Limitless Opportunities for Microbial Production of Hydroxyalkanoates Based Chemicals and Materials

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Limitless Opportunities for Microbial Production of Hydroxyalkanoates Based Chemicals and Materials

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Metabolic Engineering IX  June 3-7, 2012  Biarritz, France
I would like to thank the organizing committee, especially Professors Elmar Heinzle, Philippe Soucaille & Gregg Whited for the invitation and for giving me the opportunity to speak here, as well as the great organization efforts.

- Natural Science Foundation of China
- National High Tech 863 Grants (No. 2006AA02Z242 & 2010AA101607)
- State Basic Science Foundation 973 (No. 2012CB725200)
- Li Ka-Shing Foundation.
Content

• Introduction of PHA
• Diversity of PHA
• PHA metabolic pathways
• Pathways synthesis for PHA
  • Metabolic Engineering for PHA and monomer production
    – Homopolymers PHA
    – Block copolymers PHA
    – Random copolymers PHA
    – Chiral hydroxyalkanoic acids
• Conclusion

Macro-structures

Micro-structures
What are Polyhydroxyalkanoates (PHA)?

PHA: intracellular biopolymesters, are produced up to 96% cell dry weight

Approximately 30% soil bacteria can produce PHA

Sandoval et al., 2007
Introduction of PHA

**PHA Granules** *(cellular energy & carbon reserves)*

![PHB Granules Image](image)

**Formula:**

- \([O-C\#H-(CH_2)_m-C\%-O\)]

**Monomers:**

- **3HB**: short PHA monomers
- **3HV, 3HHx, 3HO, 3HD, 3HDD**: medium PHA monomers

**Problems:** Intracellular BioPolyesters with Diverse Structures that Are not easily Controlled in Micro- and Macro Polymer Structures
PHA Based Sustainable Industrial Value Chain

PHA Metabolism

PHA-producing bacteria
Many pathways lead to synthesis of PHA
8 Known PHA Synthesis Pathways

Pathway I
1. Sugar → Acetyl-CoA
2. Acetyl-CoA → Acetoacetyl-CoA
3. Acetoacetyl-CoA → S-3-hydroxybutyryl-CoA
4. 2-butenoyl-CoA → Butyryl-CoA
5. Butyryl-CoA → Butyric acid
6. Butyric acid → Fatty acids
7. Fatty acids → 3-Ketoacyl-CoA
8. 3-Ketoacyl-CoA → Acyl-CoA
9. Acyl-CoA → R-3-Hydroxyacyl-CoA
10. R-3-Hydroxyacyl-CoA → S-3-Hydroxyacyl-CoA
11. S-3-Hydroxyacyl-CoA → Acyl-CoA
12. Acyl-CoA → 2-cis-Enoyl-CoA
13. 2-cis-Enoyl-CoA → R-3-Hydroxyacyl-CoA
14. R-3-Hydroxyacyl-CoA → 3-Ketoacyl-CoA
15. 3-Ketoacyl-CoA → Acyl-CoA
16. Acyl-CoA → 4,5-Hydroxyacyl-CoA
17. 4,5-Hydroxyacyl-CoA → 4-Hydroxybutyryl-CoA
18. 4-Hydroxybutyryl-CoA → 4-Hydroxybutyrate
19. 4-Hydroxybutyrate → 6-Hydroxyhexanoate
20. 6-Hydroxyhexanoate → 6-Hydroxyhexanoyl-CoA
21. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA
22. Succinyl-CoA → Succinic semialdehyde
23. Succinic semialdehyde → 4-Hydroxybutyrate
24. 4-Hydroxybutyrate → 6-Oxohexanoate
25. 6-Oxohexanoate → Hexanedioic acid
26. Hexanedioic acid → Adipoyl-CoA
27. Adipoyl-CoA → Hexanedioic semialdehyde
28. Hexanedioic semialdehyde → 6-Hydroxyhexanoate

Pathway II
13. R-3-Hydroxyacyl-CoA → 3-Ketoacyl-CoA
14. 3-Ketoacyl-CoA → Acyl-CoA
15. Acyl-CoA → 4,5-Hydroxyacyl-CoA
16. 4,5-Hydroxyacyl-CoA → 4-Hydroxybutyryl-CoA
17. 4-Hydroxybutyryl-CoA → 4-Hydroxybutyrate
18. 4-Hydroxybutyrate → 6-Hydroxyhexanoate
19. 6-Hydroxyhexanoate → 6-Hydroxyhexanoyl-CoA
20. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA
21. Succinyl-CoA → Succinic semialdehyde
22. Succinic semialdehyde → 4-Hydroxybutyrate
23. 4-Hydroxybutyrate → 6-Oxohexanoate
24. 6-Oxohexanoate → Hexanedioic acid
25. Hexanedioic acid → Adipoyl-CoA
26. Adipoyl-CoA → Hexanedioic semialdehyde
27. Hexanedioic semialdehyde → 6-Hydroxyhexanoate

Pathway III
1. R-3-Hydroxyacyl-CoA → 3-Ketoacyl-CoA
2. 3-Ketoacyl-CoA → Acyl-CoA
3. Acyl-CoA → 4,5-Hydroxyacyl-CoA
4. 4,5-Hydroxyacyl-CoA → 4-Hydroxybutyryl-CoA
5. 4-Hydroxybutyryl-CoA → 4-Hydroxybutyrate
6. 4-Hydroxybutyrate → 6-Hydroxyhexanoate
7. 6-Hydroxyhexanoate → 6-Hydroxyhexanoyl-CoA
8. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA
9. Succinyl-CoA → Succinic semialdehyde
10. Succinic semialdehyde → 4-Hydroxybutyrate
11. 4-Hydroxybutyrate → 6-Oxohexanoate
12. 6-Oxohexanoate → Hexanedioic acid
13. Hexanedioic acid → Adipoyl-CoA
14. Adipoyl-CoA → Hexanedioic semialdehyde
15. Hexanedioic semialdehyde → 6-Hydroxyhexanoate

Pathway IV
1. S-3-hydroxybutyryl-CoA → 2-butenoyl-CoA
2. 2-butenoyl-CoA → Butyryl-CoA
3. Butyryl-CoA → Butyric acid
4. Butyric acid → Fatty acids
5. Fatty acids → 3-Ketoacyl-CoA
6. 3-Ketoacyl-CoA → Acyl-CoA
7. Acyl-CoA → R-3-Hydroxyacyl-CoA
8. R-3-Hydroxyacyl-CoA → S-3-Hydroxyacyl-CoA
9. S-3-Hydroxyacyl-CoA → Acyl-CoA
10. Acyl-CoA → 2-cis-Enoyl-CoA
11. 2-cis-Enoyl-CoA → R-3-Hydroxyacyl-CoA
12. R-3-Hydroxyacyl-CoA → 3-Ketoacyl-CoA
13. 3-Ketoacyl-CoA → Acyl-CoA
14. Acyl-CoA → 4,5-Hydroxyacyl-CoA
15. 4,5-Hydroxyacyl-CoA → 4-Hydroxybutyryl-CoA
16. 4-Hydroxybutyryl-CoA → 4-Hydroxybutyrate
17. 4-Hydroxybutyrate → 6-Hydroxyhexanoate
18. 6-Hydroxyhexanoate → 6-Hydroxyhexanoyl-CoA
19. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA
20. Succinyl-CoA → Succinic semialdehyde
21. Succinic semialdehyde → 4-Hydroxybutyrate
22. 4-Hydroxybutyrate → 6-Oxohexanoate
23. 6-Oxohexanoate → Hexanedioic acid
24. Hexanedioic acid → Adipoyl-CoA
25. Adipoyl-CoA → Hexanedioic semialdehyde
26. Hexanedioic semialdehyde → 6-Hydroxyhexanoate

Pathway V
1. TCA cycle → Succinyl-CoA
2. Succinyl-CoA → Succinic semialdehyde
3. Succinic semialdehyde → 4-Hydroxybutyrate
4. 4-Hydroxybutyrate → 6-Hydroxyhexanoate
5. 6-Hydroxyhexanoate → 6-Hydroxyhexanoyl-CoA
6. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA

Pathway VI
1. PHA → 3-Ketoacyl-CoA
2. 3-Ketoacyl-CoA → Acyl-CoA
3. Acyl-CoA → 4,5-Hydroxyacyl-CoA
4. 4,5-Hydroxyacyl-CoA → 4-Hydroxybutyryl-CoA
5. 4-Hydroxybutyryl-CoA → 4-Hydroxybutyrate
6. 4-Hydroxybutyrate → 6-Hydroxyhexanoate
7. 6-Hydroxyhexanoate → 6-Hydroxyhexanoyl-CoA
8. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA
9. Succinyl-CoA → Succinic semialdehyde
10. Succinic semialdehyde → 4-Hydroxybutyrate
11. 4-Hydroxybutyrate → 6-Oxohexanoate
12. 6-Oxohexanoate → Hexanedioic acid
13. Hexanedioic acid → Adipoyl-CoA
14. Adipoyl-CoA → Hexanedioic semialdehyde
15. Hexanedioic semialdehyde → 6-Hydroxyhexanoate

Pathway VII
1. 4-Hydroxybutyryl-CoA → 4-Hydroxybutyrate
2. 4-Hydroxybutyrate → 6-Hydroxyhexanoate
3. 6-Hydroxyhexanoate → 6-Hydroxyhexanoyl-CoA
4. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA
5. Succinyl-CoA → Succinic semialdehyde
6. Succinic semialdehyde → 4-Hydroxybutyrate
7. 4-Hydroxybutyrate → 6-Oxohexanoate
8. 6-Oxohexanoate → Hexanedioic acid
9. Hexanedioic acid → Adipoyl-CoA
10. Adipoyl-CoA → Hexanedioic semialdehyde
11. Hexanedioic semialdehyde → 6-Hydroxyhexanoate

Pathway VIII
1. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA
2. Succinyl-CoA → Succinic semialdehyde
3. Succinic semialdehyde → 4-Hydroxybutyrate
4. 4-Hydroxybutyrate → 6-Hydroxyhexanoate
5. 6-Hydroxyhexanoate → 6-Hydroxyhexanoyl-CoA
6. 6-Hydroxyhexanoyl-CoA → Succinyl-CoA

Chen GQ (Ed) Plastics from Bacteria. Wiley (2010)
How to Control PHA Macrostructures?

Homopolymers
- Same monomers

Copolymers
- Various monomers

Block copolymer: various monomers

$\left[ \text{CH} - (\text{CH}_2)_m - \text{C} - \right]_n$
Chen GQ. Curr Opin Biotechnol (2011)
Lee SY. Curr Opin Biotechnol (2011)

PLA: Polylactic acid
PHP: Poly3-hydroxypropionate
Gao X., Chen GQ. Curr Opin Biotechnol (2011)
Homopolymers and Copolymers of 3-Hydroxypropionate and 4-Hydroxybutyrate (PHP) and PHP4HB by *E. coli* Containing A Synthetic Pathways using Glucose, 1,3-PDO or/and 1,4-BDO as Substrates

Cargill is working on a chemical way to make P3HP

Zhou et al. Met Engn (2012)
Controllable P(3HP-co-4HB)

Meng et al. Metab Eng (2012)

<table>
<thead>
<tr>
<th>PDO (g/l)</th>
<th>BDO (g/l)</th>
<th>CDW (g/l)</th>
<th>PHA (g/l)</th>
<th>PHA/CDW (wt%)</th>
<th>3HP (mol%)</th>
<th>4HB (mol%)</th>
</tr>
</thead>
<tbody>
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<td>5</td>
<td>0</td>
<td>7.55 ± 0.09</td>
<td>2.29 ± 0.06</td>
<td>30.28 ± 0.92</td>
<td>100</td>
<td>0</td>
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<tr>
<td>10</td>
<td>0</td>
<td>8.13 ± 0.16</td>
<td>2.58 ± 0.45</td>
<td>31.68 ± 5.31</td>
<td>100</td>
<td>0</td>
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<tr>
<td>0</td>
<td>5</td>
<td>6.76 ± 1.26</td>
<td>0.86 ± 0.35</td>
<td>12.41 ± 3.27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>8.62 ± 0.25</td>
<td>2.10 ± 0.14</td>
<td>24.37 ± 1.08</td>
<td>0</td>
<td>100</td>
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<tr>
<td>1</td>
<td>10</td>
<td>8.63 ± 0.22</td>
<td>2.47 ± 0.55</td>
<td>28.73 ± 6.92</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>7.09 ± 0.33</td>
<td>3.27 ± 0.40</td>
<td>35.55 ± 3.02</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>10.13 ± 0.29</td>
<td>4.57 ± 0.30</td>
<td>45.19 ± 2.93</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>9.16 ± 0.22</td>
<td>5.16 ± 0.25</td>
<td>56.30 ± 1.38</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>3.65 ± 1.89</td>
<td>0.98 ± 1.28</td>
<td>20.38 ± 19.04</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>10.18 ± 0.12</td>
<td>5.16 ± 0.08</td>
<td>50.65 ± 1.42</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>9.92 ± 0.30</td>
<td>6.21 ± 0.35</td>
<td>62.70 ± 5.21</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>2.43 ± 0.55</td>
<td>0.16 ± 0.11</td>
<td>5.99 ± 3.16</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>1.73 ± 0.11</td>
<td>0.04 ± 0.01</td>
<td>2.30 ± 0.18</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2
P(3HP-co-4HB) production from PDO and/or BDO by recombinant E. coli grown in shake flasks.

Recombinant E. coli harboring pZQ01 and pZQ03-orfZ were cultivated in Terrific Broth medium supplemented with different concentration of BDO and/or PDO for 48 h. Data shown are the averages and standard deviations of three parallel experiments. Abbreviations: PDO, 1,3-propanediol; BDO, 1,4-butanediol; CDW, cell dry weight.
Copolymers of 3-Hydroxybutyrate and 4-Hydroxybutyrate (P3HB4HB) by *E. coli* Containing A Synthetic Pathways using Glucose as the Only Carbon Source

From Glucose To New Polymers

Li Zhengjun

Li ZJ et al Metab Engn (2010)
Production of Homopolymers of 4-Hydroxybutyrate [Poly(4HB)] by *E. coli* Containing A Synthetic Pathways using Glucose as the Only Carbon Source

Poly(4HB) suture was approved by FDA in Feb/2007 for clinical applications

Poly(4HB) was produced from Expensive 1,4-butanol, now From cheap glucose

Zhou XY et al Microbial Cell Factories (2012)
PHA Homopolymers

• However, except Poly-R-3-hydroxybutyrate (PHB), no wild type bacterium has been reported to produce any PHA homopolymer.

![Same monomers](image)

• Most bacteria produce copolymers consisting of more than two different monomers.

![Various monomers](image)
For each β-oxidation cycle, fatty acid loses one acetyl-CoA, e.g. C12 becomes C10
Deletion or weakening the β-oxidation pathways should allow fatty acid to come to PHA synthesis directly.

Complete removal of β-oxidation activity?
A series of β-oxidation Mutants were created

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Mutant genes (deleted genes in <em>P. putida</em> KT2442)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KTOY08-G</td>
<td>△fadAB △fadAx △fadB2x △phaG</td>
</tr>
<tr>
<td>KTQQ05</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP2051</td>
</tr>
<tr>
<td>KTQQ06</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP1377</td>
</tr>
<tr>
<td>KTQQ07</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP3280</td>
</tr>
<tr>
<td>KTQQ08</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP3280 △PP2051</td>
</tr>
<tr>
<td>KTQQ09</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP3280 △PP1377</td>
</tr>
<tr>
<td>KTQQ10</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP3280 △PP3754</td>
</tr>
<tr>
<td>KTQQ11</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP3280 △PP 0582</td>
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<td>KTQQ12</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP3280 △PP1377 △PP4636</td>
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<td>KTQQ17</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP3280 △PP1377 △PP4636 △PP0582 △PP3754 △PP3355 △PP2047-△PP 2048</td>
</tr>
<tr>
<td>KTQQ18</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP2047</td>
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<td>KTQQ19</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP2048</td>
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<tr>
<td>KTQQ20</td>
<td>△fadAB △fadAx △fadB2x △phaG △PP2047 △PP2048</td>
</tr>
</tbody>
</table>

Luo Ke Zhang Xinrong
The β-oxidation Mutants produced much more C12 when lauric acid (C12) was added compared with the wild type

<table>
<thead>
<tr>
<th>Strain</th>
<th>CDW (g/L)</th>
<th>PHA content (wt%)</th>
<th>PHA monomer content (mol%)</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>HHx</td>
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<tr>
<td>KTOY08-G</td>
<td>4.06±0.54</td>
<td>51.25±9.38</td>
<td>2.61±0.37</td>
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<td>KTQQ05</td>
<td>0.65±0.05</td>
<td>6.69±2.02</td>
<td>--</td>
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<tr>
<td>KTQQ06</td>
<td>2.42±0.14</td>
<td>28.00±1.39</td>
<td>5.18±1.87</td>
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<td>KTQQ07</td>
<td>3.15±0.99</td>
<td>87.00±11.91</td>
<td>1.56±0.17</td>
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<td>0.67±0.04</td>
<td>7.26±0.78</td>
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<td>2.00±0.26</td>
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<td>KTQQ10</td>
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<td>39.76±4.84</td>
<td>4.70±0.01</td>
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<tr>
<td>KTQQ11</td>
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<td>43.89±12.88</td>
<td>4.23±0.81</td>
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<td><strong>1.97±0.50</strong></td>
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<td>5.43±0.77</td>
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<td>35.56±6.97</td>
<td>6.69±0.74</td>
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<td>6.45±0.53</td>
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<tr>
<td>KTQQ16</td>
<td>4.60±0.46</td>
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<td>6.55±0.24</td>
</tr>
<tr>
<td>KTQQ17</td>
<td>0.81±0.05</td>
<td>7.57±1.18</td>
<td>--</td>
</tr>
<tr>
<td>KTQQ18</td>
<td>1.73±0.14</td>
<td>16.22±2.09</td>
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<tr>
<td>KTQQ19</td>
<td>5.32±0.14</td>
<td>20.27±2.33</td>
<td>--</td>
</tr>
<tr>
<td><strong>KTQQ20</strong></td>
<td><strong>1.03±0.01</strong></td>
<td><strong>9.19±1.20</strong></td>
<td>--</td>
</tr>
</tbody>
</table>

Lauric acid (C12) was used as precursor for PHA production
Metabolic Engineering of non-β-Oxidation Pathways for Production of Other PHA Homopolymers by \textit{P. putida}

- Replacing PHA Synthase PhaC of \textit{P. putida} by PhaC\textsubscript{Ah} of \textit{Aeromonas hydrophila}
- Deleting PhaG to stop supplying Other PHA monomers

Wang HH
Metabolic Engineering of PHA Pathways for Production of Homopolymers Using Genome Reduced Pseudomonas sp.

Wang et al Appl Microbial Biotechnol (2011); Liu et al Met Engn (2011); Chung et al Biomacromolecules (2011)
Microbial Production of Block Copolymers via a Two Stage Process

Sugars → 3-Ketoacyl-CoA Enoyl-CoA Acyl-CoA (S)-3-Hydroxyacyl-CoA PHAs

Fatty Acids → Acyl-CoA → 3-Ketoacyl-CoA Enoyl-CoA

Hexanoate (C6) → 3-Hydroxyacyl-CoA

Dodecanoate (C12) → Butyrate (C4) 4HB → Acetyl-CoA

Malonyl-CoA Malonyl-ACP (R)-3-Hydroxyacyl-CoA

Acetyl-CoA

Metabolic Engineering of PHA Pathways for Production of Homopolymers and Chemicals

Sugars

Fatty Acids with functional group

Acetyl-CoA

Malonyl-CoA

Malonyl-ACP

Fatty Acids with functional group

3-Ketoacyl-CoA

Enoyl-CoA

Fatty Acids

Acyl-CoA

3-Ketoacyl-CoA

(S)-3-Hydroxyacyl-CoA

(R)-3-Hydroxyacyl-CoA

Hu et al. Biomacromolecules (2011)

Chen et al. Biomacromolecules (Revision)
Industrial Production of Homopolymers and Random Copolymers and Block Copolymers by the *Pseudomonas* spp Platform

- Fatty acid A
- Fatty acid B
- Fatty acids A+B
- Fatty acid A

**Diagram:**
- **Homopolymer** Poly(hydroxy**Fatty acid A**)
- **Random copolymer** Poly(hydroxy**Fatty acid A -co-hydroxy**Fatty acid B)
- **Block copolymer** Poly(hydroxy**Fatty acid A**)

**Other Diagram Elements:**
- Motor
- pH controller
- Acid-base reservoir and pump
- Viewing port
- Cooling jacket
- Sparger (air)
- Sterile air
A Controlable PHA and Monomers Production Platform
Gao X...Chen GQ. Curr Opin Biotechnol (2011)

Fatty acids

Sugar

Copolymers
Various monomers

Block copolymer
Various monomers

Homopolymers
Same monomers

Pure (R)-3-Hydroxyalkanoic Acids

(R)-3-Hydroxyalkanoic Methyl Esters

Biofuels
Chiral 3-hydroxyalkanoates (3HAs) are promising precursor or intermediates for the synthesis of various fine compounds including pharmaceuticals, antibiotics, food additive, fragrances and vitamins.

- Only (R)-3-hydroxynonanoic acid and (R)-3-hydroxytetradecanoic acid are commercialized;

- 3-hydroxytetradecanoic acid (3HTD) is a natural component of lipid A, the endotoxic principle of lipopolysaccharide (LPS) embedded in the cell surface of Gram-negative bacteria.

Figure 1. The structure of a typical bacterial lipid A
Production of Chiral 3HA

P. entomophila LAC31: ΔfadB, ΔfadA, ΔPSEEN 0664, ΔPSEEN 4635, ΔPSEEN4636, ΔphaC, by JHL, ZM

S-3-Hydroxyacyl-CoA dehydrogenase: fadB and fadB2x;
3-ketothiolase: fadA;
acetyl-CoA acetyltransferase: fadAx, PSEEN 0664, PSEEN 2543, PSEEN 2795, PSEEN 3197, PSEEN 4635;
Enoyl-CoA hydratase: phaJ
PHA synthase: phaC
Production of Chiral 3HA

\[ \text{fatty acids} \rightarrow \text{1}\text{0C FA} \]

\[ \text{Acetyl-CoA} \rightarrow \text{Acyl-CoA} \rightarrow \text{Enoyl-CoA} \rightarrow \text{3-Ketoacyl-CoA} \]

\[ \text{S-3-OH-acyl-CoA} \leftrightarrow \text{R-3-OH-acyl-CoA} \]

\[ \text{R-3-hydroxyacyl acids} \]

*P.* entomophila

LAC31: ΔfadB, ΔfadA, ΔPSEEN 0664, ΔPSEEN 4635, ΔPP 4636, ΔphaC, by JHL, ZM

Thioesterase: tesB, from E. coli tesI, from yeast

by JHL, ZM
**Pure extracellular 3HTD, 3HDD or 3HD can be obtained using same chain length fatty acids as substrates**

Extracellular 3HAs production by *Pseudomonas* spp. LAC31 harboring *tesB* or *tesl* with related fatty acid as the carbon source

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>thioesterase</th>
<th>CDW(gL⁻¹)</th>
<th>3HA (gL⁻¹)</th>
<th>3HA fraction (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3HHx (C6)</td>
<td>3HO (C8)</td>
</tr>
<tr>
<td><strong>14C</strong></td>
<td>TesB</td>
<td>2.17 ± 0.40</td>
<td>3.49 ± 0.19</td>
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</tr>
<tr>
<td></td>
<td>Tesl</td>
<td>1.75 ± 0.26</td>
<td>2.65 ± 0.13</td>
<td></td>
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<tr>
<td><strong>12C</strong></td>
<td>TesB</td>
<td>0.49 ± 0.03</td>
<td>2.55 ± 0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tesl</td>
<td>0.84 ± 0.05</td>
<td>2.92 ± 0.99</td>
<td></td>
</tr>
<tr>
<td><strong>10C</strong></td>
<td>TesB</td>
<td>0.96 ± 0.04</td>
<td>0.75 ± 0.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tesl</td>
<td>1.08 ± 0.01</td>
<td>1.49 ± 0.62</td>
<td></td>
</tr>
</tbody>
</table>

Bacteria were culture one-step at 30°C for 48h with 12g/L related fatty acid supplemented in 4YLB medium.

3HA, 3-hydroxycarboxylic acids; 3HHx, 3-hydroxyhexanoate; 3HO, 3-hydroxyoctanoate; 3HD, 3-hydroxydecanoate; 3HDD, 3-hydroxydodecanoate; 3HTD, 3-hydroxytetradecanoate;
Limitless Opportunities for Microbial Production of Hydroxyalkanoates Based Chemicals and Materials
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