N-190 Genetic Elucidation of Metabolic Diversity in *Dechloromonas aromatica* strain RCB

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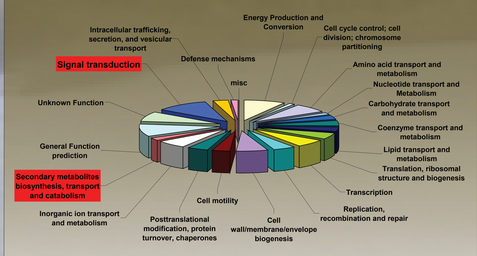
Abstract: *Dechloromonas aromatica* is a beta class proteobacteria found ubiquitously in soil environments. A facultative anaerobe, capable of aromatic hydrocarbon degradation and perchlorate reduction, it was first isolated from BTEX contaminated Potomac river sediments. Due to its metabolic versatility, strain RCB was selected for complete genome sequencing at the DOE Joint Genome Institute. The completed sequence consists of a single circular chromosomal DNA structure containing a total of 4.5 Mbases of DNA with a G+C content of 60%. Initial draft annotation was conducted at Oak Ridge National Laboratories and the Virtual Institute for Microbial Stress and Survival (http://escalante.lbl.gov) identifying approximately 4000 open reading frames (ORFs). The closest relative with a completed genome sequence is *Ralstonia solanacearum*, also a gram negative proteobacteria of the Beta sub-class. *Dechloromonas aromatica* and *Ralstonia* possess very similar genomic content, duplicative 23S and 16S RNA regions, and identical operon structures for many families of genes. In *Ralstonia*, the catabolic capabilities leading to degradation of aromatic compounds are thought to be concentrated within plasmid DNA structures. However, *D. aromatica* appears to lack a large genomic plasmid element (*R eutropha's* pGMI1000MP plasmid is 2.09Mb in size), which indicates its adaptability in degradation of aromatic and toxic compounds is not dependent on a highly transmissible and mutable plasmid structure. Genes have been annotated for InterPro domains, EC assignments, TIGRfams, KEGGS pathways, and GO ontologies. Genes that likely reflect the versatility of *D. aromatica* within the environment include a remarkably high number of two-component sensors and regulators (11% of the annotated ORFs), placing it in the top 5% of microbial genomes in this capability. The *D. aromatica* genome has been found to contain RuBisCo, indicating the ability to fix carbon dioxide. Conversely, central catabolic pathway enzymes for monoaromatic degradation such as BSS are not present, suggesting the presence of novel aromatic degradation pathways in this organism.

What's Different about *Dechloromonas aromatica*?

- A disproportionately high number of two-component sensors and phosphorelay proteins (11% of the genome).
- A highly versatile metabolism, allowing *D. aromatica* to reduce nitrate, oxidize aromatic BTEX compounds, and reduce perchlorate -- all harmful environmental contaminants.
- The genomic sequence indicates *D. aromatica* contains a CO_2 fixation pathway, with the presence of RuBisCo, as well as the ability to fix N_2. The presence of these capabilities predicts currently unexplored physiological capabilities for this novel organism.
- Though the initial recruitment to established gene families indicates a low number of transport proteins, further analysis indicates a fairly large group of transporters, but they do not belong to the nucleotide permease families expressed in other microbial genomes.

Overview of Gene Function



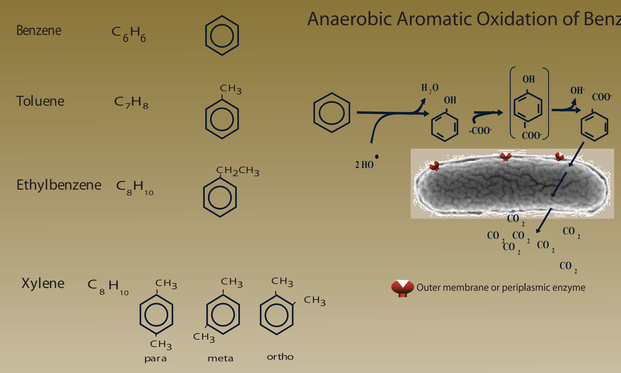
The *Dechloromonas aromatica* genome has been finished. *D aromatica's* genome is made up of 4,501,104 nucleotides, composed into a single circular chromosome. No evidence of plasmid DNA was seen.

Analysis of the Perchlorate Reduction Pathway

Perchlorate, a predominately man-made contaminant found in the environment, has been shown to interfere with thyroid uptake of iodide in humans as well as animal populations in the wild. Genomics based inquiry into the perchlorate reducing ability of *Dechloromonas aromatica* has allowed testing of knock-out mutants of two genes, perchlorate reductase A (pcrA) and chlorite dismutase. The gene cluster structure for perchlorate reductase/chlorite dismutase is shared by *Dechloromonas aromatica* and *Azospira suillum*. The origin of the genetic capability for perchlorate reduction is unclear, though it is evident that this operon belongs to the nitrate reductase family of genes. Interestingly, the highest scoring bidirectional best-hit for the pcrB gene is to the beta subunit of *Azoarcus sp* EbN1's ethylbenzene dehydrogenase (Genbank accession # YP_160314).

Dechloromonas aromatica perchlorate reductase gene cluster

Aerobic and Anaerobic Aromatic Degradation



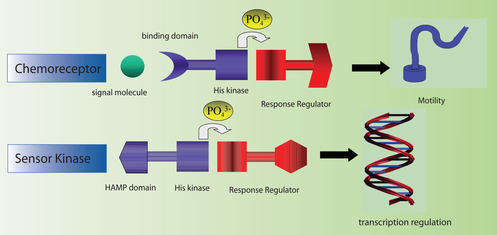
Inquiries into a novel mechanism: *Dechloromonas* strains JJ and RCB are currently the only pure culture isolates capable of complete benzene degradation in an anaerobic environment, and are able to completely degrade benzene to CO_2 under anaerobic conditions. *D. aromatica* is also capable of degrading other members of this class of aromatics, including toluene, ethylbenzene, and xylene. Though ***Dechloromonas aromatica*** **can degrade aromatic compounds both anaerobically and aerobically, it appears to lack key enzymes in known aromatic catabolic pathways. Benzylsuccinate synthase (comprised of BssA, BssB, BssC) mediates a fumarate addition reaction during the anaerobic metabolism of toluene, yet is not present in the *Dechloromonas* genome (though various upstream and downstream genes of this KEGG pathway are present). This suggests novel enzymatic steps are present in the *Dechloromonas* genome.**

Reference: Chakraborty, R. and Coates, J.D. (2005). *Hydroxylation and carboxylation – two crucial steps of anaerobic benzene degradation by Dechloromonas strain RCB. Applied and Environmental Microbiology* (in press).

Signal Transduction: Interaction with the Environment

- *D. aromatica* strain RCB encodes an unusually large number of proteins having the histidine kinase motif (1/40th of the genome; 100 open reading frames (ORFs) of 4000 total).
- This includes a large number of chemoreceptors thought to alter motility (22 methyl-accepting chemotaxis proteins) and an even larger set of sensor kinases that are predicted to modify transcription (83 ORFs).
- The histidine kinase-containing ORFs show both cytosolic and transmembrane motifs, ranging in size from 202 amino acids to 1866 amino acids (see graphic to right).
- All ORFs with four or more predicted transmembrane helices (TMs) are not yet classified with respect to known signal transduction proteins.
- A large number of pilus assembly proteins are also present (19 ORFs annotated thus far), suggesting extensive interaction with the surrounding environment (though the nature of this interactions is yet to be determined).
- Studies in this laboratory have demonstrated *D. aromatica* displays a taxic response to external stimuli including nitrate, perchlorate, and acetate.

Model Signal Transduction Proteins

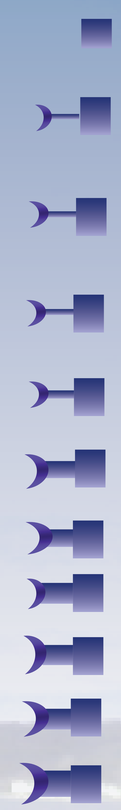


Signal Transduction: Comparison with other Genomes

Dechloromonas aromatica	*Desulfovibrio desulfuricans G20*[2]	*Geobacter metallireducens*[2]	*Ralstonia solanacearum*[3]	*Bacillus subtilis*[1]	*Streptomyces coelicolor*[1]	*Shewanella oneidensis*[2]	
4019	3294	3316	5211	4233	8183	4655	TOTAL NUMBER OF ORFs ANNOTATED
2439	2144	2225	3143	2478	4126	2448	GO:0003673: Gene_Ontology
2046	1693	1778	2578	2007	3341	2009	GO:0008150: biological_process
736	1520	1545	2204	681	1039	648	GO:0009987: cellular process
296	210	245	180	111	217	186	GO:0007154: cell communication
269	205	233	168	102	193	174	GO:0007165: signal transduction
4	2	4	7	7	15	6	GO:0000007166: cell surface receptor linked signal transduction
0				0	0	0	GO:0001560: interpretation of external signals that regulate cell growth
11	8	15	6	10	10	8	GO:0007242: intracellular signaling cascade
0				0	0	0	GO:0009966: regulation of signal transduction
165	136	134	91	45	106	88	GO:0000160: two-component signal transduction system (phosphorelay)

In general, the number of environmental or redox stimuli an organism can respond to is directly proportional to the number of sensor kinase/two-component signal transduction genes present in its genome. Analysis indicates that *D. aromatica* strain RCB is likely to be highly responsive to its environment. The number of chemotaxis/signal transduction/two-component transduction ORFs in *D. aromatica* exceeds that of other microbial whole genome sequences in the VIMSS database.
Comparisons are made to other organisms noted for 1) their versatility and expanded pool of signal transduction genes, 2) from similar environments with similar capabilities, or 3) close phylogenetic neighbors.

Histidine Kinases in *D. aromatica* RCB: cytosolic and transmembrane proteins



Soluble (no predicted TM):
small (<500aa): 6
medium (500-100aa): 13
large (1000 - 2000aa): 4
includes two cheA-like proteins and glnL (involved in nitrogen regulation)

One predicted TM:
small (<500aa): 1
medium (500-100aa): 5
large (1000 - 2000aa): 3
includes phoR and barA

Two predicted TMs (most typical):
small (<500aa): 11
medium (500-1000aa): 27
large (1000 - 2000aa): 9
includes 6 copies of the quorum sensor qseC, envZ, rstB, narX and two barA homologs

Three predicted TMs:
small (<500aa): 2
medium (500-1000aa): 3
includes arcB, and kdpD

Four predicted TMs:
small (<500aa): 1
medium (500-1000aa): 3

Five predicted TMs:
small (<500aa): 2
medium (500-1000aa): 4
large (1000 - 2000aa): 1

Seven predicted TMs:
medium (800aa): 1

Eight predicted TMs:
medium (535aa): 1

Nine predicted TMs:
medium (704aa): 1

Twelve predicted TMs:
medium (903aa): 1

Fourteen predicted TMs:
large (1131aa): 1

Conclusions

- We have finished the *Dechloromonas aromatica* complete genome, and are currently annotating the resulting gene set.
- *D. aromatica* has a large number of two-component signal transduction proteins, suggesting it has the capacity for sensitive interaction with its environment.
- Conversely, *D. aromatica* appears to have fewer enzymes for nucleotide metabolism and transport than other microbes sequenced thus far. The reason for this is unknown.
- Pathway analysis indicates the ability to fix CO_2 and N_2 exists in this organism.
- Several members of currently known catabolic pathways for aromatic degradation have not been found by analysis of the completed genome, suggesting the presence of novel enzymes for aromatic catabolism.

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