Xylan structural variation: Effects on the fermentation profiles of *Plantago* species seed mucilage

George N. Mondoh

Co-authors:
A/Prof Rachel Burton
Dr. Matthew Tucker
Cell walls & Dietary fibre

- Cell walls Composition

(Doblin et al., 2010)
(Burton et al. 2010).
Dietary fibre

- Soluble (dissolves in gut)
  - Reduce absorption of fats and sugar
    e.g. Legumes, Barley, Oat bran etc.

- Insoluble (absorbs)
  - Prevent and treat constipation, IBS etc
    e.g. wheat, corn, psyllium, fruits and vegetables etc.

- Benefits; Prevent cardiovascular diseases & colonic cancer etc
Plantago species

- What are Plantago species?
- Over 400 known species.
- *Plantago* extensively used to study xylan synthesis.
- *Plantago ovata* seed husks (PSH) also used as an important source of xylan rich dietary fibre (DF). (Metamucil).
- PSH xylans are poorly fermented by colonic bacteria.
- Fermentation of DFs by colonic bacteria yields health beneficial end-products like Short Chain Fatty Acids (SCFA).
- Is there a readily fermentable *Plantago* species?
**Plantago species seed mucilage diversity**  
(Phan 2012)

- **Mucilage extrusion profiles**

- **Xylan structure**

- **Mucilage composition**

<table>
<thead>
<tr>
<th>Species</th>
<th>Xylan % in mucilage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. ovata</em></td>
<td>88%</td>
</tr>
<tr>
<td><em>P. coronopus</em></td>
<td>48%</td>
</tr>
<tr>
<td><em>P. cunninghamii</em></td>
<td>85%</td>
</tr>
<tr>
<td><em>P. lanceolata</em></td>
<td>66%</td>
</tr>
</tbody>
</table>
Heteroxylan structure in *Plantago ovata* seed mucilage

In both *in-vivo* and *in-vitro* fermentation models xylanases digest the structure by cleaving at specific points.

(Van Craeyveld et al., 2009).
Project Aims

To investigate the effect of the differences in *Plantago* species seed mucilage composition and xylan structures on their fermentability and influence on gut microbial composition.

Specific Objectives

- To determine the fermentation kinetics of mucilage from four *Plantago* species.
- To identify and measure key fermentation end-products i.e ammonia and short chain fatty acids (SCFA).
- To determine gut bacterial population shifts after fermentation of mucilage from four *Plantago* species.
Results and discussion

- **In vitro batch fermentation process**
- **Plantago** species used:
  
  \[P.\ ovata,\ P.\ coronopus,\ P.\ lanceolata\ \text{and}\ P.\ cunninghamii.\]

- **Design**
  
  Five replicates of the four **Plantago** species were fermented in an *in vitro* batch fermentation process using bacteria inoculum extracted from porcine faeces.
Comparison of fermentation kinetics after 48 hours

Gas production profiles

Cumulative gas production parameters after 48 hours (Tukey’s Studentized test)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>RMAX (ml/h)</th>
<th>TMAX (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. cunninghamii</td>
<td>5.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>P. coronopus</td>
<td>8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>P. lanceolata</td>
<td>5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>P. ovata</td>
<td>3.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
### Comparison of fermentation end-products after 48 hours

<table>
<thead>
<tr>
<th>Substrates</th>
<th>NH$_3$</th>
<th>Acetic BCR</th>
<th>Propionic</th>
<th>Butyric</th>
<th>Total SCFA</th>
<th>AcTot</th>
<th>PropTot</th>
<th>ButTot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mMol/gDM</td>
<td>mMol/gDM</td>
<td>mMol/gDM</td>
<td>mMol/gDM</td>
<td>mMol/gDM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. cunninghamii</td>
<td>15.44$^b$</td>
<td>3.0$^c$</td>
<td>2.1$^a$</td>
<td>0.07$^b$</td>
<td>0.12$^b$</td>
<td>5.8$^a$</td>
<td>51.92$^c$</td>
<td>36.97$^a$</td>
</tr>
<tr>
<td>P. coronopus</td>
<td>19.26$^a$</td>
<td>3.41$^b$</td>
<td>1.85$^{ab}$</td>
<td>0.084$^a$</td>
<td>0.14$^a$</td>
<td>6.06$^a$</td>
<td>56.33$^b$</td>
<td>30.53$^c$</td>
</tr>
<tr>
<td>P. lanceolata</td>
<td>17.20$^{ab}$</td>
<td>3.86$^a$</td>
<td>1.74$^b$</td>
<td>0.069$^b$</td>
<td>0.12$^b$</td>
<td>6.30$^a$</td>
<td>61.31$^a$</td>
<td>27.65$^d$</td>
</tr>
<tr>
<td>P. ovata</td>
<td>16.44$^b$</td>
<td>3.11$^{bc}$</td>
<td>2.07$^a$</td>
<td>0.070$^b$</td>
<td>0.13$^{ab}$</td>
<td>5.94$^a$</td>
<td>52.42$^c$</td>
<td>34.81$^b$</td>
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<tr>
<td>P value</td>
<td>0.0020</td>
<td>0.0001</td>
<td>0.0081</td>
<td>0.0001</td>
<td>0.0022</td>
<td>0.2057</td>
<td>0.0001</td>
<td>0.0001</td>
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<tr>
<td>MSD</td>
<td>2.3495</td>
<td>0.3654</td>
<td>0.3161</td>
<td>0.007</td>
<td>0.018</td>
<td>0.6764</td>
<td>1.4887</td>
<td>1.8839</td>
</tr>
</tbody>
</table>
Bacterial population shift analysis

- Ion torrent sequencing
- Ion tag PCR for 16S rRNA (using modified locus specific primers)
- PCR product purification (HPLC)
- Submission to AGRF

- Sequence read output: **4,748,104**

- Phylogenetic analysis using MG-RAST software
  (Metagenomics Rapid Annotation using Subsystem Technology) [www.metagenomics.anl.gov](http://www.metagenomics.anl.gov)
  - Dereplication
  - Screening
  - Dynamic trimming
  - Sequences aligned to greengenes (16S database)

Ion torrent Personal Genome Machine

316 chip (100Mbp)

Courtesy AGRF Adelaide
Post-fermentation microbial species distribution

Bacterial species

- Lactobacillus murinus
- Lactobacillus agilis
- Bifidobacterium pullorum
- Bacteroides ovatus
- Bacteroides xylanisolvens
- Bacteroides fragilis
- Clostridium haemolyticum
- Clostridium xylanolyticum
- Prevotella copri
- Prevotella buccalis

Normalised values

- P. ovata
- P. coronopus
- P. lanceolata
- P. cunninghamii
- Initial inoculum
Conclusion and Recommendations

• Distinct fermentation patterns were observed between *Plantago* species.

• *P. coronopus* mucilage identified as the most readily fermentable substrate.

• Clear differences were identified in relative bacterial abundance.

• It is not clear what mucilage components were being fermented.

• Amounts of fermented xylans were not determined.

• Assays to determine exact amount of xylans fermented are required.

• Comparison with other xylan rich producing grasses necessary.
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References


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