Analyses of Cell Wall Glycans Using Glycome Profiling in Two Commercially Important Lignocellulosic Fiber Raw Materials

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Abstract

Lignocellulosic fibers are plant-based bio-fibers that are sourced from terrestrial and non-terrestrial plants that also include agricultural by-products. In textile industry, being a renewable and sustainable resource, these fibers have attained market potential with their special qualities including biodegradability, applicability in green chemistry, ecofriendly properties and cost effectiveness in processing. Lignocellulosic fibers mainly comprise plant cell walls that are essentially composed of cellulose, lignin, pectin, hemicelluloses and waxes. Numerous studies have been conducted to explain the commercial performance, mechanical properties and sustainability of these lignocellulosic fibers. Although several work have been conducted on the cell wall compositional aspects of these fibers, majority of the studies have been focused on understanding the cellulose and lignin constituents. Hence, there is an increasing need for more studies to be conducted on non-cellulosic cell wall components in these fibers. In this study, we performed a novel immunological approach namely glycome profiling on two commercially important lignocellulosic fibers from aquatic and terrestrial plants namely duckweed and hemp, respectively. Our studies demonstrated differences in the extractability of cell wall glycans from biomass materials from these plants. Duckweed biomass had significantly higher abundance of extractable pectins in their walls. However, cell walls from biomass raw materials of terrestrial plant, hemp had lesser amounts of pectin with significantly higher amounts of xylans that are easily extractable. Duckweed fibers had significantly higher proportion of lignin-associated wall glycans in comparison to that of hemp. These results demonstrate that lignocellulosic fiber raw materials from varied plant sources are different in non-cellulosic cell wall glycan compositions and comprehending these variations could potentially be instrumental in commercial processing of these fibers.

Keywords: Lignocellulosic fibers; Cell wall glycans; Glycome profiling

Introduction

Natural fibers, that are commercially important, are fibers derived from natural sources including plants, animals and minerals [1]. Fibers originating from plant-based sources are called plant fibers also popularly known as lignocellulosic fibers. In future, lignocellulosic fibers will potentially be the most important textile composites for its commercial performance and ecofriendly properties. Lignocellulosic fibers are mainly available from primary natural resources such as plants (e.g., crops, grass, woody plants and non-terrestrial plants) and other secondary resources originating from recycled plant based bio-products (e.g., paper and pulp industrial waste, municipal waste and Agricultural waste). Commercially, lignocellulosic fibers are used in the manufacture of garment, home and industrial textile products. Additionally, lignocellulosic fibers are also used in paper, pulp, chemical, agricultural, poultry, medicine, biomaterials, nanomaterials and pharmaceutical industries. The lignocellulosic materials can be also recycled or converted to biofuels such as bioethanol and biodiesel. Thus, currently much emphasis is given to research and applications involving lignocellulosic raw materials.

Lignocellulosic fibers are mainly composed of plant cell walls mainly constituted by cellulose, hemicellulose, lignin, and pectin. Relative proportions of these cell wall components determine their commercially relevant physical properties including density, tensile strength, crystallinity, and modulus. Cell walls, constituting the major bulk of lignocellulosic biomass, immensely differ in their composition and structure depending on the plants, organs, tissues, and even cell types. This heterogeneity and compositional dynamics makes the research on lignocellulosic fibers from diverse resources challenging.

Majority of the previous studies on lignocellulosic fibers focused on the influence of cellulose and lignin content with respect to the fiber properties [2]. The fiber characteristics including moisture content, water absorption, modulus, tensile strength, crystallinity are also well studied [3]. The hemicelluloses and pectin components in commercial lignocellulosic fibers (non-cellulosic cell wall glycans) are yet to be studied in detail with respect to their overall composition, structure and how they influence the fiber properties.

Here, we employ a powerful immunological approach, glycome profiling for the first time to conduct a comprehensive analyses of non-cellulosic cell wall components in two commercially relevant plant fiber raw materials from duckweed and hemp. The analyses described here promises to be an instrumental approach for moderate through put to high through put analyses of any lignocellulosic fiber raw materials. Thus far, very limited numbers of studies have been done on these two lignocellulosic materials or crops. In one such study, the distributions of various plant cell wall glycans were analyzed among different tissues using immunolocalization approaches [4]. These studies were done mainly in situ and thus do not directly correlate to the cell wall properties pertaining to actual commercially relevant biomass raw materials. However, few studies were conducted on isolated hemp fibers including those on fibers that are modified by chemical
treatment prior to using them in synthesis of biocomposites [2] and those subjected to the physicochemical treatments [5]. In yet another study, the hemicellulose and lignin contents of the hemp were analyzed for its use in biocomposites [6,7]. Most of the other studies were done to investigate the physical characteristic [8] and visual characteristics of the hemp fibers [9,10]. In the case of duckweed, again limited numbers of studies were conducted to detail cell wall glycans and these mainly include the study on solubilization and structures of pectic polysaccharides examined using NMR spectroscopy [11,12]. Hence, there is a further need to comprehensively understand structural and compositional aspects of non-cellulosic cell wall glycans in these two commercially important lignocellulosic raw materials.

Materials and Methods

Plant fibers

Duckweed sample was collected from Ven consulting, LLC in Melbourne, FL. Duckweed (genus Lemna) was grown and harvested. Duckweed was dried using forced convection dryer at 70°C with moisture content of 11%. The sample was then knife milled (SM2000/1690 Utm, retsch GmbH, Germany) with 0.25 mm screen.

Hemp fiber sample was obtained from University of Mississippi where it was retted using the traditional process. The sample was then grinded using knife milled (SM2000/1690 Utm, retsch GmbH, Germany) with 0.25 mm screen.

Glycome profiling

Preparation of alcohol insoluble residues (primarily containing cell walls) and glycome profiling of cell wall extracts were carried out as described earlier [13]. Total sugar estimation was done using phenol-sulphuric acid method [14].

Plant cell wall glycan-directed monoclonal antibodies were obtained from laboratory stocks (CCCR, JIM and MAC series) at the Complex Carbohydrate Research Center (available through CarboSource Services; http://www.carbosource.net) or were obtained from BioSupplies (Australia) (BG1, LAMP). Details of the McAbs used can be seen in the Supporting Information (Supplementary table 1).

Results and Discussion

Biomass materials from hemp and duckweed plants chosen here represented two commercially relevant model resources for terrestrial and non-terrestrial plant fibers, respectively. These fibers are known sources of cellulotic fibers that are used in commercial synthesis of biocomposites and nanocellulose [15,16]. The main objective of this study was to systematically analyze the broad spectrum of non-cellulosic cell wall glycans present in duckweed and hemp biomass raw materials. As mentioned in the method section, raw biomass from duckweed and hemp plants were harvested and subjected to set processing conditions to give rise to the final residues (that is the primary raw material for commercial applications) that were analyzed in the current study. A powerful immunological approach, glycome profiling was employed to analyze these biomass materials. The method glycome profiling involves isolating the cell walls (the primary component of plant biomass) and generating a set of sequential extracts from them with increasingly harsh reagents. These extracts are thus selectively enriched with various classes of cell wall glycans based on the tightness with which they are integrated in to the cell walls [13]. The extracts generated were then probed with a comprehensive suite of cell wall glycan directed monoclonal antibodies (McAbs) that can monitor variations in the abundance of glycan epitopes comprised in most major cell wall polysaccharides [13,17]. A number of recent studies have reported analyses of diverse plant biomass samples at various contexts applying glycome profiling as the primary tool [18-20].

Cell wall glycan extractability and composition of duckweed biomass material, sourced from an aquatic environment were revealed by glycome profiling analyses (Figure 1). Cell wall extracts generated by least harsh reagents (oxalate and carbonate) were found to contain only pectin, pectic-arabinogalactans and arabinogalactans as indicated by the significant binding of homogalacturonan backbone-1, Rhamnogalacturonan-I (RG-1)/Arabinogalactan (AG) and AG-2 groups of McAbs. More severe alkaline extracts, such as 1M KOH and 4 M KOH, released highest amounts of extracted carbohydrate materials (Figure 1-Bar graph on top) that contained higher proportions of hemicellulosic polysaccharides such as xylodogulcans (XG) and xylans as revealed by the higher binding of various XG and xylans groups of McAbs (Figure 1). During chlorite extraction, most of the lignin is fragmented and removed with lignin-associated glycan being released in to the extract generated. Chlorite extract of duckweed biomass contained xylans and pectins as denoted by the binding of xylan-5, xylan-7, homogalacturonan backbone-1, RG-1/AG and AG-2 groups of McAbs (Figure 1). The post chlorite alkaline extract (4 M KOH PC) that further extracts out the remaining glycans after lignin removal was similar in composition to other alkaline extracts with higher proportions of hemicellulosic polysaccharides, xyloglucans and xylans.

Glycome profiling analyses of lignocellulosic raw materials from hemp (a terrestrial plant) demonstrated distinct cell wall properties in terms of glycan extractability and composition. Over all, among various extracts a significantly reduced abundance of pectic epitopes was evident in hemp biomass materials. This is an expected result as these biomass materials were subjected to retting after harvest where in most of the pectic glycans could have been dissolved out by microbial action. However, significant amounts of hemicellulosic polysaccharides were extracted out among all cell wall extracts except chlorite extracts. Xylans were extracted out under all extraction conditions except chlorite extraction as indicated by the binding patterns of various xylan groups of McAbs (Figure 1). Xyloglucans extractability in hemp was similar to that of duckweed samples with higher extractability in all KOH extracts. Again, in hemp highest amounts of cell wall carbohydrates were extracted out by 1 M and 4 M KOH extractions and these reagents essentially extracted out hemicelluloses.

Overall, glycome profiling analyses done here demonstrated variations in the cell wall glycomes of commercially applicable biomass raw materials produced from duckweed and hemp. It is understood that there is a difference in the way in which these biomass are processed after harvest. For example, duckweed biomass went through a drying procedure at 70°C for a prolonged duration to reduce the moisture content while hemp materials underwent processes such as retting and drying. Hence, these samples are not only from different plants sources but also are processed differently. Thus, there is higher possibility of introducing variation in the cell walls constituting these biomass materials at structural and compositional level. Recent studies demonstrated that pretreatment regimes for bio-fuel applications induce significant structural alterations in cell walls constituting plant biomass such as poplar and switch grass [18,21]. It is here that moderately to high through put analytical tools such as glycome profiling become instrumental for analyses. Supporting this, and as explained earlier, the glycome profiling analyses demonstrated significant variation in the composition and extractability of non-cellulosic glycans between the two lignocellulosic raw materials studied here. As cell wall components
Figure 1: Glycome profiling of cell walls isolated from duckweed and hemp lignocellulosic materials (see materials section). Note: Sequential extracts of cell walls were made from duckweed and hemp lignocellulosic materials (see materials section). These sequential extracts were screened with 155 McAbs directed against most major plant cell wall glycans (See supplementary Table). The ELISA binding response data are denoted as heatmaps with white-yellow-red-purple-blue-black scale indicating the strength of the ELISA signal (white, red and black colors depict strong, medium, and no binding, respectively). The groupings of McAbs are based on their specificity to various cell wall glycans as shown in the panel at right hand side of the figure. The bar graph on top depicts the mg soluble carbohydrate (glucose equivalent) recovered per gram biomass (Supplementary table 1).
like hemicelluloses and pectin do influence biomass’s physical/mechanical/chemical features and thus the downstream applicability of these raw materials, a detailed understanding on composition and relative proportions of these cell wall components (such as non-cellulosic glycans) is highly valuable.

Thus far, the structure and composition of cell walls constituting lignocellulosic fibers have been studied largely using chemical analytical tools such as Fourier transform infrared spectroscopy (FTIR), Nuclear magnetic resonance (NMR), mass spectrometry (MS) and microscopic tools such as transmission electron microscopy (TEM) [2,22,23]. Chemical analytical tools such as NMR and MS are often done on isolated cell wall fractions and require prolonged sample preparation and optimization times. Again, the data so obtained are limited to a single or small group of cell wall glycans. Hence, these tools are often preferred for very few through put analyses. Further, data from the analysis such as FTIR and TEM cannot be quantified without perfect model. The glycome profiling analyses, on the other hand, while efficiently monitoring most major non-cellulosic glycans in plant biomass materials, can also function in a moderately to high throughput manner with relatively lesser sample preparation time.

ELISA based glycome profiling is a semi quantitative method. The strength of McAb binding directly corresponds to the abundance of glycan epitopes (that they are specific to) present in a given cell wall polymer. ELISA binding values obtained here cannot be used directly to assess the accurate quantitative measurement of a given cell wall polymers as McAbs recognizing them are epitope specific rather than polymer specific. To conduct glycome profiling, cell wall extracts are loaded on to the ELISA plates on an equal carbohydrate basis [17]. The raw ELISA response values of each McAbs corresponds to the abundance of glycan epitopes (that they are specific to) present in a given cell wall extract and thus, indirectly provide an assessment of relative abundance/proportion of that glycan in that extract. Additionally, it also provide the actual amounts of total carbohydrate materials released during each extraction steps as a bar graph (top part of the Figure 1). Correlating the ELISA response values with the total amounts of carbohydrate materials recovered in each extract can provide an indirect quantitative assessment of specific cell wall glycans. The raw values for ELISA responses of all McAbs and carbohydrate recovered in each extracts are provided in the supplementary table 1.

In summary, our studies for the first time demonstrated the effective application of glycome profiling to comprehensively analyze most major non-cellulosic glycan in duckweed and hemp lignocellulosic fiber raw materials. The studies delineated interesting variations in the composition, extractability and relative proportions of pectin and hemicellulosic components in these two biomass materials. The results from these studies could potentially be instrumental in fine-tuning the downstream processing of these materials for commercial applications.

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References