Identifying Novel Archaeal Species From Anaerobic Digester Isolates

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Abstract

Methanogens are members of the domain archaea that live in anaerobic conditions and grow using substrates including carbon dioxide, hydrogen, methanol, and acetate. They are important members of the global methane cycle, contributing between 350 and 420 Tg of methane per year to the atmosphere, and can be used as sources of renewable energy. Additionally, methanogens are present in anaerobic digesters, which break down organic wastes to produce methane which can be harvested for energy.

The Buan lab has isolated 26 strains from anaerobic digester and manure samples. 16S subunit DNA sequencing, which targets the highly conserved gene for the 16S subunit of ribosomal RNA, can be used for the identification of the species. Through DNA sequencing of their genomes 16S region, the methanogens in these cultures can be identified and placed in a phylogenetic tree so their relationships to each other and their evolutionary history can be better understood.

Background

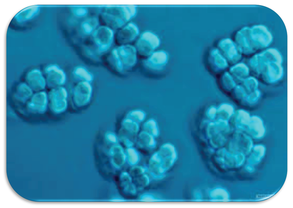


Fig. 1 *Methanosarcina acetivorans.* Methanogens can be found growing in many anaerobic environments, including anaerobic digesters, ocean sediment, and the digestive systems of many animals, including humans. The methane produced as they grow could become an important source of renewable energy.

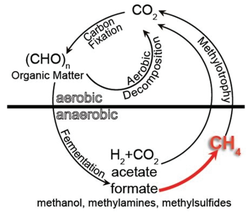


Fig. 2 Methanogens play an important role in the global carbon cycle as they break down metabolic products, such as acetate, of other organisms. In turn, methylotrophs feed off of the methane produced by methanogens. For every mole of substrates consumed, methanogens produce less than two moles of ATP.

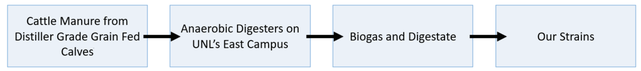


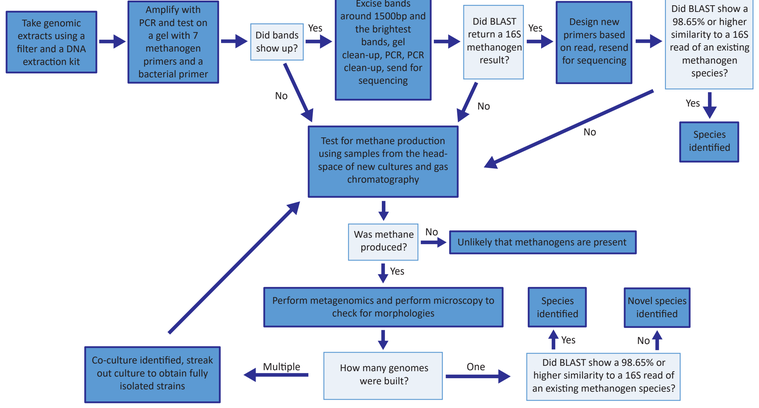
Fig. 3 Our strains were obtained from anaerobic digesters on UNL's East Campus. The digesters were fed with manure from cattle who were fed distiller grade grain. Digesters were run for 42 days at 100˚C. Digestate was passaged twice into low-salt formate/acetate media then plated and streaked to obtain cultured isolates.

Research Questions

- What species of archaea are present in cultured isolates from anaerobic digester samples?
- Are the isolates axenic? If not, which species can be found growing together?
- How are the identified species phylogenetically related?

Methods

Project Overview



Extraction

- Strains were grown anaerobically in balch tubes and serum bottles
- Cultures were filtered to maximize the number of cells available for genoimc DNA extraction due to the low density the cells grow to in the low-salt formate/acetate media
- Genomic DNA was extracted using the PureLink Genomic DNA Mini kit

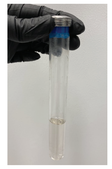


Fig. 4 A balch tube filled with media used for the growth of our isolates.

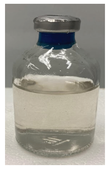


Fig. 5 A serum bottle filled with media used for the growth of our isolates.

Fig. 6 Clamped vacuum filter holder used to collect cells on a 0.2 micron filter.

16S rRNA Identification

- Seven different primers designed for various archaea and methanogen genera were tested with each sample to see which amplified the 16S rRNA gene best
- Potential 16S bands were excized with sterile technique and a gel clean-up was performed
- Products were amplified again using the same primers and purified using a PCR clean-up kit

Strain	Primer 1	Primer 2	Primer 3	Primer 4	Primer 5	Primer 6	Primer 7
277	1	2	2	1	3	1	6
278	1	1	2	1	2	1	5
279	0	0	0	1	1	1	0
280	1	3	4	0	4	1	6
282	0	2	2	1	1	1	5
283		3					
284	2	1	3	1	2	2	3
285	1	3	0	0	1	2	1
286	3	5	3	1	2	3	6
290	1	2	0	1	1	1	6
292	1	2	0	1	3	2	7
293	1	1	1	0	1	1	3
295	1	1	5	1	3	1	9
345	1	2	4	1	4	1	4
380	1	2	3	1	1	1	4
381	1	1	3	0	1	1	5

Fig. 7 Primers that showed amplification for each strain are shown highlighted in blue. The numbers in the highlighted boxes represent the number of bands that were amplified, and red circles enclose the primers used to characterize the identified strains.

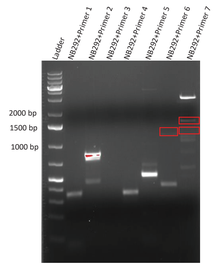


Fig. 8 NB292 was tested with multiple primer sets to find amplification around 1500bp. Excized bands are outlined in red. Primers 1-7 are designed for 16S amplification of *methanosarcinales*, archaea, *methanococcales, methanobacteriales, methanomicrobiales, methanosarcinaceae,* and *methanosaetaceae* respectively.

Sequencing and Microscopy

- Samples were sent for Sanger sequencing, and results were analyzed using BLAST
- New primers were designed for those reads which showed less than a 98.65% identity to an existing archaeal species
- Microscopy was used to verify the presence of methanogens

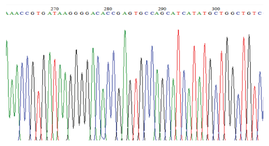


Fig. 9 Partial sequencing results of NB280.1.

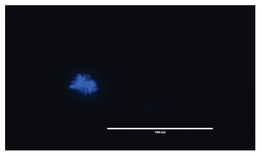


Fig. 10 Microscopy of NB295. Methanogens fluoresce blue under certain light due to the cofactor F420.

Contamination

- Several sequencing reads returned as *E. coli* genomic DNA, yet *E. coli* should not be able to grow in our media
- Through negative controls, the presence of *E. coli* contamination was confirmed
- The contamination was traced to the DNA extraction kit being used
- The identities of the methanogens found remain unaffected, but an extra emphasis on running controls is being placed on the project for its remainder

Conclusions

Identified Strains

Species (≥ 98.65% Identity)

Strain	Species
NB295	*Methanobacterium formicicum*
NB280	*Methanosarcina mazei*
NB277	*Methanobacterium subterraneum*
NB286	*Methanoculleus marisnigri*

Genera (≥ 94.50% Identity)

Strain	Genera
NB283	*Methanoculleus*
NB292	*Methanobacterium Methanosarcina*
NB277	*Desulfovibrio*

16S Phylogenetic Tree

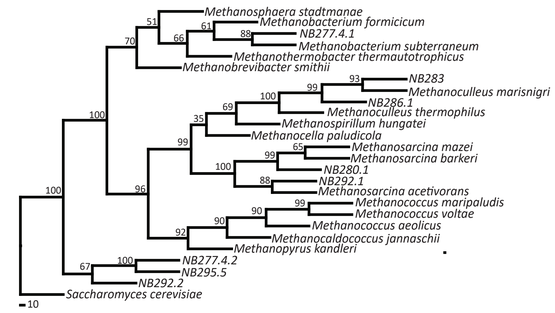


Fig. 11 The values on the tree show the number of times the shown branches occured out of 100 trees. The tree was created using the neighbor joining method with identified strains and sequences pulled from GenBank.

- NB277 shows a co-culture of a methanogen and sulfate-reducing bacteria
  - Sulfate-reducing bacteria outcompete methanogens on acetate, formate inhibits bacterial reducing abilities
- NB292 shows a co-culture of methanogens, potentially with each growing off of only one available substrate
  - *Methanobacterium formicicum* utilizes formate and acetate to grow
  - *Methanosarcina mazei* utilizes only acetate
  - *Methanobacterium subterraneum* utilizes only formate
- A phylogenetic tree reinforces the identification of NB277 as *Methanobacterium subterraneum*, of NB283 as *Methanoculleus*, and NB292 as *Methanosarcina*

Future Directions

- Continue to culture the isolates, test primers with samples, and analyze sequencing results
- Test cultures for methane production
- Perform metagenomics on cultures that do not share a 98.65% or greater identity with an existing species

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