Microbial Ecology

Colony-Forming Analysis of Bacterial Community Succession in Deglaciated Soils Indicates Pioneer Stress-Tolerant Opportunists

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Abstract

We investigated the response of bacterial communities inhabiting two deglaciated soils (10 and 100 years post-deglaciation) to two stimuli: (i) physical disruption (mixing), and (ii) disruption plus nutrient addition. PCR/DGGE analysis of 16S rRNA genes extracted from soil during a 168-h incubation period following the stimuli revealed that more bacterial phylotypes were stimulated in the 10-y soil than in the 100-y soil. In addition to 10-y and 100-y soils, two additional soils (46 and 70 y) were further differentiated using colony-forming curve (CFC) analysis during a 168-h incubation period, which revealed that younger soils contained a higher proportion of rapidly colonizing bacteria than successively older soils. ''Eco-collections'' of CFC isolates that represented colonies that formed ''fast'' (during the first 24 h) and ''slow'' (final 36 h) were harvested from 10-y and 100-y soils and differentiated according to response to three stress parameters: (i) tolerance to nutrient limitation, (ii) tolerance to temperature change, and (iii) resistance to antibiotics. The tested parameters distinguished ''fast'' from ''slow'' bacteria regardless of the age of the soil from which they were isolated. Specifically, eco-collections of ''fast'' bacteria exhibited greater nutrient- and temperature-stress tolerance as well as more frequent antibiotic resistance than ''slow'' bacteria. Further DGGE analysis showed that several eco-collection phylotype bands matched (electrophoretically) those of soil phylotypes enriched by mixing and nutrient stimulus. Overall, the results of this study indicated that the succession of colony-forming bacteria was differentiated by bacterial opportunism and temporal response to stimuli. Furthermore, although stress tolerance strategies are associated with opportunistic bacteria regardless of successional age, it appears that the proportion of opportunistic bacteria distinguishes early vs late succession forefield bacterial populations.

Introduction

Over the past 100 years, atmospheric warming has led to global deglaciation [28], which has affected large landmasses more or less uniformly, resulting in vast amounts of newly exposed land known as glacier forefields. The forefield environment represents a chronosequence of physical, chemical, and biological gradients that facilitates the study of several decades of natural succession over the distance of a few hundred meters. Much of our current knowledge of ecological succession has resulted from studies of plant populations [13, 21], especially within the forefield ecosystem [4, 9], in which the distribution and dispersivity of individuals [3, 4] longevity, and ultimate size [8] have been central themes. However, bacteria are the initial colonizers of most primary succession environments, especially high-latitude/high-altitude environments [10, 37], and recent studies of forefield bacterial population diversity and evenness revealed trends that were different from those of plants [33, 35].

Ecologists have labeled organisms that possess the ability to rapidly respond and dominate following a disturbance event as ''opportunists'' [27] whereas ''maintenance''-type organisms are characterized by a slower response. Although this categorization has been historically directed toward plant populations, bacteria populations can be characterized in a similar manner. Opportunistic bacteria (r-strategists) should be able to rapidly take advantage of transient alterations in their environment, while less opportunistic types (K-strategists) will tend to respond relatively slow. One proposed indicator of opportunism among succession bacteria is the degree of culturability on a ''nonselective'' medium, which has been used to demonstrate competitive differ-

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ences between rhizosphere-colonizing bacteria [11]. Using this approach we showed that late-succession bacterial populations were less opportunistic than those of early succession, providing evidence that the ability to quickly respond to potentially transient, advantageous growth conditions is an important attribute of early succession bacteria [35].

Although it is possible to assess some broad functional characteristics of bacteria communities through cultivation-based methods, a complementary assessment of soil microbial populations can be achieved by applying molecular methods [15]. Specifically, genetic fingerprinting of 16S rRNA genes extracted from microbial assemblages has enabled researchers to investigate a larger subset of the microbial community than was previously possible with culture-dependent techniques. For example, the method of denaturing gradient gel electrophoresis (DGGE; reviewed by Muyzer and Smalla [26]) has greatly facilitated ecological studies of microbial systems and has been frequently used to assess the diversity of dominant microbial communities in various succession environments [7, 17, 33, 35].

Since our previous investigations revealed a greater degree of opportunism among early succession populations than those of later succession, we were interested in further exploring this difference by characterizing the forefield succession in terms of the community's response to disturbance. In addition to investigating the overall disturbance response, we determined how the population distribution (evenness vs dominance) changed with succession. We hypothesized that bacteria possessing the ability to (i) respond quickly to a disturbance event and (ii) tolerate stress will be most abundant in the youngest soil where conditions favoring r-strategists prevail [35]. Conversely, the oldest soil was hypothesized to harbor less opportunistic, more stress-susceptible populations that are slower to respond to environmental disturbance.

Materials and Methods

Site Description and Sampling. Soils were harvested from the forefield of the Dammaglacier (8° E 27′ 30″, 46° N 38′ 00″) in the Canton of Uri, Switzerland (soil chemical and physical properties are described in detail in [33–35]). Approximately 2 kg of soil was collected from each of four sites along a 500-m transect that ran parallel to the direction of the glacial recession. Sampling sites were located 60, 100, 350, and 500 m from the glacier terminus, representing soils deglaciated for approximately 10-, 46-, 70-, and 100-y [3]. At least six subsamples were harvested to a depth of ~4 cm from within a 10-m-diameter circle surrounding each sampling point and pooled. The soils were sieved (2 mm) on site and either processed within 24 h (nucleic-acid-based analyses), or maintained for 3 days at 12°C (other analyses), which was the average soil temperature at the time of sampling.

Colony-Forming Curve Analysis. Triplicate soil samples (0.5 g, dry weight) were suspended in 5 mL of 5 mM sodium phosphate buffer (pH 7), vortexed for 1 min, diluted to 10^{-4}, and plated (50 μL) in quadruplicate onto 0.1× Difco Nutrient Agar (pH 7) (Difco Labs, Detroit, Michigan) containing 100 ppm cycloheximide. Plates were incubated in the dark at 25°C. A colony-forming curve (CFC [14]) was generated for each soil by counting newly visible colonies every 12 h for a 168-h incubation period and plotting the cumulative number of colonies at each time point.

Eco-collections. Collections of bacterial colonies, termed "eco-collections" [12] were harvested at random from the 10-y and 100-y CFC plates. These collections comprised four distinct groups of isolates based on two criteria: (i) the age of the soil from which they were isolated, and (ii) the lag time between plating and initiation of growth. For example, isolates that initiated growth during the first 24 h of incubation were arbitrarily termed "10-y-fast" or "100-y-fast" eco-collections. Conversely, those initiating growth during the last 36 h of incubation were termed "10-y-slow" or "100-y-slow" eco-collections.

The distribution of phylotypes within each eco-collection was determined by RFLP analysis of the near-full-length 16S rRNA gene of each isolate according to previously described methods [23] using a combination of two tetrameric restriction enzymes, *Hae* III and *Taq* I. Phylotypes were grouped according to the RFLP pattern and the Shannon evenness index (E) of each distribution was calculated [22]. Phylotype rank abundance plots were constructed and compared among eco-collections using analysis of covariance (ANCOVA). A single representative isolate of each phylotype was selected for further analysis and was maintained in a glycerol stock at −80°C throughout the duration of the experiments.

Response of Eco-collection Isolates to Stress. Tolerance of the isolates to nutrient stress was determined by plating each isolate on 0.01× and 0.001× Nutrient Agar and visually assessing growth (with a binocular microscope, as needed) following incubation at 25°C for either 24 h (10-y and 100-y fast eco-collections) or 168 h (10-y and 100-y-slow eco-collections). Tolerance was scored as positive (signs of colony formation) or negative (no formation).

Growth of the phylotypes at a range of temperatures was assessed by plating each isolate on 0.1× Nutrient Agar and incubating the cultures at 4, 12, 25, 37, or 42°C as described for the nutrient assay. Colony formation (growth) was classified as strong, intermediate, weak, or

none, relative to colony formation at the original isolation temperature (25°C). The ability of sensitive isolates to recover following incubation at a temperature unsuitable for growth was assessed by returning the isolate to 25°C and incubating as described above. Recovery was scored as positive (colony formation) or negative (no formation).

To assay potential antibiotic resistance, each isolate was grown in 0.1× Nutrient Broth (Difco) to an approximate OD of 0.5 (600 nm) and 120 μL of the culture was spread on Nutrient Agar. Within 15 min, paper disks impregnated with antibiotics including ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), erythromycin (10 μg), novobiocin (30 μg), and trimethoprim (5 μg) (Oxoid AG, Basel) were applied. Following incubation as described above, strain susceptibility was classified as sensitive, intermediate, or resistant by comparing the diameter of the zone of inhibition with values in a diagnostic chart (supplied with the antibiotic disks) specific to the culture conditions.

The results of each assay were normalized by a weighted comparison of eco-collections based on the abundance of each phylotype in the four eco-collections.

Analysis of the Soil Community Response to Disturbance. The 10-y and 100-y soils were analyzed using a culture-independent approach to determine the soil community response following two stimuli; (i) vigorous mixing (termed "mixing only"), which simulated physical disruption, and (ii) vigorous mixing in combination with nutrient addition (termed "mixing/nutrient"), which simulated physical disruption and a nutrient pulse. Plate counts on Nutrient Agar (as described above) revealed that 4.8×10^5, 4.6×10^5, 2.0×10^6, and 2.4×10^6 cells g^{-1} dry soil were culturable from the 10-, 46-, 70-, and 100-y soils, respectively. Based on these numbers, nutrients were added in the form of Nutrient Broth powder (Difco) at a rate of ~150 μg C 10^{-5} cells, which was equivalent to 1 mg g^{-1} 10-y soil (dry mass equivalent) and 5 mg g^{-1} 100-y soil. The powder was mixed into bulk samples of each soil for exactly 1 min with a sterile spatula. The "mixing only" treatment was prepared identically, but without nutrient amendment. Negative control samples (no mixing or nutrient amendment) were also prepared. For the 100-y soil 1-g aliquots of each soil were placed into triplicate 2-mL microcentrifuge tubes. Previous extractions of the 10-y soil resulted in low concentrations of DNA [33]. Therefore, six subsamples per triplicate were prepared. Tubes were incubated in the dark at 25°C and extracted for DNA following 0, 6, 12, 24, 48, 96, and 168 h of incubation. Subsamples of DNA from like soils were pooled prior to final DNA precipitation and quantification. Partial bacterial 16S rRNA genes were amplified with primers 341f-GC (5′-GC clamp-ATT ACC GCG GCT GCT GG-3′) and 534r (5′-CCT ACG GGA GGC AGC AG-3′) and DGGE was performed at 67 V for 15 h according to the method of Muyzer et al. [25]. For each lane, the number of bands was determined using Quantity One image analysis software (version 4.0, Bio-Rad Laboratories) under the assumption that a single DGGE band served as a surrogate for a specific soil bacteria population (phylotype).

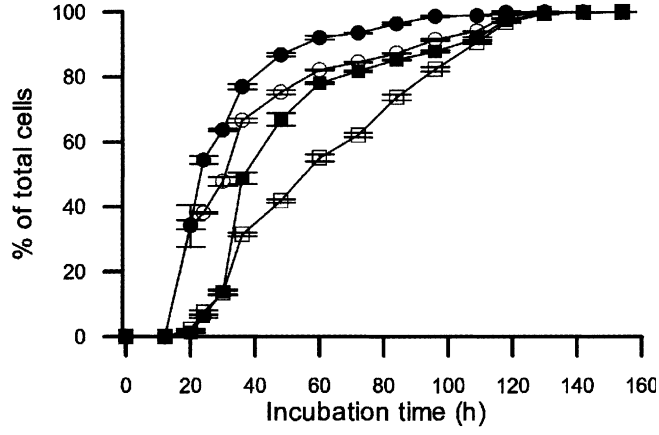

Figure 1. Colony-forming curves (CFCs) for glacier forefield bacteria inhabiting ●: 10-y; ○: 46-y; ■: 70-y; □: 100-y soil. CFCs were based on the accumulation of 155, 462, 234, and 324 CFUs per plate (average of quadruplicate platings) for 10-, 46-, 70-, and 100-y soils, respectively. Error bars represent the standard error among four replicates.

In order to determine if any of the soil bacteria community members that were affected by the disturbance treatments were also represented among the eco-collection phylotypes, DNA from each eco-collection phylotype was PCR amplified as described above and compared by DGGE to a representative soil sample from each treatment. We putatively identified the eco-collection phylotype's concurrent presence in the treated soil by noting if comigration of soil community DGGE bands and eco-collection phylotype DGGE bands occurred. To avoid mistakenly matched phylotypes, we only considered bands that were clearly separated by DGGE analysis.

Results

Colony-Forming Curve Analysis. The CFCs indicated that culturable bacteria populations inhabiting the four soils differed in their colony formation behavior and that the accumulation of colonies was soil age dependent, predictable, and sequential in nature (Fig. 1). The CFCs suggested that the 10-y soil harbored the highest proportion of cells that were capable of rapid colonization of the medium (54% of the total cells formed in the first 24 h), while the 100-y soil harbored the lowest proportion (6%). Accordingly, analysis of the 46-y and 70-y soils resulted in CFCs that indicated intermediate levels of

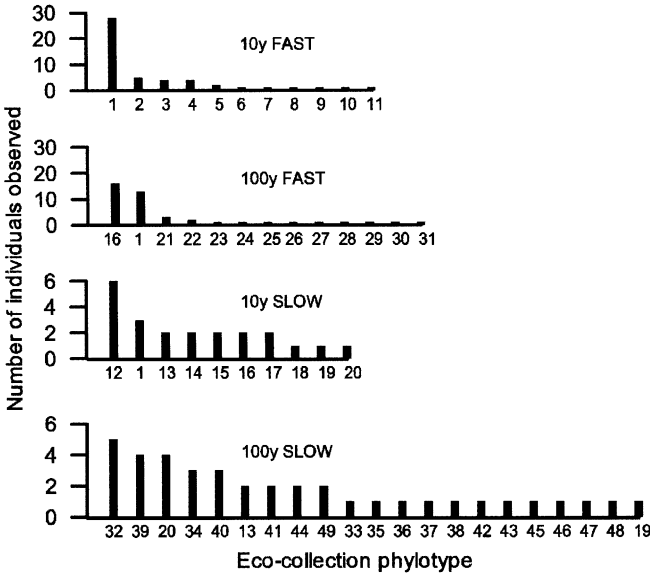

Figure 2. Rank abundance distribution of 10-y-fast, 100-y-fast, 10-y-slow, and 100-y-slow eco-collections. Numbers along the *x*-axis represent the number assigned to the eco-collection phylotype. Note the difference in *y*-axis values between fast- and slow-forming phylotypes.

colony accumulation (38% and 7%, respectively) with respect to the 10-y and 100-y soils.

Eco-collection Phylotype Distribution. Our goal was to collect 50 isolates for each eco-collection, but in three of the four collections (100-y-fast, 10-y-slow, and 100-y-slow) fewer than 50 total isolates initiated growth during the time windows described in Materials and Methods. Therefore, a total of 161 isolates were recovered (50, 40, 22, and 49 isolates comprising 10-y-fast, 100-y-fast, 10-y-slow, and 100-y-slow eco-collections, respectively). Phylogenetic grouping of the isolates using RFLP analysis indicated the presence of 11, 13, 10, and 21 distinct phylotypes in the 10-y-fast, 100-y-fast, 10-y-slow, and 100-y-slow eco-collections, respectively (Fig. 2). The Shannon evenness index (E) and ANCOVA of phylotype rank abundance data indicated a significantly higher evenness ($P < 0.05$) among slow-colonizing phylotypes ($E = 0.93$ and 0.94 for 10-y- and 100-y-slow, respectively) than among fast-colonizing phylotypes ($E = 0.65$ and 0.72 for 10-y- and 100-y-fast phylotypes, respectively). Conversely, the difference in E between either set of "fast" or "slow" isolates harvested from 10-y or 100-y soils was nonsignificant ($P = 0.572$ and 0.234, respectively).

Analysis of Eco-collection Isolate Stress Tolerance. All fast-forming isolates grew on 0.01× and 0.001× Nutrient Agar, whereas slow-forming isolates were inhibited at both dilutions with the exception of those from the 10-y soil plated onto 0.01× medium (Fig. 3A). Specifically, 56% of the 100-y slow-forming isolates exhibited growth on 0.01× medium, whereas at the 0.001× dilution, 68% (10-y) and 31% (100-y) of the slow-forming isolates grew.

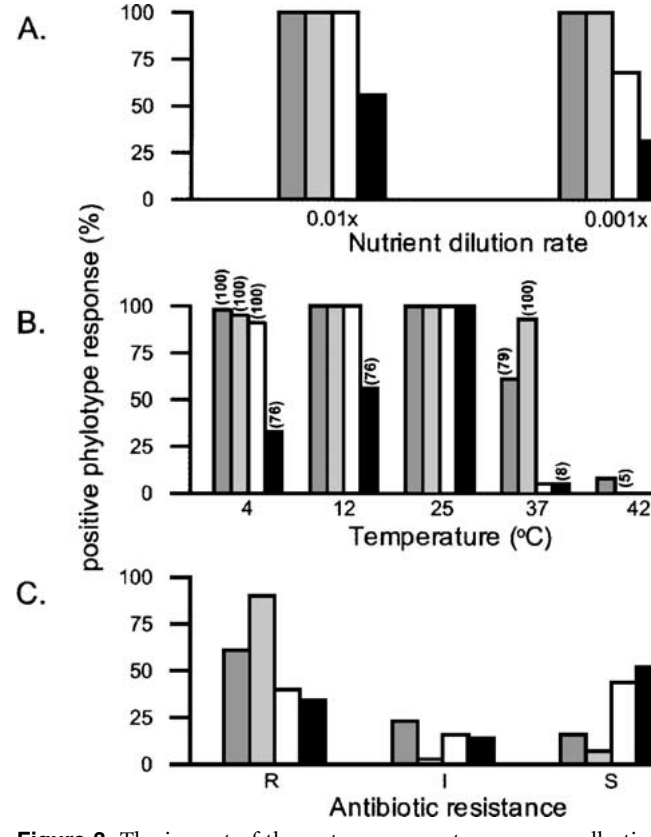

Figure 3. The impact of three stress parameters on eco-collection phylotype growth. (A) Nutrient dilution rate; (B) temperature (parenthetical numbers indicate the percentage of phylotypes that were classified as "non-growing" at the assay temperature but recovered following transfer to 25°C, see text for details); and (C) antibiotic resistance (R: resistant; I: intermediate; S: susceptible). Dark gray bars: 10-y fast; light gray bars: 100-y fast; white bars: 10-y slow; black bars: 100-y slow.

We observed a similar trend in response to temperature, as the negative impact of temperature stress on growth was greater on the slow-forming isolates than on fast-forming isolates. This was reflected by the notable decrease in viability of the slow-forming isolates at both low (4 and 12°C) and high (37 and 42°C) temperatures, relative to the initial culturing temperature (25°C) (Fig. 3B). Recovery of nongrowing isolates was also enhanced among the fast-forming isolates, as on average, 68 and 49% (10-y and 100-y, respectively) of fast-forming isolates recovered the capacity for growth at 25°C, while 29 and 33% (10-y and 100-y, respectively) of slow-forming isolates were able to resume growth.

An average of 75% of the fast-forming isolates were resistant to each of the six antibiotics tested in this study,

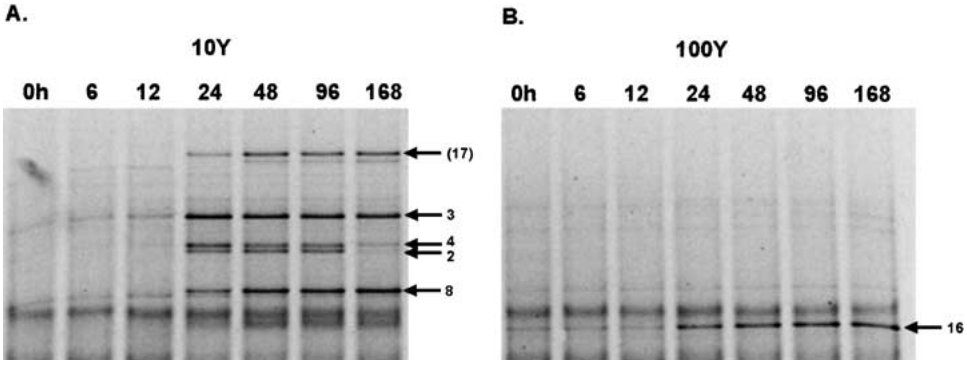

Figure 4. Time series DGGE analysis of (A) 10-y and (B) 100-y soils following mixing disturbance and incubation for 0, 6, 12, 24, 48, 96, and 168 h. Arrows refer to the migration distance of DGGE bands representing eco-collection phylotypes assayed as described in Materials and Methods. Arrow labels refer to the same phylotypes as in Fig. 2. All arrows refer to fast-forming phylotypes with the exception of "17" (in parentheses), which is a slow-forming phylotype.

whereas 37% of the slow-forming isolates were resistant. The balance of the isolates exhibited susceptibility or intermediate resistance (Fig. 3C).

Analysis of the Soil Community Response to Disturbance. DGGE analysis revealed that bacteria communities of the 10-y and 100-y soils responded differently to both mixing and mixing/nutrient addition with regard to response quality (the number of phylotypes stimulated) and response time (the length of time between the disturbance and the detection of phylotype stimulation). In both soils, the enhancement of the bacteria populations was detected 24 h following the mixing-only treatment. However, the populations in the 10-y soil responded with greater quality to mixing, as evidenced by seven enhanced soil phylotypes (Fig. 4A), whereas in the 100-y soil, one soil phylotype was stimulated (Fig. 4B).

Based on the intensity of DGGE bands, the mixing/nutrient treatment resulted in a greater number of stimulated phylotypes than mixing alone, especially in the 100-y soil (Fig. 5A, B). Image analysis indicated that 13 and 17 enhanced phylotypes were detected following 24 h of incubation in 10-y and 100-y soils, respectively. Although the 100-y soil exhibited a notable burst in phylotype number in the 24-h sample, enhancement of the 10-y soil phylotypes was detected as early as 6 h following the treatment, indicating a faster response on the part of the 10-y soil community. We did not observe any stimulation of the bacteria communities from the negative control samples (no mixing or nutrient amendment), which produced fingerprints similar to those of the 0-h samples of treated soils throughout the duration of the experiment (not shown).

Linking the Soil Community to Eco-collection Phylotypes. Following DGGE analysis, several bands that represented stimulated soil community members could be matched with one or more corresponding bands generated by the individual eco-collection phylotypes for all treatment/soil combinations (Fig. 4 and 5). For both disturbance treatments, the majority of the phylotypes were observed to respond simultaneously (after 24 h)

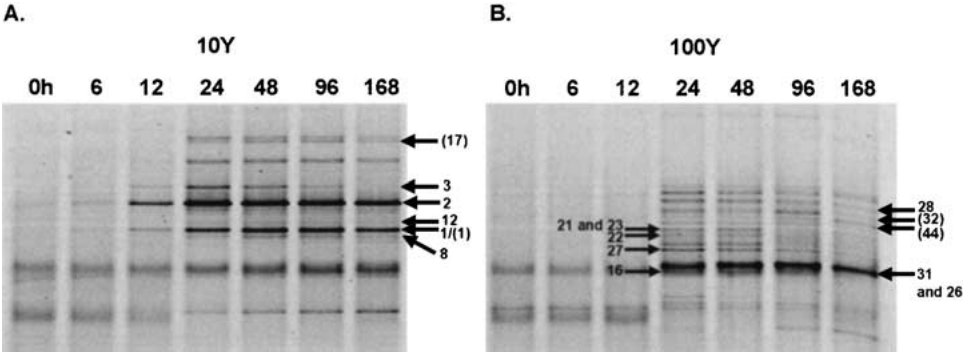

Figure 5. Time series DGGE analysis of (A) 10-y and (B) 100-y soils following mixing disturbance combined with nutrient addition and incubation for 0, 6, 12, 24, 48, 96, and 168 h. Arrows refer to the migration distance of DGGE bands representing eco-collection phylotypes assayed as described in Materials and Methods. Arrow labels refer to the same phylotypes as in Fig. 2. All arrows refer to fast-forming phylotypes with the exception of those in parentheses, which represent slow-forming phylotypes.

Table 1. General relationships between bacteria traits and forefield succession stage, as well as the experimental level at which the relationships were observed

Bacterial trait	*Forefield succession stage*		*Experimental level*[a]
	Early (10-y)	*Late (100-y)*	*(relevant figure)*
Opportunism[b]	High	Low	Eco-collection (Fig. 1) Soil community (Figs. 4, 5)
Community structure	Dominance over evenness	Evenness over dominance	Eco-collection (Fig. 2)
Stress response			
Nutrient	Tolerant	Sensitive	Eco-collection (Fig. 3A)
Temperature	Tolerant	Sensitive	Eco-collection (Fig. 3B)
Recovery	High	Low	Eco-collection (Fig. 3C)
Antibiotic resistance	Resistant	Susceptible	Eco-collection (Fig. 3D)
Physical disturbance	Fast	Slow	Soil community (Figs. 4, 5)

[a]Eco-collection: experiments referring to CFCs and/or eco-collection isolates; soil community: experiments referring to DGGE community analysis.
[b]Expressed as cell formation rate.

and were not discernable based on response time. However, bands corresponding to fast-forming eco-collection phylotypes were observed to be among the first to exhibit enhancement in the mixing/nutrient treatment (phylotypes 1, 2, and 3; Fig. 5A). Furthermore, several DGGE bands that represented fast-forming phylotypes (2 and 4, Fig. 4A; 21/23, 27, and 28, Fig. 5B) appeared to decrease in intensity following the initial response (24 h). Conversely, the stimulation of soil phylotypes that matched with two slow-forming eco-collection phylotypes (17, Fig. 4A, and 44, Fig. 5B) appeared to be delayed until 48 and 168 hours following the treatments, respectively.

Discussion

Petraitis et al. [30] predicted that no single species could tolerate both the disruption associated with disturbance and the ensuing competition of recolonization. In the glacier forefield, this trade-off appears to result in a succession of phylotypes possessing differing degrees of opportunism (Table 1). Based on the ratio of culturable to total cells we previously showed that the degree of opportunism among culturable bacteria decreased with succession age [35]. In the current study, we confirmed this finding through colony-forming-curve (CFC) analysis [14], which indicated that the 10-y soil harbored a higher proportion of opportunistic (fast-forming) cells than successively older soils (Fig. 1). This finding also partially explained the enhanced microbial response to the disturbance treatments in the 10-y soil relative to the 100-y soil as detected by DGGE (Fig. 4 and 5). This conclusion was supported at the eco-collection level as well, as DGGE analysis putatively matched several soil community members that responded either fast or slowly to the disturbance treatments to members of fast or slow eco-collections, respectively. We are aware of the evidence that single DGGE bands can represent more than one phylotype due to the comigration of differing DNA sequences. This proved to be the case in two specific examples in this study (phylotypes 21 and 23, and 31 and 26; Fig. 5B). Because Nutrient Agar was used as the carbon source in the mixing/nutrient treatment and CFC platings, it is likely that similar organisms were stimulated in each experiment. Although not coupled by a common carbon source, the eco-collection phylotypes that matched with enhanced soil community members from nonamended soil samples were matched through similar visual placement in the DGGE gels relative to other diagnostic DGGE bands. Therefore, it is reasonable to assume that the majority of the enhanced soil community DGGE bands do represent eco-collection phylotype bands, although it must be noted that further sequencing of these bands is necessary to confirm this, as comigrating bands in DGGE fingerprints might not always represent identical bacteria [6].

Although there is evidence of enhanced rhizosphere colonization potential (a proxy for opportunism) by bacteria tolerant to specific environmental stresses [18, 20], the relationships among succession, community structure, and bacteria stress tolerance are not well understood. In the current study, we observed a greater stress tolerance among fast-forming isolates than slow-formers. Since the proportion of fast-forming phylotypes was greatest in the 10-y soil and decreased with succession age, our results indicated that early succession communities were characterized by dominance of a few robust, opportunistic phylotypes, whereas late succession communities exhibited a more even distribution of less tolerant phylotypes.

The eco-collection stress assays we selected are representative of stresses common to the glacial environment. For example, the availability of soluble nutrients in porous, young forefield soils is often limited by abundant rainfall and seasonal melting events [24]. Therefore, it is conceivable that the high proportion of nutrient stress-tolerant

phylotypes inhabiting the 10-y soil is indicative of a community adapted to periods of transient nutrient availability. Similarly, early-succession alpine soils contain little organic matter and low water-holding capacity, resulting in low temperature buffering capacity and diurnal temperature fluctuations of up to 40°C [19]. Bennett and Lenski [2] showed that the overall fitness and resource use efficiency of temperature-fluctuation-adapted *Escherichia coli* populations was greater than that of nonadapted populations. Our results suggest that the ability of forefield bacterial populations to tolerate and recover from such temperature variance plays an important role in their survival, especially in the younger soils, where a greater proportion of fast-forming cells (and thus, temperature-range-tolerant cells) were present. Although we observed frequent antibiotic resistance among all eco-collections, it seems counterintuitive that such a high frequency should be necessary under the relatively density-independent conditions of the 10-y soil. However, in this case, antibiotic resistance might allow for a rapid, competitive response to a nutrient pulse or to resulting transient density-dependent conditions, analogous to previously observed impacts of antibiotic production on the competitive fitness of biological control agents [32].

Overall, fast-forming phylotypes were better able to withstand nutrient depletion and temperature extremes and were more frequently resistant to selected antibiotics than were slow-forming phylotypes, irrespective of the soil from which they were isolated. We regard the broad stress tolerance of fast-forming phylotypes as an indicator of adaptive ability, and we maintain that the proportion of organisms able to tolerate stress represents an important parameter that differentiates early- and late-succession communities. In general, this strategy could translate into increased colonization potential considering that pioneer organisms must often possess heightened tolerance to environmental extremes [36] despite the potentially high energetic cost of tolerating environmental stresses [1]. Such waste appears not of immediate concern to r-type organisms and, in fact, is regarded as a normal facet of early succession colonization strategy [16, 29].

Pianka [31] described early succession environments as unstable favoring opportunistic assemblages of organisms (r-strategists), while late succession was characterized by relatively stable, specialized populations (K-strategists). In this study of a glacier forefield succession, we extended this characterization by showing that early- and late-succession soils were discernable based upon the response of the soil bacteria community and individual isolates to stimuli. We recognize that our analyses involve the representation of only a subset of the total microbial community. However, as significant advances in traditional ecology have been produced through studying subsets of "macro" communities, the study of microbial community subsets can provide meaningful information as well. Although it is currently impossible to sample the entire breadth of a complex community, this study has shown that coupling the two approaches and describing the agreements between them (e.g., the matching of soil-community and eco-collection phylotype DGGE bands) can provide an effective means of characterizing microbial community succession.

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