Characterization of Aromatic Compound Degradation Pathways in *Rhodopseudomonas palustris* with Stable Isotope Labeling Quantitative Shotgun Proteomics and Microarray

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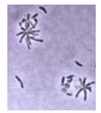
OVERVIEW

Research Objectives:

- Elucidation of *R. palustris* gene expression regulation for aromatic compound degradation.
- Discovery of novel genes and pathways involved in aromatic compound degradation.
- Comparison of protein level profile and mRNA level profile in *R. palustris.*



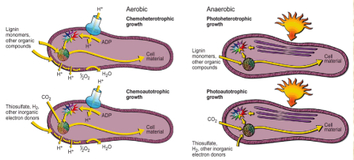
Research Focus:

- Comparison of three carbon sources growth: p-coumarate, benzoate and succinate.
- Quantitative proteomics measurements with metabolic stable isotope labeling.
- Affymetrix microarray measurements from the same isotopically labeled cultures.

INTRODUCTION

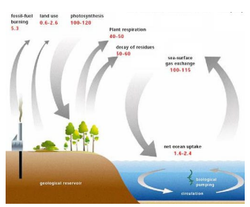
Rhodopseudomonas palustris

- ***R. palustris*** **is a purple photosynthetic bacterium capable of the four modes of metabolism (photoautotrophic, photoheterotrophic, chemoheterotrophic and chemoautotrophic)**
- **The complete genome of *R. palustris* has been sequenced and annotated (Larimer F.W. et la, Nature Biotechnology 22, 55-61 (2003)).**
- **4936 genes are predicted from the genome sequence**



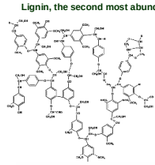
Aromatic Compound Degradation

- **Aromatic compound degradation is a vital link in the global carbon cycle, involved in the process of plant residue decay.**
- **Large quantities of industrially generated aromatic contaminants in the environment are cleaned up by biodegradation.**

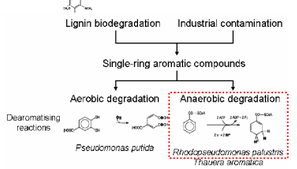


The global carbon cycle (Numbers are billions of tons of carbon, GbC)

Lignin, the second most abundance carbon polymer on Earth

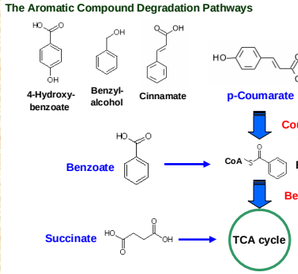


In the process of plant residue decay, large quantities of lignin monomers in diverse forms of simple aromatic compounds are generated and released into anoxic environments, such as aquifers, aquatic sediments and submerged soils.



EXPERIMENTAL

The Aromatic Compound Degradation Pathways



Known aromatic compounds that *R. palustris* degrades

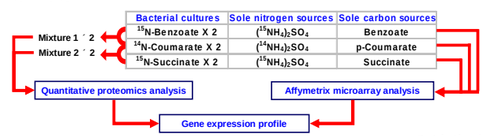
The diverse aromatic compounds are hypothesized to be converted to the central intermediate, benzoyl-CoA, via peripheral pathways

Benzoyl-CoA is degraded via 14-step benzoate pathway into acetyl-CoA, which enters TCA cycle. In contrast, succinate can enter TCA cycle directly.

Experimental Methods

1. ***R. palustris*** **cultures were anaerobically grown to O.D. = 0.5 with ample light on different defined minimum media. Two biological replicates of mixture A and mixture B was prepared as shown in the table.**
2. **Each mixture was analyzed in replicate by shotgun proteomics with a 12-step MudPIT method on an LTQ-MS instrument. Proteins were identified with SEQUEST and DTSelect [Xcorrs of at least 1.8 (+1), 2.5 (+2), and 3.5 (+3)]. The ProRata program was used to estimate the proteins' abundance ratios and their confidence intervals.**
3. **The isotopically labeled bacterial cultures were also analyzed with Affymetrix microarrays. The raw data were analyzed with the Cyber-T program**

Bacterial cultures	Sole nitrogen sources	Sole carbon sources
^{15}N-Benzoate X 2	$(^{15}NH_4)_2SO_4$	Benzoate
^{14}N-Coumarate X 2	$(^{14}NH_4)_2SO_4$	p-Coumarate
^{15}N-Succinate X 2	$(^{15}NH_4)_2SO_4$	Succinate



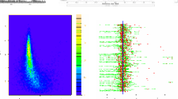
ProRata: A free quantitative proteomics program.

Download:
http://www.MSProRata.org

Full Scans **Ion Chromatograms** **Protein Abundance Ratio Confidence Interval**

Screenshots

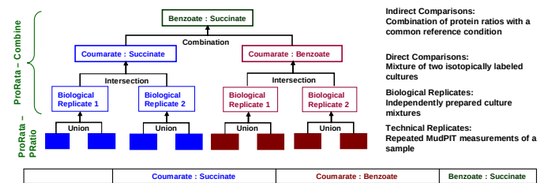
Estimation of peptide log-ratios in a 1:5 standard mixture



Estimation of protein log-ratios in a 1:5 standard mixture

RESULTS

Quantitative Proteomics Measurement Results



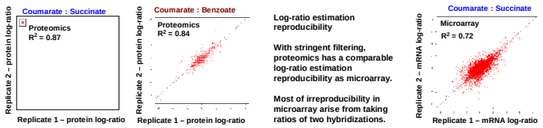
Indirect Comparisons:
Combination of protein ratios with a common reference condition

Direct Comparisons:
Mixture of two isotopically labeled cultures

Biological Replicates:
Independently prepared culture mixtures

Technical Replicates:
Repeated MudPIT measurements of a sample

	Coumarate : Succinate			Coumarate : Benzoate			Benzoate : Succinate
Measurements	Biological Replicate 1	Biological Replicate 1	Intersection	Biological Replicate 1	Biological Replicate 1	Intersection	N/A
Identified Proteins	2,385	2,172	2,012	2,058	1,979	1,801	N/A
Quantified Proteins	1,859	1,790	1,539	1,785	1,730	1,627	1,151

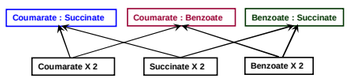


Log-ratio estimation reproducibility

With stringent filtering, proteomics has a comparable log-ratio estimation reproducibility as microarray.

Most of irreproducibility in microarray arise from taking ratios of two hybridizations.

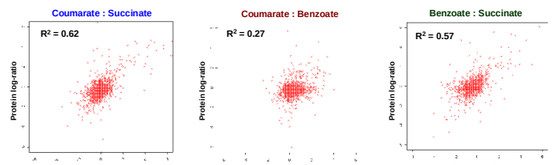
Affymetrix Microarray Measurement Results



Comparisons of the mRNA signals measured from cultures of different growth conditions.

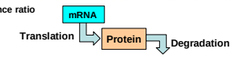
Replicate measurements of the mRNA profiles of the same isotopically labeled cultures as ones used for quantitative proteomics

Correlation Of Protein Log-ratio With mRNA Log-ratio



Possible explanations for the difference between mRNA and protein abundance ratio

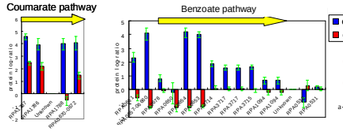
- **Measurement error from both microarray and proteomics**
- **Post-transcriptional regulation.**
- **Differential protein degradation rate.**



Pathway Analysis With Proteomics Results

Coumarate pathway

Benzoate pathway



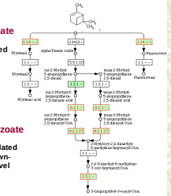
- **Supports the putative proteins in coumarate pathway.**
- **Reveals the regulation of these two pathways under coumarate and benzoate conditions.**

a-pinene degradation up-regulated in the coumarate condition

Up-regulated pathways in coumarate	Up-regulated pathways in benzoate
Ascorbate and aldarate metabolism	Benzoate degradation via CoA ligation
Limonene and pinene degradation	Bacterial chemotaxis
gamma-Hexachlorocyclohexane degradation	Tetrachloroethene degradation
Fluorene degradation	Selenoamino acid metabolism
Stilbene, coumarine and lignin biosynthesis	Benzoate degradation via hydroxylation
Bile acid biosynthesis	Methionine metabolism

Coumarate : Benzoate

Enrichment of regulated proteins in the KEGG pathways



Coumarate : Benzoate

Genes with up-regulated mRNA level and down-regulated protein level

Locus	mRNA change		Protein Change		Description
	LogRatio	P value	LogRatio	CI	
RPA0537	2.1	3.78E-05	-1.5	[-1.7, -1.3]	coenzyme A transferase
RPA0652	1.6	1.12E-04	-1.1	[-1.3, -1.0]	cyclohexanecarboxyl-CoA dehydrogenase
RPA0657	1.8	6.24E-08	-1.2	[-1.3, -1.1]	benzoyl-CoA reductase subunit
RPA0658	1.1	1.06E-06	-1.4	[-1.5, -1.3]	benzoyl-CoA reductase subunit
RPA1561	1.7	6.50E-04	-1	[-1.1, -0.8]	cbbX protein homolog
RPA2930	1.3	6.36E-04	-1	[-1.4, -0.6]	conserved unknown protein
RPA3309	1.8	2.33E-04	-1	[-1.2, -0.8]	conserved unknown protein
RPA3390	2.3	5.89E-06	-2.7	[-3.4, -0.9]	phosphoribosyl c-AMP cyclohydrolase
RPA4343	2	1.33E-03	-1	[-1.6, -0.5]	possible excinuclease ABC subunit B

CONCLUSIONS

- **The reliability of protein quantification with quantitative proteomics has been validated with standard mixtures and with known pathways in the biological samples.**
- **The down-regulation of part of benzoate pathway in the coumarate condition relative to the benzoate condition suggests that the metabolism rate of the benzoate pathway is perhaps turned down to match that of the coumarate pathway.**
- **The proteins in the coumarate pathway are maintained at a relatively high level even in the benzoate condition, which indicates that enzymes involved in coumarate degradation have a wide range of substrate specificity**
- **Aromatic compound degradation is accompanied with the regulation of many pathways in carbon metabolism, cross-membrane transportation and stress response.**
- **The global correlation between protein level and mRNA level is weak, but the genes in the benzoate and coumarate pathways showed good linear relationship between their mRNA level and protein level.**

ACKNOWLEDGEMENTS

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