Short-Term Response of Oak Ridge Groundwater Microbes to Different Carbon Sources

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Abstract

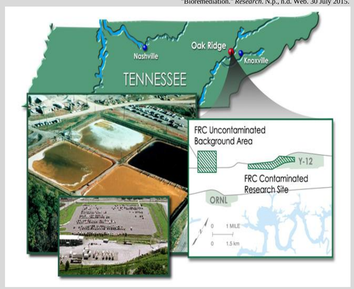
The purpose of this study is to explore the effects of different carbon sources on the growth of microbial communities present in groundwater from well FW305 at the Oak-Ridge Field Research Center, Tennessee. Six different carbon sources were chosen: acetate, glucose, benzoate, casamino acids, cell lysate, and FW305 sediment extracts. Microbes were isolated using two dilutions of three different media. Isolated colonies were identified using 16S rDNA gene sequencing. The results illustrated that different microbial species were enriched, highly influenced by specific carbon sources available to them. Each carbon source enriched different bacteria and this was confirmed by the 16S sequencing. Some novel strains of bacteria were isolated, and further work needs to be done to characterize these strains.

Introduction – Oak Ridge Tennessee

Contaminated site:
- Extreme pH range 3-10
- Volatile organic compounds
- High and low nitrate
- Uranium, Thorium, Technetium

Uncontaminated site:
- pH 7
- No nitrate
- No radioactive

"Bioremediation." *Research*. N.p., n.d. Web. 30 July 2015.



Our research investigated the uncontaminated site to have a full understanding when investigating the contaminated site.

Materials and Methods

Carbon Sources-10% volume
Acetate (5 mM)
Glucose (5 mM)
Benzoate (0.5 mM)
Casamino Acids (10 µg/mL)
Cell lysate
Sediment Extract
Mixture of the 6 C sources

Solid Media Types:
1/10 dilution *LB
1/10 dilution *TSA
1/10 dilution *R2A
1/25 dilution LB
1/25 dilution TSA
1/25 dilution R2A

* Luria broth
* Tryptone Soy Agar
* Reasoner's 2A Agar

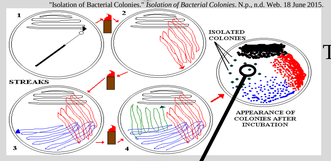
Procedure

80% Filter-sterilized groundwater
10% Unfiltered groundwater
10% Carbon source (7 types)

Two weeks growth in culture tubes

16S rRNA metagenomics

We want to use metagenomics to get the information of all microbes present, regardless of cultivability.

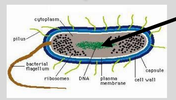


Two days growth on solid media

Selecting pure bacterial colonies to inoculate in liquid media
(Same media type as solid)

One week growth in liquid media

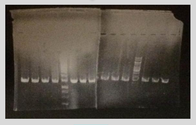
When culture is grown, DNA Isolation (Extraction, Purification)



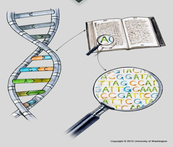
DNA

*PCR
(Amplification and product Purification)

*Polymerase Chain Reaction



Identify Bacterium by 16S rDNA Sequence (Sequence Facility)



16 rDNA

Results

Summary of the Enrichment in Each Carbon Source

Acetate	Glucose	Cell Lysate	Sediment Extracts
Cupriavidus basilensis	*Cupriavidus basilensis*	*Cupriavidus basilensis*	*Acidovorax caeni*
Cupriavidus necator	*Cupriavidus necator*	*Janthinobacterium agaricidamnosum*	*Cupriavidus basilensis*
Cupriavidus numazuensis	*Janthinobacterium lividum*	*Leptothrix discophora*	*Massilia plicata*
Pelomonas saccharophila	*Pelomonas aquatica*	*Pseudacidovorax intermedius*	*Nocardia coeliaca*
Pseudomonas azotoformans	*Pelomonas saccharophila*	*Pseudomonas frederiksbergensis*	*Pseudomonas extremorientalis*
Pseudomonas baetica	*Pseudomonas frederiksbergensis*	*Rugamonas rubra*	*Pseudomonas poae*
Variovorax paradoxus	*Undibacterium pigrum*	*Undibacterium pigrum*	*Rhodococcus erythropolis*
		Variovorax paradoxus	*Variovorax paradoxus*

Benzoate	Casamino Acids	Mixture
Arthrobacter aurescens	*Cupriavidus basilensis*	*Cupriavidus basilensis*
Cupriavidus necator	*Cupriavidus necator*	*Ideonella azotifigens*
Janthinobacterium lividum	*Cupriavidus numazuensis*	*Ideonella dechloratans*
Pelomonas saccharophila	*Nocardia coeliaca*	*Pelomonas saccharophila*
Pseudomonas chlororaphis	*Pelomonas saccharophila*	*Pseudomonas brassicacearum*
Rhodococcus erythropolis	*Pseudomonas azotoformans*	*Pseudacidovorax intermedius*
	Pseudomonas chlororaphis	*Pseudomonas corrugata*
	Pseudomonas entomophila	*Pseudomonas frederiksbergensis*
	Pseudomonas mosselii	*Variovorax paradoxus*
	Rhodococcus erythropolis	
	Variovorax paradoxus	

Summary:
- 6-11 strains were isolated from each carbon source
- The most isolations (11) were obtained from casamino acid
- The least isolations (6) were obtained from benzoate
- *Cupriavidus* and *Pseudomonas* were present in every carbon source, indicating that those strains were not carbon specific and can utilize more types carbon sources than other strains.
- Some strains were only found in one specific carbon source, such as: *Pseudomonas baetica*, *Pelomonas aquatic*, *Janthinobacterium agaricidamnosum*, *Leptothrix discophora*, *Pseudomonas extremorientalis*, *Pseudomonas poae*, *Arthrobacter aurescens*, *Pseudomonas entomophila*, *Pseudomonas mosselii*, *Ideonella azotifigens*, *Ideonella dechloratans*, *Pseudacidovorax intermedius*, *Pseudomonas corrugata*, indicating that these strains may prefer a specific carbon source. (Highlighted in table)

Conclusion

Each carbon source enriched different bacteria as evidenced by their appearance on the petri dish and this was confirmed by the 16S sequencing. Therefore the change in carbon source does enrich different types of bacteria. Also, some novel strains of bacteria were isolated, and further work needs to be done to characterize these strains. While metagenomic data is not available at this time, comparison can be made of the total microbial diversity present with each carbon source, and the total diversity of microbes cultivable. Future questions: Do the bacteria favor a specific carbon source? Does one bacterium dominate the other? Does the presence of different carbon sources influence the metabolism of bacteria? Does the rate of metabolism differ from one bacterium to the other? What are the new strains that grew after the isolation date?

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