TROPICAL BACTERIAL PLASTIC TERMINATOR

Bogor Agricultural University - Indonesia -

Consumption Plastic per Capita (Kg/Year)

(Panda et al. 2010)
Less Awareness About Plastic

Al-Salem et al. (2010)
Disadvantages of Plastic Wastes
what is PET?

esterases
lipases
polyhydroxyalkanoate depolymerases

(Braaz et al. 2003, Calado et al. 2004)

Methodology
Modelling

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Result

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**Isolate Collection**

- (NH₄)₂SO₄, 1g/L
- NaNO₃, 1g/L
- K₂HPO₄, 1g/L
- KCl, 1g/L
- MgSO₄, 0.2g/L
- Yeast Extract, 0.1g/L
- Glucose, 0.5 g/L
- Bacto Agar 22.23g/L
- Plastic Powder (PE; PP) 2 gr/L
- Distillate water 1L

20 spots from Garbage dumping area Galuga

Σ 20 plates, enrichment medium

6 potential isolates

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**Potential Isolates**

6 selected potential isolates

A | B | C

D | E | F

C and F have the widest clear zone

Enzyme activity of both isolates?
Depolymerase enzymes activity

The Enzyme Activity (24 h) from C and F isolates to degrade PET

$\Delta W/24 \text{ h} (%)$

$\Delta W = \text{Weight loss of Plastic}$

Isolate Identification

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query coverage</th>
<th>E value</th>
<th>Max Ident</th>
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</thead>
<tbody>
<tr>
<td>Bacillus subtilis strain IAM-H-63 16S rDNA gene, partial sequence</td>
<td>1050</td>
<td>1020</td>
<td>88%</td>
<td>0.0</td>
<td>94%</td>
</tr>
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<td>Bacillus sp. MEG-20101 strain H44 16S rDNA gene, partial sequence</td>
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<td>1030</td>
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<tr>
<td>Bacillus subtilis strain NCK-C2 complete genome</td>
<td>1037</td>
<td>1022</td>
<td>86%</td>
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<td>94%</td>
</tr>
<tr>
<td>Bacillus subtilis strain AKB4 16S rDNA gene, partial sequence</td>
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<tr>
<td>Bacillus subtilis strain 16S rDNA gene, partial sequence</td>
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<td>1022</td>
<td>86%</td>
<td>0.0</td>
<td>94%</td>
</tr>
<tr>
<td>Bacillus subtilis strain 16S rDNA gene, partial sequence</td>
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<td>1022</td>
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<td>94%</td>
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<td>Bacillus subtilis strain DF6116S rDNA gene, partial sequence</td>
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<tr>
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<td>1022</td>
<td>86%</td>
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</tr>
<tr>
<td>Bacillus subtilis strain IAM-H-63 16S rDNA gene, partial sequence</td>
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<td>1022</td>
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</tbody>
</table>

Max Ident: 94%

Bacillus subtilis

New Strain?

Lipase (Asheeba et al. 2010)

Cutinase (Ban et al. 2008)
Cutinase-like gene isolation

Note:
M: 1 Kb Marker
C, D, F: Isolate code

**Primer...**
Forward: 5'ACGCGCGGCGGTACCGAGCA'3
Reverse: 5'ACGCGTCGTCGCGTCAGGCGCA'3

Prospect Result

- Cutinase-like gene
- DNA Ligation with pGEM-T
- *E. coli* DH5α Transformation
- pGEM-T recombinant Isolation
- Ligation PET 14B-promotor & terminator with pSB1C3
- Ligation of pGEM-T recombinant with pSB1C3
- The Insertion of pSB1C3 recombinant to *E. coli* DH5α
- Plastic Terminator
Conclusion

PET degradation enzyme activity
- C: 1.093%/day
- F: 0.43%/day

Tropical bacterial has ability on degrade PET which is having 94% maximum identity with *Bacillus subtilis* (new strain)

Cutinase-like gene is potential to produced in high number and degrade the PET

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