













## A HIGH DEGREE OF GENOMIC DIVERSITY MIGHT SUPPORT FLEXIBILITY IN INTERACTION PARTNERS

The 33 acl actinobacterial MAGs from our 6 mixed cultures can be assigned to 3 of the 13 recognized tribes, acl-A1, acl-B2, and acl-B4 (Fig. S1). Interestingly, even when our cultures harbored reduced community-level diversity, they all contained more than one acl genotype and even multiple discrete populations as defined by 97% average nucleotide identity (ANI). This reflects a high diversity at the population level of this common and dominant free-living freshwater bacterium (9).

As previous studies with complete genomes have calculated, the acl core genome is about 800 genes (4). Assuming around 1,600 genes per acl organism (21), this means that about half of the whole acl genome belongs to the flexible genome. This is similar to the proportion reported for the family of “*Candidatus Pelagibacter*” (22). Thus, both acl and “*Ca. Pelagibacter*” organisms have larger flexible genomes than photoautotrophic free-living aquatic bacteria like *Prochlorococcus* (8). Just as for the family of “*Ca. Pelagibacter*” (22), the high number of auxiliary genes in populations of streamlined acl genomes is likely to render populations of this free-living bacterial lineage functionally versatile. This versatility combined with auxotrophy creates a fascinating paradox: acl members can do many different things, but they cannot do any of them alone.

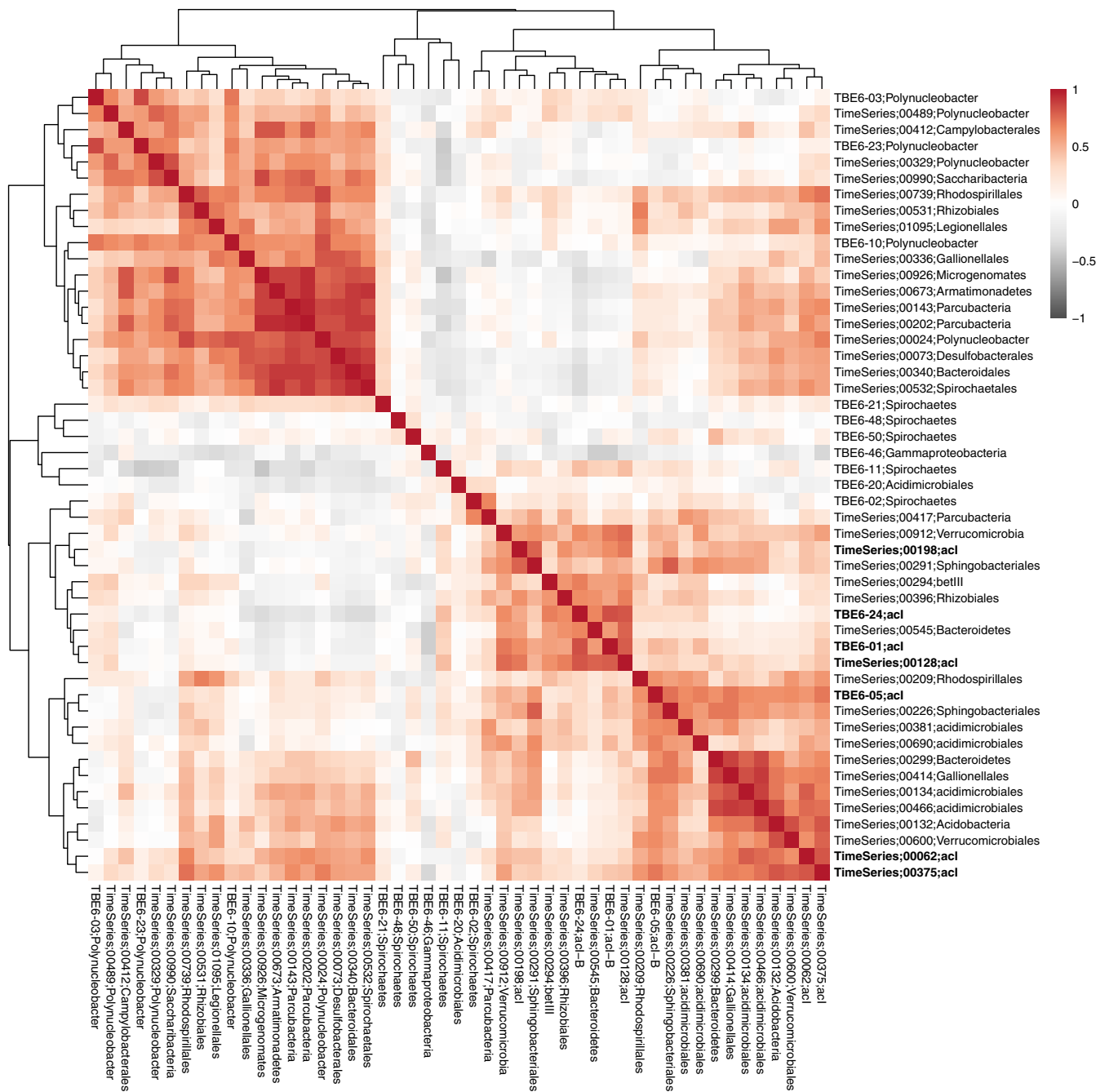
## SOME FREE-LIVING MICROORGANISMS FULFILL THEIR METABOLIC NEEDS FROM THOSE THAT THEY HAPPEN TO ENCOUNTER

By diluting and obtaining viable mixed cultures, we first obtained evidence that these plankton community partners represent diverse sets of community members. The variety of different phylotypes growing together with acl hints at a nonspecific metabolic dependence of acl actinobacteria on other abundant freshwater bacteria.

Since six mixed cultures are not sufficient to generalize the character of the observed associations, we correlated the abundance of mixed-culture TBE6 MAGs with newly assembled MAGs from a 9-year shotgun metagenome time series from the mixed-culture’s source environment, Trout Bog Lake (23). If acl actinobacteria do indeed have a nonspecific metabolic dependence on other freshwater bacteria, we expected to see a larger number of positive correlations between the abundance of acl organisms and the abundance of other freshwater bacteria than in the culture. In contrast, a number of positive associations exclusive to taxa recruited in the cultures would indicate highly specific dependencies for acl organisms.

In the Trout Bog Lake epilimnion, we recovered 36 MAGs that correlated with the MAGs recovered from the TBE6 culture (Fig. 2). These included four acl MAGs, three *Polynucleobacter* MAGs, and a single *Spirochaetes* MAG that were different (<90% ANI) from those recovered from the mixed culture. The rest of the MAGs had diverse taxonomic affiliations, such as *Saccharibacteria*, *Parcubacteria*, *Verrucomicrobia*, and *Bacteroidetes*, among others. Several positive and negative correlations were observed. In the first quadrant of Fig. 2, positive interactions can be observed, mostly between different taxonomic groups and the *Polynucleobacter* organisms of our TBE6 mixed culture. A second block of positive interactions can be observed between the acl MAGs from TBE6 and diverse taxonomic groups from the lake. Moreover, a third block of positive interactions was detected between one of the acl MAGs from TBE6 and diverse other taxonomic groups, including several distinct acl actinobacteria. Thus, it appears that the positive correlations do not exclusively occur between similar taxonomic groups.

A similar analysis was repeated for the hypolimnion of Trout Bog Lake (see Fig. S2 in the supplemental material) and the combination of both layers (Fig. S3), and similar results were observed. Results from previous cooccurrence studies support the idea that free-living streamlined bacteria have very high connectivities in their environments and are critically dependent on metabolites that might be provided by other planktonic community members (7, 24, 25). Moreover, our analysis shows that in nature, we observe many more interaction partners than we can observe in a single dilution



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**FIG 2** Spearman correlation of normalized relative abundances between MAGs from TBE6 and taxa in the epilimnion of the environment of origin (Trout Bog Lake). Metagenome samples from 45 time points were used. Correlations are sorted using hierarchical clustering. MAG names that start with TBE6 refer to the MAG number from the mixed-culture. MAG names starting with the words “time series” represent bins from a 9-year metagenome time series (23). The acI MAGs are in bold. To view the correlation matrix that was derived from time series metagenomic sequencing of the hypolimnion and from both the epilimnion and the hypolimnion combined, see the supplemental material (Fig. S2 and S3).

culture. Some future work to confirm the nature of these interactions might include more cultures with the respective supporting time series data and metabolic networks that will confirm the promiscuity of the interactions in free-living microorganisms.

In conclusion, acI actinobacteria appear to depend on numerous other abundant microorganisms for metabolic handouts (i.e., some vitamins, amino acids, and reduced sulfur). This kind of dependence seems to be non-taxon specific or promiscuous since highly specific exclusive cooccurrences could not be observed in the mixed cultures or in the time series metagenomes. This, we hypothesize, might also be the case with



other free-living streamlined organisms. Paradoxically, with a small genome size, the large proportion of accessory genes renders them functionally versatile at the population level. Our results suggest that this metabolic versatility facilitates interactions with a variable set of community members. In natural systems with a high temporal and spatial variability in environmental drivers, this might be one of the keys to the competitiveness of streamlined “free-living” microorganisms in highly dynamic microbial communities.

**Accession number(s).** The raw shotgun metagenome reads are publicly available in the JGI portal, and the assembly is available in the IMG database under the submission numbers 26656, 26658, 26650, 29729, 29808, and 50227. The bacterial metagenome-assembled genomes (MAGs) are also available through IMG. The MAGs assembled from Trout Bog Lake (GGBR00000000) and fungal MAGs have been deposited in DDBJ/ENA/GenBank. For taxon operational identifiers or accession numbers of MAGs from mixed cultures, see the supplemental material.

### SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/mSphere.00202-18>.

**TEXT S1**, DOCX file, 0.2 MB.

**FIG S1**, PDF file, 0.2 MB.

**FIG S2**, PDF file, 0.03 MB.

**FIG S3**, PDF file, 0.02 MB.

**DATA SET S1**, XLSX file, 0.03 MB.

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S.L.G., M.B., K.D.M., and A.E. conceived the research. S.L.G. and H.-P.G. collected and prepared the samples. S.L.G., M.B., J.J.H., C.W., and A.E. analyzed the data. All authors wrote and/or revised the manuscript.

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