Functional ecology of an Antarctic Dry Valley

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The McMurdo Dry Valleys are the largest ice-free region in Antarctica and are critically at risk from climate change. The terrestrial landscape is dominated by oligotrophic mineral soils and extensive exposed rocky surfaces where biota are largely restricted to microbial communities, although their ability to perform the majority of geobiological processes has remained largely uncharacterized. Here, we identified functional traits that drive microbial survival and community assembly, using a metagenomic approach with GeoChip-based functional gene arrays to establish metabolic capabilities in communities inhabiting soil and rock surface niches in McKelvey Valley. Major pathways in primary metabolism were identified, indicating significant plasticity in autotrophic, heterotrophic, and diazotrophic strategies supporting microbial communities. This represents a major advance beyond biodiversity surveys in that we have now identified how putative functional ecology drives microbial community assembly. Significant differences were apparent between open soil, hypolithic, chasmaendolithic, and cryptoendolithic communities. A suite of previously unappreciated Antarctic microbial stress response pathways, thermal, osmotic, and nutrient limitation responses were identified and related to environmental stressors, offering tangible clues to the mechanisms behind the enduring success of microorganisms in this seemingly inhospitable terrain. Rocky substrates exposed to larger fluctuations in environmental stress supported greater functional diversity in stress-response pathways than soils. Soils comprised a unique reservoir of genes involved in transformation of organic hydrocarbons and lignin-like degradative pathways. This has major implications for the evolutionary origin of the organisms, turnover of recalcitrant substrates in Antarctic soils, and predicting future responses to anthropogenic pollution.

The largest ice-free regions on the Antarctic continent are the McMurdo Dry Valleys, designated by international treaty as an Antarctic Special Managed Area (1) to reflect their environmental significance. The Dry Valleys are among the most threatened environments from climate change due to their polar location and unique ecology (2, 3). The terrestrial landscape is dominated by oligotrophic mineral soils (4) and extensive rocky outcrops with life restricted mainly to microbial communities due to the extreme environmental stress (5).

Extensive recent research has focused on elucidating the biodiversity of Dry Valleys landscapes (5–16). Classical microbiological studies identified edaphic Antarctic taxa by morphology and revealed a general recalcitrance to cultivation (17, 18). Molecular interrogations greatly expanded understanding of taxonomic community structure (5–16, 19) and speculated on the origin of inocula such as from streams that freeze dry and subsequently are dispersed by wind in winter (5).

These and other studies have provided major insight into the biodiversity of soil and rock niches largely from the rRNA gene perspective, although a caveat to any solely molecular study concerning biodiversity and functionality is that DNA can be isolated from nonviable propagules (20) or from inactive material blown in from elsewhere such as desiccated microbial mats on streams, dry lake beds, glaciers, coastal ice, and wetlands or brought in by snowfall (21). A limited number of in situ respirometry studies also indicated microbial contributions to carbon and nitrogen transformations may be significant (22, 23). A picture of the extent of bacterial colonization has emerged, but also soil and rock niches support algae (24), fungi (20), lichen (25), mosses (26), and invertebrates (27). A high degree of niche specialization in terms of rRNA gene-defined community assembly has been recorded between open soil, hypolith (colonized ventral surface of quartz), chasmaendolith (colonized cracks and fissures in sandstone and granite), and cryptoendolith (colonized pore spaces in weathered sandstone) (5).

Despite this, almost nothing is known about the contribution of Dry Valleys microbial communities in soils and rocks toward metabolic processes essential to biological mineral transformation and the stress tolerance mechanisms that allow them to flourish in such harsh extremes. The least extreme sub-Antarctic and maritime peninsula Antarctic locations received relatively greater attention, and the GeoChip-based functional gene arrays were successfully applied to indicate pathways and taxonomic identity of soil microbial carbon and nitrogen utilization (28–31) but they represent a fundamentally different biome compared with the extreme polar desert of the Dry Valleys ecosystem (32).

Here, we present findings from a comprehensive study using GeoChip to address key issues related to microbial contributions in geobiological processes, including carbon cycling genes, i.e., involved in autotrophy, acetogenesis, and methanogenesis; nitrogen cycling genes, i.e., nitrification and assimilatory and dissimilatory nitrogen reductions; and also, importantly, the stress tolerance strategies available to microbes to survive in these harsh environmental conditions. The data were compared with PCR-based diversity of 16S rRNA genes and biomass estimates and demonstrated autotrophic, heterotrophic, and diazotrophic pathways in Antarctic Dry Valleys microorganisms. Strikingly, we identified unique stress response pathways that can be directly related to environmental stressors in this Antarctic environment. Finally, we highlighted soil microbial pathways that indicate a potential ability to transform lignin-like molecules and anthropogenic pollutants and discussed this in view of the evolutionary origin of the microorganisms, increasing human exposure, and potential future contamination in the Dry Valleys system.

Results

Overall Differences of Microbial Communities. Communities that displayed niche-specific metabolic potential output from the array data were grouped into functional categories related to major metabolic processes. The level of redundancy in large
number of pathway-specific GeoChip oligonucleotides allowed a high degree of confidence in signal recovery inferring occurrence of any given pathway (33). GeoChip 4 contains about 84,000 50-mer oligonucleotide probes covering 152,000 gene variants (i.e., individual sequences from a gene) from 401 distinct functional gene categories involved in major biogeochemical, ecological, and other processes. Among these probes, 35,858 probes were derived from genes involved in carbon, nitrogen cycling, and stress responses. Hybridization of DNA from the four niches was achieved with an average of 45.1% of the 84,000 probes, covering an average of 91.8% of the targeted genes of interest on GeoChip 4 (Tables S1 and S2). For determining diversity, GeoChip utilized the highly specific probes targeting DNA gyrase subunit B (gyrB), which are functional genes with higher evolutionary rates than 16S rRNA genes. GyrB can achieve taxonomic resolution at the species-strain level (34–37), which is higher than that of 16S rRNA gene at the genus-family level. GeoChip hybridization revealed higher phylogenetic diversity in the samples than the 16S rRNA gene cloned library (Table S3). The results differ between the two techniques due to probe specificity.

The microarray analyses revealed that all colonized niches (open soil, hypoliths, chasmoendoliths, and cryptoodoliths), encountered in McKelvey Valley, supported a functional diversity that included photoautotrophic, heterotrophic, diazotrophic, and stress response pathways. Ordination and cluster analyses of the overall functional gene profiles indicated significant variations between the communities from each of the edaphic niches analysis of similarity (ANOSIM) global $R = 0.784$, $P = 0.0001$, $n = 14$, permutation $= 9,999$] (Fig. 1). The two most exposed niches (hypothillic and chasmoendolithic) shared greatest similarity. Pairwise ANOSIM comparisons indicated the most significant differences occurred between soil and the three lithic niches ($P < 0.01$). A permutational multivariate analysis of variance (PERMANOVA) test also supported the same conclusion that overall soil metabolic potential was statistically different from all three lithic niches ($P < 0.05$). A similarity percentage (SIMPER) analysis was performed to assess which genes were primarily responsible for the observed difference between soils and the lithic niches. Overall, the most striking differences (59% of variation) between soil and lithic samples were due to the presence in soil communities of pathways involved in transformation of complex aromatic compounds (Tables S1 and S2 and Fig. S1), and variation in bacteriophage, metal resistance, and stress response gene categories each contributed around 7–10% of the total difference (Tables S1 and S2 and Fig. S1).

Because many probes on GeoChip 4 are designed on the basis of the gene sequences from pure cultures of known phylogeny or from environmental sequences of known taxonomic groups based on the National Center for Biotechnology Information database, the hybridization data can be used to assess phylogenetic composition and structure of microbial communities. For example, for nitrogenase reductase gene ($nifH$), there are 764 sequence-specific probes and 460 group-specific probes, which covered 2,223 coding sequences. About 89.1% of the probes were from 4,332 bacterial strains (27 phyla), 3.1% from 188 archaeal strains (4 phyla), 6.1% from 420 eukaryotic strains (15 fungal phyla), and 1.3% from 273 bacteriophages (five orders). Thus, the phylogenetic nature of the data acquisition also enabled confident assignment of metabolic capabilities to specific bacterial, archaeal, or eukaryal phyla in each niche and biodiversity estimates that were compared with sequence-based assessments from extensive clone libraries of the same samples (5). Although many different types of genes were detected, in this study, we are most interested in microbial diversity important to carbon, nitrogen cycling, and survival strategies.

Carbon Utilization. In an oligotrophic environment such as the Dry Valleys (samples from McKelvey Valley had total organic carbon mean ~0.15 g/100 g soil and negligible carbon in rock interspace; Table S4), carbon sequestration is a major challenge and hence autotrophy is important. The key enzymes in autotrophic carbon fixation pathways used to indicate autotrophy include the ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCo) in the Calvin–Benson–Bassham cycle, the propionyl-CoA/acetyl-CoA carboxylase (pcc) in the 3-hydroxypropionate/malyl-CoA cycle, the ATP citrate lyase ($aclB$), and the carbonmonoxide dehydrogenase (CODH) in the reductive acetyl-CoA pathway; these genes were detected in all of the samples (Fig. S2). We assigned possession of Functional Form I RubisCo as indicative of photoautotrophic potential, which was largely indicated as cyanobacteria (Fig. 2A). Functional Forms II and III RubisCo were indicated as mainly in Archaea, Actinobacteria, and Proteobacteria (Fig. 2A) and suggested a significant capability in chemomautotrophy.

We identified the presence of genes indicating acetogenesis particularly in Actinobacteria, Chloroflexi, Gammaphyceae, and Spirochaeae (Fig. 2B). In addition, we identified C1 pathways involving methanogenesis (Methanococci, Methanomicrobia, and unidentified archaeal taxa, Fig. 2C) and proteobacterial methane oxidation (Fig. 2D). The ability to transform various organic polymers including starch, pectin, wood polymers, and complex aromatic substrates was indicated and the ability to carry out simple and complex carbohydrate catabolism was present in 34 phyla across all domains (Fig. 2E), whereas the ability to catabolize complex aromatic compounds was indicated mainly by Actinobacteria, Deinococci, proteobacterial phyla, and both ascomycete and basidiomycete fungi (Fig. 2F). The range of detected pathway-specific genes indicate the potential to transform lignin-like compounds and other naturally occurring polyaromatics and also a range of xenobiotic aromatics including halogenated compounds, a capability demonstrated by white-rot basidiomycetes (38).

Nitrogen Utilization. Soil-combined nitrogen levels for McKelvey Valley samples were in the order of 0.05 g/100 g soil and undetectable in rock interspaces (Table S4). Our functional analysis detected genes critical to all of the major pathways of the nitrogen cycle (Fig. 3 and Fig. S3). The data indicated diazotrophic
ability and, therefore, nitrogen input to this Dry Valleys system largely by Actinobacteria, Cyanobacteria, and Alpha- and Epsilonproteobacteria (Fig. 3A). Mineralization ability (introduction of nitrate via decomposition) was indicated by basidiomycete fungi as well as Actinobacteria, Deinococci, and Alpha- and Betaproteobacteria (Fig. 3B).

The most abundant nitrifiers (oxidation of ammonium ions from nitrogen fixation and mineralization into nitrate) were Actinobacteria, Cyanobacteria, and Alpha- and Betaproteobacteria (Fig. 3B). Anoxic pathways that lead to nitrate loss from soils were also detected, as well as anoxic nitrogen assimilation (Fig. 3D). Dissimilatory nitrate reduction was indicated largely by Betaproteobacteria (Fig. 3F). The potential for soil nitrate removal via denitrification and anaerobic ammonium oxidation (annamox) pathways was also indicated, although these were very phylum-specific to Bacteriodetes and Deltaproteobacteria (denitrifiers, Fig. 3F) and Betaproteobacteria and Planctomycetes (annamox, Fig. 3G).

Stress Response. Almost nothing is known of stress response in edaphic Antarctic microbial communities. This study provides unique evidence that across niches a range of pathways are present in colonists, which include pathway-specific genes and σ-factors for response to osmotic, radiation (desiccation), cold-shock, heat-shock, and nutrient stress (Fig. 4 and Fig. S4). Osmotic shock pathways were largely attributed to Actinobacteria and the Proteobacteria and were abundant in rocky substrates (Fig. 4A). In soils and hypoliths in soil contact, a broader range of phyla possessed these genes. Radiation stress genes were used as a proxy for desiccation-tolerance pathway genes, and these generally occurred in phyla that were common in all niches.

Hypolithic communities supported the most diverse range of taxa with desiccation-tolerance genes (Fig. 4B). Interestingly, cold-shock genes in these Antarctic communities were detected exclusively among Actinobacteria, Firmicutes, and the Alpha- and Betaproteobacteria (Fig. 4C). Heat-shock pathway genes were more common among phyla, including bacteria and fungi (Fig. 4D).

Genes indicative of pathways that respond to nitrogen limitation were relatively widespread, but particularly abundant among...
Functional metagenomic approaches have been successfully applied to understand temperate and tundra soils (40–42) but the present evidence is unique for functional ecology among the full range of edaphic niches in polar desert. Given that we have also previously shown that some phyla in these niches experience very limited gene flow at the rRNA level over extended timescales (43) and consequently a high degree of stochastic demography (44), the source of variation in functional genes and pathways should be considered. It is not possible to quantitively the level of lateral gene transfer in these communities on the basis of our data, although the comparison between rRNA-defined taxonomy and functional diversity indicates that a high degree of predictive power is possible in terms of using taxonomic markers as indicators of functionality within a community, as recently observed in other ecosystems (45, 46). The level of exposure to environmental extremes and fluctuation appears to be a strong driver of functional ecology.

We have clearly shown that stress response pathways are more abundant and diverse in above-ground exposed niches (hypoliths and chasmoendoliths) compared with sheltered soils and cryptendolithic substrates, and this can be related to the measurements of thermal and moisture stress and UV exposure (47). Diverse mechanisms of adaptation were also demonstrated in hyporheic cyanobacterial mats in the Arctic and Antarctic, with the former showing dominance of copper homeostasis genes and the latter showing more sigma B genes, as we observed, which were considered to be due to the severe osmotic stress during freeze-up of Antarctic ponds (48). This indicates that recruitment to exposed niches is strongly based on selection for stress tolerance traits and that these communities have a more diverse “arsenal” of responses with which to counter environmental extremes than more sheltered niches. These offer tangible clues to the mechanism behind the enduring success of microorganisms in this seemingly inhospitable terrain.

The level of similarity in core primary carbon and nitrogen metabolism between all niches supports the notion of a “blanket” of microbial productivity covering desert surfaces (39). We conclude that the Cyanobacteria, although ubiquitous, are likely also accompanied by proteobacterial and algal photoautotrophy in these systems. High abundance of a member of the Alphaproteobacteria was previously identified in cryptendolithic cyanobacteria from Beacon sandstone in the McMurdo Dry Valleys (19). Our analysis revealed that several common edaphic phyla also possessed pathways for chemosynthesis, expanding the knowledge of autotrophic carbon input sources in this oligotrophic system. This observation has parallels with observations of other autotrophic microbial habitats, e.g., in oligotrophic oxygen-minimum zones of the ocean (49). Clearly, the most abundant phyla in the Dry Valleys comprise both heterotrophic and autotrophic strategies to mitigate low organic availability in this system.

A further limitation to colonization is nitrogen transformation, because this is a limiting nutrient in these systems’ input attributed to cyanobacterial diazotrophy (5, 23, 50) and also snow, as demonstrated in the East Antarctic snowpack (51). We identified that a “complete” pathway for nitrogen metabolism occurs, which indicates that during environmental conditions favorable to annamox and denitrification, net microbially mediated nitrogen loss from the system may occur. These may be envisaged to occur during the austral summer in waterlogged soils (from snowmelt). Cyanobacterial diazotrophy has traditionally been viewed to occur as a “dark” reaction due to oxygen inhibition during photosynthesis (52). However, this clearly cannot occur during a 24-h daylight cycle of the austral summer, and so interesting regulations on this pathway are likely to emerge. On going research into circadian systems and their influence on this may shed additional light (53). Anannox pathways have received significant attention in other habitats, and it is of note that Antarctic soils also have potential for this via the same restricted...
group of Betaproteobacteria and Planctomycetes (and Archaea) as in other temperate and tropical habitats (54, 55).

The range of stress response pathways revealed by our study suggests how the Antarctic soil microflora are adapted to environmental extremes. We highlight not only stress responses directly related to moisture, radiation, and thermal shock but also others that may offer clues to community regulation. For example, the identification of antibiotic resistance genes indicates indirect pathways of competitive interactions between taxa. Similarly, shotgun metagenomic sequencing analyses of Antarctic soil metagenomes also demonstrated the presence of antibiotic resistance genes (16).

We also identified a large frequency of phage signals that suggest viral controls on population numbers may be important in this system where grazers (and hence controls via higher trophic levels) are largely absent, a role previously indicated by metaproteogenomic analysis of a hypersaline Antarctic lake (56). This notion has also been suggested as a regulatory process in hydothermal systems, where microbial communities also dominate. This notion has also been suggested as a regulatory process in hydrothermal systems, where microbial communities also dominate (56).

Conclusions

Antarctic landscapes have emerged as far more biodiverse than previously envisaged as a result of microbial surveys. We conclude that rRNA estimates identified the dominant and endemic phylotypes that are underrepresented in databases, whereas GeoChip detected greater diversity at a broader taxonomic rank. Taken together, the two estimates are complementary and together yield a more complete view of diversity in this system. Critically, this study identified the functional traits of organisms that drive community assembly and interactions. The insights also expand our knowledge of how such communities tolerate extreme environmental stress and their capacity to respond to future changes, including climate and human impacts.

Materials and Methods

Field Sampling and Abiotic Variables. Edaphic colonization in McKelvey Valley (central valley coordinates 77°26’S, 161°33’E) was surveyed during Antarctica New Zealand Event K021 in January 2008. Surface soils, sandstone, and quartz substrates were surveyed as previously described (5) and frequency of colonization for chasmoendoliths, cryptoendoliths, and hypoliths was recorded (n = 400). Colonized soil and rock samples were recovered aseptically and stored in sterilized plastic containers with no headspace at ~80 °C until processed. A suite of abiotic variables including moisture content (gravimetric), pH (potentiometric), soluble salts (potentiometric), total organic carbon, and total nitrogen (gas chromatography-thermal conductivity detector at 900 °C) was measured for each substrate as previously described (5).

GeoChip Analysis. Recovery of environmental DNAs was with a protocol optimized for edaphic desert microorganisms (64). similarity between communities was assessed using nonmetric multidimensional scaling of Bray–Curtis similarities from terminal restriction fragment length polymorphism analysis of 16S and 18S rRNA genes as previously described (5). Independent replicates from each niche that were most representative for a given niche were then selected for GeoChip (version 4.0) analysis (n = 14). GeoChip 4.0 contained 83,992 50-mer oligo probes that covered 152,414 gene variants from 401 distinct functional gene categories involved in major biogeochemical, ecological, and other processes (12 categories: biochemical cycling of carbon, nitrogen, phosphorus, and sulfur; resistance to metal and antibiotics; energy process; organic compound remediation; stress response; bacteriophage related; virulence related; and others) (65, 66). About 20 ng community DNAs from each sample was amplified (67) and 1 μg of the amplified sample was used for GeoChip hybridization as previously described (65, 66). The normalized hybridization output data were then reorganized on the basis of functional categories.

Output was analyzed using the multivariate statistical software package PRIMER-E v6 (Plymouth Marine Laboratory). Alpha diversity indices (species richness, Shannon’s index, Simpson’s diversity index, and Pielou’s evenness) were calculated using untransformed data. Nonmetric multidimensional scaling (NMDS) ordinations were used to visualize Bray–Curtis Similarities. ANOVA, AMOVA, ANOSIM, PERMANOVA, and SIMPER analyses were performed to indicate confidence in similarities/differences observed. Visualization of different phylum-level and/or class-level contributions to each metabolic pathway was achieved using spider dendrograms, where each arm of the plot was specific to a given phylum/class.

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