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Replacing a suite of commercial pectinases with a single enzyme, pectate lyase B, in *Saccharomyces cerevisiae* fermentations of cull peaches

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Abstract Fermentation of pectin-rich biomass with low concentrations of polysaccharides requires some treatment of the pectin, but does not need complete degradation of the polysaccharide to reach maximum ethanol yields. Cull peaches, whole rotten fruits that are not suitable for sale, contain high concentrations of glucose (27.7 % dw) and fructose (29.3 % dw) and low amounts of cellulose (2.8 % dw), hemicellulose (4.5 % dw) and pectin (5.6 % dw). Amounts of commercial saccharification enzymes, cellulase and cellobiase can be significantly decreased and commercial pectinase mixtures can be replaced completely with a single enzyme, pectate lyase (PelB), while maintaining ethanol yields above 90 % of the theoretical maximum. PelB does not completely degrade pectin; it only releases short chain oligogalacturonides. However, the activity of PelB is sufficient for the fermentation process, and its addition to fermentations without commercial pectinase increases ethanol production by ~12 %.

Keywords *Saccharomyces cerevisiae* · PelB · Glycome profile · Ethanol

Introduction

For the US, to become petroleum independent, a wide array of technologies and biomass types must be considered. Biomass produced on small scales can provide bioethanol to niche markets. For example, cull peaches, rotten fruit unfit for commercial sale, that accumulate at peach orchards can be fermented into ethanol and used on site. These peaches are already collected and stored in bulk and because of their low lignin content do not require harsh pretreatments like many other lignocellulosic biomasses. Currently, cull peaches are landfilled, which can be environmentally hazardous because of the peach's high organic content.

In 2011, the US was the fourth largest producer of peaches (1.17 million tons, MT), behind China (11.53 MT), Italy (1.64 MT) and Spain (1.33 MT), according to the UN's Food and Agricultural Organization (http://faostat.fao.org/). The USDA reports US peach production in 2011 as slightly lower (1.07 MT) with 28,810 tons going unutilized (www.nass.usda.gov/). Based on the FAO and USDA data, over 423,000 tons of peaches go to waste each year worldwide. Assuming the biomass is 17 % dry weight (dw) and contains 60 % fermentable sugars, over 7 million gallons of ethanol could be produced from this biomass.

For production of ethanol from plant biomass to be an economically viable solution to US energy needs, the production price must decrease. One of the most expensive components of the fermentation process is the addition of

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commercial saccharification enzymes. Since most of the sugars being fermented into ethanol in cull peaches are present as free monomeric carbohydrates, it is possible to decrease the loading of commercial enzymes and thus the cost of the entire process. Comparison of ethanol production from fresh and enzymatically treated cull peaches has shown that ethanol concentrations increased by 43.1 % upon the addition of cellulase and hemicellulase from *Aspergillus niger* when fermented with distillers yeast [19]. Therefore, some commercial enzymes will be required to reach maximum ethanol production. This study seeks to elucidate the lowest enzyme loading at which maximum ethanol production can be achieved by fermentation of cull peaches using *S. cerevisiae* XR122 N.

Saccharomyces cerevisiae XR122 N does not naturally produce any saccharification enzymes. However, a novel pectate lyase (PelB) from *Paenibacillus amylolyticus* strain 27C64 has been described that has properties of both pectate lyases and pectin lyases [2]. As a natural hybrid, the production of this single enzyme could replace the use of the commercial pectinase mixtures.

Materials and methods

Peach preparation

Cull peaches were donated by Lane Southern Orchards (Fort Valley, GA). Peaches were cut into eighths and juiced using a Pulp Ejector Juicer model 110.5 (L'EQUIP, St. George, UT). All of the solid material and liquid were pooled and stored at −20 °C. Peach biomass was thawed at room temperature for fermentation and saccharification preparations.

S. cerevisiae XR122 N simultaneous saccharification and fermentation

Fermentations were conducted as described [6] with all deviations noted below. Briefly, 15 % (dw/v) peach fermentations were conducted in 500 mL bioreactors at a final volume of 200 mL, with a final concentration of 1X tryptic soy broth (TSB). All fermentations were completed in triplicate. Peach dw was determined using a Denver Instrument IR 35 moisture analyzer (Denver, CO). Peaches (30 g dw) and any additional water needed were autoclaved at 121 °C and 1 atm for 20 min. After autoclaving, the pH was adjusted to 5.0 using 10 M KOH and 6 N HCl. Varying amounts of commercial enzymes were added and will be specified in the Results and Conclusions. The enzymes used were cellulase (Novozymes 5013, Novozymes, Franklin, NC), pectinase (Pectinex P2736, Novozymes) from *Aspergillus niger* and cellobiase (Novozymes 188; Novozymes). Fermentations were inoculated with 2 g/L lyopholized *S. cerevisiae* XR122 N (North American Bioproducts Corp., Duluth, GA).

After inoculation the fermentations were placed in water baths at 35 °C and mixed with magnetic stir bars. The pH was maintained at 5.0 throughout the fermentation. Samples were taken at the time of inoculation and every 24 h, out to 72 h. Samples were centrifuged at 14,000 rpm for 7 min. The supernatant was collected and centrifuged in a 0.22-μm filter (Corning Inc., Corning, NY). The supernatant was collected and stored at −20 °C for further analysis.

S. cerevisiae XR122 N simultaneous saccharification and fermentation with PelB

Saccharomyces cerevisiae XR122 N fermentations containing 1.88 filter paper units cellulase (FPU)/g dw biomass and 6.5 cellobiase units (CBU)/g dw biomass were performed essentially as described above, except TSB was added to a final concentration of 0.5X. The volume of the fermentation was raised to 200 mL through the addition of supernatant from an overnight culture of *E. coli* LY40A + pWEB, a vector containing *pelB* [2].

Supernatant from *E. coli* LY40A + pWEB was prepared by reviving the organism from freezer stocks on Luria-Bertani (LB) agar plates containing 2 % (w/v) glucose, 40 μg/mL chloramphenicol, and 50 μg/mL ampicillin and incubating at 37 °C overnight. After 24 h, 100 mL of M9 minimal media with 40 μg/mL chloramphenicol and 50 μg/mL ampicillin was inoculated with a single colony and grown overnight at 37 °C with shaking. The overnight culture was centrifuged at 5,000 rpm for 10 min, and the supernatant was collected and filtered through a 0.22-μm Millipore Express PLUS (PES) filter (Millipore Co., Billerica, MA). Enzyme activity was measured in the supernatant using a pectate lyase assay at 37 °C and pH 9.5 [5, 22]. Polygalacturonic acid (2.4 g/L in Tris-HCl, pH 9.5) and PelB (LY40A + pWEB supernatant) were heated to 37 °C and mixed (5:1). The change in absorbance was measured over 180 s reading every 5 s at 235 nm. The units of activity per milliliter were determined, and a Bradford assay was used to determine the protein concentration [3]. Specific activity of PelB was calculated to be 73.5 ± 4.2 IU/mg protein.

Peach saccharification

Fifteen mL of 15 % (dw/v) peach was prepared and autoclaved in screw cap tubes, and the pH was adjusted to 5.0. TSB was added to a final concentration of 1X, and commercial enzymes, listed above, were filter sterilized and loaded at varying concentrations. Saccharifications were incubated in a water bath at 35 °C for 24 h; the pH was maintained at 5.0 through the addition of KOH and HCl as

necessary. After 24 h, samples were taken as described for fermentations and stored at −20 °C until analyzed.

Sample analysis

Ethanol concentrations were measured using gas chromatography (GC) performed on a Shimadzu (Columbia, MD) GC-8A instrument as previously described [6]. Sugar analysis was conducted using high-performance liquid chromatography with refractive index detection (HPLC-RID). Chromatographic separation was achieved on a Shimadzu Prominence LC-20AT liquid chromatographic system (Shimadzu Scientific Instruments, Columbia, MD) using a Bio-Rad (Hercules, CA) Aminex HPX-87H 300 MM by 7.8-mm column with a Bio-Rad Cation H guard column; 5 mM H_2SO_4 was used as the mobile phase to perform an isocratic run at 0.6 mL/min with the column temperature at 60 °C.

Glycome profiling

Glycome profiling [17] was conducted on 15 % (dw/v) peach treated with no enzymes, 60 polygalacturonase units (PGU)/g dw pectinase, 7.5 PGU/g dw pectinase or 0.22 IU/g dw pectate lyase (PelB) at pH 5.0 for 24 h. Samples containing approximately 1 g dw of peach biomass were removed from saccharification experiments. The samples were washed sequentially at room temperature with 80 % (v/v) ethanol (overnight), 100 % ethanol (2 h) and 100 % acetone (10 min). After washing, only the alcohol insoluble residues (AIR) remained. AIR was dried in a vacuum oven for 72 h. Subsequent extractions were performed with 10 mg/mL suspensions based on the initial weight of the AIR. First, 50 mM ammonium oxalate (pH 5.0) was added to the AIR and allowed to incubate at room temperature with shaking. After 24 h, samples were centrifuged at 4,000 rpm for 15 min at 25 °C. The supernatant was decanted and saved as the ammonium oxalate extract. The pellet was washed with 35 mL diH_2O. After the wash step, the wash supernatant was discarded, and the pellet was used for the next extraction. The process was repeated sequentially with 50 mM sodium carbonate (pH 10) containing 0.5 % (w/v) sodium borohydride ($NaBH_4$), 1 M KOH containing 1 % (w/v) $NaBH_4$ and 4 M KOH containing 1 % (w/v) $NaBH_4$. The 1 M KOH and 4 M KOH extracts were neutralized on ice using glacial acetic acid. All samples were dialyzed (using 3,500 Da molecular weight cutoff tubing, no. S632724, Spectrum Laboratories Inc., CA, USA) in diH_2O (with an approximate 1:60 sample to diH_2O ratio) at room temperature for a total of 48 h, changing the diH_2O 4 times.

The dialyzed samples were lyophilized for 72 h, and the remaining material was weighed; 2.0–2.5 mg of sample was removed and dissolved to a final concentration of 0.2 mg/mL. Total sugar was determined using a phenol–sulfuric acid micro plate assay [7, 14]. ELISA plates [Corning 384 well clear flat bottom polystyrene high bind microplate (product no. 3700), Corning Life Sciences, Tewksbury, MA] were then coated with samples at a final concentration of 20 μg/mL and allowed to dry overnight at 37 °C. ELISAs using a comprehensive suite of 155 cell wall glycan-directed monoclonal antibodies (McAbs) were done as described previously [16, 17]. The ELISA responses were used to produce heat maps using a modified version of R-Console software (R Development Core Team, 2006) [18].

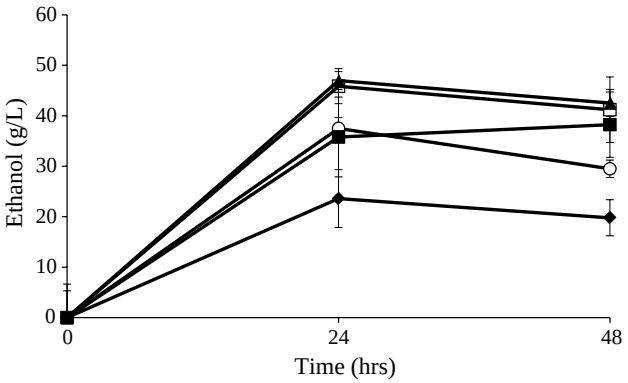

Fig. 1 Ethanol production from 15 % (dw/v) peach fermentations conducted with *S. cerevisiae* XR122 N with varying commercial enzyme loadings. *Filled triangle*: 15 FPU cellulase/g dw, 60 PGU pectinase/g dw and 50 CBU cellobiase/g dw; *white square* 1.88 FPU cellulase/g dw, 7.5 PGU pectinase/g dw and 6.25 CBU cellobiase/g dw; *filled square*: 1.41 FPU cellulase/g dw, 5.63 PGU pectinase/g dw and 4.69 CBU cellobiase/g dw; *circle*: 0.94 FPU cellulase/g dw, 3.25 PGU pectinase/g dw and 3.13 CBU cellobiase/g dw; (*filled diamond*) no additional commercial enzymes

Results and discussion

Decreasing commercial enzymes cellulase, cellobiase and pectinase

Due to the low concentrations of polysaccharides and *S. cerevisiae* XR122 N's inability to metabolize all of the sugars released from the polysaccharides, it is likely that only low concentrations of commercial enzymes will be necessary to achieve maximum ethanol production using culled peach biomass. Commercial enzyme loadings in fermentations of 15 % (dw/v) peach were sequentially reduced until ethanol production began to decrease. Figure 1 displays ethanol production over time from fermentations treated with selected enzyme concentrations.

Table 1 Sugars remaining from 15 % (dw/v) peach saccharifications after 24 h

Enzyme loading (U/g dw)	Sugar concentration (g/L)			
	Glucose	Fructose	Cellobiose	Galacturonic acid
15 FPU, 60 PGU, 50 CBU	50.1 ± 2.3	50.4 ± 1.3	6.6 ± 0.5	9.8 ± 0.5
1.88 FPU, 7.5 PGU, 6.25 CBU	46.4 ± 1.2	50.1 ± 0.5	4.4 ± 0.2	7.5 ± 0.3
1.88 FPU, 0 PGU, 6.25 CBU	45.7 ± 1.2	50.7 ± 0.7	4.1 ± 0.1	4.6 ± 0.1
0 FPU, 0 PGU, 0 CBU	43.7 ± 1.8	50.1 ± 1.2	ND[a]	3.9 ± 0.2

[a] Not detected

Fermentations conducted with enzyme loadings between 15 FPU cellulase/g dw, 60 PGU pectinase/g dw, 50 CBU cellobiase/g dw and 1.88 FPU cellulase/g dw, 7.5 PGU pectinase/g dw, 6.25 CBU cellobiase/g dw all produced the highest concentration of ethanol by 24 h, 47.0 ± 1.8 g/L ethanol and 45.9 ± 5.5 g/L ethanol, respectively. The next lowest enzyme loading tested, 1.41 FPU cellulase/g dw, 5.63 PGU pectinase/g dw, 4.69 CBU cellobiase/g dw, only reached 35.8 ± 7.9 g/L and required 48 h to reach this ethanol concentration. The lowest concentration of ethanol was produced when no commercial enzymes were added (23.6 ± 5.5 g/L).

Maximum theoretical yield was calculated based on the amount of free sugars released during the total saccharification of 15 % (dw/v) peach with the highest enzyme concentrations (50.1 g/L glucose, 50.4 g/L fructose, 6.6 g/L cellobiose and 9.8 g/L galacturonic acid) and the capability of *S. cerevisiae* XR122 N to ferment specific sugars. The maximum theoretical yield was 49.2 g/L ethanol, assuming 90 % conversion efficiency of sugar to ethanol. The highest enzyme loading reached 95.5 % of the maximum theoretical yield, making it unlikely that the addition of more commercial enzymes would increase ethanol production. When the enzyme loading was decreased to 1.41 FPU cellulase/g dw, 5.63 PGU pectinase/g dw, 4.69 CBU cellobiase/g dw, the ethanol production at 24 h dropped below 80 % of the theoretical maximum. With no commercial enzymes, the conversion efficiency was only 51 %.

The effects of the concentration of commercial enzymes on the release of sugars from the peach biomass were monitored through 24 h saccharification experiments. No fermenting organism is present, so sugars were retained in solution and could be quantified via HPLC. The highest enzyme loading had the highest concentrations for all sugars measured (glucose, fructose, cellobiose and galacturonic acid) (Table 1). Lowering the enzyme loading to 1.88 FPU/g dw, 7.5 PGU/g dw, 6.25 CBU/g dw decreased the production of cellobiose and galacturonic acid. However, these polysaccharide degradation products were still present in large quantities compared to saccharifications performed with no additional enzymes. There was a slight increase in glucose levels in the presence of commercial enzymes due to the degradation of cellulose, but no change in fructose, which is found as a free sugar in the peach cell wall. Overall, the addition of commercial enzymes even at low concentrations significantly changed the sugar profile of the 24 h peach saccharifications, supporting the fermentation results. The decrease in sugars when enzyme loading was cut could be the result of end-product inhibition of the saccharification enzymes [8]. End-product inhibition would not be a problem during fermentation because *S. cerevisiae* would consume the sugars as they were being released.

Removal of commercial pectinase

Since *S. cerevisiae* XR122 N is not capable of fermenting galacturonic acid, this acidic sugar builds up throughout the fermentation without being metabolized (data not shown). To determine whether or not degradation of pectin is necessary for maximum ethanol production from fermentations of 15 % (dw/v) peach with *S. cerevisiae* XR122 N, pectinase was completely removed from the enzyme loading. Only 1.88 FPU cellulase/g dw and 6.25 CBU cellobiase/g dw were added to the fermentation. Ethanol production without pectinase only reached 82.5 % of the maximum theoretical yield. Similar results have been shown during the fermentation of citrus waste with *S. cerevisiae*. However, citrus biomass has a higher pectin content and requires higher concentrations of pectinase for maximum ethanol production [20, 24].

When pectinase is removed from saccharification experiments there is a slight drop in glucose and cellobiose production. As expected, galacturonic acid levels are more similar to the no enzyme saccharification than they are to saccharifications containing pectinase. As seen earlier, fructose levels do not change with the addition of enzymes (Table 1).

All of the polysaccharides in the plant cell wall (cellulose, hemicellulose and pectin) form large, complex and likely intertwined networks. Pectin is believed to form a pectic network that can covalently bond with hemicellulose, specifically xyloglucan and/or xylan. Xyloglucan also forms hydrogen bonds with the cellulosic network [4]. The degree of entanglement of cellulose, hemicellulose and pectin likely necessitates the addition of pectinase enzymes to the fermentation for efficient cell wall deconstruction. Without these enzymes, cellulose would not be easily accessible to cellulase degradation enzymes; therefore, the glucose contained in cellulose would not be available for fermentation by *S. cerevisiae* XR122 N. The addition of

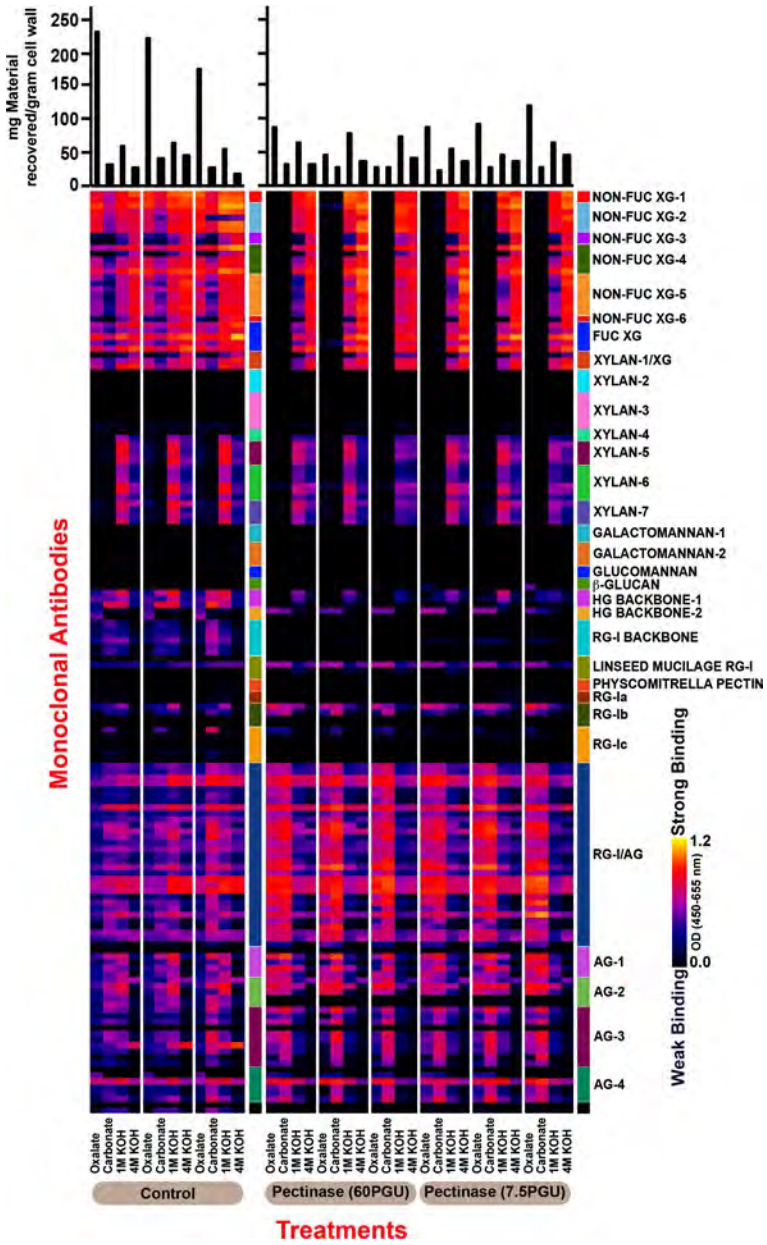

Fig. 2 Glycome profiling of 15 % (dw/v) peach treated for 24 h without any pectinase, with 60 PGU/g dw pectinase or 7.5 PGU/g dw pectinase: The sequential cell wall extracts derived from peach cell wall samples (see "Materials and Methods") were screened by ELISA using 155 McAbs directed against most major plant cell wall glycans (see Online Resource 1). The ELISA response data are represented as heat maps with a *white–red–black* scale indicating the strength of the ELISA signal (*white*, *red* and *black* colors depict strong, medium and no binding, respectively). The McAbs are grouped based on the cell wall glycans they recognize as depicted in the panel at the *right hand side* of the figure. The amounts of materials extracted from the walls in each extraction step are depicted in the *bar graph* at the top of the heat maps

pectinase to an enzyme mixture has been shown to increase enzymatic digestion of potato pulp [11] and fiber hemp [15]. Pectin decreases cell wall digestion by decreasing the accessibility of saccharification enzymes to cell wall polysaccharides. For example, removal of pectin increased the porosity of the cell wall in soybeans [1]. There is evidence that homogalacturonan interacts directly with cellulose [23]. Thus, pectins could interfere with cellulase access to cellulose, thereby decreasing enzymatic digestion of the cell wall [12, 13, 23].

The effects of pectin on the peach cell wall were tested at 60 and 7.5 PGU/g dw pectinase using glycome profiling. This method involves sequential extractions of plant cell walls using increasingly harsh reagents (ammonium oxalate, sodium carbonate, 1 and 4 M KOH) and subsequent fully automated semiquantitative detection of the polysaccharide epitopes in the extracts using a comprehensive collection of cell wall glycan-directed McAbs [17]. Glycome profiling provides information about the glycan epitope composition of the walls as well as an assessment of how tightly various cell wall glycans are integrated into the wall. Thus, more tightly integrated wall glycan components are extracted with the harsher extractives (1 M and 4 M KOH), and loosely integrated glycans are extracted under milder extraction conditions (oxalate and carbonate).

Glycome profiling analyses were conducted on cell walls isolated from untreated (control) and pectinase treated 15 % (dw/v) peach samples (Fig. 2). In general, the pectinase

treatments prominently affected the overall extractabilities of xyloglucan, pectin and pectic arabinogalactan epitopes (Fig. 2). As expected, pectinase at either the low (7.5 PGU) or high (60 PGU) concentration almost completely removed pectin from the carbonate, 1 and 4 M KOH peach cell wall extracts, as indicated by significant reductions in the binding of homogalacturonan backbone (HG backbone-1 and -2) and rhamnogalacturonan backbone (RG-I backbone) groups of McAbs. More surprisingly, the pectinase treatments almost completely removed xyloglucan epitopes from the first two extractions (ammonium oxalate and sodium carbonate). This was also reflected in the decreased amount of material recovered (see the bar graphs at the top of Fig. 2 depicting mg material recovered/gram cell wall) in the sodium oxalate extracts of all pectinase-treated peach biomass compared with the controls. There are two possible explanations for these results: (1) the pectinase preparation used in these experiments is an enzyme cocktail with a variety of enzymatic activities, one of which could be xyloglucan endoglucanase, or (2) the easily extractable xyloglucan is associated with pectin in the peach cell wall, and upon degradation of the pectin this subfraction of xyloglucan is also removed from the cell wall.

There was also a significant increase observed in the abundance of pectic arabinogalactan (as denoted by the binding of RG-I/AG groups of McAbs) and arabinogalactan (as denoted by the binding of various AG groups of McAbs) epitopes in the oxalate and carbonate extracts. This was accompanied by a decrease in signals from these groups of antibodies in the harsher alkali extracts, suggesting that pectinase treatment resulted in the loosening of the association of arabinogalactans within the cell wall matrix. Two McAbs in the AG-2 antibody clade, CCRC-M133 and CCRC-M107, showed no binding to any of the extracts isolated from pectinase-treated peach cell walls; in comparison, these two antibodies showed binding in the carbonate and 1 M KOH extracts in the controls. The other antibodies in this clade with previously demonstrated arabinan and arabinogalactan specificities (JIM14 [9], JIM19 [10] and JIM12 [21]) were not affected by the addition of pectinase. These results suggest that the epitope structures recognized by CCRC-M133 and CCRC-M107 are susceptible to the action of pectinase enzymes used in this study.

Overall, the glycome profiling studies demonstrated that pectinase treatment significantly affected the plant cell wall, and its impact was not limited to pectin degradation. This was true for both the high and low pectinase treatments.

Supplementation of commercial cellulase and cellobiase with PelB

A novel pectate lyase, PelB, from *P. amylolyticus* strain 27C64 was used to supplement the saccharification

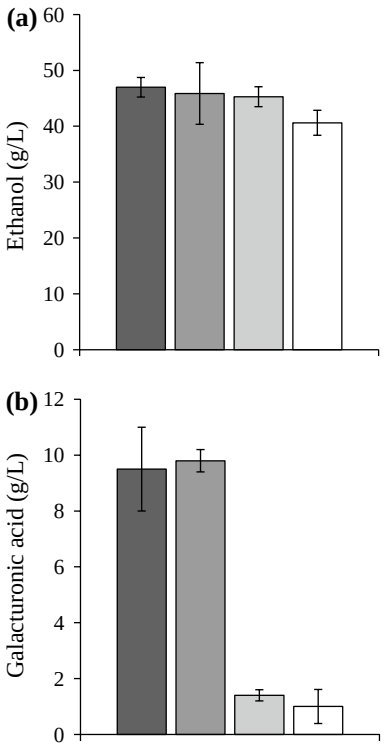

Fig. 3 Ethanol production at 24 h (**a**) and galacturonic acid production (**b**) from 15 % (dw/v) peach fermentations conducted with *S. cerevisiae* XR122 N with varying commercial enzyme loadings; 15 FPU cellulase/g dw, 60 PGU pectinase/g dw, 50 CBU cellobiase/g dw (*black*), 1.88 FPU cellulase/g dw, 7.5 PGU pectinase/g dw, 6.25 CBU cellobiase/g dw (*dark gray*), 1.88 FPU cellulase/g dw, 0 PGU pectinase/g dw, 6.25 CBU cellobiase/g dw and PelB (*light gray*), 1.88 FPU cellulase/g dw, 0 PGU pectinase/g dw and 6.25 CBU cellobiase/g dw (*white*)

enzymes, 1.88 FPU cellulase/g dw and 6.25 CBU cellobiase/g dw. The addition of PelB allowed the fermentation to reach 92.1 % of the maximum theoretical ethanol production. Without PelB, the same fermentation only produced 40.6 ± 2.2 g/L ethanol, with the supplementation ethanol production increased to 45.3 ± 1.8 g/L (Fig. 3a). When PelB was added there was no increase in free galacturonic acid in the fermentation after 72 h because PelB releases oligogalacturonides, not monomeric sugars (Fig. 3b). This demonstrates that pectin did not need to be broken down entirely to monomeric sugars to allow cellulase and cellobiase access to the cellulose in the biomass or allow the yeast access to the fermentable sugars. Therefore, a single enzyme that partially degrades pectin can be used to replace the suite of commercial pectinases needed to enhance conversion of the peach biomass.

The glycome profiles of peach cell wall treated with PelB did not show any major differences from the controls (Fig. 4). A subtle increase in the abundance of some xyloglucan epitopes was noted in the oxalate and carbonate

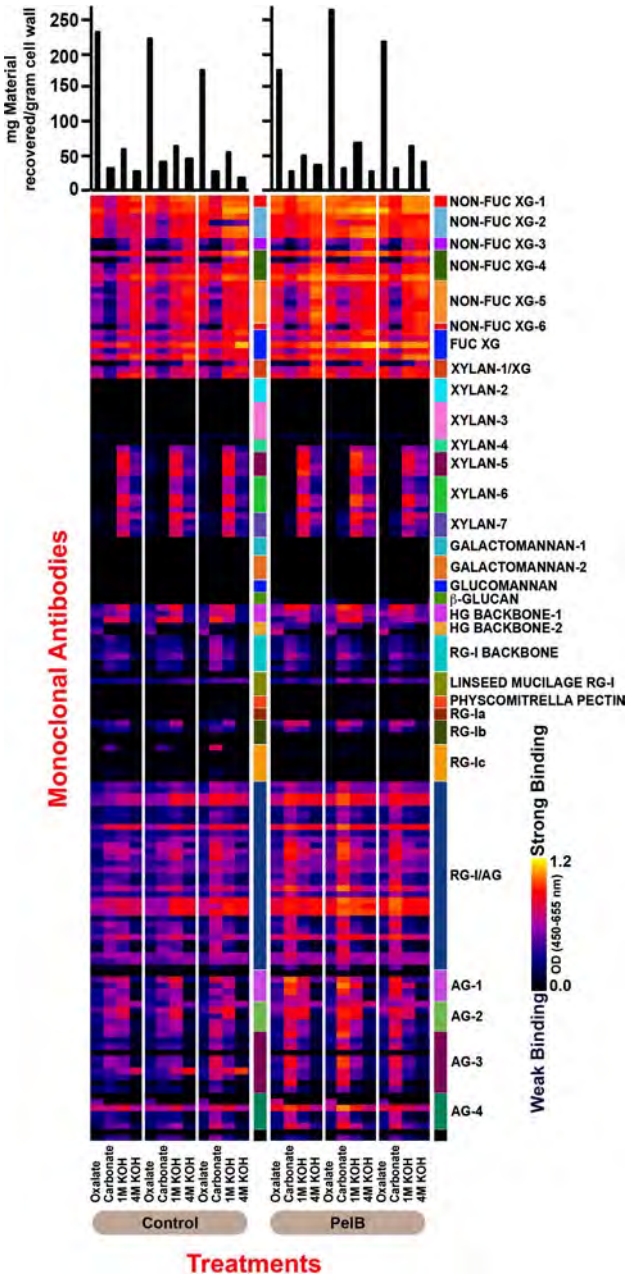

Fig. 4 Glycome profile of 15 % (dw/v) peach treated for 24 h without any enzymes or with PelB: The sequential cell wall extracts derived from peach cell wall samples (see "Materials and Methods") were screened by ELISA using 155 McAbs directed against most major plant cell wall glycans (see Online Resource 1). The ELISA response data are represented as heat maps with a *white–red–black* scale indicating the strength of the ELISA signal (*white*, *red* and *black* colors depict strong, medium and no binding, respectively). The McAbs are grouped based on the cell wall glycans they recognize as depicted in the panel at the *right hand side* of the figure. The amounts of materials extracted from the walls in each extraction step are depicted in the *bar graph* at the top of the heat maps

extracts from PelB-treated peach well walls. Other groups of antibodies also showed such subtle increases in antibody binding in extracts from PelB-treated cell wall samples, such as the HG-backbone-2 antibodies (especially in oxalate extracts), RG-I/AG antibodies (oxalate and carbonate extracts) and AG-1 through AG-4 antibodies (especially in carbonate extracts). These results suggest that a subtle loosening of the associations within the walls resulted from PelB treatment, leading to slight increases in the extractability of subpopulations of these polysaccharides.

These data suggest that PelB increased the fermentability of cull peaches through a process dissimilar from that of the commercial pectinase mixture. Most of the pectin remained in the cell wall, but became relatively less tightly bound. The degradation of pectin into smaller oligomers reduced the cell wall integrity and freed up other polysaccharides (demonstrated by their increased extractability in the glycome profile) and facilitated access of the commercial cellulases and cellobiase to the plant cell wall.

Conclusions

The necessity for pectinase in the fermentation of pectin-rich biomass has been shown previously [20, 23]. In this report, we demonstrate that an entire suite of pectinolytic enzymes can be replaced with a single enzyme, PelB. Commercial pectinase enzymes and PelB can serve the same purpose in the fermentation of cull peaches; however, they act in different manners. Commercial pectinase completely eliminates pectin and loosely bound xyloglucan from the walls. The activity of PelB is less dramatic, but equally efficient in its effects on ethanol production. Instead of removing pectin from the cell wall, PelB opens up the cell wall, making many of the polysaccharides, including some xyloglucans, rhamnogalacturonan I, arabinogalactans and the homogalacturonan backbone, relatively easier to extract. This effectively increases the accessibility of free sugars and polysaccharides located in the peach cell wall to commercial cellulase, cellobiase and *S. cerevisiae* XR122 N without completely degrading the pectin. Accessibility of these sugars is required for maximum ethanol production. The addition of 7.5 PGU/g dw pectinase or PelB increases ethanol production from 40.6–45.9 and 45.3 g/L, respectively.

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