Biodegradation of Polystyrene Foam by the Microorganisms from Landfill

Pat Pataranutaporn

Assistant prof. Savaporn Supaphol
prof. Amornrat Phongdara
Sureeporn Nualkaew
Hi, I would like to invite you to take a look on my research.
Pat
Introduction

“Styrofoam”
Polystyrene

Disadvantage

- Non-biodegradable in the environment
- Made from non-renewable petroleum products
- Chronic, low-level exposure risks undetermined

Physical Properties

- chemical formula is \((C_8H_8)_n\)
- monomer styrene
- Thermoplastic
- blowing agents
Bacteria nutritional requirements

- Energy source
- Carbon source
- Nitrogen source
- Minerals
- Water
- Growth factors

Polystyrene structure

Possibly work?

http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit6/metabolism/growth/factors.html
Aims of the research

‣ To identify the microbe that able to growth in the condition that polystyrene is a sole carbon source

‣ To study the changing of microbe community structure in the selective culture which polystyrene is a sole carbon source

‣ To observe the biodegradability of polystyrene
To analyse the by product of polystyrene after degradation
Methodology
Methodology

1. Microbe sampling
2. Screening Cultivation
3. 2 months later
4. 16s Ribosomal RNA identification
5. Agar cultivation
6. Community fingerprint
7. Molecular cloning
8. Phylogenetic tree
9. Degradability observation (SEM)
Methodology

Microbe sampling & cultivation

1. Microbe sampling
2. Screening Cultivation
3. 2 months later

- Degradability observation (SEM)
- Agar cultivation
- Community fingerprint
- 16s Ribosomal RNA identification
- Molecular cloning
- Phylogenetic tree

Microbial sampling & cultivation process diagram.
landfill that was contaminated by Polystyrene foam in Pattani, Thailand

Methodology

Community structure analysis

Styrofoam in the landfill

Contaminated soil
Methodology

Community structure analysis

**F**
- MSM broth
- Sterile Polystyrene
- Landfill styrofoam

**S**
- MSM broth
- Sterile Polystyrene
- Landfill soil

**Control**
- MSM broth
- Sterile Polystyrene

**Sterile Polystyrene**

**MSM broth**
- K$_2$HPO$_4$
- KH$_2$PO$_4$
- (NH$_4$)$_2$SO$_4$
- MgSO$_4$
- FeSO$_4$.2H$_2$O
- MnCl$_2$.4H$_2$O
- CoCl$_2$.6H$_2$O
- CuCl$_2$.2H$_2$O
- NiCl$_2$.6H$_2$O
- Na$_2$MoO$_4$.2H$_2$O
- ZnSO$_4$.7H$_2$O
- H$_3$BO$_3$
Methodology

Community structure analysis

Shake in shaker for 1 month then inoculate to new fresh broth for sub culture.
Every week, the cell suspension in particular flask was taken to the eppendorf then stored at 2°C for stop bacteria growth. This solution used to monitor the changing of bacteria population.
Methodology

Community structure analysis

![Diagram showing time (week) and transfer culture process]

- 0: Place 400 µl.
- 1: Transfer culture
- 2, 3, 4, 6, 7, 8, 9: Further cultures
Methodology

Community structure analysis

- Microbe sampling
- Screening Cultivation
- 2 months later
- Agar cultivation
- Degradability observation (SEM)
- 16s Ribosomal RNA identification
- Community fingerprint
- Molecular cloning
- Phylogenetic tree
Cell suspensions collected from each week of cultivation.

DNA Extraction
(Methode: QIAamp Protocol)

Polymerase Chain Reaction (PCR)
16S rRNA gene Amplification
Primer VFC & VR

16s Ribosomal RNA identification

Denature Gradient Gel Electrophoresis (DGGE)
Community fingerprint

Community structure analysis
DNA Replication: PCR (TopTaq Master Mix Kit)

16S rRNA gene Amplification

by using Primer VR (Medlin et al., 1998) & VFC (Muyzer et al., 1993)

CONSERVED REGIONS: unspecific applications

VARIABLE REGIONS: group or species-specific applications
Community structure trend

Microbe diversity

Dominant species

Each DNA band represents 1 microbe

DGGE
Denature Gradient Gel Electrophoresis

Looking for survival!
**Result**

Community structure analysis

**DGGE 26/04/55**

Running 300 minute from PCR product 24/04/55 template use 8 µl.

- **Continuing band & found in control**
- **Continuing band**
- **Non-continuing band**
## Methodology

### Molecular cloning & identification

<table>
<thead>
<tr>
<th>Bacteria from soil</th>
<th>Bacteria from styrofoam</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
<td>Marker</td>
<td>Marker</td>
</tr>
<tr>
<td>Soil week 1</td>
<td>Foam week 1</td>
<td>Control week 7</td>
</tr>
<tr>
<td>Soil week 5</td>
<td>Foam week 5</td>
<td>Control week 8</td>
</tr>
<tr>
<td>Soil week 6</td>
<td>Foam week 6</td>
<td>Marker</td>
</tr>
<tr>
<td>Soil week 20</td>
<td>Foam week 7</td>
<td>Control week 7</td>
</tr>
<tr>
<td>Soil week 8</td>
<td>Foam week 8</td>
<td>Marker</td>
</tr>
</tbody>
</table>

Selected DNA

- Bacteria from soil
- Bacteria from styrofoam
- Control

Selected for cloning
**Methodology**

**DNA from S week 7, F week 7 and con week 7**

**Polymerase Chain Reaction (PCR)**

16S rRNA gene Amplification

Primer AF1 & 1541R

**16s Ribosomal RNA identification**

**Molecular cloning & identification**

Ligate with pGEM T-Easy Plasmid

Transfer Plasmid to the competent cell (E.Coli) + Propagate

extracted Plasmid + cutcheck with EcoR1

Purify Plasmid + cutcheck with EcoR1

Nucleotide sequencing

**Phylogenetic tree**

Blasting + Neighbourhood joining tree contracting
**PCR Product**

**16S rRNA gene Amplification**

Primer AF1 & 1541R

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**Molecular cloning & identification**

- Extracted Plasmid + cut check with EcoR1
- Purify Plasmid + cut check with EcoR1

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**Gel electrophoresis**
Molecular cloning & identification

Sequence report - Electropherogram

F3
F10
S7

Control 4
Control 7
F5
## Sequence blasting

<table>
<thead>
<tr>
<th>Sample</th>
<th>Length (bp)</th>
<th>Similar sequence</th>
<th>Max iden</th>
<th>Max score</th>
<th>E.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F10</td>
<td>444</td>
<td>Herbasprillium.sp</td>
<td>98%</td>
<td>753</td>
<td>0.0</td>
</tr>
<tr>
<td>F3</td>
<td>504</td>
<td>Massialia aerilata</td>
<td>97%</td>
<td>830</td>
<td>0.0</td>
</tr>
<tr>
<td>S7</td>
<td>485</td>
<td>Caulobacter segnis ATCC 21756</td>
<td>98%</td>
<td>830</td>
<td>0.0</td>
</tr>
<tr>
<td>Control4</td>
<td>1,055</td>
<td>Azohydromonas australica</td>
<td>83%</td>
<td>1297</td>
<td>0.0</td>
</tr>
<tr>
<td>Control7</td>
<td>1,006</td>
<td>Ochrobactrum rhizosphaerea</td>
<td>82%</td>
<td>1193</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Molecular cloning & identification

Herbaspirillum chlorophenolicum
Herbaspirillum frisingense
Herbaspirillum seropedicae
F10
Collimonas arenae
Herminiimonas glaciei
Janthinobacterium lividum
Janthinobacterium agaricidamnosum
Janthinobacterium agaricidamnosum(2)
Massilia brevitalea
Naxibacter varians
Naxibacter haematophilus
F3
Massilia aerilata
Methylibium petroleiphilum PM1
Schlegelella thermodedeopolymerans
Azohydromonas lata
Rubrivivax gelatinosus IL144
Aquincola tertiaricarbonis
Control4
Azohydromonas australica
Brevundimonas nasdae
Streptomyces longisporoflavus
Mycoplasma bullata
S7
Caulobacter segnis ATCC 21756
Phenyllobacterium koreense
Rhizobium alamii
Ensifer adhaerens
Sinorhizobium fredii NGR234
Brucella ovis ATCC 25840
Ochrobactrum haematophilum
Control7
Ochrobactrum rhizosphaerae
out group

Neighbourhood joining tree contract from the Specimen DNA sequence
## Result

<table>
<thead>
<tr>
<th>Information</th>
<th>Found in soil culture(S)</th>
<th>Found in foam culture(F)</th>
<th>Found in control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taxonomy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caulobacter segnis</td>
<td>Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Caulobacter</td>
<td>Bacteria; Proteobacteria; Alphaproteobacteria; Burkholderiales Oxalobacteraceae Massilia</td>
<td>Bacteria; Proteobacteria; Alphaproteobacteria; Burkholderiales Oxalobacteraceae Herbaspirillum</td>
</tr>
<tr>
<td>Massilia aerilata</td>
<td>Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales Oxalobacteraceae Massilia</td>
<td>Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales Oxalobacteraceae Herbaspirillum</td>
<td>Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Brucellaceae; Ochrobactrum</td>
</tr>
<tr>
<td>Herbaspirillum seropedicae</td>
<td>Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales Oxalobacteraceae Herbaspirillum</td>
<td>Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Brucellaceae; Ochrobactrum</td>
<td>Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales Alcaligenaceae Azohydromonas</td>
</tr>
<tr>
<td>Ochrobactrum sp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azohydromonas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Morphology &amp; classification</strong></td>
<td>Negative, Bacilli, Aerobic, Mesophilic</td>
<td>Negative, Bacilli, Aerobic</td>
<td>Negative, Bacilli</td>
</tr>
<tr>
<td><strong>Styrene degradation</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Aromatic compound degradation</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Carbon fixation</strong></td>
<td>-</td>
<td>Na</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Polycyclic aromatic degradation</strong></td>
<td>✓</td>
<td>Na</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Chlorophenol degradation</strong></td>
<td>✓</td>
<td>Na</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Nitrogen metabolism</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Other pathway</strong></td>
<td>Cellulose degradation pathway</td>
<td>Polyhydroxybutyrate (PHB) production</td>
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Methodology

Degradability Observation

1. Microbe sampling
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   (SEM)
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Methodology

Degradability Observation

The microscopic techniques

- Test Method Used: In house method refer to WI-RES-SEM-Quanta-001 and WI-RES-SEM-001
- Test Equipment: Scanning Electron Microscope, Quanta40, FEI, Czech Republic
- Test Technique: Electron micrograph
- Test Condition
  - Mode: low vacuum
  - Detector: Large Field Detector (LFD)
  - High Voltage: 15.00, 20.00 kV
Methodology

Degradability Observation

Control: Polystyrene in MSM broth without bacterial source.

Regular polystyrene foam that didn’t use in experiment.
Methodology

Degradability Observation

Polystyrene in Medium with bacteria from **Styrofoam in the landfill.**

Regular polystyrene foam that didn’t use in experiment.
Polystyrene in Medium with bacteria from soil in the landfill.

Regular polystyrene foam that didn’t use in experiment.
Polystyrene in Medium with bacteria from soil in the landfill.
Methodology

Agar Cultivation

Microbe sampling → Screening Cultivation → 2 months later → Degradability observation (SEM) → 16s Ribosomal RNA identification → Molecular cloning → Phylogenetic tree → Community fingerprint

1. Agar Cultivation
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**Methodology**

**MSM broth**
- $\text{K}_2\text{HPO}_4$
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- $\text{CoCl}_2.6\text{H}_2\text{O}$
- $\text{CuCl}_2.2\text{H}_2\text{O}$
- $\text{NiCl}_2.6\text{H}_2\text{O}$
- $\text{Na}_2\text{MoO}_4.2\text{H}_2\text{O}$
- $\text{ZnSO}_4.7\text{H}_2\text{O}$
- $\text{H}_3\text{BO}_3$

**MSM + Agar**
No carbon source

**Control**
- MSM + Agar
- No carbon source

**MSM + Agar**
- + Polystyrene-coacrylic acid (PSA)
  (particles diameter 500 nm)
The purpose is to isolate the single colony of the bacteria prior culture in the liquid broth.

Problem: Agar is also the carbon source for bacteria result in unable to created selective condition.

Using filter paper for bacteria attachment surface.
Methodology

Agar Cultivation

- No bacteria colony grow on the filter paper
- Bacteria colony not separate well on the plate
- Bacteria density in the plate with PS is more than plate with out PS
Conclusion & Discussion

Control

Soil source

Styrofoam source
## Conclusion & Discussion

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The highest degradation trade was made by the bacteria from the styrofoam in the landfill, relate to the dominance species that were present in the continuous bold DNA band in the DGGE gel.

The DNA sequence reveals that the bacteria that were in the consortium, some have a metabolism to degrade styrene and aromatic-hydrocarbon.
Research Achievements

13th NCSC, Jaipur India 2011

Youth summit 2012, Dubai UAE

BYEE, Leverkuzen Germany
present to the princess of Thailand


Scholarships
If I have seen further it is by standing on the shoulders of giants.

- Isaac Newton -
RIP
My brave Bacteria