Review of: "Trophic and symbiotic links between obligate-glacier water bears (Tardigrada) and cryoconite microorganisms"

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The study examined relationships between microorganisms and tardigrades inhabiting cryoconite holes on glacier surfaces. It compared the community structure of bacteria (16S rDNA), fungi (ITS1), and microeukaryotes (18S rDNA) associated with different host group (cryoconite, fully fed and starved tardigrades). High throughput sequencing of different marker genes was conducted for the same samples, and the total number of samples taken allowed the authors to identify various significant relationships between hosts and associated microorganisms. An important finding of this work is that some microbial taxa were potential commensals (typical of both fully fed and starved tardigrades), as well as a few taxa were potential food source for tardigrade (typical of both cryoconite and fully fed tardigrades). Common microbial taxa in all three host groups were also identified. These interesting results can serve as a basis for revealing more complex patterns of relationships and functional features of these microbial communities.

1. To identify synchronous changes in the structure of communities of bacteria, microeukaryotes, and fungi associated with different hosts, a coinertia analysis can be performed. The analysis (https://doi.org/10.18637/jss.v022.i04) will show among which communities there is a higher correlation and, accordingly, a closer relationship: bacteria with microeukaryotes, bacteria with fungi, or microeukaryotes with fungi.

2. The authors revealed the relationship between different microorganisms with each host by identifying common and specific taxa of microorganisms. However, it is also possible to identify relationships among individual taxa within each microbial group.

a. Clustering (https://doi.org/10.1038/ismej.2009.108) and correlation analysis with subsequent clustering of the correlation matrix (https://doi.org/10.1038/ismej.2011.119) are used for identify co-occurrence patterns and potential relationships between microorganisms in various ecosystems. These methods will reveal potential positive (mutualism) or negative relationships (competition, parasitism) between taxa within bacteria-bacteria, microeukaryote-microeukaryote, and fungi-fungi pairs.

b. It would be interesting to apply these methods to show relationships between bacteria, fungi and other microeukaryotes. However, in this case, one should carefully interpret the results obtained and compare them with literature data on the relationships between microorganisms in order not to draw erroneous conclusions. This should be done cautiously as it is still not clear how correct it is to detect correlations between reads of different genetic markers (for example, 16S rRNA, 18S rRNA, or ITS1). Yet such studies are ongoing (https://doi.org/10.1016/j.watres.2020.116020).

c. Based on the data of a correlation matrix, it is possible to build a co-occurrence network of different taxa. Analysis of the network will unveil its various features, as well as the keystone taxa of microorganisms in this network (https://doi.org/10.1038/nrmicro2832). Keystone taxa potentially play an important role in the community and can contribute to its sustainability (https://doi.org/10.1038/s41579-018-0024-1).

d. When using the correlation analysis, it is necessary to apply methods that take into account the compositional features of data on the structure of communities. It means that the relative abundance (ratios or proportions of marker genes of certain taxa) of microorganisms are considered rather than their absolute abundance. Alternatively, firstly it is better to eliminate compositionality, and then build correlations. Of course, more samples are needed in order to conduct a more exacting statistical analysis, but this can be taken into account in future studies.

3. The authors showed the relative abundance (shares) of taxa of bacteria, fungi, and other microeukaryotes, which were subdivided into commensals and food sources of tardigrades. The relative abundance of microeukaryotic taxa belonging to the functional groups of autotrophs, heterotrophs, and potential parasites were also presented.

a. It would be interesting to know how the proportions and composition of microeukaryotes associated with cryoconite and fully fed and starved tardigrades differ not only at the level of taxa, but also at the level of functional groups. Detection of changes in the proportions of functional groups can open up additional knowledge about trophic relationships in this niche.

b. To generalize and visually demonstrate the most significant findings, it would be quite useful to build a summary scheme that allow other researchers to better understand the results and will help create new hypotheses.

4. Although the work is self-sufficient in comparing the structure of microbial communities, there are a few notes:

a. The study lacks information about physico-chemical conditions in the sampling area: what the water temperature and pH in the cryoconite holes, and the range of air temperatures during the sampling period were. If this has not been measured, then cite literature data. Are there any data on the concentrations of the main nutrients (phosphates, nitrates, silicon) in the water of the cryoconite holes? The amount of nutrients is likely to depend on the composition of the cryoconite at the bottom of the cryoconite hole.

b. The results of the analysis of fungi (Fig. 4. Heatmap of fungi in different host groups) includes green algae (Trebouxiophyceae sp. 1, Trebouxiophyceae sp. 2, Raphidonema sp. 3). It would be more correct to remove algae when processing the data of fungi.