

Metagenomic and proteomic analyses to elucidate the mechanism of anaerobic benzene degradation

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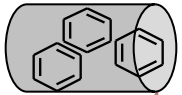
ISMOS-3 Calgary, June 13-15, 2011

Overview

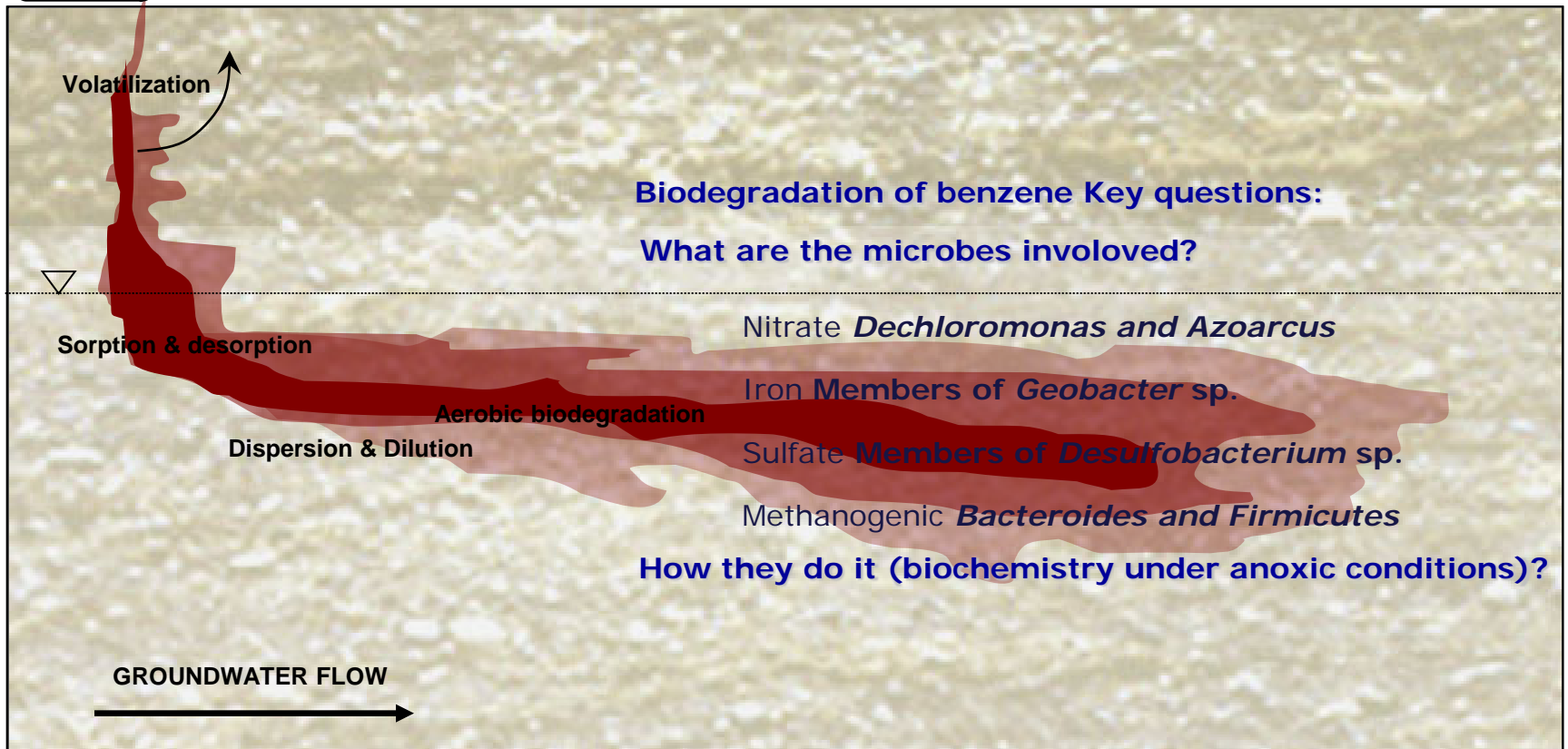
- Introduction
- Methods
- Iron-reducing enrichment culture BF
 - Physiological and molecular properties
 - Metagenome analysis
- Identification of specific benzene-expressed proteins
- Describing the initial activation mechanism
- Summary

Introduction

Benzene degradation in the contaminated aquifers



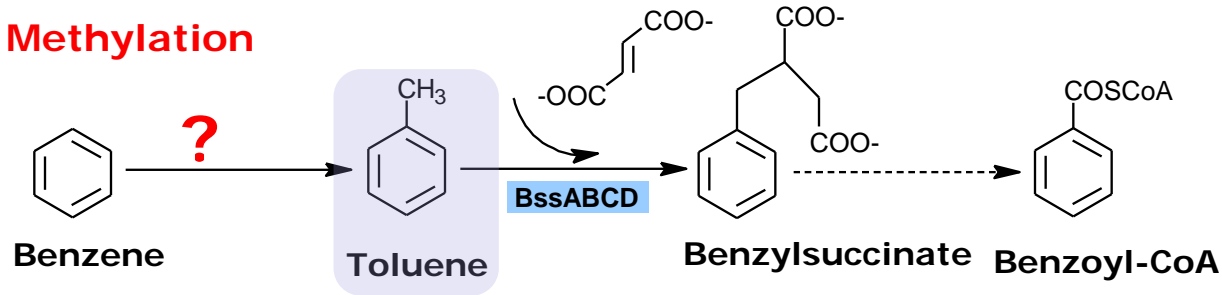
Industrial processes



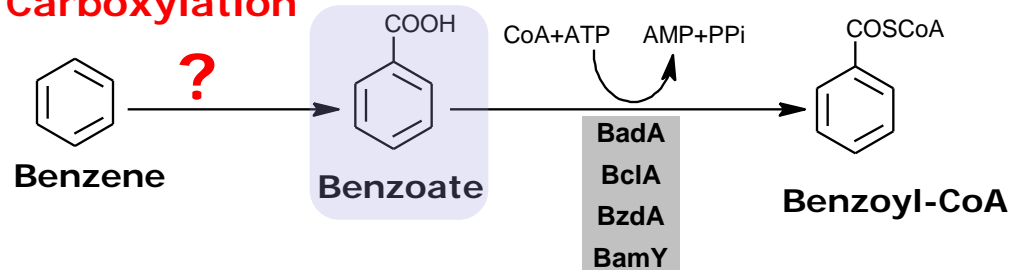
Hypotheses

Proposed initial activation mechanisms of benzene under anaerobic conditions

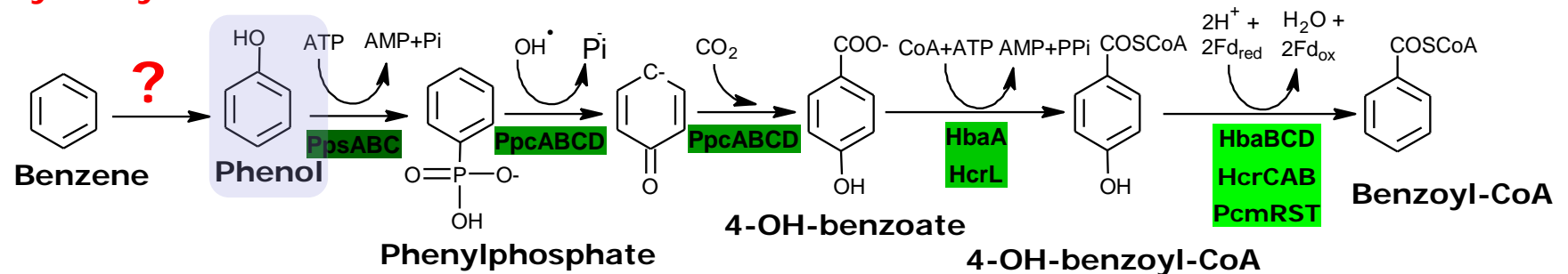
Methylation



Carboxylation

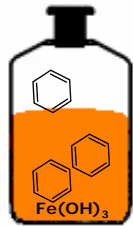


Hydroxylation



Methods

Molecular characterization



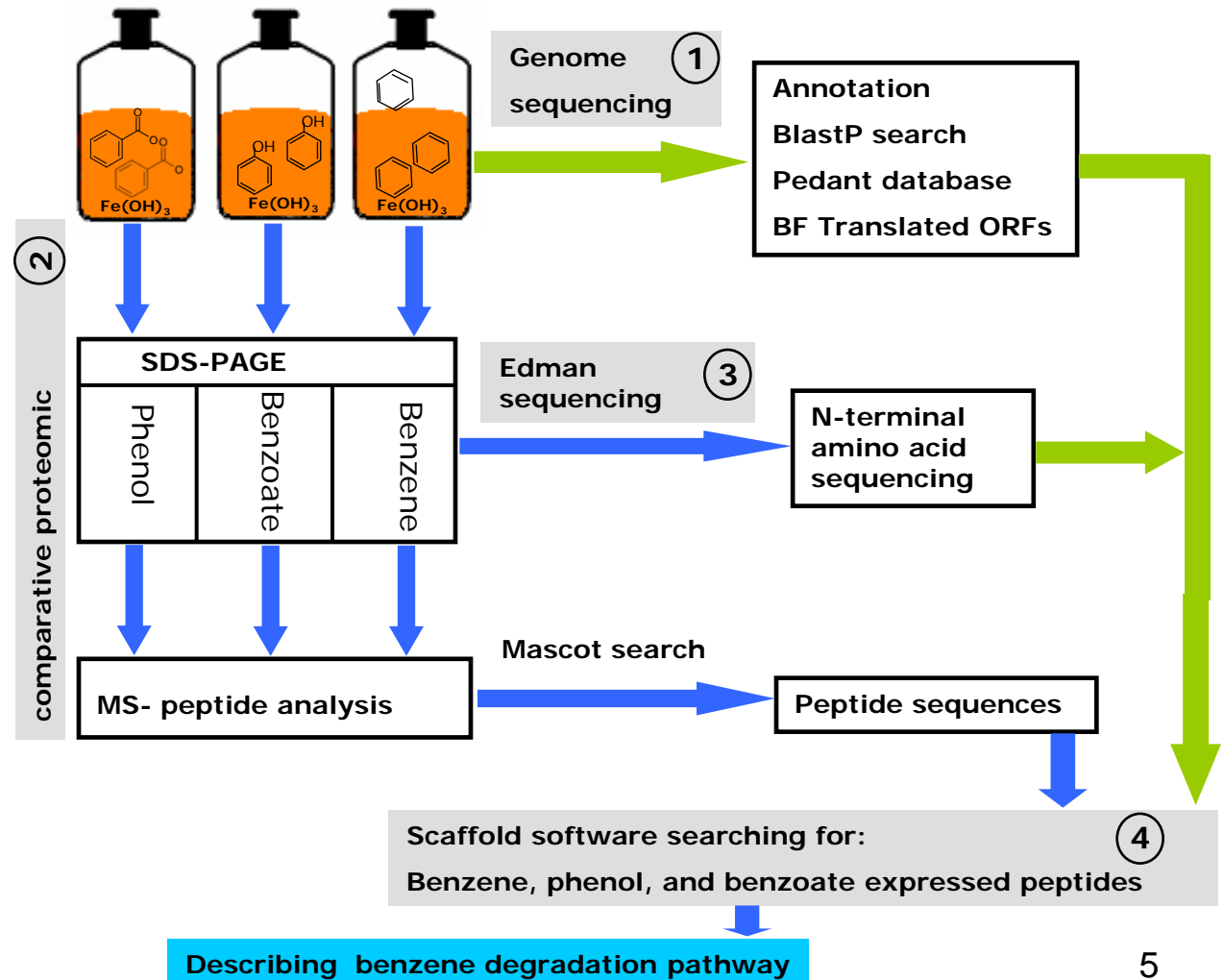
16S rRNA T-RFLP analysis

16S rRNA Cloning & sequencing

16S rRNA FISH

Novel benzene degrader

Identification of benzene-degrading enzymes

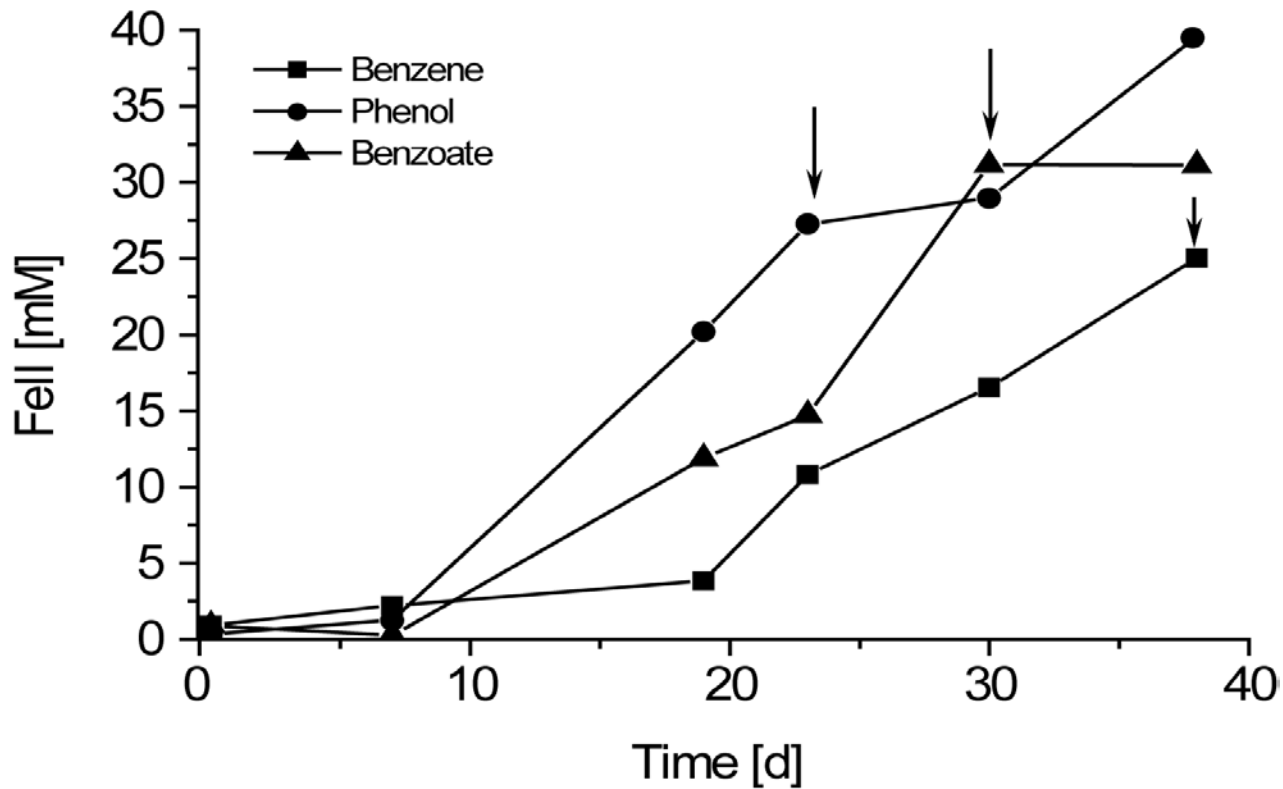


Iron-reducing enrichment culture BF

Characteristics	Enrichment culture BF
<u>Physiological</u>	
Gram stain	Positive & negative
Aromatic substrates	Benzene/ Phenol/ OH-benzoate/ Benzoate
Electron acceptor	Ferrihydrite [Fe(OH) ₃]
Special requirements	0.5 mM Na ₂ S
<u>Molecular</u>	
16S rRNA T-RF	Dominant 289-bp
Phylogentic affiliation	92 % <i>Therminocola ferriacetica</i>
Association	Syntrophic with <i>Desulfobulbaceae</i>

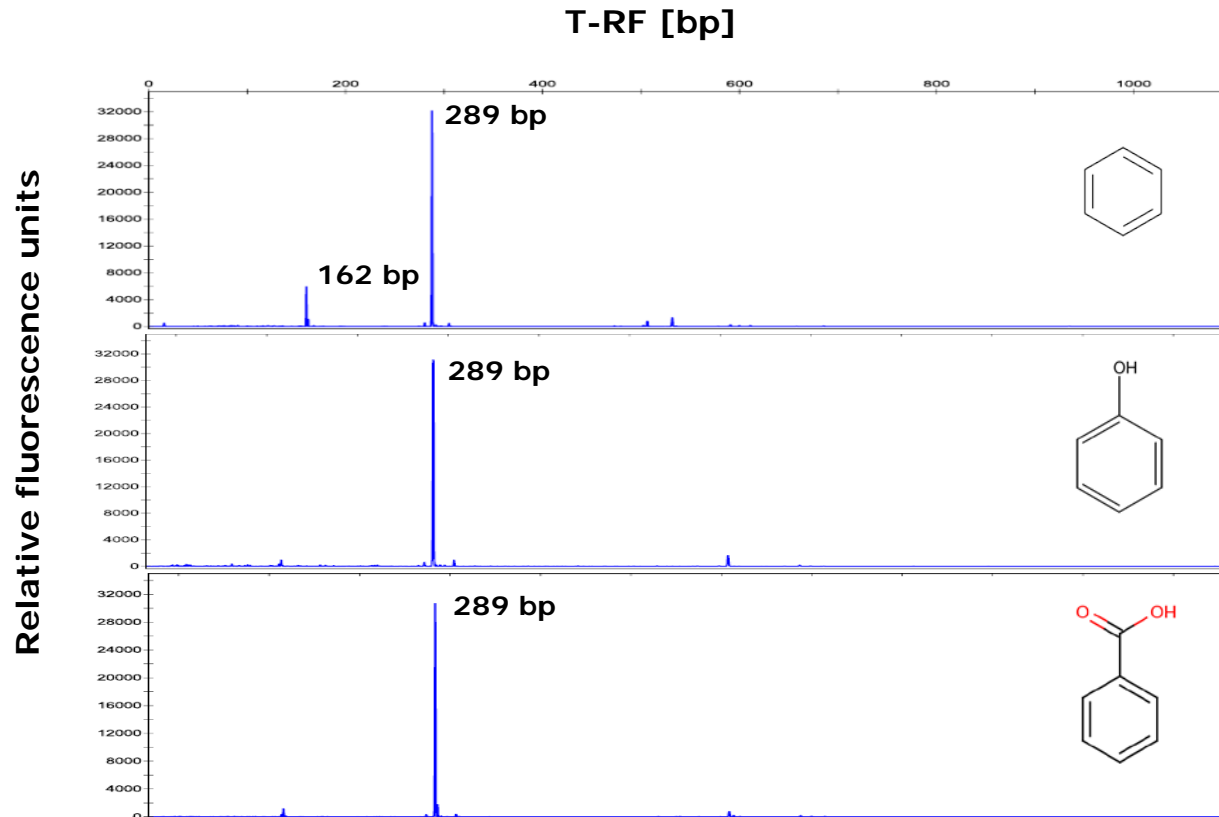
Iron-reducing enrichment culture BF

Growth of bacteria on benzene, phenol or benzoate as organic substrates



Iron-reducing enrichment culture BF

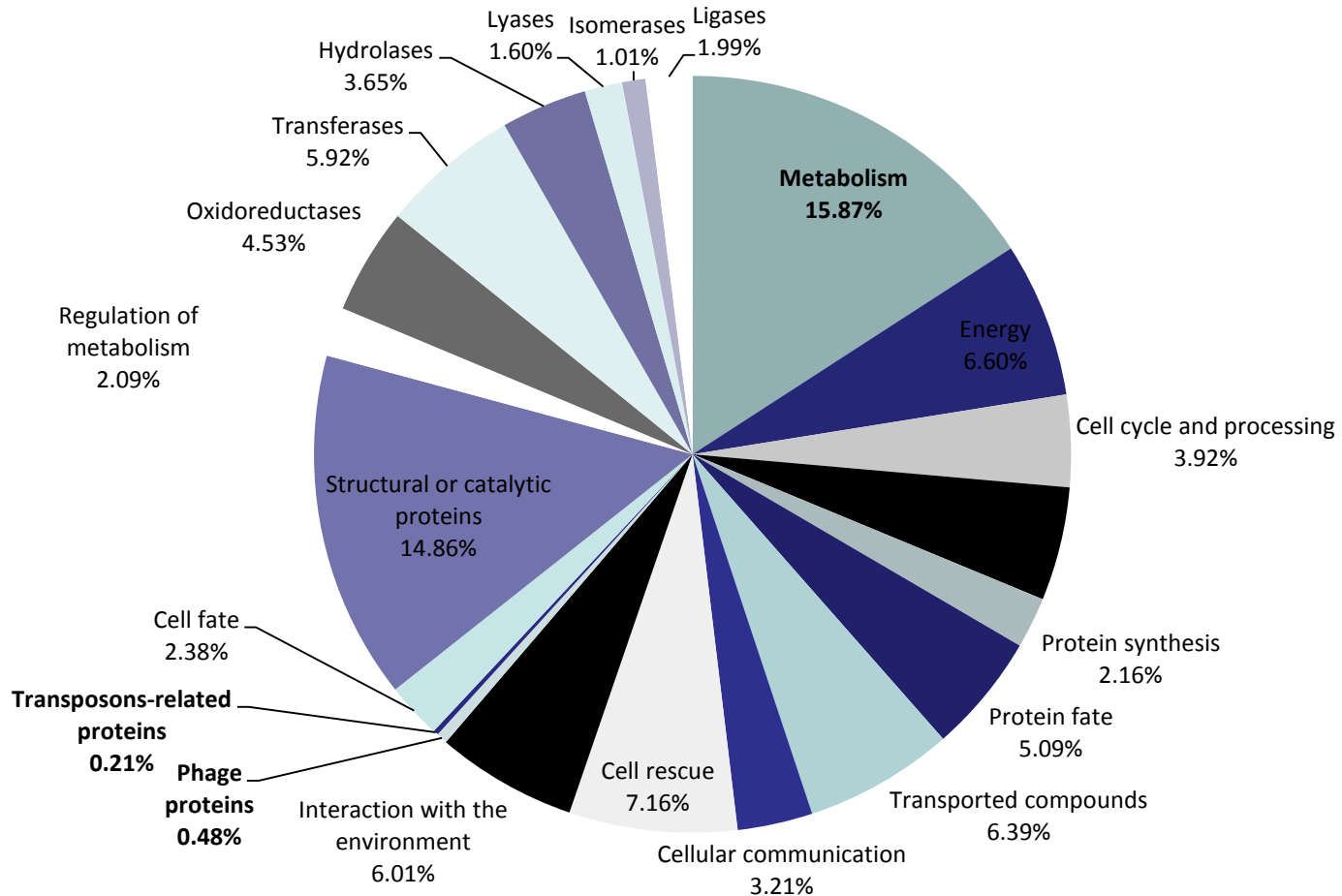
T-RFLP analysis of BF culture grown on benzene, phenol, and benzoate



Metagenome analysis of iron-reducing culture BF

Bacteria clustering within *Peptococcaceae* and *Desulfobulbaceae*

Total size 10.13 Mb / 14270 ORFs/ 5832 contigs size range 0.07-537.4 kb



Metagenome analysis of benzene-degrading culture BF

Putative mobile elements genes identified in the aromatic-degrading contigs

Contig no.	Mobile element ORF no. ^a	Gene type	Function of best protein match (SIMAP)	Putative aromatic-degrading genes located in the contig ^b
BF_5874	BF_5874_28992 BF_5874_31814 BF_5874_38427 BF_5874_42081	Phage-gene Phage-gene Phage-gene Phage-gene	Mu-like prophage protein Conserved hypothetical phage protein Putative phage terminase protein, large subunit DnaC, DNA replication, recombination, and repair	<i>ppsA</i> ^d
BF_5932	BF_5932_1	Phage-gene	DNA breaking-rejoining enzyme (integrase-like)	<i>hbaB</i> ^c , <i>hbaD</i> ^c
BF_11345	BF_11345_125129 BF_11345_509350	Transposon-gene Phage-gene	Cell wall-associated hydrolases Predicted transcriptional regulator	<i>bamB-bamI</i> ^e , <i>hbaD</i> ^c , <i>hcrB</i> ^c
BF_11393	BF_11393_35649	Phage-gene	Phage integrase family protein	<i>bamB</i> ^e , <i>bamM</i> ^e , <i>bzdY</i> ^d , <i>bzdW</i> ^d , <i>bzdX</i> ^d
BF_11402	BF_11402_16417 BF_11402_30654 BF_11402_50586 BF_11402_87968 BF_11402_104993	Phage-gene Phage-gene Phage-gene Phage-gene Phage-gene	Spore cortex-lytic enzyme SleB Glycoside hydrolase family 18 Peptidase M23B Methyltransferase domain family Glycosyltransferases involved in cell wall biogenesis	<i>hcrC</i> ^c
BF_11425	BF_11425_36288	Phage-gene	Ssb, Single-stranded DNA-binding protein [DNA replication, recombination, and repair]	<i>bamY</i> ^e
BF_11429	BF_11429_966 BF_11429_30881 BF_11429_38373	Phage-gene Phage-gene Phage-gene	A, Site-Specific Recombinase, XerD [tyrosine recombinase] AbiF, Abortive infection bacteriophage resistance protein [Defense mechanisms] Transcriptional regulator, XRE family	<i>bamD</i> ^e , <i>ppcD</i> ^d
BF_11460	BF_11460_113289	Transposon movement-gene	Transposase, IS605 OrfB family [DNA replication, recombination, and repair]	<i>bamB</i> ^e , <i>bamC</i> ^e , <i>bamM</i> ^e , <i>pcmR</i> ^e , <i>PcmS</i> ^e
BF_11494	BF_11494_43580 BF_11494_47335 BF_11494_76645	Transposon-gene Phage-gene Phage-gene	DNA segregation ATPase FtsK/SpolIIE and related proteins Transposase, IS605 OrfB family [DNA replication, recombination, and repair] LysM repeat [Cell envelope biogenesis, outer membrane]	<i>bbsA-bbsG</i> ^d
BF_11500	BF_11500_2	Transposon-gene	Transposase, IS605 OrfB family [DNA replication, recombination, and repair]	<i>hbaD</i> ^c , <i>hbaC</i> ^c , <i>pcmT</i> ^e , <i>bamY</i> ^e
BF_11563	BF_11563_1 BF_11563_37691	Transposon-gene Phage-gene	Transposase, IS605 OrfB family [DNA replication, recombination, and repair] Glycoside hydrolase family 2	<i>pcmS</i> ^e , <i>pcmT</i> ^e

^a The number of the ORF for predicted proteins from contigs bzn_200901 PEDANT database.

^b The abbreviations of the genes are: *ppsA*, phenylphosphate synthase alpha subunit; *bbsA-bbsG*, beta-oxidation of benzylsuccinate; *hbaD/hcrB/pcmR*, 4-hydroxybenzoyl-CoA reductase beta subunit; *hbaC/hcrA/pcmT*, 4-hydroxybenzoyl-CoA reductase alpha subunit; *hbaB/hcrC/pcmS*, 4-hydroxybenzoyl-CoA reductase gamma subunit; *bamY*, benzoate-CoA ligase; *bamB-bamI*, benzoyl-CoA reductase analogue; *bzdY*, 6-oxocyclohex-1-ene-1-carbonyl-CoA hydratase; *bzdW*, enoyl-CoA hydratase; *bzdX*, 6-hydroxycyclohex-1-ene-1-carboxyl-CoA dehydrogenase.

^c gene is more similar to those of facultative anaerobes of *alpha-proteobacteria*.

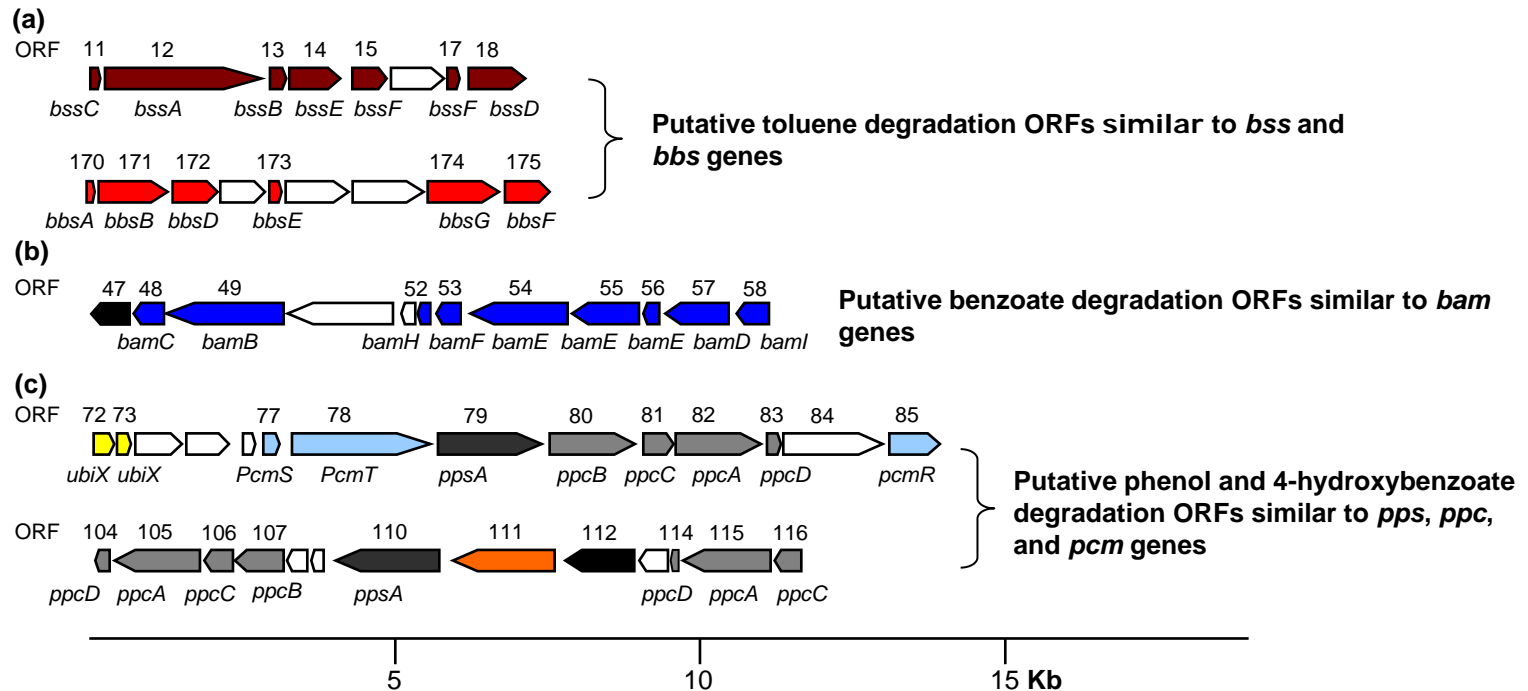
^d gene is more similar to those of denitrifying of *beta-proteobacteria*.

^e gene is more similar to those of strict anaerobes of *delta-proteobacteria*.

Metagenome analysis of benzene-degrading culture BF

More than 205 genes related to aromatic compounds degradation

Putative aromatic-degrading gene clusters



- Benzylsuccinate synthase
- β -oxidation of benzylsuccinate
- Benzoate degradation *bamB-I*
- Benzoate-CoA ligase
- 3-octaprenyl-4-hydroxybenzoate carboxy-lyase
- Phenylphosphate carboxylase
- Phenylphosphate synthase
- 4-hydroxybenzoyl-CoA reductase
- Sigma 54 specific transcriptional regulator
- Putative uncharacterized protein

(Abu Laban *et al.*, EMI 2010)

Metaproteomic analysis of iron-reducing cultures BF

SDS-PAGE for identification of specific benzene-expressed proteins

ORF 138: 57.4 kDa, 43% identity to PpcA

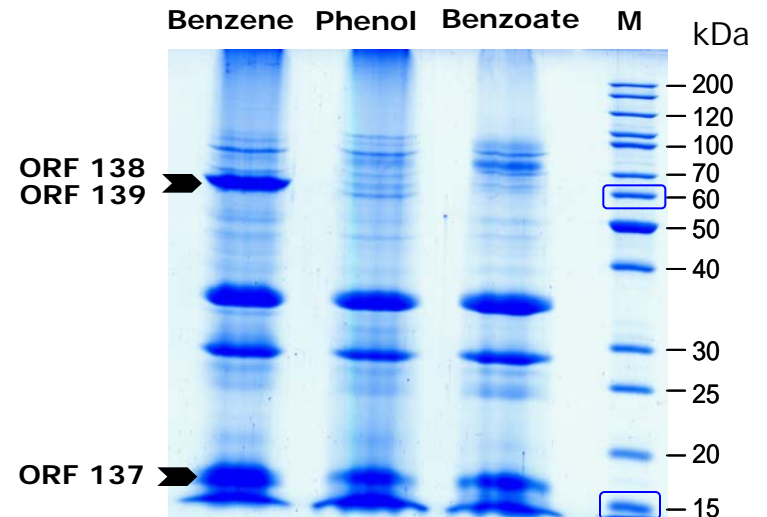
Aromatoleum aromaticum EbN1

ORF 139: 60.7 kDa, 56% identity to BamY

Geobacter metallireducens

ORF 137: 14.7 kDa, 37% identity to PpcD

Aromatoleum aromaticum EbN1



Phenylphosphate carboxylase PpcD-, and PpcA-like proteins specifically expressed in benzene. We named **anaerobic benzene carboxylase AbcD and AbcA.**

Benzoate-CoA ligase BamY named BzIA.

(Abu Laban *et al.*, EMI 2010)

Summary

- ❖ Gram-positive bacteria were shown to be involved in benzene degradation under iron-reducing condition.
- ❖ About 205 genes were identified to have closest sequence similarity to genes encoding enzymes and transcriptional regulators known to be involved in anaerobic aromatic hydrocarbon degradation.
- ❖ Specific proteins of anaerobic degradation pathway were detected from benzene-grown cultures, whereas no enzymes of anaerobic toluene or phenol degradation could be identified.
- ❖ Putative anaerobic benzene carboxylase (AbcDA) and Benzoate-CoA ligase (BzIA) proteins specifically expressed in benzene.
- ❖ These findings support our recent metabolite-based analysis where carboxylation was favored as initial biochemical mechanism of anaerobic benzene degradation.
- ❖ The catalysis mechanism of the putative anaerobic benzene carboxylase needs further investigations.

Acknowledgements

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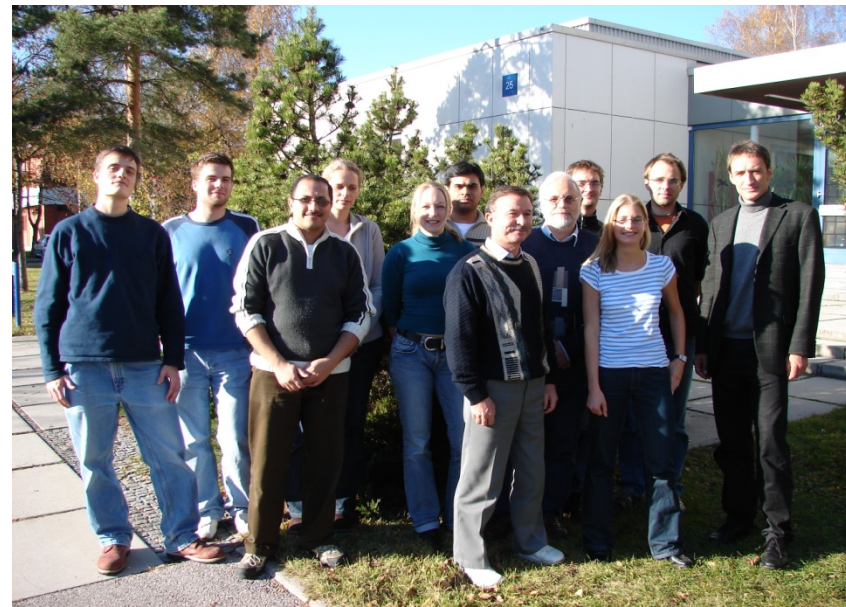
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Recent work poster “Identifying Key Microbes in Methanogenic Hydrocarbon-Degrading Enrichment Cultures from Oil Sands Tailings”.



Thank you for your attention

Questions and suggestions