Nitrite Reducing Bacteria Isolated from Groundwater and Sediment from the Oak Ridge Field Research Center

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Abstract: Microbes' and microbial communities' impact on their ecosystems are not well understood. We work to isolate novel strains of bacteria from their environments and study their physiology. In this research, nitrite respiring bacteria are isolated from core sediment through enrichment for carbon utilization under anaerobic conditions. Furthermore, three naturally co-existing strains of *Pseudomonas fluorescens*, which have already been isolated from groundwater, were cultivated in mono-cultures and co-cultures under denitrifying conditions to study their growth and explore their tolerance and sensitivity to nitrite. These cultures were exposed to different concentrations of nitrite in 48 well plates and its growth was monitored throughout its period of incubation. We study the effects of nitrite on the system because it is the first intermediate in the pathway for nitrate reduction and is also known to be toxic to bacteria. Currently there is a knowledge gap, so these results have implications for future research and studies on how microbes interact under other conditions or in different microbiomes.

Background

- This project is part of a larger multi-institutional collaboration funded by the US Department of Energy called ENIGMA
- The ultimate goal is to advance our understanding of microbial biology and the impact microbial communities have on their ecosystems
- Environmental microbes studied were collected from the sediment and groundwater at the Oak Ridge Field Research Center.
- Bacteria studied here are under denitrifying conditions and grown in the presence of nitrite which not only is the first intermediate in the nitrate reduction pathway, but is also known to be toxic to bacteria.

Isolations

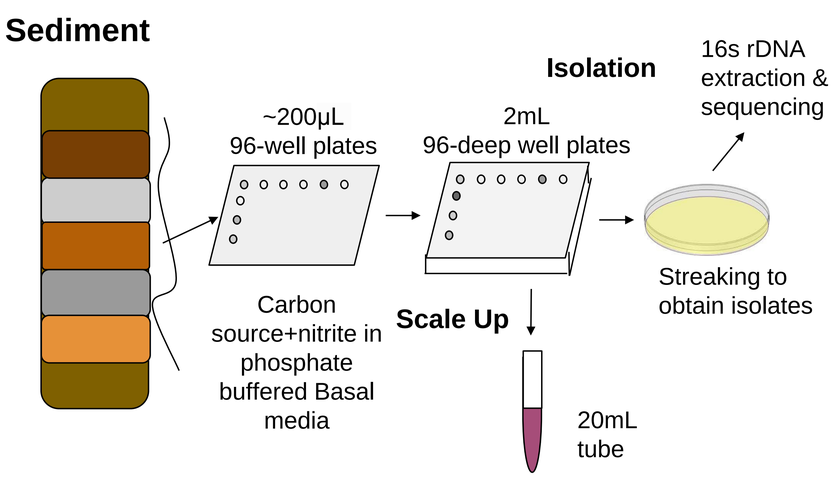


Figure 1. Carbon Enrichments. Each well contains a different carbon source for the enrichment. The carbon is utilized or consumed for the bugs to grow and thrive. Communities that are able to respire a specific carbon will dominate in the well containing that carbon.

	1	2	3	4	5	6	7	8	9	10	11	12
A	a-Cyclodextrin	D-Galactose	D-Glucose	D-Maltose monohydrate	D-Mannose	D-Raffinose pentahydrate	D-Ribose	a-Ketoglutaric acid disodium salt hydrate	DMSO	Beta-Lactose	D-Arabinose	D-Fructose
B	D-Xylose	Sucrose	L-Arabinose	L-Rhamnose monohydrate	L-Sorbose	p-Hydroxybenzoic Acid	5-Keto-D-Gluconic Acid potassium salt	Citric Acid	D-Gluconic Acid sodium salt	D,L-Malic Acid	Glycolic Acid	
C	Itaconic Acid	Sodium butyrate	Sodium D-Lactate	Sodium D,L-Lactate	Sodium Formate	Sodium Fumarate dibasic	Sodium L-Lactate	Sodium octanoate	Sodium propionate		Potassium acetate	Potassium oxalate monohydrate
D	Sodium pyruvate	Vanillic acid	Sodium succinate dibasic hexahydrate	Syringic acid	Trisodium citrate dihydrate	Casamino acids	D-Alanine	D-Serine	Gly-Glu	Glycine	L-Alanine	
E	L-Arginine	L-Asparagine	L-Glutamine	L-Histidine	L-Isoleucine	L-Leucine	L-Lysine	L-Methionine	L-Phenylalanine	L-Proline	L-Aspartic Acid	L-Cysteine hydrochloride monohydrate
F	L-Glutamic acid monopotassium salt monohydrate	L-Serine	L-Valine	L-Threonine	L-Tryptophan	L-tyrosine disodium salt	Amylose	D-Cellobiose	Lignin CMC	NOM (Sediment Extract)	Tannic Acid	Gelatin
G	Tween 20	2-Deoxy-D-Ribose	Adenine hydrochloride hydrate	Adenosine	Coniferyl alcohol	Starch	Cytosine	D-Glucosamine Hydrochloride	D-Mannitol	D-Salicin	D-Sorbitol	Ethanol
H	Carnitine Hydrochloride	Glucuronamide	Glycerol	Inosine	L-Citrulline	m-Inositol	N-Acetyl-D-Glucosamine	Parabanic Acid	Putrescine Dihydrochloride	Uridine	Xylitol	

Figure 2. Carbon utilization at different depth of the sediment. Count of number of carbon species within the class that displayed an increased OD (optical density).

Depth	0'-3'	3'-5'	5'-8'	8'-10'	10'-13'	13'-15'	15'-18'
Sugar	12	8	3	8	6	9	6
Organic Acids	12	10	12	5	4	7	3
Amino Acids	11	6	19	4	3	10	7
Polymers	2	2	7	2	1	4	4
Natural Products	8	11	8	12	8	10	12

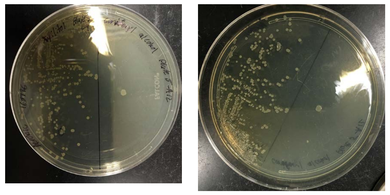
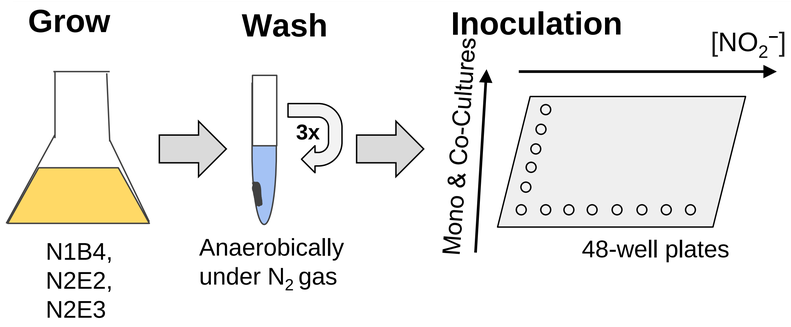


Figure 3. Growth observed on aerobic plate streaked with culture enriched in xylitol and found in sediment 10'-13' below ground. DNA was extracted from one colony and went through a process of amplification and purification.

Conclusion

- Scaled up core plates are used for community analysis/metagenomics
- *Pseudomonas* can be studied further and other aspects of the bacteria's physiology can be tested.
- Testing *Pseudomonas* and its tolerance at different levels of nitrite gives us a better understanding of microbial communities present in anaerobic conditions and thriving in the presence of nitrite.
- Determining whether or not these bugs will grow at certain levels of nitrite can pave way to selecting certain bug combinations for future analysis.
- We now can also better predict and have a good understanding of how these *Pseudomonas* will react in the environment, when the soil/water becomes contaminated with nitrate from the nearby contaminated waters at Oak Ridge.
- This research is a tiny step in furthering our understanding of microbes and microbial communities in environmental microbiomes.

Nitrite Tolerance



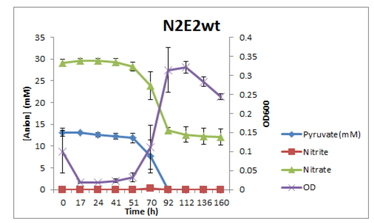
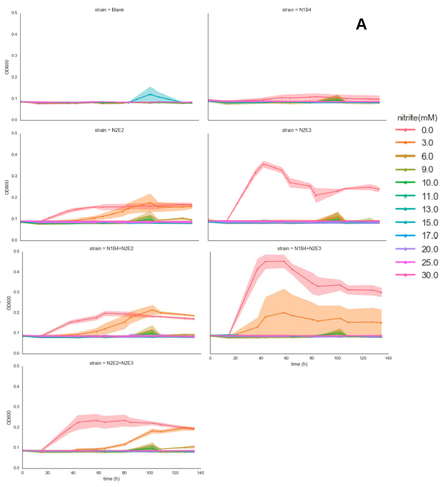


Figure 4. Metabolism and growth of N2E2 mono-culture. Consumption of the electron donor, pyruvate, corresponds to the utilization of the electron acceptor, nitrate and the growth(OD) of the bacteria. No significant relationship with nitrite is demonstrated here.



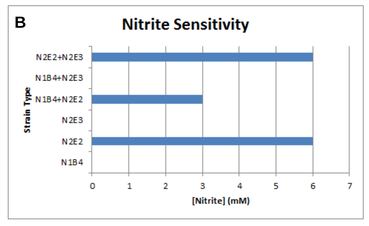


Figure 5. Mono-cultures and co-cultures. In N1B4 co-cultures, the other strain dominates. N2E2+N2E3 is observed to have a positive interaction as the end OD at 3mM and 6mM are greater than its monocultures(A). Strains sensitive to nitrite demonstrates no growth. This is also true with its co-culture pair(B).

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