Rapid evolution drives increased function of a complex microbial community

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Abstract

Evolution occurring over ecological time scales can affect community functions both directly and indirectly, via changes in community composition. This interplay between ecology and evolution makes identifying the role of rapid evolution in shaping the functioning of complex natural communities challenging. We used a methanogenic community from an anaerobic digester to investigate the potential importance of rapid evolution in shaping community methane production. To disentangle evolutionary and ecological processes, we pre-adapted the community to a novel feedstock in laboratory anaerobic fermenters for 6 weeks and then inoculated 1% back into the ancestral community. This allowed the introduction of any within-taxa genetic changes that occurred over the pre-adaptation period, while minimally altering the taxonomic composition of the community. The addition of the pre-adapted inoculum increased biogas production by ~10% over 6 weeks compared to a control treatment without the addition, with the corresponding minor changes in community composition unable to explain this increase. These findings suggest that rapid adaptive evolution can play an important role in shaping the structure and function of natural microbial communities and provide a novel approach to enhancing microbial community functions.

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Abstract

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To disentangle evolutionary and ecological processes, we pre-adapted the community to a novel feedstock in laboratory anaerobic fermenters for 6 weeks and then inoculated 1% back into the ancestral community. This allowed the introduction of any within-taxa genetic changes that occurred over the pre-adaptation period, while minimally altering the taxonomic composition of the community. The addition of the pre-adapted inoculum increased biogas production by ˜10% over 6 weeks compared to a control treatment without the addition, with the corresponding minor changes in community composition unable to explain this increase.

These findings suggest that rapid adaptive evolution can play an important role in shaping the structure and function of natural microbial communities and provide a novel approach to enhancing microbial community functions.

Main Text

Introduction

Evolution occurring over ecological time scales can potentially affect community functions both directly and indirectly; the latter via changes in composition . Community composition and function in turn shapes evolution . Disentangling the effect of evolution from purely ecological dynamics on community function therefore requires manipulation of evolutionary changes independently of ecological changes. In simple laboratory communities of readily culturable taxa, this can be achieved by evolving taxa within communities, and then comparing the function of evolved and ancestral communities reassembled to have the same starting taxonomic composition . This approach is however impractical for most natural communities, where there is extensive diversity and many taxa are hard to culture in isolation. An alternative approach involves a degree of separation of evolutionary and ecological processes in time. Community members can be pre-adapted for different amounts of time, added back into a community and the compositional and functional consequences determined (14). The pre-adaptation step will likely result in changes in community composition, which might then influence the composition of ancestral community to which it is returned. The impact of this can be greatly minimised by the addition of only very small amounts of the pre-adapted community.

We used this approach to determine the importance of rapid evolution on the composition and function of a methanogenic community. Methane production, which is of both major environmental and biotechnological significance, is the result of interactions between many different community members, with the breakdown of complex polymers to methane involving a number of biochemical steps carried out by different taxa. This diversity makes it a parsimonious system to investigate the potential role of rapid evolution, as large amounts of ecological trait variation are predicted to impede rapid evolutionary change . Furthermore, methane production typically increases rapidly during propagation on a novel feedstock , providing an unambiguous community level phenotype for which the role of rapid evolution can be determined. We initiated 12 replicate laboratory fermenters using a methanogenic community isolated from an industrial anaerobic digestor. These communities were "pre-adapted" for 6 weeks, while the ancestral community was kept at 4°C to minimise any compositional and evolutionary changes. The pre-adapted communities were then inoculated at a low frequency (1%) into new reactors set up from the ancestral community ("adaptation" treatment), and gas production and composition over 6 weeks compared with "control" reactors initiated with the ancestral community only (Fig. 1).

Results

Gas Production

Total gas production over the 6 week-period was approximately 10% greater in adapted communities compared to both control and pre-adapted communities (Figure 2A, one-way ANOVA, $F_{(2,32)}=12.9, P$ value <

.0001, Tukey HSD: Pre-adapted – Adapted, mean = 353.7, mean difference 95% CI [-529.8, -177.5], p<0.001, Control - Adapted, mean = 245.9, mean difference 95% CI [-422.0, -69.7], $P = 0.005$). The effect of community treatment on gas production varied over time (Figure 2B, LMM; effect of time × adaptation treatment on gas production: $\chi^2_{(10)} = 66.03$, P value < 0.001), with gas production being significantly higher in the adapted communities from 3 weeks onwards (see Supplemental Table 1 for contrasts). This delay in the increase in gas production argues against the possibility that gas production increased in the adapted communities because of physiological or nutritional changes resulting from the 1% addition of the pre-adapted communities. Instead, the delay suggests community members introduced from the pre-adapted communities that contributed to higher gas production increased in frequency from rare.

Compositional changes

We next explored if the increased gas production could be explained by compositional differences resulting from the addition of the pre-adapted inocula. At the end of the experiment, community composition had changed in parallel across both the control and adaptation treatments (and the pre-adaptation source communities for the adaptation treatment), indicative of strong selection associated with the transition to a novel environment. There was a 60% drop in the mean number of taxa detected per community, compared to the ancestral community, but we did not find a significant difference in diversity loss across treatments (one-way ANOVA on ASV reduction from the ancestral sample; $F_{(2,32)}$=2.93, P value = 0.07; Figure 3A). In particular, there were reductions in the abundance of taxa belonging to the Firmicutes, Proteobacteria, Spirochaetes and Tenericutes.

Despite these parallel changes, treatments differed in terms of their community composition (Figure 3B, PERMANOVA on Bray-Curtis dissimilarities: $F_{(2,32)} = 15.8$, $R^2 = 0.49$, Unweighted UniFrac: $F_{(2,32)} = 5.6$, $R^2 = 0.26$, Weighted UniFrac: $F_{(2,32)} = 26.9$, $R^2 = 0.63$, p-value = 0.001 and, 999 iterations per test in all cases), with the frequency of methanogens, the organisms directly responsible for methane production, remaining constant across treatments. Most notably, control samples had a higher fraction of Bacteriodia and Gamma-proteobacteria compared to both the Pre-Adaptation and Adaptation samples (Figure 4). We note however that the magnitude of compositional differences between control and adapted communities are similar to that observed between pre-adapted versus control communities, which did not differ in gas production.

The compositional changes and associated increases in gas production resulting from the addition of the pre-adapted inocula could have arisen through selection acting on novel ecological composition (ecology), genetic variants (evolution) or both. Given that directly attributing causal changes in composition and function to evolution in such complex communities is not possible, we instead sought to determine if ecological processes were sufficient to explain these changes. First, our experimental design - adding only 1% of the pre-adapted communities - should have had only a minimal direct impact on the ecology of the recipient communities. To confirm this, we simulated the addition of 1% of the pre-adapted communities to control communities and unsurprisingly found that this caused non-significant changes in community composition (adonis2, $F_{1,23} = 0.017$, $R^2<0.001$ $P = 1$). Furthermore, adapted communities were no more similar to directly linked pre-adapted communities, compared to unlinked (randomly selected pre-adapted community) or control communities (Fig. 2C).

Previous work has shown that rare taxa can play an important role in methane production in methanogenic and other communities . As such, while adding only 1% of the pre-adapted community had no detectable direct impact on community composition, it could have had a direct effect on gas production if functionally important rare taxa increased in frequency by orders of magnitude during pre-adaptation. To investigate this possibility, we first identified taxa that had significantly greater abundance in adapted versus control communities, as their presence could explain between-treatment differences in gas production. Next, we determined whether any of those taxa increased between the start (i.e., the ancestral community) and end of the pre-adaptation treatment to an extent where they could have enriched the adaptation treatment. Using this two-pronged approach, we found one candidate taxon, belonging to the Ruminococcus genus (Table 1) – this taxon had a higher frequency in the adaptation versus control treatment and was enriched over the

course of the pre-adaptation treatment. It is however highly unlikely that this organism could explain the difference in gas production between control and adaptation treatments: it was only detected in a subset of replicates in the adaptation treatment (10 out of 12), so its presence cannot contribute to all observed increases in gas production. The taxon is also present in the pre-adaptation treatment (in 6 replicates) and we did not find a significant difference in its abundance when comparing pre-adapted versus adapted communities ($t_{(21)}$ = -0.21; $P = 0.84$, means of 92.8 for pre-adaptation and 104.5 for adaptation treatment, 95% CI -138.1 to 114.1). Despite that, there is a significant difference in gas production between those two treatments.

Discussion

Here, we attempted to determine the role of very rapid (co)evolution of taxa within a complex methanogenic community in shaping community methane production. Methane production was increased by the addition of a pre-adapted community, and this increase could not be explained by direct changes in community composition.

Taken together, our findings data strongly suggests that only rapid evolution, i.e within taxon variation, can explain the observed increase in gas production, either directly, or indirectly through the resultant changes in community composition. We emphasise that taxa here are defined as 16S rRNA Amplicon Sequence Variants with <99% similarity in the 254 bp V4 region of the gene. Within-ASV variation, on which selection acted, may have been standing variation or have arisen*de novo* by mutation or horizontal gene transfer, including infection by mobile genetic elements. Unfortunately, current sequencing and analytical approaches preclude identification of anything but a tiny subset of genetic changes associated with microevolution within such complex communities in the absence of target genes or multiple reference genomes of each taxon , so we are unable to directly quantify the extent of molecular evolution of individual community members.

It is unclear how far our results are generalisable to different systems. Rapid evolution could potentially play an even more important role in high nutrient, aerobic environments, where microbial population growth rates are much faster because of greater energy availability . That said, we used this experimental system because we anticipated rapid evolution would play a particularly important role in altered community functions. Methanogenic communities have a linear biochemistry, funneling all the substrates to methane, and as a consequence selection on many taxa likely acts in the same direction to ultimately increase methane production, especially when a community is introduced to a novel environment .

Irrespective of whether our findings are limited to methanogenic communities, our results have relevance for understanding how methane production may change in the face of environmental change. In addition, from a more practical point, our novel approach could be used to enhance industrial biogas production by, for example, pre-adapting industrial communities to a feed shift. If our findings can be confirmed in other non–AD systems, it opens doors for further applications, including pre-adaption of gut microbiomes to help coping with gut-related illnesses, rather than recruiting already adapted species by random processes like fecal transplant , as a tool in harnessing rhizosphere microbes to improve agricultural yields or to study the impact of rapid evolution on adaptation to the warming planet .

Materials and Methods

Biological material

Biological material used in the experiment was collected from an Anaerobic Digester plant in South West region of UK. After being collected, the material was stored at 4°C for the duration of the experiment.

Experimental design

Communities were cultured for eight weeks in 500 ml bottles (total volume with headspace: 600 ml, Duran) using Automated Methane Potential Test System (AMPTS II, Bioprocess Control Sweden AB to measure CO_2-stripped biogas (called biogas in this paper) production.

Each replicate was started using 300 mL of AD sample. The communities were put into the fermenters in full carrying capacity. Communities were acclimatised in the fermenters for a week in order to make sure any residual biogas production did not obscure the final results. Starting with the day one of the second week, each community was fed 2g of feed composed of 3.53% casein, 1.17% peptone, 1.17% albumen, 47.07% dextrin and 47.07% sucrose (all compounds – Sigma). Feed was suspended 1:10 in sterile water. At the start of the experiment the community was supplemented with 0.3 mL 1000x Trace Metal Stock (1 gl^{-1} $FeCl_2$. $4H_2O$, 0.5 gl^{-1} $MnCl_2$. $4H_2O$, 0.3 gl^{-1} $CoCl_2$. $4H_2O$, 0.2 gl^{-1} $ZnCl_2$, 0.1 $gl^{-1}NiSO_4$. $6H_2O$, 0.05 gl^{-1} Na_2MoO_4 . $4H_2O$, 0.02 $gl^{-1}H_3BO_3$, 0.008 $gl^{-1}Na_2$ WO_4 . $2H_2O$, 0.006 gl^{-1} Na_2SeO_3 . $5H_2O$, 0.002 gl^{-1} $CuCl_2$. $2H_2O$).

The experiment (see Fig. 1) was conducted in two stages, with three treatment regimens (12 replicates each): Stage one, where we cultivated 12 replicates of the community ('pre-adaptation') and stage two – testing the pre-adaptation success – where we cultivated 12 replicates of the original community and 12 replicates of the original community supplemented with 1% (w/w) of the pre-adapted community from stage one. The second stage was started a week before the first was finished to starve the communities for a week, such that any residual carbon sources that could be metabolised, were metabolised and did not interfere with the later gas measurements. After a week of pre-incubation, 1% (w/w) of the communities from the first stage were transferred to the adaptation treatments and 1% w/w of stock community was transferred to the control treatment.

Measuring methane content of biogas

All resulting lab-scale reactors inoculated with the samples were monitored for six weeks after the first feeding at 37°C using an AMPTS II (BPC Instruments) system that measures the volume of biogas stripped from CO_2 using sodium hydroxide. We confirmed that the resulting stripped biogas was >95% methane by comparing the composition of the produced biogas pre- and post-stripping using GC-MS (Agilent, 7890A) and comparing these with a standard curve made using methane standard (Sigma).

DNA extractions

The DNA was extracted using FastDNA SPIN Kit for Soil (MP). For the qPCR, the 1:10 and 1:100 dilutions of the samples were used for extraction. The quality and quantity of the extractions was confirmed by 1% agarose gel electrophoresis and dsDNA BR (Qubit) respectively. The extracted DNA was used later to determine the cell densities using qPCR and for sequencing.

Amplicon library construction and sequencing

For the DNA extractions we used DNeasy PowerSoli kit (Qiagen). 16S rRNA gene libraries were constructed using primers designed to amplify the V4 region (Supplementary Table S1) and multiplexed. Amplicons were generated using a high-fidelity polymerase (Kapa 2G Robust) and purified using the Agencourt AMPure XP PCR purification system and quantified using a fluorometer (Qubit, life technologies). The purified amplicons were then pooled in equimolar concentrations by hand based on Qubit quantification. The resulting amplicon library pool was diluted to 2 nM with sodium hydroxide and 5 µl transferred into 995 µl HT1 (Illumina) to give a final concentration of 10 pM. 600 µl of the diluted library pool was spiked with 10% PhiX Control v3 and placed on ice before loading into Illumina MiSeq cartridge following the manufacturer's instructions. The sequencing chemistry utilised was MiSeq Reagent Kit v2 (500 cycles) with run metrics of 250 cycles for each paired end read using MiSeq Control Software 2.2.0 and RTA 1.17.28. One of the Pre-adaptation samples (P12) failed in the sequencing run.

Analyses of sequenced samples

Sequencing data were analysed in R (v 3.3.2) using the packages *'dada2'* , '*phyloseq'* , and '*vegan* ' . Using a full-stack workflow we estimated the error rates, inferred and merged sequences, constructed a sequence table, removed chimeric sequences, and assigned taxonomy to each amplicon sequence variant (ASV). Greengenes database provided the taxonomy information based on a previously published pipeline . The phylogenetic

tree was estimated using the R package '*phangorn* ' . We constructed a neighbour-joining tree, and then fit a Generalized time-reversible with Gamma rate variation maximum likelihood tree using the neighbour-joining tree as a starting point. We used UniFrac distance (weighted and unweighted) and Bray-Curtis as our measures of compositional dissimilarity . In case of alpha diversity, we calculated species richness and evenness .

Data Analyses

For all further analyses, we used *R* Version 4.0.3 (R Development Core Team; http://www.r-project.org). In general, models were compared by sequentially deleting terms and comparing model fits using F-tests or χ^2-tests (where appropriate), after which pairwise contrasts were computed using the ' emmeans' packages , with $\alpha < 0.05$. We checked residual behaviour using the 'DHARMa' package . All plots were produced using the 'ggplot2' package .

To test for the effect of evolution treatment on temporal changes in gas production, we used a linear mixed model (LMM) with treatment $\times$ time as fixed explanatory categorical variables, as well as their interaction. To account for non-independency of observations over time, we fitted random intercepts for each reactor. Based on the obtained simulation-based residual plots, we included a dispersion parameter for levels of treatment and time, using the *'glmmTMB'* function in the*'glmmTMB'* package . Based on the full model, we calculated pairwise treatment contrasts for each week, adjusted for multiple testing using the 'tukey' method in the emmeans package.

To look at the impact of the 1% transfer on ecological changes, we looked at (1) dissimilarity of linked pre-adapted versus adapted communities (i.e. those that received 1% enrichment from a linked pre-adapted community, n = 12 samples), (2) dissimilarity of pre-adapted and adapted communities that were not directly linked through 1% transfer (n = 132 non-linked samples) and (3) dissimilarity of the pre-adapted versus control communities (n = 144 samples).

We also simulated potential impact of the 1% enrichment by in silico adding a 1%-rarefied pre-adaptation samples to the control treatments and comparing their composition with the original control samples via PERMANOVA (adonis).

To determine which taxa differed in abundance in the control versus evolution treatments, we fitted a negative binomial GLM to the sequencing data using the '*DESeq'* function in the R package '*DESeq2* ' . Focussing on the 100 most common taxa, we calculated significant differences in the abundance of taxa using Wald tests and corrected P –values for multiple testing using the 'frd' method.

Acknowledgments

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References

Figures and Tables

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Figure 1. Experimental design. Communities were kept in the fermenters for one week without feeding prior to the start of the experiment to remove any residual gas production. DNA was isolated at the start (Week 0 for Pre-Adaptation treatment (grey) and 6 for Adaptation (Blue) and Control (Red) treatments, respectively) and end (Week 7 and 13) of each treatment. Arrows on top of bottles indicate times communities were fed.

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Figure 2.

(A) Cumulative gas production over a 7-week period, with treatments depicted in different colours. Significance estimated using one-way ANOVA with Tukey HSD contrast and (B) Temporal changes in gas production (mean +/- SE) across treatments. After 3 weeks, Adaptation samples showed increased gas production.

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Figure 3.

Alpha Diversity of each treatment at end point and start point (P_{start} and A/C_{start}) (A); NMDS plot displaying Bray-Curtis dissimilarity (stress = 0.04) (B); Bray Curtis dissimilarity difference between samples from linked and unlinked pre-adaptation and adaptation treatments, and between adaptation and control samples (C).

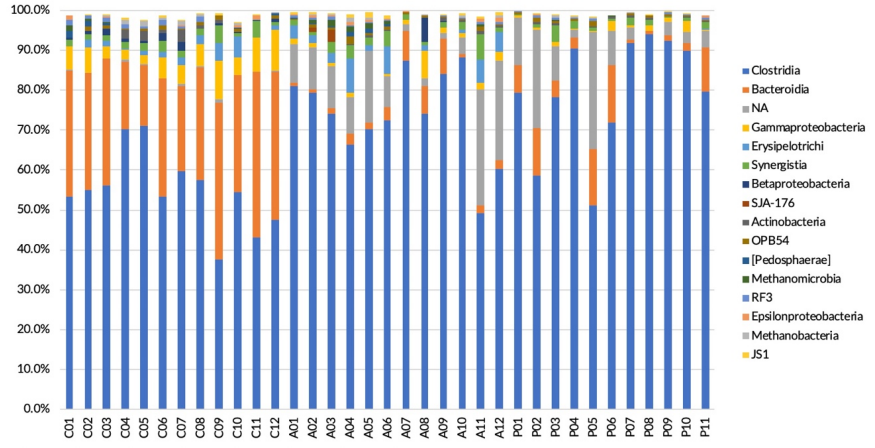

Figure 4.

Composition of the communities at class level, only classes present at over 1% in at least one replicate indicated. C – control treatment, A – adaptation treatment, P – pre-adaptation treatment. Numbers indicate the replicate number of the sample.

Table 1. Taxa showing taxa with a significant increase in abundance in the adaptation versus control treatments, as quantified using the DESeq package. Last column estimates the fraction of total reads in C_{Start} that 1% addition of P_{end} sample introduces for each of those taxa.

Species	log2 Fold Change Adaptation vs Control	Counts in P_{Start}	Count in C_{Start}	Mean Counts
Unknown Firmicute	10.5	211	below detection	363
Ruminococcus species	8.9	below detection	below detection	101
Unknown Bacterium	7.9	37	8	16
Defluviitalea saccharophila	7.5	1872	below detection	1077
Defluviitalea saccharophila	7.3	1577	below detection	1227
OPB54	6.3	30	47	1.6
Unknown Firmicute	6.2	183	56	346