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The cell walls of green algae: a journey through evolution and diversity

David S. Domozych1*, Marina Ciancia2, Jonatan U. Fangel3, Maria Dalgaard Mikkelsen3, Peter Ulvskov3 and William G. T. Willats3

1 Department of Biology and Skidmore Microscopy Imaging Center, Skidmore College, Saratoga Springs, NY, USA
2 Cátedra de Química de Biomoléculas, Departamento de Biología Aplicada y Alimentos, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina
3 Department of Plant Biology and Biochemistry, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

The green algae represent a large group of morphologically diverse photosynthetic eukaryotes that occupy virtually every photic habitat on the planet. The extracellular coverings of green algae including cell walls are also diverse. A recent surge of research in green algal cell walls fueled by new emerging technologies has revealed new and critical insight concerning these coverings. For example, the late divergent taxa of the Charophycean green algae possess cell walls containing assemblages of polymers with notable similarity to the cellulose, pectins, hemicelluloses, arabino-galactan proteins (AGPs), extensin, and lignin present in embryophyte walls. Ulvophycean seaweeds have cell wall components whose most abundant fibrillar constituents may change from cellulose to β-mannans to β-xylans and during different life cycle phases. Likewise, these algae produce complex sulfated polysaccharides, AGPs, and extensin. Chlorophycean green algae produce a wide array of walls ranging from cellulose–pectin complexes to ones made of hydroxyproline-rich glycoproteins. Larger and more detailed surveys of the green algal taxa including incorporation of emerging genomic and transcriptomic data are required in order to more fully resolve evolutionary trends within the green algae and in relationship with higher plants as well as potential applications of wall components in the food and pharmaceutical industries.

Keywords: cell walls, scales, green algae, sulfated polysaccharides, glycoproteins

INTRODUCTION

The emergence of green algae (Chlorophyta and Streptophyta, Viridiplantae; sensu Leliaert et al., 2012) onto land roughly 470 million years ago represents one of the most important events in the history of life on the planet. Their successful colonization of land and subsequent evolution into modern land plants significantly altered the atmosphere, changed terrestrial substrates and paved the way for the evolution of other biota. Today, humans ultimately depend on the evolutionary “offshoots” of green algae (i.e., embryophytes or “land plants”) for food, textiles, building materials, pharmaceuticals, and fuels. Yet these events and applications represent only parts of a much larger story of green algae. Contemporary green algae are ubiquitous. They are important members of the ocean’s phytoplankton, common and sometimes nuisance seaweeds of coastal marine habitats, peculiar symbionts of lichens and flatworms, and inhabitants of just about any freshwater ecosystem ranging from ponds, rivers, lakes, wetlands, and snow banks. In the 1.5 billion years since they first appeared (Lewis and McCourt, 2004; Becker and Marin, 2009; Finet et al., 2010; Leliaert et al., 2011; Wodnick et al., 2011), green algae have successfully adapted to virtually all photic zones of the planet.

Similar to their land plant offspring, the vast majority of extant green algae today are covered by a very large assortment of types of extracellular matrix (ECM). These external coverings are products of complex biosynthetic machineries that often make use of the bulk of the alga’s photosynthetically fixed carbon. The ECM is integral to growth and development, affords the alga physical protection and defense against microbial attack, is involved in cell–cell and cell–substrate adhesion and in some cases, is involved in sexual reproduction. Some green algae are covered by multiple layers of intricately sculpted scales while others have crystalline glycoprotein coverings or thick multilaminate fibrillar cell walls. A few taxa though have cell walls with remarkable structural and biochemical similarity to cell walls found in land plants (Sørensen et al., 2010, 2011). How did these diverse extracellular coverings arise and what are the evolutionary links between them? Many analytical approaches and technologies are now being used to study green algal ECMs and are providing new and critical insight into structure, chemistry, and evolution of these coverings (Table 1). Nevertheless, we are only in an infancy stage in our understanding of the green algal extracellular coverings. In this review, we describe some of these recent discoveries and comment on future directions for study of the cell walls of green algae.

THE CURRENT STATE OF STUDY OF GREEN ALGAL CELL WALLS AND EXTRACELLULAR COVERINGS

While the green algae display a large and diverse array of ECM-coverings, only a few taxa have been studied in detail. It is widely accepted that taxa of the Ulvophyceae and the Charophycean green algae (CGA) possess fibrillar cell walls (Popper et al., 2011) consisting of various polysaccharide and proteoglycan constituents while other taxa, especially those of the Prasinophyceae,
Table 1 | Major methodologies used today in the study of green algal coverings.

| Methodology            | Technical aspects                                      | Data obtained/status                                      | Reference                                                                 |
|------------------------|--------------------------------------------------------|-----------------------------------------------------------|----------------------------------------------------------------------------|
| **Biochemical**        | Chemical and enzymatic fractionation; methylolation analysis-GC/MS; NMR; electrophoresis | Monosaccharide composition, glycosidic linkage composition, conformational studies, molecular weights of various cell wall polysaccharides | Popper et al. (2011), Popper and Fry (2003), Estevez et al. (2009)          |
| **Carbohydrate microarrays** | Sequential extraction of polysaccharides; immobilization onto nitrocellulose, mAb probing | Early divergent CGA walls differ from late divergent taxa walls; late divergent taxa possess HGA, RG-I, MLG, various hemicelluloses, AGPs, extensins | Sørensen et al. (2010, 2011), Möller et al. (2007)                         |
| **Immunocytochemistry** | Immunofluorescence and immunogold labeling of live cells and sections of fixed cells | Wall polymer mAbs may be used in live cell studies; *Coleochaete* walls possess lignin-like epitopes | Domozych et al. (2009, 2011), Eder and Lutz-Meindl (2010), Sørensen et al. (2011) |
| **FTIR microspectroscopy** | IR spectral arrays obtained from microscopically imaged covering | Analysis of presence and distribution of polymers in the cell wall of Ulvophyceae | Estevez et al. (2009), Fernández et al. (2011a), Carpita et al. (2001) |
| **Molecular**           | Transcriptome and genome acquisition; annotation of genes | Genomes sequenced in Volvox carteri, *Chlamydomonas reinhardtii*, *Micromonas* sp. RCC299, *Ostreococcus* tauri, and *Ostreococcus lucimarinus* (see http://bioinformatics.psb.ugent.be/plaza/) and *Chlorella variabilis* NC64; several transcriptomes analyzed | Timme and Delwiche (2010), Vannierum et al. (2011), Timme et al. (2012) |

mAb, monoclonal antibody; RG-I, rhamnogalacturonan-I; MLG, mixed linkage glucans; AGP, arabinogalactan protein; FTIR, Fourier transform infrared; CGA, Charophycean green algae.

Chlorodendrophyceae, and some taxa of the Chlorophyceae, produce coverings that are structurally and biochemically unique. Presently, extant green algae are classified into six distinct clades (Delwiche and Timme, 2011; Leliaert et al., 2011, 2012). The following represent brief synopses of the current state of knowledge concerning the ECM of these groups (see also Table 2).

THE PRASINO PHYCEAEAN MATRIX: SUBTLE TO THE SPECTACULAR!

The Prasinophyceae or prasinophytes represent a group of motile and non-motile unicells that are presently classified in four clades (Leliaert et al., 2012) and are most commonly found in marine habitats. In photic zones of oceans, picoplanktonic prasinophytes (perhaps the smallest extant eukaryotes; 0.8 μm cell size), like *Ostreococcus* and *Micromonas*, exist in very large numbers. Previous microscopy-based research has shown that taxa like these are either covered with scales or do not have any discernable matrix at all (i.e., they are naked; Piganeau et al., 2011). However, recent analysis of the *Ostreococcus* genome plus immunocytochemical investigations in our laboratories together raise questions as to the nakedness of this picoc alga. Other prasinophytes are significantly larger and covered with layers of thousands of distinctly shaped scales coating both the cell and flagellar membrane surfaces (Moestrup and Walne, 1979). Biochemical analyses have shown that these scales are comprised primarily of neutral and acidic sugars including 2-keto sugars such as 3-deoxy-lyxo-2-heptulosonic acid (DHA; Becker et al., 1991, 1994). All scales of prasinophytes are believed to be processed in the Golgi apparatus, packaged in secretory vesicles and secreted to the cell surface near the flagellar apparatus or to vacuole-like scale reservoirs before release to the cell surface.

CHLORODENDROPHYCEAE: WALLS FROM A FUSION

Taxa of the small Chlorodendrophyceae group of green algae consist of motile or non-motile and sometimes stalked unicells (*Tetraselmis* and *Schewiella*) that are covered by a single cell wall or layers of cell walls. Furthermore, the wall or theca consists of regular repeating subunits and unlike the cell walls of other green algae, this wall is believed to be a product of fused scales. The subunits of the theca are scale-like and are processed in the Golgi apparatus like those of scaly prasinophytes. The acid sugars, 2-keto-3-deoxy-D-manno-octulosonic acid, 5-O-methyl 2-keto-3-deoxy-D-manno-octulosonic acid, and DHA comprise 60% of the sugars present in the theca (Becker et al., 1991).

TREBOUXIO PHYCEAE: WALLS OF UNUSUAL POLYMERS

The Trebouxiophyceae consists of an assemblage of primarily freshwater and terrestrial forms that exhibit diverse phenotypes ranging from unicells to colonies to filaments as well as representing most of the photosynthetic green algae of lichens (e.g., *Trebusia*). Some are considered highly attractive candidate genera for use in algal biofuel production (e.g., *Chlorella*; Rodrigues and da Silva Bon, 2011). Most members of this group possess cell walls but surprisingly little is known about their biosynthesis, composition, or architecture. In *Chlorella*, the cell wall contains cellulose and in some species, the wall is coated by a highly resistant outer stratum consisting of “algaeenan,” an aliphatic polymer containing long polymethylene chains that are decorated with amide and N-alkyl substituted pyroles (Rodrigues and da Silva Bon, 2011). In *Tebouxia* isolated from lichens and grown separately from its fungal partner, β-galactofuranan has been demonstrated, a polysaccharide previously found in fungi but not known from green algae (Cordeiro et al., 2006).
Table 2 | Summary of the composition of extracellular coverings in green algae.

| Taxon                      | Covering type                  | Biochemical composition                                                                 | Reference                                                                 |
|----------------------------|--------------------------------|-----------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Prasinophyceae             | “Scales,” coatings             | 2-Keto sugars (e.g., DHA), mannans, glycoproteins                                        | Becker et al. (1991, 1994), Moestrup and Weine (1979)                     |
| Chlorodendrophyceae        | Wall of fused scales           | 2-Keto sugars (e.g., DHA), proteins                                                     | Becker et al. (1991)                                                      |
| Trebouxiiphyceae           | Cell walls                     | Cellulose, algaenan, β-galactofuranan                                                   | Rodrigues and da Silva Bon (2011), Cordeiro et al. (2006)                 |
| Chlorophyceae              | Crystalline glycoprotein walls; fibrillar cell walls | Hyp-rich glycoproteins, cellulose pectins, AGP, extensin                                | Voigt et al. (2001, 2007), Kirk (1998), Estevez et al. (2008)             |
| Ulvophyceae                | Cell walls                     | Cellulose, β-mannans, β-xylans, sulfated (sometimes pyruvylated) polysaccharides or sulfated rhamnogalacturonan, AGP, extensin | Cianza et al. (2012), Estevez et al. (2009), Percival (1979), Lahaye and Robic (2007) |
| Charophyceae-early divergent clades | Scales, cell walls         | 2-Keto sugars, cellulose, homogalacturonan, 1,3 β-glucans, AGP                          | Sørensen et al. (2011), Domozych et al. (1991)                            |
| Charophyceae-late divergent clades | Cell walls                  | Cellulose, homogalacturonan, RG-I xyloglucans, mannans, xylans, mixed linkage glucans, 1,3 β-glucans, AGP, extensin, lignin | Sørensen et al. (2011, 2012), Popper and Tuohy (2010)                      |

For further detailed information, key references are provided. AGP, arabinogalactan proteins; Hyp, hydroxyproline.

**CHLOROPHYCEAE: GLYCOPROTEINS AND CELLULOSE**

The Chlorophyceae are the largest group of green algae and exhibit great morphological diversity ranging from motile unicells to large filaments to blade-like thalli. The extracellular coverings of the Chlorophycean algae are also very diverse and consist of a distinct assortment of “cell walls.” In Oedogonium, the cell wall resembles those of higher plants in containing microfibrillar cellulose, homogalacturonans and rhamnogalacturonan-I, extensin, and arabinogalactan constituents (Estevez et al., 2008). Many of the polysaccharides that are common to embryophyte walls are thought to have evolved within the CGA, so these results were unexpected. Further biochemical study and a much wider screening of this and other Chlorophycean taxa will be required to ascertain the similarity of these polymers with those of the CGA and embryophytes. However, in the *Chlamydomonas–Volvox* assemblage (i.e., volvocalean flagellates), the cell wall does not contain cellulose but is made of crystalline glycoproteins, specifically one based upon aggregates of hydroxyproline-rich glycoproteins (HRGPs) and glycine-rich glycoproteins (Imam et al., 1985; Adair et al., 1987; Kirk, 1998; Voigt et al., 2001, 2007). Extensins of plants are a group of cell wall glycoproteins that probably share at least some glycosylation motives and a common ancestry with the HRGPs of *Chlamydomonas–Volvox* assemblage. The glycosylation motives that govern extensin-type glycosylation comprise the SPPPPP sequence (i.e., serine-proline-proline-proline-proline) usually occurring several times. The prolines are hydroxylated by prolyl hydroxylases prior to glycosylation. Showalter et al. (2010) used SPPPSPPP to define the class of extensins in their bioinformatic classification of HRGPs. The genetic encoding of the repetitive structures allows for substantial genetic drift without loss of function (Kieliszewski and Lamport, 1994), this being the reason why clear orthologies between individual vascular plant organisms, including angiosperms and invertebrates (Aquino et al., 2014). These sulfated wall constituents may be classified into one of two groups as originally designated by Percival (1979): (1) uronic acid-rich polysaccharides also containing rhamnose, xylose, and sometimes galactose, and (2) uronic acid-limited polysaccharides consisting of major quantities of galactose, arabinose and, in some cases, xylose. The first group is represented by Ulva.
Cell walls from the first group as represented by several *Ulva* species comprise two major polysaccharide components, soluble ulvans and cellulose, and two minor ones, an alkali-soluble linear xyloglucan and a gluconuran. Ulvan is the family of sulfated polysaccharides that consist of large quantities of glucuronic acid and rhamnose with the main repeating disaccharide being \( \beta \)-d-GlcAp-(1→4)-\( \alpha \)-l-Rha. Ulvan is remarkable that the *Chara* transcriptome (courtesy of Ger-not Glöckner, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin) as well as that of *Nitella hyalina* (Timme et al., 2012) each have a putative member in family GT77 clade D, but none in clade A or C. The transcriptome of *Nitella*, but not that of *Chara* comprises putative prolyl hydroxylases allowing for the existence of AGPs even though extensins are missing. It thus appears that members of Charales have evolved and separated themselves significantly from other members of the CGA, notably the Zygmenatales and Coleochaetales which feature cell walls that more closely resemble that of vascular plants and which are known to express enzymes involved in cell wall metabolism akin to that observed in angiosperms (e.g., Vannerum et al., 2011). These latter similarities suggest that late divergent taxa of the CGA (i.e., their ancestors 470 million years ago) may have possessed cell wall characteristics that pre-adapted them for successful emergence onto and life on land.

**THE NEXT GOALS?**

More detailed characterization of the various taxa will be required before we can fully understand the evolution of extracellular coverings of green plants as well as adaptations to ECM chemistry in response to life in marine, freshwater, and terrestrial habitats. Some specific questions and areas of focus for future study include:

1. Insights into the polysaccharide biosynthetic machinery of CGA are and will at the same time offer insights into the evolutionary events that accompanied adaptation to life on land. This will require full genomic sequencing as transcriptomic analysis is useful for demonstrating the functional expression of genes, but not for the absence. This endeavor offers many challenges as many relevant CGA genomes are estimated to be as big as if not bigger than that of *Arabidopsis* (Kapraun, 2007).

2. Detailed analysis of the roles of cell wall polymers in the CGA is critical. Although the CGA share many cell wall constituents with their embryophyte descendents, it is not clear if they are used in equivalent roles.

3. Sulfated polysaccharides from ulvophyte seaweeds have only recently been reexamined and have yet to be widely used in the hydrocolloid industry. Ulvan has been investigated as...
potential dietary fiber for human diet (Lahaye and Robic, 2007) due to its medical properties and potentially profitable extraction yields from harvested specimens. In addition, ulvans have some interesting biological activities, including acting as antioxidants, modifying certain macrophage activities, and serving as potential anti-hyperlipidemic agents (Wijesekara et al., 2011). Sulfated polysaccharides from the Bryopsidales are not obtained in large yields (Ciancia et al., 2007, 2012), but have piqued interest as bioactive compounds with several potential pharmacological applications (Ohta et al., 2009; Ciancia et al., 2010; Costa et al., 2010; Lee et al., 2010). More detailed characterization of these molecules is needed in order to fully recognize their potential.

(4) Comprehensive chemical and functional screening of the large but virtually unknown polysaccharide complexes secreted outside the cell walls of many Zygnematalean taxa (Domozych et al., 2005) will be important for understanding their physiology and importance to ecosystem dynamics.

The study of green algal cell walls and other coverings has now entered a truly exciting phase whereby new methodologies especially from the biochemical and molecular fronts are allowing for detailed resolution of wall polymers. While evolutionary and cell biology-based studies have been and will continue to be driving forces for this, the study of green algal coverings represents a critical step for emerging applied technologies as green algae are being, or will be, used as food sources for humans and domesticated animals, hydrocolloids for the food and pharmaceutical industry, bioactive compounds for medicinal use and starting material for biofuels.

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REFERENCES
Adair, W. S., Steinmetz, S. A., Mattson, D. M., Goodenough, U. W., and Heuser, J. E. (1987). Nucleated assembly of Chlamydomonas and Volvox cell walls. J. Cell Biol. 105, 2373–2382.
Aquino, R. S., Gratitivol, C., and Mourão, P. A. S. (2011). Rising from the sea: correlations between sulfated polysaccharides and salinity in plants. PLoS ONE 6, e18862. doi:10.1371/journal.pone.0018862.
Becker, B., Becker, D., Kamerling, J. P., and Melkonian, M. (1991). 2-Keto-sugar acids in green flagellates: a chemical marker for prasinophycean scales. J. Phycol. 27, 498–504.
Becker, B., and Marin, B. (2009). Streptophyte alga and the origin of embryophytes. Ann. Bot. 103, 999–1004.
Becker, B., Marin, B., and Melkonian, M. (1994). Structure, composition, and biogenesis of prasinophyte cell coverings. Protoplasma 181, 233–244.
Bilan, M. I., Vinogradova, E. V., Shashkov, A. S., and Usov, A. I. (2007). Structure of a highly pyruvated galactan sulfate from the Pacific green alga Codium yezoense. Carbohydr. Res. 342, 586–596.
Blanc, G., Duncan, G., Agarkova, I., Borodovsky, M., Gurnon, J., Kuo, A., Lindquist, E., Lucas, S., Panglioni, J., Polle, J., Salamov, A., Terry, A., Yamada, T., Dunigan, D. D., Grigoriev, I. V., Claverie, J. M., and Van Etten, J. L. (2010). The Chlorella variabilis NC64A genome reveals adaptation to photosymbiosis, coevolution with viruses, and cryptic sex. Plant Cell 22, 2943–2955.
Carpita, N. C., Defreznè, M., Findlay, K., Wells, B., Shoue, D. A., Catchpole, G., Wilson, R. H., and McCann, M. C. (2001). Cell wall architecture of the elongating maize coleoptile. Plant Physiol. 127, 551–565.
Cassolato, J. E. F., Noseda, M. D., Pujol, C. A., Pelizzari, F. M., Damonte, E. B., and Duarte, M. A. R. (2008). Chemical structure and antiviral activity of the sulfated heterorhamn isolated from the green seaweed Gynalia oxyserpina. Carbohydr. Res. 343, 3085–3095.
Chattopadhyay, K., Mandal, P., Lerouge, P., Alberghina, J., Arata, P. A., and Tosso, M. (2011). Cell wall polysaccharides of the red alga Gratilaria crassicostata. Protoplasma 244, 233–244.
Ciancia, M., Quintana, I., and Cerezo, A. S. (2007). Polysaccharides from the green seaweed Codium fragile and C. vermiculare with controversial effects on hemostasis. Int. J. Biol. Macromol. 41, 641–649.
Cordeiro, L. M., Carbonero, E. R., Sasaki, G. L., Reis, R. A., Stocker-Worgotter, E., Gorin, P. A. J., and Iacomini, M. (2006). A fungus-like β-galactofuranan in the cultivated Trebouxia photobiont of the lichen Ramalina gracilis. FEMS Microbiol. Lett. 244, 193–198.
Costa, L. S., Fidelis, G. P., Cordeiro, S. L., Oliveira, R. M., Sabry, D. A., Cámara, R. B. G., Nobre, L. T. D. B., Costa, M. S. S. P., Almeida-Lima, J., Farias, E. H. C., Leite, E. L., and Rocha, H. A. O. (2010). Biological activities of sulfated polysaccharides from tropical seaweeds. Biomed. Pharmacother. 64, 21–28.
Delwiche, C. F., and Timme, R. E. (2011). Plants. Car. Biol. 21, R417–R423.
Domozych, D. S., Brechka, H., Britton, A., and Tosso, M. (2011). Cell wall growth and modulation dynamics in a model unicellular green alga – Penium margaritaceum: live cell labeling with monoclonal antibodies. J. Bot. doi:10.1155/2011/632165.
Domozych, D. S., Kort, S., Benton, S., and Yu, T. (2005). The extracellular polymeric substance of the green alga Penium margaritaceum and its role in biofilm formation. Biofms 2, 1–6.
Domozych, D. S., Lambiase, S., Kiemle, S. N., and Gretz, M. R. (2009). Cell-wall development and bipol lar growth in the desmid Penium margaritaceum (Zygmenapthecaceae, Streptophyta). Asymmetry in a symmetric world. J. Phycol. 45, 879–893.
Domozych, D. S., Serfis, A., Kiemle, S. N., and Gretz, M. R. (2007). The structure and biochemistry of chryophycean cell walls. I. Pectins of Penium margaritaceum. Protplast 230, 99–115.
Domozych, D. S., Wells, B., and Shaw, P. J. (1991). The basket scales of the green alga Mesotaenium viride: chemist ry, immunology and ultrastructure. J. Cell Sci. 100, 397–407.
Dunn, E. K., Shoue, D. A., Huang, X., Kline, R. E. L., Mackay, A. L., Carpita, N. C., Taylor, I. E. P., and Mandoli, D. F. (2007). Spectroscopic and biochemical analysis of regions of the cell wall of the unicellular ‘mannan weed’, Acetabularia acetabulum. Plant Cell Physiol. 48, 122–133.
Eder, M., and Lutz-Meindl, U. (2010). Analyses and localization of pectin-like carbohydrates in cell wall and mucilage of the green alga Nertium digitus. Protozool 243, 25–38.
Eder, M., Tenhaken, R., Driouich, A., and Lutz-Meindl, U. (2008). Occurrence and characterization of arabinogalactan-like proteins and hemicelluloses in Micros disse (Streptophyta). J. Phycol. 44, 1221–1234.
Egelund, J., Obel, N., Ulvskov, P., Geshi, N., Pauly, M., Bacin, A., and Petersen, B. L. (2007). Molecular characterization of two Arabidopsis thaliana glycosyltransferase mutants, ral and rra2, which have a reduced residual arabinose content in a polymer tightly associated with the cellulose wall residue. Plant Mol. Biol. 64, 439–451.
Keskiaho, K., Hieta, R., Sormunen, R., Kapraun, D. F. (2007). Nuclear DNA

Imam S. H., Buchanan, M. J., Shin, Huizing, H. J., and Rietema, H. (1975).

Gotteli, L. B., and Cleland, R. (1968).

Finet, C., Timme, R. E., Delwiche, C.

Fernández, P. V., Ciancia, M., and Fernández, P. V., Ciancia, M., and

Farias, E. H. C., Pomin, V. H., Valente, A. P., Nader, H. B., Rocha, H. A. O., and Mouroá, P. A. S. (2008). A preponderantly 4-sulfated, 3-linked galactan from the green alga Codium idiothecoides. Glycobiology 18, 250–259.

Fernández, P. V., Ciancia, M., and Estevez, J. M. (2011a). Cell wall variability in the green seaweed Codium vermilaria (Bryopsidales Chlorophyta) from the Argentine coast. J. Phycol. 47, 802–810.

Fernández, P. V., Ciancia, M., Miravallès, A. B., and Estevez, J. M. (2011b). Cell-wall polymer mapping in the coenocytic macroalga Codium vermilaria (Bryopsidales, Chlorophyta). J. Phycol. 46, 456–465. Finet, C., Timme, R. E., Delwiche, C. F., and Marlézat, F. (2010). Multi-gene phylogeny of the green lineage reveals the origin and diversification of land plants. Curr. Biol. 20, 2217–2222.

Gottelli, L. B., and Cléland, R. (1968). Differences in the occurrence and distribution of hydroxyproline-proteins among the algae. Am. J. Bot. 55, 907–914.

Huizing, H. J., and Rietema, H. (1975). Xylan and mannan as cell wall constituents of different stages in the life histories of some siphonaceous green algae. Br. Phycol. J. 10, 13–16.

Huizing, H. J., Rietema, H., and Sietema, H. J. (1979). Cell wall constituents of several siphonaceous green algae in relation to morphology and taxonomy. Br. Phycol. J. 14, 25–52.

Imam S. H., Buchanan, M. J., Shin, H.-C., and Snell, W. J. (1985). The Polysaccharides of green, red, and brown seaweeds: their basic structure, biosynthesis and analysis of hydroxyproline-rich glycoproteins. Carbohydr. Res. 11, 128.

Kappraff, D. (2007). Nuclear DNA content estimates in green algal lineages: Chlorophyta and Streptophyta. Ann. Bot. 99, 677–701.

Keskiahko, K., Hieta, R., Sormunen, R., and Myllyharju, J. (2007). Chlamydomonas reinhardtii has multiple prolyl 4-hydroxylases, one of which is essential for proper cell wall assembly. Plant Cell 19, 256–269.

Kieselwski, M. I., and Lampert, D. T. A. (1994). Extensin: repetitive motifs, functional sites, posttranslational modifications and phylogeny. Plant J. 5, 157–172.

Kirk, D. L. (1998). Vulcanos. New York: Cambridge University Press.

Lahaye, M., and Robic, A. (2007). Structure and functional properties of ulvan, a polysaccharide from seaweeds. Biomacromolecules 8, 1764–1776.

Lee, J.-B., Ohta, Y., Hayashi, K., and Hayashi, T. (2010). Immunostimulating effects of a sulfated galactan from Codium fragile. Carbohydr. Res. 345, 1452–1454.

Leläert, F., Smith, D. R., Moreau, H., Kirk, D. L. (1998). “Algal polysaccharides of green, red, and brown seaweeds: their basic structure, biosynthesis and function.” Br. Phycol. J. 14, 103–117.

Pericival, E., and McDowell, R. H. (1981). “Algal walls. Composition and biosynthesis,” in Encyclopedia of Plant Physiology, Vol. 13B, eds W. Tanner and F. A. Loewus (Berlin: Springer), 277–316.

Petersen, B. L., Faber, K., and Ulvskov, P. (2011). “Glycosyltransferases of the GT77 family,” in Annual Plant Reviews: Plant Polysaccharides, Biosynthesis, and Bioengineering, ed. P. Ulvskov (New York: Blackwell), 305–320.

Pignanou, G., Grimsley, N., and Moreau, H. (2011). Genome diversity in the five extant marine photosynthetic eukaryotes. Res. Microbiol. 62, 570–577.

Popper, Z. A., and Fry, S. C. (2003). Primary cell wall composition of bryophytes and charophytes. Ann. Bot. 91, 1–12.

Popper, Z. A., Michel, G., Herve, C., Domozhy, D. S., Willats, W. G. T., Tuohy, M. G., Klosseg, B., and Stengel, D. B. (2011). Evolution and diversity of plant cell walls: from algae to flowering plants. Annu. Rev. Plant Biol. 62, 567–590.

Popper, Z. A., and Tuohy, M. G. (2010). Beyond the green: understanding the evolutionary puzzle of plant and algal cells. Plant Physiol. 153, 373–383.

Rantungarten, C., Ebert, B., Herter, T., Petzold, C. J., Ishi, T., Mukhopad- hyay, A., Usadel, B., and Scheller, H. V. (2011). The interconversion of UDP-Arabinofuranose and UDP-Arabinofuranose is indispensable for plant development in Arabidopisi- sis. Plant Cell 23, 1373–1390.

Ray, B. (2006). Polysaccharides from Enteromorpha compressa: isolation, purification and structural features. Carbohydr. Polym. 66, 408–416.

Rodrigues, M. A., and da Silva Bon, E. P. (2011). Evaluation of Chlorilla (Chlorophyta) as source of fermentable sugars via cell wall enzymatic hydrolysis. Enzyme Res. doi:10.4061/2011/450603.

Showalter, A. M., Keppler, B., Lichten- berg, J., Gu, D., and Welch, L. R. (2010). A bioinformatics approach to the identification, classification, and analysis of hydroxyproline-rich glycoproteins. Plant Physiol. 153, 485–513.

Sørensen, I., Domozhy, D., and Willats, W. G. T. (2011). How have plant cell walls evolved? Plant Physiol. 153, 366–372.

Sørensen, I., Pettolino, F. A., Bacic, A., Ralph, J., Lu, F., O’Neill, M. A., Fei, Z., Rose, J. K. C., Domozhy, D. S., and Willats, W. G. T. (2011). The Charo- phycean green algae offer insights into early origin of plant cell walls. Plant J. 153, 366–372.

Sørensen, I., Rose, J. K. C., Doyle, J. J., Domozhy, D. S., and Willats, W. G. T. (2012). The Charophycean green algae as model systems to study plant cell walls and other evolutionary adaptations that gave rise to land plant. Plant Signal. Behav. 7, 1–3.

Timme, R. E., Bachvaroff, T. R., and Delwiche, C. F. (2012). Broad phylogenomic sampling and the sister lineage of land plants. PLoS ONE 7, e29696. doi:10.1371/jour- nal.pone.0029696.

Tijs, E. R., and Delwiche, C. F. (2010). Uncovering the evolution- ary origin of plant molecular processes: comparison of Coleochaete (Coleochoetales) and Spirigryra (Zygnematales) transcriptomes. BMC Plant Biol. 10, 96. doi:10.1186/1471-2229-10-96.

Vannenrum, K., Huysman, M. J. J., De Rycke, R., Vuyts, P., Lelièvre, F., Pollier, J., Lutz-Meinl, U., Gillard, J., Deveylden, L., Goossens, A., Inze, D., and Veymeren, W. (2011). Transcriptional analysis of cell growth and morphogenesis in the unicellular green alga Micrasterias (Streptophyta), with emphasis on the role of expansins. BMC Plant Biol. 11, 128. doi:10.1186/1471-2229-11-128.

Velasquez, S. M., Ricas, M. M., Doros, J. G., Fernandez, P. V., Nadra, A. D., Pol-Fachin, L., Egelund, J., Gille, S., Harholz, J., Ciancia, M., Verhi, H., Paul, M., Bacic, A., Olsen, C. E., Ulvskov, P., Petersen, B. L., Somerville, C., Isem, N. D., and Estevez, J. M. (2011). O-glycosylated cell wall proteins are essential in root hair growth. Science 332, 1401–1403.

Voigt, J., Liebich, I., Kieß, M., and Frank, R. (2001). Subcellular distribution of 14-3-3 proteins in the unicellular green alga Chlamydomonas reinhardtii. Eur. J. Biochem. 268, 6449–6457.

Voigt, J., Woestemeyer, J., and Frank, R. (2007). The chaetosoluble glycoprotein GP2 is a precursor of the insoluble glycoprotein framework of the Chlamydomonas cell wall. J. Biol. Chem. 282, 30381–30392.

Wysewala, I., Pangestuti, R., and Kim, S.-K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carbohydr. Polym. 84, 14–21.
Wodnick, S., Brinkmann, H., Glockner, G., Heidel, A. J., Philippe, H., Melkonian, M., and Becker, B. (2011). Origin of land plants: do conjugating green algae hold the key? BMC Evol. Biol. 11, 104. doi:10.1186/1471-2148-11-104

Wutz, M., and Zetsche, K. (1976). Zur Biochimie und Regulation des Heteromorphen Generationswechsels der Grünalge. Planta 129, 211–216.

Yamagaki, T., Maeda, M., Kanazawa, K., Ishizuka, Y., and Nakamichi, H. (1997). Structural clarification of Caulerpa cell wall β-(1→3)-xylan by NMR spectroscopy. Biosci. Biotechnol. Biochem. 61, 1077–1080.

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