HERBASPIRILLUM SEROPEDICAE: STUDY OF POLYHYDROXYALKANOATES PRODUCTION

Molecular Microbiology Laboratory-Institute of Biological Research “Clemente Estable” (IIBCE)
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Project PHAs
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Origin: Isolated in Brazil by the group of Prof. J. Döbereiner.

Beta-Proteobacterium isolated from rice, corn or wheat root systems first described in 1986: Baldani JI et al. (1986) "Characterization of Herbaspirillum seropedicae gen. nov. sp. nov. a root-associated nitrogen-fixing bacterium." Int. J. Syst. Bacteriol.

Strickly aerobic organism, flagellar motility, diazotroph. It can grow microaerophilically.

Soil bacterium and endophyte, colonizes roots (rice, sugar cane, maize, coffee, etc).

Plant growth-promoting and non-pathogenic.
H. seropedicae: General background

- Public access to the genome of strain Z78 (Univ Paraná, Curitiba- Brasil) (Genopar Project, responsible: F. Pedrosa).

- Carbon metabolism: we determined enzymatic activities for ED and TCA. It was not possible to determine the presence of Phosphofructokinase (EMP) and Phosphogluconate dehydrogenase (Pentose Phosphate).

- Curitiba's group studied N and arabinose metabolism.

- No special growth requirements (optimal growth temp. 34º C).
H. seropedicae: PHAs producer

- Classic pathway for PHB synthesis.
- Acumulates PHB and copolymer P(3HB-co-3HV) with different carbon sources.
- Acumulates up to 60 % cell dry weight of PHB when consumes glucose as sole carbon source (under optimized conditions).

<table>
<thead>
<tr>
<th>Carbon Source</th>
<th>Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>PHB</td>
</tr>
<tr>
<td>Xylose</td>
<td>PHB</td>
</tr>
<tr>
<td>Mannitol</td>
<td>PHB</td>
</tr>
<tr>
<td>Galactose</td>
<td>PHB</td>
</tr>
<tr>
<td>Arabinose</td>
<td>nd*</td>
</tr>
<tr>
<td>Succinate</td>
<td>PHB</td>
</tr>
<tr>
<td>Nonanoic acid</td>
<td>P(3HB-co-3HV)</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>nd</td>
</tr>
</tbody>
</table>

Z67  Z69  Z78

*nd.: Not determinated
We studied the accumulation of PHB in batch cultures and have obtained a yield of 50% cell dry weight.
OBJECTIVE

Characterization of xylose metabolism and optimization of PHB production when xylose is used as the carbon source
Xylose metabolism

Pathways described in different microorganisms

Xylose Isomerase pathway

D-xylose

xylose reductase

NAD(P)H → NAD(P)

D-xylose

xylose isomerase

xylitol dehydrogenase

NADH + H

D-xylulose

xylulose kinase

D-xylulose-5-Phosphate

Pentose Phosphate

Oxo-reductive pathway

NAD+

xylitol dehydrogenase

NADH + H

D-xylulose

Weimberg-Dahms pathway

Stephens et al. 2007
Biochemical strategy:
Determination of enzymatic activities

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity (nmol/min.mg prot.)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extracts Glucose 30g/l</td>
<td>Extracts Xylose 10 g/l</td>
<td>Extracts Xylose 30g/l</td>
<td>Extracts Xylitol 10g/l</td>
</tr>
<tr>
<td>Xylose deshydrogenase</td>
<td>15.7</td>
<td>29.2</td>
<td>30.4</td>
<td>0</td>
</tr>
<tr>
<td>Xylitol deshydrogenase</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>46.5</td>
</tr>
</tbody>
</table>

These results suggest the presence of the Dahms-Weimberg pathway for xylose degradation.
L-Arabinose Metabolism in *Herbaspirillum seropedicae*

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FIG. 2. Pathway of L-arabinose metabolism in *H. seropedicae*.
**Metabolic strategy:**

Metabolic pathway analysis using elementary modes (EM)

The EM represent metabolic pathway involved in conversion of the substrate into the products:

Continuous culture experiment

<table>
<thead>
<tr>
<th>D (h⁻¹)</th>
<th>Xt (g/l)</th>
<th>Xr (g/l)</th>
<th>PHB (g/l)</th>
<th>PHB (%)</th>
<th>Xyl initial (g/l)</th>
<th>Xyl residual (g/l)</th>
<th>Xyl consumed (g/l)</th>
<th>NH₄Cl initial (mM)</th>
<th>NH₄Cl residual (mM)</th>
<th>Yₓₓr/xil (g/g)</th>
<th>YₓₓPHB/xil (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.021</td>
<td>0.96</td>
<td>1.35</td>
<td>0.88</td>
<td>65</td>
<td>0</td>
<td>7.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>0.53</td>
<td>0.97</td>
<td>65</td>
<td>0</td>
<td>7.11</td>
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<td></td>
<td>0</td>
<td>0.074</td>
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<tr>
<td></td>
<td>1.47</td>
<td>0.47</td>
<td>1.00</td>
<td>68</td>
<td>0</td>
<td>7.11</td>
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<td></td>
<td></td>
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<td>0.066</td>
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<tr>
<td></td>
<td>1.57</td>
<td>0.64</td>
<td>0.93</td>
<td>69</td>
<td>0</td>
<td>7.11</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0.090</td>
</tr>
</tbody>
</table>
Metatool

Input File

**Metabolic network:** ED, Weimberg-Dahms, anaplerotic pathway (Gluconeogenesis and Phosphate Pentose), aerobics respiration

**Constraints**
67% cell dry weight PHB accumulation

$XT = X_r + PHB$
Output File:

1:  ATP = ADP
2:  20241.4 XYLext + 301819 ADP + 103446 O = 301819 ATP + 55999.8 CO2 + XT
3:  XYLext + 29 ADP + 10 O = 29 ATP + 5 CO2
4:  34046.6 XYLext + 715976 ADP + 241498 O = 715976 ATP + 125026 CO2 + XT
5:  20241.4 XYLext + 274208 ADP + 103446 O = 274208 ATP + 55999.8 CO2 + XT

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<thead>
<tr>
<th>N° EM</th>
<th>$Y_{Xr/xil}$ (g/g)</th>
<th>$Y_{PHB/xil}$ (g/g)</th>
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<tbody>
<tr>
<td>2</td>
<td>0.108</td>
<td>0.221</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.064</td>
<td>0.131</td>
<td>0.075</td>
<td>0.133</td>
</tr>
<tr>
<td>5</td>
<td>0.108</td>
<td>0.221</td>
<td></td>
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</table>
Catabolic pathways
• The Weimberg pathway
• TCA

Anabolic pathways
• Gluconeogenesis
• Pentoses Phosphate
These results strongly suggest that *H. seropedicae* uses the Weimberg pathway to metabolize xylose.
- EM N°4 does not have the highest yield transformation of carbon source into polymer compared with those calculated from EM N° 2 and N°5.

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</table>
It involves the Weimberg and Dahms pathways and part of the Glioxylate cycle.

It does not involve the enzymes CK1 (citrate synthase) and CK2 (aconitase) of the TCA.
It involves the Weimberg, ED pathways.

It does not involve the enzymes CK1 (citrate synthase) and CK2 (aconitase) of the TCA.
Citrate synthase: key enzyme as a target for mutagenesis

In *H. seropedicae*, this enzyme is encoding by *prpC* gene.

Site directed mutagenesis in *prpC* gene using the method described by Sukdeo and Charles.

We designed the primers and performed the PCR.

We obtained a fragment of the expected size cloned and sequenced it to verify that the sequence is correct.
Once obtained *H. seropedicae* *prpC* mutant, we will analyze metabolic pathways and its performance.

Production assays using hemicellulose hydrolizate as carbon source.
Montevideo
Thank you!