Genomic Analysis of *Acetoanaerobium noterae* VLB-1

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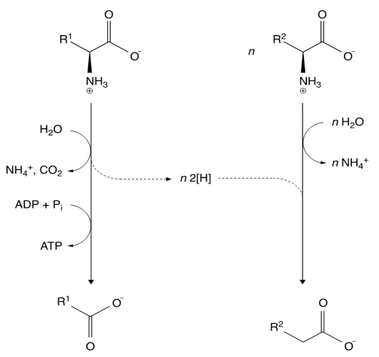
Abstract

Genomic and physiological characteristics of an anaerobic, environmental bacterial isolate, *Acetoanaerobium* sp. strain VLB-1, were determined from the assembled annotated 2.57 megabase-pair draft genome. Strain VLB-1 was isolated from an anaerobic, alkaline, saline methanogenic enrichment initiated from soils collected from the Eastern Saline Wetlands in Lincoln, NE. With this isolate, an investigation into elemental and amino acid cycling via the Stickland reaction and the Wood-Ljungdahl pathway was conducted to determine possible metabolic products. The Stickland reaction is a relatively newly discovered pathway, observed in the genus *Clostridium*. *A. sticklandii* is the main model for this method of anaerobic amino acid fermentation and the new way to generate energy. The genome of our isolate was sequenced, using long read sequencing techniques from Novogene with a goal to close the genome for a complete reading. The genome had a close relation to *Acetoanaerobium sticklandii* strain DSM 519 and *Acetoanaerobium noterae* strain NOT-3, each with a similarity of 98.48%. With the analysis of the genome performed, the organism appears to use the Stickland reaction to oxidize amino acids and the Wood-Ljungdahl pathway to fix carbon, which is characteristic of many *Acetoanaerobium* and *Clostridium* species.

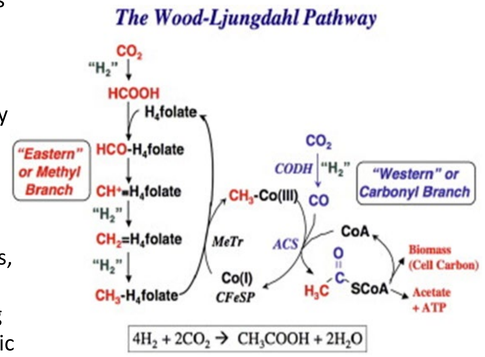
Background



General mechanism for Stickland fermentation reaction.

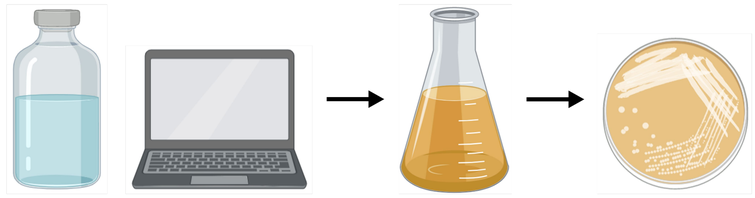
The Wood-Ljungdahl pathway is also invaluable to many Acetoanaerobium spp. to produce acetic acid via acetogenesis. In this pathway, energy is conserved by converting carbon dioxide and carbon monoxide into acetyl-CoA which is then used for mass energy production in the citric acid cycle. All in all, they create around 1013 kg of acetic acid per year, far greater than the world's commercial production. Acetogens, with methanogens and sulfate reducers are crucial in maintaining the alkaline pH that is characteristic of the Eastern Saline Wetlands.

One common feature among Acetoanaerobium and the genus Clostridium is their special role in amino acid degradation with the Stickland reaction which preferentially oxidizes an amino acid, while reducing its pair. However, not much is known about the Stickland reaction or its attributes to growth or providing energy to organisms. Amino acids are used as the main carbon and thus energy sources. In this process, ATP is formed by substrate-level phosphorylation. A. sticklandii, which is closely related to strain VLB-1, uses the amino acids threonine, arginine, lysine, and serine. Along with these, aromatic and branched amino acids can also be degraded, however, the pathways and processes are still unknown.



General mechanism for Wood-Ljungdahl Pathway from
https://www.sciencedirect.com/science/article/abs/pii/S1570963908002574

Approach

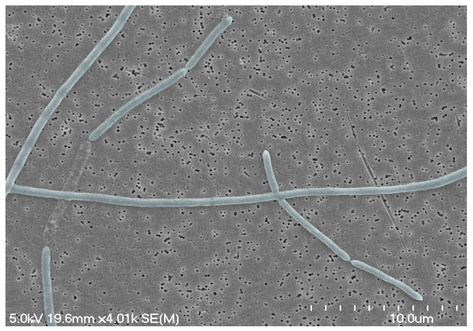


Demonstration of the agar deep method where an organism is isolated via known metagenomic information.

Isolation
- grown in minimal, semi-freshwater saline wetland culture medium (argon headspace with H_2 added via syringe, pH 8.3) with calcium carbonate as a sole source of carbon
- aliquot of enrichment culture transferred into anaerobic (100% argon) DSMZ Medium 38 (without sulfide, pH 7.5)
- serial dilution performed, cultured via the agar deep method
- five colonies isolated from the agar, of which VLB-1 was one

DNA Extraction
- harvested cell mass mid log-phase and extracting nucleic acid using the Griffith's method
- Illumina sequencing with Novogene
- assembled with MEGAHIT, binned with CONCOCT, MetaBat, and MaxBin 2, and binning results joined with DAS Tool
- Ribosomal Database Project (RDP) Classifier used to verify the taxonomy of *Acetoanaerobium* sp. VLB-1 based on the 16S rRNA sequence

Results

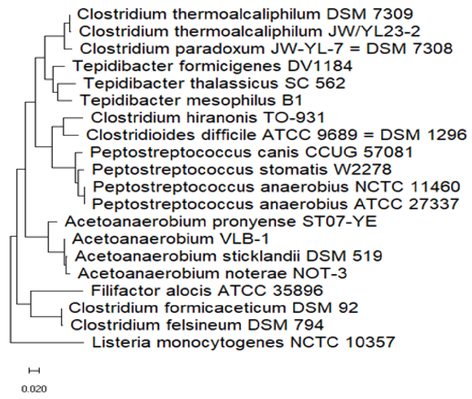


***Acetoanaerobium* sp. VLB-1 under scanning electron microscopy.**

The resulting genome was estimated to be 98.46% complete with nine contigs and a size of 2,574,940 base pairs with only 1.40% contamination. The Ribosomal Database Project (RDP) Classifier was used to verify the taxonomy of Acetoanaerobium sp. VLB-1 based on the 16S rRNA sequence.

With a confidence threshold of 80%, VLB-1 is classified in the phylum Firmicutes, class Clostridia, order Clostridiales, family Peptostreptococcaceae, and genus Acetoanaerobium by 100%. Through using the Type (Strain) Genome Server which calculates the average nucleotide identity of the genome against other Acetoanaerobium species, sp. VLB-1 was identified as Acetoanaerobium noterae with a 97.65% percent similarity, followed by A. sticklandii with a 96.14% similarity.

A. sticklandii, a classic example of an organism applying the Stickland reaction, uses the amino acids threonine, arginine, lysine, and serine and various other branched and aromatic amino acids. VLB-1 can metabolize amino acids arginine via the arginine succinyltransferase pathway and threonine, serine, and cysteine via their respective biosynthesis pathways. The Shikimate pathway is used to degrade aromatic amino acids. Threonine and leucine are metabolized via their respective biosynthesis pathways. There does appear to be multiple glycine reductase complex component B subunits, hinting at a possible way to reduce glycine, however, the pathway is unknown.



MEGAX constructed phylogenetic tree based on 16 rRNA gene sequences using MUSCLE alignment of DNA including strain VLB-1 and closely related taxa in the Family Clostridiales. (Listeria monocytogenes was used as an outgroup.

VBL-1 contains most genes in the Wood-Ljungdahl pathway except one: K22015 or formate dehydrogenase, which is paired with iron hydrogenase HydA2 and the FeS-containing electron transfer protein. The supposed absence of this gene could be attributed to the less accurate nature of the draft genome.

Conclusions

Acetoanaerobium sp. strain VLB-1 has the metabolic potential for autotrophy or acetogenesis and amino acid fermentation via the Wood-Ljungdahl pathway and the Stickland reaction respectively. The Wood-Ljungdahl pathway is environmentally crucial in the balance between acetogenesis and methanogenesis, with methanogens acting as hydrogen sinks. Due to the unique environment this organism was found in, understanding elemental cycling and metabolism is crucial to understanding its role in the preservation in the Nebraska Saline Wetlands. In keeping with environmental practices, the biotechnological potential of these organisms could be used to generate acetic acid and possibly diminish methane production affecting climate change and microbial-induced corrosion.

Acknowledgments

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