Attempts to cultivate bacteria from deep subsurface aquifers and mountaintop plant communities

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Abstract
In the late 1990s, the limits of life were pushed even further when microorganisms were discovered thriving 2.5 km below the surface of the Earth in deep South African gold mines. These very simple communities were dominated by a single species of bacteria from within the phylum, Firmicutes. *Desulforudis audaxviator* remains unique to a sizeable portion of the South African deep subsurface. At depths below 2.5km, it comprises well over 99% of all organisms present, which presents a unique circumstance in which the environment has provided a natural pure culture. From this naturally occurring pure culture, environmental genomics was applied to obtain the complete *D. audaxviator* genome and thus its biological functions were established. This presents a unique opportunity to now attempt to grow a previously uncultured organism using its genome as a road map to design a specific cultivation approach for *D. audaxviator*. The genome combined with precise chemical analysis of its native environment has yielded invaluable insights such as the organism’s ability to form spores, to reduce sulfate, to fix nitrogen and use ammonia, along with many other unique traits all of which will lead to successful cultivation. Here we describe the genome-enabled cultivation of this to date uncultured microorganism.
Attempts to Cultivate Bacteria from Deep Subsurface Aquifers and Mountaintop Plant Communities

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INTRODUCTION

In the late 1990s, the limits of life were pushed even further when microorganisms were discovered thriving 2.5 km below the surface of the Earth in deep South African gold mines (1). These very simple communities were dominated by a single species of bacteria from within the phylum Firmicutes. Desulfurococcus autotrophicus remains unique to a small portion of the South African deep subsurface. At depths below 2.5 km, it comprises over 99% of all organisms present; essentially a natural pure culture. An environmental genomic approach was applied by collaborators to obtain the complete D. autotrophicus genome (Figure 1) (1) and then its biological functions (Figure 2) (1) were established. The genome, combined with chemical analysis of its native environment, has yielded valuable insights informing potential cultivation strategies. These include the ability to form spores, reduce sulfur, and utilize formate or CO2 as carbon and energy sources. Here we describe an attempt to perform genome-enabled cultivation of this to date uncultured microorganism.

MATERIALS & METHODS

Basal Media Preparation

Mean concentrations of major ions (1) from boreholes where D. autotrophicus was previously identified (1) were utilized to develop a basal medium. Six variations were prepared: three with FeSO4 as an M3 source and three without. Formate and acetate or formate plus acetate acids were utilized as potential C sources or sources of formate (Table 1). Lysine, HCl and urea were added and pH adjusted to 8.6. Media were dispensed into Batch tubes and autoclaved. The tubes were then immediately placed into a Coy Type B anaerobic chamber maintained with N2, CO2, H2 (70/20/10), and sealed with butyl rubber stoppers.

Table 1: Basal media variations and gas mixtures

<table>
<thead>
<tr>
<th>Ionic Medium</th>
<th>Gas Mixture</th>
<th>H2 (% N2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES+KH2PO4</td>
<td>70/20/10</td>
<td>0.3</td>
</tr>
<tr>
<td>Lysine+HCl</td>
<td>70/20/10</td>
<td>0.3</td>
</tr>
<tr>
<td>Urea+HCl</td>
<td>70/20/10</td>
<td>0.3</td>
</tr>
<tr>
<td>Formate+HCl</td>
<td>70/20/10</td>
<td>0.3</td>
</tr>
<tr>
<td>Formate+Acetate</td>
<td>70/20/10</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Inoculation

Six separate samples of African mine borehole water, previously confirmed to contain D. autotrophicus spores and cells, were chosen as inocula for each variation of the basal medium. Aliquots of each sample were heat shocked at 80°C for 10 min before inoculation. Four additional series of inoculations were included with 55% M3, as in the headline (Table 1). The inoculated tubes were incubated in the dark at 50°C.

RESULTS

- No definitive growth as of yet.

Table 2: D. autotrophicus Clones

<table>
<thead>
<tr>
<th>Name</th>
<th>Sample</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>MSP106</td>
<td>11g</td>
</tr>
<tr>
<td>B</td>
<td>MSP123K</td>
<td>11g</td>
</tr>
<tr>
<td>C</td>
<td>EV1816K</td>
<td>11g</td>
</tr>
<tr>
<td>D</td>
<td>EV1816K</td>
<td>11g</td>
</tr>
<tr>
<td>E</td>
<td>EV1816K</td>
<td>11g</td>
</tr>
<tr>
<td>F</td>
<td>VRN505C1</td>
<td>11g</td>
</tr>
</tbody>
</table>

* Tangential flow filtration concentrate.
** As judged by viable turbidity

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REFERENCES


MOUNTAIN METHYLOBACTERIA

INTRODUCTION

Methylobacterium are a genus of facultative methylo trophic bacteria, capable of surviving off the methane emitted from the drums on the surface of leaves. These bacteria are commonly referred to as PFPEs (pink/pigmented facultative methylo trophic bacteria). Identification of a distinct pink pigmen that produces (1). Esterolate plants from Buzona Peak, 16,230 ft, 1998 m in the spring mountainous peaks of NV were collected to determine if specific plant species have unique pink pigmentation populations.

RESULTS

Leaves taken from Buzona Peak plants were macerated using a steel tissue grinder and a 1x phosphate-buffered homogenate was streaked over isolation cold, defined media with 0.075% methanol and added to sole C source. After incubation at room temperature for 4 weeks, conspicuous pink colonies were further purified by transfer to fresh plates.

DISCUSSION / FUTURE WORK

- D. autotrophicus may have a doubling time of ~400 years.
- D. autotrophicus may be an obligate aerophile.
- Mutations on media could be developed.
- Culturing of this organism would enable experiments relevant to emerging fields such as astrobiology.

REFERENCES