Development and application of a suite of polysaccharide-degrading enzymes for analysing plant cell walls

Stefan Bauer

Imperial College, London
29.02.2008
overview

• structure of plant cell wall polysaccharides
  cellulose, hemicellulose, pectins

• polysaccharide analysis

• development of enzyme collection

• examples of enzymes
  enzymes acting on xyloglucan and xylan

• application of the collection
  identification of cell wall defects in A. thaliana irx8 and irx9
cell wall polysaccharides

- cellulose
- hemicellulose
- pectins
- AGPs
cellulose: $\beta(1\rightarrow4)$-linked glucan chains
hemicelluloses: $\beta(1\rightarrow4)$-linked backbone (1)

xylans

mannans

hemicelluloses: $\beta(1\rightarrow4)$-linked backbone (2)

xyloglucans

mixed-linked glucans $\beta(1\rightarrow3)/\beta(1\rightarrow4)$

pectins: $\alpha(1\rightarrow4)$-linked galacturonic acid

homogalacturonan

rhamnogalacturonan I (RG I)

arabinan

arabinogalactan

pectins: $\alpha(1\rightarrow4)$-linked galacturonic acid

rhamnogalacturonan II (RG II)

arabinogalactan proteins (AGPs)
model of the plant cell wall


model of the plant cell wall

model of the plant cell wall

composition of *Arabidopsis thaliana* cell walls

Characterization of the Cell-Wall Polysaccharides of *Arabidopsis thaliana* Leaves¹

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Complex Carbohydrate Research Center and Department of Biochemistry and Molecular Biology, University of Georgia, 220 Riverbend Road, Athens, Georgia 30602–4712

Localisation and characterisation of cell wall mannan polysaccharides in *Arabidopsis thaliana*

Michael G. Handford · Timothy C. Baldwin
Florence Goubet · Tracy A. Prime · Joanne Miles
Xiaolan Yu · Paul Dupree


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Original Article

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<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>homo-galacturonan</td>
<td>23%</td>
</tr>
<tr>
<td>rhamno-galacturonan I</td>
<td>11%</td>
</tr>
<tr>
<td>rhamno-galacturonan II</td>
<td>8%</td>
</tr>
<tr>
<td>xyloglucan</td>
<td>20%</td>
</tr>
<tr>
<td>celluloset</td>
<td>14%</td>
</tr>
<tr>
<td>protein</td>
<td>4%</td>
</tr>
<tr>
<td>pectins</td>
<td>42%</td>
</tr>
<tr>
<td>glucuronoarabinoxylan</td>
<td>6%</td>
</tr>
<tr>
<td>glucuronoarabinoxylan</td>
<td>4%</td>
</tr>
<tr>
<td>glucuronoarabinoxylan</td>
<td>14%</td>
</tr>
<tr>
<td>cellulose</td>
<td>14%</td>
</tr>
</tbody>
</table>
cell wall composition is tissue specific

Richmond & Somerville Plant Mol. Biol. 47 (2001) 131-143
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cell wall analysis

sequential extraction of polysaccharides from the alcohol insoluble residue (AIR)

crude extracts are fractionated by size-exclusion chromatography

fractions are further investigated by partial degradation

resulting oligomers are separated by HPAEC-PAD or CE and structure elucidation is performed by standard methylation analysis, MALDI-TOF and NMR
cell wall analysis

monosaccharide composition - (‘sugar analysis’)
GC, HPAEC-PAD

IR-spectroscopy

IR-spectroscopy

tissue staining
specific dyes, antibodies...

partial degradation
MALDI-TOF, CE
<table>
<thead>
<tr>
<th>Acidic Hydrolysis</th>
<th>Enzymatic Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial degradation</td>
<td>Much more specific and under less drastic conditions</td>
</tr>
<tr>
<td>Not completely specific</td>
<td>Many commercially available enzymes are products from fungal culture filtrates and are rather complex mixtures</td>
</tr>
<tr>
<td>Requires optimised conditions in every particular case due to different acidic stabilities of glycosidic linkages</td>
<td>The only feasible way to obtain reproducible access to enzymes with properties required is to clone the genes of interest into a suitable host</td>
</tr>
</tbody>
</table>
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cloning of enzymes

Aspergillus nidulans

Pichia pastoris

pPICZα C
3.6 kb
cloning of enzymes

5' AOX1 α-factor coding sequence myc 6x his zeocin 3'

NH₂ α-factor amino acid sequence myc 6x his COOH

translation

secretion

NH₂ amino acid sequence myc 6x his COOH
Amplification of genes from cDNA of *A. nidulans* grown on various polysaccharides containing media

Cloning into *Pichia pastoris*

Clones are tested for expression levels in the supernatants using anti-C-myc antibody

Optimisation of expression using different media

large scale expression
enzyme purification – affinity chromatography
- 74 *Pichia pastoris* clones

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucans</td>
<td>18 (4)</td>
</tr>
<tr>
<td>Xyloglucan</td>
<td>4</td>
</tr>
<tr>
<td>Xylan</td>
<td>10</td>
</tr>
<tr>
<td>Mannan</td>
<td>9 (3)</td>
</tr>
<tr>
<td>Pectins</td>
<td>23 (2)</td>
</tr>
<tr>
<td>Others</td>
<td>10 (5)</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td>74 (14)</td>
</tr>
</tbody>
</table>
Development and application of a suite of polysaccharide-degrading enzymes for analyzing plant cell walls

Stefan Bauer*, Prasanna Vasu†, Staffan Persson*, Andrew J. Mort†, and Chris R. Somerville**

*Carnegie Institution, Stanford, CA 94305; and †Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078
Contributed by Chris R. Somerville, June 4, 2006
problems: finding the right substrate

p-nitrophenyl-glycosides (‘PNP-labeled sugar’)

“free sugar”

p-nitrophenol

405 nm

AN8149.2 (α-fucosidase): not active on PNP-α-fucopyranoside but on cotton xyloglucan subunits

AN1804.2 (β-glucosidase): active on PNP-β-glucopyranoside but not on cellobiose

(but active on other aryl-β-glucosides …)
problems: finding the right substrate

AN2206.2 ($\alpha$-rhamnosidase): not active on PNP-$\alpha$-rhamnopyranoside, not active on naringin/hesperidin

but active on rhamnogalacturonan I, releasing terminal L-rhamnose
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Tamarind xyloglucan consists of 4 repeating tetrameric subunits.

CE electropherogram (LIF detection) after APTS derivatisation.
tamarind xyloglucan digest (xyloglucanase)

xyloglucanase (Novozyme)

xyloglucanase (AN0452.2)

1 d

3 d

5 d
tamarind xyloglucan subunit digest
(β-galactosidase, Megazyme)
cotton xyloglucan contains additional subunits
(a substrate for α-fucosidase AN8149.2)
cXG subunits after $\alpha$-fucosidase incubation (AN8149.2)
oligoxyloglucan reducing end-specific
xyloglucanobiohydrolase (OREX) – AN1542.2
OREX can be used in MALDI-TOF analysis (1)
OREX can be used in MALDI-TOF analysis (2)
Ferulic acid esterase (AN5267.2) → α-arabinofuranosidases (AN1571.2 & AN7908.2) → α-acetylxylosidases (AN3294.2 & AN6093.2) → α-glucuronidase (AN9286.2) → β-xylosidases (AN2359.2 & AN8401.2) → β-acetylxylosidases (AN3613.2 & AN1818.2) → α-arabinofuranosidases (AN1571.2 & AN7908.2) → α-glucuronidase (AN9286.2) → β-xylosidases (AN2359.2 & AN8401.2) → β-acetylxylosidases (AN3613.2 & AN1818.2) → α-arabinofuranosidases (AN1571.2 & AN7908.2) → α-glucuronidase (AN9286.2) → β-xylosidases (AN2359.2 & AN8401.2) → β-acetylxylosidases (AN3613.2 & AN1818.2) → α-arabinofuranosidases (AN1571.2 & AN7908.2) → α-glucuronidase (AN9286.2) → β-xylosidases (AN2359.2 & AN8401.2) → β-acetylxylosidases (AN3613.2 & AN1818.2) → α-arabinofuranosidases (AN1571.2 & AN7908.2) → α-glucuronidase (AN9286.2) → β-xylosidases (AN2359.2 & AN8401.2) → β-acetylxylosidases (AN3613.2 & AN1818.2) → α-arabinofuranosidases (AN1571.2 & AN7908.2) 

Enzymes towards xylans
enzymes towards xylans

XylA

XylC

β-xylosidase

α-glucuronidase
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characterisation of *irregular xylem* mutants

characterisation of *irx9* cell walls
characterisation of *irx8* cell walls

**endo-galactanase**

- *irx8-1*, buffer
- *irx8-1*, endo-galactanase
- pectic galactan, endo-galactanase
- *irx8-1* + pectic galactan, endo-galactanase

**ball-milled cell walls**

- *irx8-1*, buffer
- *irx8-1*, endo-xylanase
- *irx8-1*, endo-xylanase
- Birchwood xylan, endo-xylanase

**XylC**

- AN1818.2
- Col-0, buffer
- Col-0, endo-xylanase
- Birchwood xylan, endo-xylanase

**1 M KOH fraction**

- *irx8-1*, buffer 1 M KOH
- *irx8-1*, endo-xylanase 1 M KOH
-birchwood xylan, endo-xylanase

**XylA**

- AN3613.2
- Col-0, buffer 1 M KOH
- Col-0, endo-xylanase 1 M KOH
- Birchwood xylan, endo-xylanase
characterisation of \textit{irx8} cell walls

\textbf{WT}

\textbf{Xyl C}

\textbf{Xyl A}

\textbf{irx8}
74 enzymes cloned and expressed in *P. pastoris*

clones are deposited in the Fungal Genetics Stock Center (FGSC, University of Kansas) and are freely available to the research community

these pure enzymes are a powerful toolkit for the analysis of plant cell wall polysaccharides complementing techniques such as monosaccharide analysis, IR, immunolabelling etc.
acknowledgement

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Chris Somerville
Carnegie Institution
Stanford, CA

Staffan Persson & all members of the Somerville lab(s) ...
acknowledgement

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