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Division-Specific Differences in Bacterial Community Assembly of Two Alkaline Hot Spring Ecosystems from Yellowstone National Park

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Abstract A fundamental issue in ecology is whether communities are random assemblages or, alternatively, whether there are rules that determine which combinations of taxa can co-occur. For microbial systems, in particular, the question of whether taxonomic groups exhibit differences in community organization remains unresolved but is critical for our understanding of community structure and function. Here, we used presence–absence matrices derived from bar-coded pyrosequencing data to evaluate the assembly patterns of eight bacterial divisions distributed along two Yellowstone National Park hot spring outflow channels. Four divisions (Cyanobacteria, Chloroflexi, Acidobacteria, and Cytophaga–Flavobacterium–Bacteroides) exhibited less co-occurrence than expected by chance, with phototrophic taxa showing the strongest evidence for nonrandom community structure. We propose that both differences in environmental tolerance and competitive interactions within divisions contribute to these nonrandom assembly patterns. The higher degree of nonrandom structure observed for phototrophic taxa compared with the other divisions may be due in part to greater overlap in resource usage, as has been previously proposed for plant communities.

Introduction

One of the central aims of ecology in general and the investigation of microbial diversity in particular is to

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understand how communities are assembled. The issue of whether communities are organized by specific assembly rules has been contentious, however, particularly with respect to the statistical problem of how to identify nonrandom structure in community assemblages from presence–absence data (reviewed by [1]). The mechanisms that shape patterns of community structure likewise remain poorly understood. Diamond [2] proposed that competition creates nonrandom distribution patterns in which some taxon pairs never co-occur. Others, however, have pointed out possible alternative explanations for nonrandomly structured distributions, including differences among taxa in habitat preference [3], historical factors (e.g., allopatric speciation; [3–5]), and neutral processes [6, 7].

A meta-analysis of 96 co-occurrence matrices collected for plant and animal communities indicated that approximately one half of the communities exhibited nonrandom structure [3]. An analogous meta-analysis of community structure of archaea, bacteria, and fungi for 124 microbial data sets [8] from a wide range of habitats (e.g., freshwater, marine, hot spring, coral, soil, and sediment) yielded similar results, with 56 % of communities exhibiting less co-occurrence among taxa than expected by chance. Gotelli and McCabe [3] also noted taxon-specific differences in community assembly related to physiology. Specifically, plants and homeothermic animal communities were assembled nonrandomly, while poikilotherms (with the exception of ants) were not. The authors attributed the strong nonrandom structure in plants to competition resulting from a high degree of similar resource usage. It is unknown if microbial community structure is similarly associated with physiology. Whereas it has been reported that community assembly patterns were similar among the domains Bacteria, Archaea, and Eukarya [8], it remains unclear if taxon-specific differences exist among microorganisms at levels

of phylogenetic resolution that are finer than that of the domain, such as divisions. For example, we might expect phototrophic taxa (e.g., Cyanobacteria) to be highly structured, as observed for plants, due to competition within groups for resources including light. By contrast, groups exhibiting a greater diversity of potential energy sources (e.g., Proteobacteria) may exhibit less niche overlap on average and therefore less structure.

Here, we tested for differences in the community structure of bacterial divisions in the 16S rRNA bar-coded pyrosequencing data set of Miller et al. [9], which consists of 39 samples collected from along the outflow channels of White Creek and Rabbit Creek, two Bacteria-dominated, alkaline hot springs located in the Lower Geyser Basin of Yellowstone National Park. We report that bacterial divisions in these communities vary strongly in their degree of structure, ranging from strong nonrandomness within divisions represented by phototrophic members to apparent random assembly.

Methods

Study System

We focused our investigation on operational taxonomic units (OTUs) from the eight bacterial divisions which accounted for nearly 97 % of the sequences of the V3 region of the 16S rRNA gene that were recovered in the full pyrosequencing data set of Miller et al. ([9]; $N=32,099$; Table 1). Each OTU represents a unique V3 sequence tag (i.e., all members of an OTU share 100 % sequence identity) and was assigned to a division if it exhibited sequence similarity of greater than 85 % to a cultured representative (although typically this value was much greater). Full details of the OTU identification process and the assignment of OTUs to divisions may be found in the original paper [9]. In these environments, members of three of these divisions (Cyanobacteria, Chloroflexi, and Acidobacteria) are phototrophic [9–11], whereas the remaining divisions are either principally or exclusively chemotrophic.

Statistical Analysis

To test for nonrandom structure, we used the C score [12], a presence/absence-based measure of the average co-occurrence of taxa within bacterial divisions. Data for each division were organized as a matrix table (Online Resource 1), with each row representing an individual OTU and columns indicating the presence or absence of that OTU in each of the 39 samples. For each matrix, the C score is defined as the average number of checkerboard units between all pairs of OTUs, where a checkerboard unit is defined as a submatrix of two samples and two OTUs for

which the OTUs do not co-occur. For each analysis, a null distribution was generated with EcoSim version 7.0 (Acquired Intelligence Inc. & Kesey-Bear) from 5,000 simulated matrices created using the sequential swap algorithm with row and column sums fixed. The standardized effect size (SES) is the difference between the observed C score and the mean of the simulated null model, normalized by the standard deviation of the null distribution; higher values therefore indicate a higher degree of nonrandom structure. The C score has desirable statistical properties with respect to power and vulnerability to false positives [13], whereas other co-occurrence indices (checkerboard score, number of taxon combinations) are particularly sensitive to measurement errors in the presence-absence matrix and are thereby more prone to type II error. The latter issue is a particular concern for the undersampled data sets obtained for most microbial communities. Of the eight divisions investigated from the data set of Miller et al. [9], four (Acidobacteria, Armatimonadetes, Thermotogales, Thermus) had plateaued in a rarefaction analysis and were therefore comprehensively sampled (Online Resource 2).

Results and Discussion

The C scores for four of the divisions (Cyanobacteria, Chloroflexi, Acidobacteria, Cytophaga-Flavobacterium-Bacteroides (CFB)) were highly significantly greater than their respective null distributions ($P=0$; Table 1), which indicated less taxon co-occurrence than expected by chance. We could not reject the null hypothesis of random assembly for four divisions (Proteobacteria, Armatimonadetes, Thermus, Thermotogales), however, suggesting that there are differences among divisions in community structure. Since the raw C score values are dependant on the number of OTUs included in the analysis, we compared results across divisions using the SES [3] of their C scores, which further indicated that the divisions that are exclusively or predominantly composed of phototrophs in these systems (Cyanobacteria, Chloroflexi, Acidobacteria) are the most highly structured (indicated by a greater SES; Table 1). The CFB group also exhibited significant nonrandom structure, though to a lesser degree than the predominantly phototrophic taxa (Table 1). In addition, one of the four divisions for which the null hypothesis was not rejected (Proteobacteria) had not plateaued in a rarefaction analysis (Online Resource 2). Horner-Devine et al. [8] investigated the effects of taxon undersampling on the inference of community structure and observed that the C score SES often but not always increases with increasing sample size, but that it also increased by no more than two- to threefold. Therefore, while we might expect that increased sampling would provide stronger evidence for the nonrandom

Table 1 *C* scores and their standardized effect sizes (SES) for the eight most abundant bacterial divisions in the community samples of Miller et al. [9]

Taxon	<i>N</i>	OTUs	<i>C</i> score	Null (variable)	<i>P</i>	SES
Acidobacteria	1,796	5	78.4	65.5 (0.72)	0	15.2
Cyanobacteria	10,877	41	19.0	16.5 (0.04)	0	13.0
Chloroflexi	13,108	44	23.9	22.7 (0.02)	0	7.8
CFB	2,656	33	13.3	12.6 (0.03)	0	4.3
Proteobacteria	498	41	7.2	7.0 (0.02)	0.08	1.4
Armatimonadetes	2,322	8	28.4	27.8 (0.57)	0.20	0.8
Thermus	611	7	32.2	32.3 (0.58)	0.50	0.1
Thermotogales	231	3	7.0	7.5 (0.20)	0.35	-1.1

SES is the standardized effect size of the *C* score (see “Methods” for details)

structure of Proteobacteria, we would not expect the SES to approach that observed for phototrophic divisions.

For the divisions for which we rejected the null hypothesis (Cyanobacteria, Chloroflexi, Acidobacteria, CFB), it is unlikely that any single factor is entirely responsible for taxon segregation in these hot spring systems; however, differences among taxa in physiological tolerance likely make an important contribution to community assembly through its influence on the probability of co-occurrence. In particular, environmental temperature has been shown to be strongly associated with both community similarity and taxon richness at both White Creek and Rabbit Creek [9]. Thus, although microbes are typically capable of surviving over a broad temperature range, the thermal gradients of these hot springs (~37 to 72 °C) presumably create habitat gradients that filter taxa based on their relative fitnesses at different temperatures. Examples of evidence for structuring by temperature include the higher temperature distributions at White Creek of the Cyanobacteria *Synechococcus* spp. (e.g., OTUs 4 and 6 in Online Resource 1) compared with *Mastigocladus laminosus* (cyanobacterium OTU 8 in Online Resource 1); the higher temperature distribution of Acidobacteria OTU 1 versus OTU 3 (Online Resource 1); and the higher temperature distributions of CFB OTU 3 at White Creek compared with OTUs 5, 6, 7, and 11 (Online Resource 1).

If the nonrandom structures of these matrices were due solely to differences among taxa in temperature tolerance, then we would expect to accept the null hypothesis for presence–absence matrices constructed for data pooled from samples of similar temperature, irrespective of hot spring of origin. However, because the two creeks exhibit differences in water chemistry (e.g., phosphate; [9]), we expected that other environmental parameters actually contribute to community organization. To distinguish between these alternatives, we analyzed pooled, temperature-matched samples from White Creek and Rabbit Creek. We observed highly significant nonrandom structure ($P=0$) in all cases: WC1 (39 °C) and RC1 (38 °C) (SES=6.5); WC3 (47 °C) and RC2 (47 °C) (SES=6.0); WC5 (54 °C), WC6 (57 °C), RC4 (53 °C),

and RC5 (55 °C) (SES=7.2); WC7 (61 °C) and RC6 (61 °C) (SES=19.1); and WC9 (67 °C), RC9 (67 °C), and RC10 (69 °C) (SES=4.7).

Therefore, mechanisms other than temperature alone must also contribute to the structure of these communities. These may include differences in water chemistry between springs (e.g., Rabbit Creek has greater amounts of combined nitrogen and phosphorus than White Creek [9]). Consistent with this possibility is the observation that some OTUs are abundant in one spring but absent or rare in the other. For example, the heterocyst-forming, nitrogen-fixing cyanobacterium *M. laminosus* (cyanobacterium OTU 8 in Online Resource 1) is only found in White Creek, where combined N is low. Other examples include CFB OTUs 1, 2, and 3 in White Creek and Acidobacteria OTU 5, which was unique to Rabbit Creek (Online Resource 1).

Competition for limiting resources likely also plays a key role in structuring these communities. For the strongly structured phototrophic divisions, this may include light, as has been suggested for plant communities [3]. A notable example within these geothermal systems for which there is good evidence that competition contributes to realized distributions is for the cyanobacterial genus *Synechococcus*. The observed temperature range distributions of *Synechococcus* OTUs in situ [9] are narrower than the ranges of physiological tolerance exhibited by laboratory strains [14, 15]. We interpret this disparity between potential and realized niche breadths to be the result of competitive exclusion at range boundaries.

Our analysis illustrates the potential utility of applying community ecological theory to investigate how microbial diversity is distributed in situ and to obtain insights and guide hypothesis development regarding the underlying mechanisms shaping these patterns. For example, the lower degree of structure observed for divisions for which the null hypothesis was accepted (Table 1) would traditionally be interpreted as evidence that members of these divisions generally experience less resource overlap on average than do members of the highly structured divisions, thereby increasing their probability of co-occurrence. Metagenome

data may be able to explicitly address this issue in the future: one would predict, for example, that divisions that are more flexible with respect to their resource requirements would exhibit more complex networks in metabolic reconstructions. Future investigations will also seek to address the impact of the scale of phylogenetic resolution on our inferences of community structure. For example, the ca. 100-bp V3 sequence used to define OTUs used in the present study may not be sufficient to resolve existing structure for closely related taxa. If there are taxon-specific differences among community members in their evolutionary time scales of diversification, then detection of nonrandom assembly that is the product of more recent diversification may require the analysis of more rapidly evolving loci than highly conserved rRNA genes (e.g., population genetic data from metagenomes).

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Conflict of Interest The authors declare no conflicts of interest.

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