Development of rapid technologies for the assessment of biodegradation potential in contaminated groundwater using gene array technologies

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ATWARM meeting - 1st of May 2012
1. Microbial Community Study in polluted groundwater

2. Identification of common patterns and markers of biodegradation process

3. Development of rapid technology
1. Microbial Community Study in polluted groundwater

Molecular Biology Methods:

- Amplification, DGGE and sequencing of functional genes involved in aerobic degradation of PAHs, BTEX and Alkane and anaerobic degradation of aromatic compounds!

- 16s rRNA 454 Pyrosequencing bTEFAP (Bacteria Tag-Encoded FLX Amplicon Pyrosequencing) method!
Both sites located in Northern Ireland:

**SITE1** is contaminated by Diesel and the contamination has been caused by an accidental spillage (3 sample sets).

**SITE2** is a former gaswork site and is contaminated mainly by PAHs and BTEX (2 sample sets).
Progress up-to-date: functional genes

## Primer sets (PCRs, DGGE, Sequencing)

<table>
<thead>
<tr>
<th>Gene/subunit</th>
<th>Primer set</th>
<th>Pathway</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+ α sub PAH dioxygenase</td>
<td>PAH-RHDα GP F/R</td>
<td>Aerobic – PAHs degradation</td>
<td>[1]</td>
</tr>
<tr>
<td>G- α sub PAH dioxygenase</td>
<td>PAH-RHDα GN F/R</td>
<td>Aerobic – PAHs degradation</td>
<td>[1]</td>
</tr>
<tr>
<td>Naphthalene dioxygenase</td>
<td>NAPH-1 F/R + NAPH-2 F/R DGGE</td>
<td>Aerobic – PAHs degradation</td>
<td>[2]</td>
</tr>
<tr>
<td>nahAc-type dioxygenase</td>
<td>Ac114 F/Ac596 R</td>
<td>Aerobic – PAHs degradation</td>
<td>[3]</td>
</tr>
<tr>
<td>Comamonas α sub PAH diox</td>
<td>COM1 F/R</td>
<td>Aerobic – PAHs degradation</td>
<td>[4]</td>
</tr>
<tr>
<td>Pseudomonas α sub PAH diox</td>
<td>PSE1 F/R</td>
<td>Aerobic – PAHs degradation</td>
<td>[4]</td>
</tr>
<tr>
<td>Rhodococcus α sub PAH diox</td>
<td>RHO1 F/R</td>
<td>Aerobic – PAHs degradation</td>
<td>[4]</td>
</tr>
<tr>
<td>Benzoate dioxygenase</td>
<td>Bdo F/R</td>
<td>Aerobic – BTEX degradation</td>
<td>[5]</td>
</tr>
<tr>
<td>alkB gene monooxygenase</td>
<td>AlkBw F/R</td>
<td>Aerobic – Alkane degradation</td>
<td>[6]</td>
</tr>
<tr>
<td>Benzoyl succinate synthase</td>
<td>BssA 1230 F</td>
<td>Anaerobic – BTEX degradation</td>
<td>[8]</td>
</tr>
</tbody>
</table>

### Progress up-to-date: functional genes

#### Functional Genes Amplification

<table>
<thead>
<tr>
<th>Primer set</th>
<th>Site1</th>
<th>Site2</th>
<th>+ control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAH-RHDα GP aerobic path</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PAH-RHDα GN aerobic path</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NAPH-1 aerobic path</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Ac114/Ac596 aerobic path</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>COM1 aerobic path</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PSE1 aerobic path</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>RHO1 aerobic path</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Bdo aerobic path</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AlkBw aerobic path</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BamA anaerobic path</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BssA anaerobic path</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ means a band of the proper size in the agarose gel, +/- means a band of the proper size but with a weak intensity or more than one band, - means no bands.

- **Progress up-to-date:** functional genes

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Does a Comamonas type bacterium dominate an aerobic degradation process in the contaminated area? ...probably!

Further investigation are required!

1\textsuperscript{st} cut and sequence the DGGE band

2\textsuperscript{nd} link the function with the identity: what about SIP???

3\textsuperscript{rd} compare all the P2 samples taken over the last 12 months (3 sample sets)
Progress up-to-date: functional genes

Cloning and sequencing

1st round to evaluate the primer effectiveness prior other tests (qPCR and deeper sequencing): only few clones sequenced!

*Bss and ndo gene from BH8*

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...and now? Next steps in the functional genes analysis are deeper sequencing and qPCR

**It worked properly!!**
Progress up-to-date

16s 454 pyrosequencing - bTEFAP method

DNA SAMPLES

1. TAG1
2. TAG2
3. TAG3
4. TAG4

A m p l i f i c a t i o n

Pooling

16S

454 Pyrosequencing

A specific software assigns each tag to the proper sample

Faster
Deeper coverage

PHYLOGENETIC ANALYSIS of SITE2 DNA samples

...experimental design concluded sequencing in the next weeks!

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Future plan

Microarray construction

Analysis of methanotroph community composition using a pmoA-based microbial diagnostic microarray

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6 months protocol!

Our functional genes SEQUENCES + database resources: probe design!

qPCR + samples collection: validate the effectiveness of the tool
THANK YOU!