Communities that thrive in extreme conditions captured from a freshwater lake

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Abstract

Organisms that can grow in extreme conditions would be expected to be confined to extreme environments. However, we were able to capture highly productive communities of algae and bacteria, capable of growing in acidic (pH 2), basic (pH 12) and saline (40 ppt) conditions, from an ordinary freshwater lake. Microbial communities may thus include taxa that are highly productive in conditions that are far outside the range of conditions experienced in their host ecosystem. The organisms we captured were not obligate extremophiles, but were capable of growing in both extreme and benign conditions. The ability to grow in extreme conditions may thus be a common functional attribute in microbial communities.

Introduction

Microbial life is ubiquitous on the Earth's surface, yet we are only just beginning to understand even basic macroecological patterns for this large portion of the biosphere [1]. An ecosystem would not be expected to contain microorganisms capable of growing in conditions that are far outside the range of those available in that ecosystem. There are nevertheless three processes that might account, individually or in combination, for the occurrence of extremophiles in benign environments: **1)** these organisms disperse to the host ecosystem from extreme environments; **2)** the range of conditions available in their host ecosystem is actually larger than the range measured; **3)** the latent capacity to grow in these extreme conditions does not come at a large cost in terms of growth under average conditions in the host ecosystem.

The expectation that microorganisms capable of growing in extreme conditions will be found in all ecosystems due to dispersal from extreme environments is based on a classic hypothesis that everything will be found everywhere (the so-called "*Baas Becking hypothesis"* [2]), which has recently received support from studies investigating oceanic seed banks [3] and the widespread dispersal of thermophiles across cold seabeds [4]. However, dispersal between extreme environments appears to be very limited, and extremophile communities show clear patterns of geographic endemism [5,6].

Extremophiles may be found in most ecosystems if these contain extreme microhabitats, permanent or ephemeral, that are difficult to detect. For example, soils constitute a highly heterogeneous matrix, in contrast to aquatic ecosystems such as the one investigated in

48 this study, which may explain the large abundance of alkaliphiles [7] and halophiles [8]
49 in neutral and non-saline soils.
50
51 Facultative extremophiles may thrive in benign environments if the functional attributes
52 required to grow in extreme conditions come at little or no cost for growth in benign
53 conditions. These functional attributes may be either constitutively expressed or
54 available in the genome and expressed as a plastic response. Most described
55 extremophiles, however, are obligate forms that cannot grow or grow poorly in non-
56 extreme conditions (examples [9,10]).
57
58 Finding extremophiles and identifying the mechanism for their presence in benign
59 environments provides insight into our nascent understanding of microbial functional
60 biogeography [11] and, as extremophiles are used in a number of industrial processes
61 [12], may highlight the potential of benign environments for bio-prospecting. If the only
62 extremophiles present are obligate extremophiles because of a trade-off between growth
63 in extreme and benign environments, their presence would be due to dispersal or local
64 heterogeneity and they would be expected to be very rare. This potential rarity requires an
65 innovative approach for their enrichment. To find out whether an ordinary freshwater
66 lake contained extremophiles, we used a novel method to enrich organisms in acidic,
67 basic and saline conditions from this system. To measure the cost of the ability of
68 enriched communities to grow in extreme conditions, we measured the ability of these
69 organisms to grow in both extreme and benign conditions.
70

Methods

Enrichment using an amplifying bioreactor

The amplifying bioreactor (ABR, Supplemental Material [SM] 1) is a continuous-flow vessel akin to the chemostat [13], except that, in contrast to a chemostat, the ABR receives a constant input of organisms from the environment. An ABR will amplify even extremely rare taxa that can reproduce in the vessel faster than they are washed out. ABRs will potentially amplify any organism present in the source environment based on its rate and duration of operation (SM2).

We set up 4 ABRs fed from a small mesotrophic lake draining an enclosed watershed of protected old-growth forest (Lake Hertel, 0.34 km^2 surface area; 7 m maximum depth, average chlorophyll-a concentration of 2 µg/L [14], average pH is 8.4 ± SD 0.5, no detectable salinity 0.00 ppt, conductivity 80.9 ± SD 9.1 µS/cm [15]). Our ABRs were setup to enrich for micro-algae through the provision of light (continuous ~1000 Lux of white light), mineral nutrients (SM 3) and air. Micro-algae were targeted because their productivity could sustain a complex community of heterotrophs, and because extremophilic micro-algae have far ranging biotechnological applications [12]. Target treatment conditions (SM1) were control, acidic (addition of HCl to pH 2), basic (addition of NaOH to pH 12) and saline (addition of NaCl 40 ppt). Each 100 L ABR received lake water at an exchange rate of ⅓ of the volume per day. Temperature in the lake during the experiment was 18.7 ±SD 5.1 °C and in the amplifying bioreactors 18.9 ±SD 4.6 °C.

94

95 Isolation of communities

96 After 10 weeks, 50 mL samples were taken from the ABRs. A sample for benign
97 conditions was taken directly from the lake water inlet. These sampled communities were
98 maintained in batch cultures using aseptic techniques, shaken at 350 rpm under 1000 Lux
99 of continuous light at 20°C, by transferring 0.5 mL of inoculum into 50 mL of BBM
100 medium adjusted to the target treatment conditions every ten days. To ensure that the
101 organisms identified were growing in the treatment conditions rather than being
102 continuously brought in from the environment, community sequencing was performed
103 immediately following transfers to flasks ("before") and repeated after 1.5 years of
104 culture in the lab ("after") with the reciprocal transplants.

105

106 Reciprocal transplant assay

107 Flasks of each treatment condition were inoculated with each of the enriched
108 communities. Cultures were transferred once into replicate flasks in all treatment
109 conditions before measurements started. Optical density at 660 nm was recorded daily.
110 An exponential model was fitted to the first 8 days of optical density measurements for
111 the calculation of growth rate (optical density was not linked to abundance after 8 days).

112

113 Community characterisation

114 Community composition was established by amplicon sequencing using primers for 16S,
115 18S and 23S regions on 454 GS FLX Titanium platform (as per [16] and presented in
116 SM4).

Raw sequence data has been deposited in the ENA (http://www.ebi.ac.uk/ena/data/view/PRJEB10729). Data and analysis scripts are available at dryad.org (10.5061/dryad.42r9h [17]).

Results

Capture

All treatment conditions led to the capture of communities that were highly productive in their selection environment (Fig. 1 and SM5); average intrinsic growth rates of 0.42 [SD 0.03] day^{-1} is higher than that of algae being considered for industrial biomass production [18] and is comparable to the dilution rate of the ABRs.

Reciprocal transplant

With one exception, only communities that were selected for growth in an extreme environment (resident communities) grew in that environment (Fig. 1, mean difference in growth rate between residents and transplants = 0.49 day^{-1}, Tukey-HSD- $P<0.05$). The one exception was that the community selected in the basic environment and the community selected in the saline environment grew equally well in the basic environment (mean growth rate 0.60 [SD 0.05] day^{-1}, Tukey-HSD- $P= 0.999$). All selected communities grew as well in benign control environment as in their environment of selection (mean growth rate in benign environment 0.71 [SD 0.22] day^{-1}, Tukey-HSD- $P>0.05$) and all communities grew equally well in the benign environment ($F_{3,8}=3.568$, $P=0.125$).

Community characterisation

Each extreme environment enriched a different community (Fig 2, SM7). 61.5 % of OTUs enriched in the extreme environment were not detected in benign culture conditions or in the lake and are thus rare in the lake. In both saline and basic communities, the dominant autotrophs were chlorophytes of the genus *Chlorella* including *Chlorella variabilis* and *Chlorella sorokiniana*, and also contained Coccomyxaceae. The saline condition also contained a diatom in the family Thalassiosiraceae and the cyanobacterium *Synechococcus*, both known to contain marine species. In the acidic conditions, the dominant autotrophs were chlorophytes from the *Koliella*/*Pabia* clade, these two genera being closely related phylogenetically [19], the family Oocystaceae and the genus *Apatococcus*. Both *Chlorella* and *Koliella*/*Pabia* were also enriched by the benign conditions and detected in the lake sample.

Discussion

The lack of a strong trade-off between growth in the selected extreme conditions and growth in the benign environment indicates that none of the communities that thrived in extreme conditions were obligate extremophiles (Fig. 1). This may explain why the functional breadth of biodiversity held in a benign ecosystem includes the capacity to grow in extreme conditions. Although our findings do not preclude the presence of obligate-extremophiles in the lake, if present they have a lower fitness in the ABRs than the captured facultative extremophiles.

Some of the taxa captured by the ABRs include species known to have very wide functional breadth or contain extremophile species consistent with their enrichment conditions. However, currently documented functional breadth and plasticity of identified taxa are insufficient to explain growth in the conditions used or to explain the specificity of the capacity to grow in only a single extreme condition (SM7 for discussion of heterotrophs). Strains of *Chlorella* are known to grow across a wide pH range, from pH 3 to pH 10.5 at 25°C [20]. However, the communities assembled at high pH that contained *Chlorella* grew poorly, if at all, in acidic conditions (Fig. 1 and 2). The salt tolerance of *Chlorella* varies among species and even strains [21]. Some species can grow between 10 to 50 ppt [22], whereas *Chlorella sorokiniana,* which is found in our saline communities, is inhibited by salt concentrations as low as 11 ppt although it can grow in concentrations as high as 26 ppt [23]. Many of the enriched organisms may depend on ecological interactions for survival, including heterotrophic consumption of algal exudates, and some of the organisms we found, such as the Rickettsiales, may be endosymbionts protected from extreme conditions by living within a host [24].

The treatments used in this experiment are all forms of ionic stress so that the strong trade-offs detected between the ability to grow in different treatments were not necessarily expected. Though we did not specifically enrich in combinations of stressors that would benefit poly-extremophiles, the existence of a strong trade-offs between treatments suggests that poly-extremophiles are outcompeted when a single stressor is applied. The physiochemical boundaries of life may be different when extremes are imposed separately or in combination [25].

186

187 The ability of ABRs to sort extremely large and diverse communities efficiently suggests
188 that the systematic deployment of ABRs would allow us to probe the functional breadth
189 of biodiversity held in a range of ecosystems and to describe the biogeography of
190 extremophiles [11]. Finding organisms that can thrive in extreme conditions in an
191 ordinary lake suggests that organisms of biotechnological importance may even be found
192 in a backyard pond.

193

194

195 **Authors' contributions**

196 ELD carried out field/lab work, data analysis, led the design of the study and drafted the
197 manuscript; AJD carried out the sequencing analysis; GB and GFF contributed to the
198 design of the study. All authors contributed to the editing of the manuscript and gave
199 final approval for publication and all authors agree to be held accountable for the content
200 of the article.

201 **Competing interests**

202 We have no competing interests.

203 **Ethical statement**

204 None of the activities described in this article required ethical approval.

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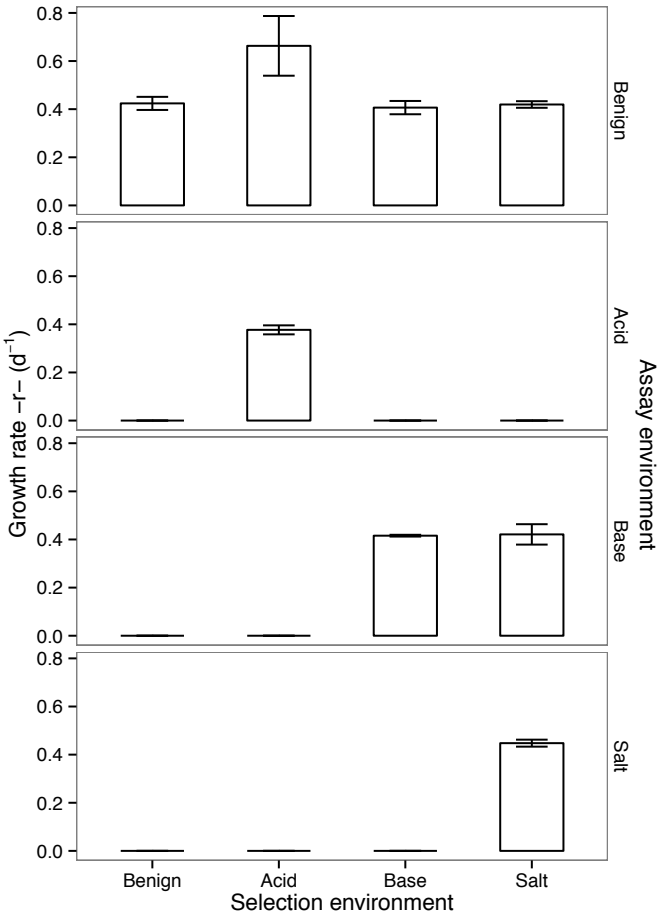
Figures and Figure Legends



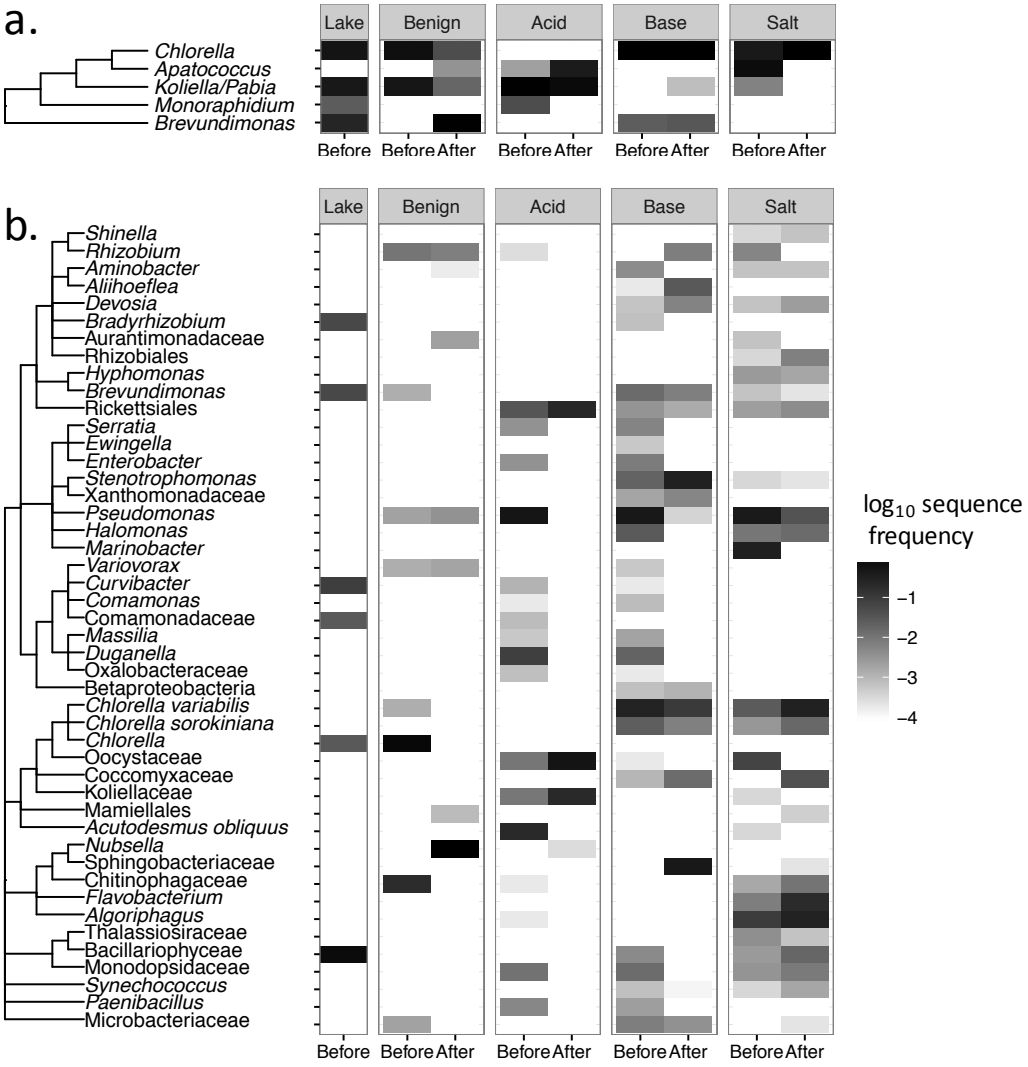
215

216 **Figure 1:** Growth rate of enriched communities in the reciprocal transplant assay (-*r*-

217 day^{-1}, error bars: 95 % confidence interval).

218

219



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221 Figure 2: Taxonomic composition (SM7) using primers a. 23S and b. 16S. Tree indicates

222 taxonomic relationship; labels are name of highest identifiable taxonomic level.

223

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