversity and the predicted benefit. It will provide us a better view on the effect of rhzobia densities if they show a density-response curve describing the relationship between rhizobia densities and other characteristics such as community composition,

strain diversity.

4. The result that relative fitness rankings are similar across communities of increasing complexity was very interesting, but they only showed that the relative ranking between rhizobia is not affected by other rhizobia. In fact, the absolute frequency of each rhizobia strain and the full community composition change a lot. I am not sure whether the conclusion that higher-order interactions between strains are rare in the context of host nodule formation and development is appropriate. Furthermore, the main obstacle of biological inoculants is the difference in competitiveness among strains, thus the impact of rhizobia community complexity on the relative ranking of strains is not significant to solve the obstacle of biological agents. In a complexity communities, you still can't predict which strain will dominate in nodule.
5. The discussion section should be reorganized or revised. I suggest the authors to discuss the key points following the order of results, that is, logically starting with main factors such as the effects of host genetic variations and rhizobia density on strain fitness and beneficial interactions.

Minors

1. Nitrogen treatments were not clearly described in the method section.
2. The types of citations in the manuscript should be consistent throughout the text.