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NOTE

THE CELL WALL POLYSACCHARIDES OF A PHOTOSYNTHETIC RELATIVE OF APICOMPLEXANS, *CHROMERA VELIA*[1]

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Chromerids are a group of alveolates, found in corals, that show peculiar morphological and genomic features. These organisms are evolutionary placed in-between symbiotic dinoflagellates and parasitic apicomplexans. There are two known species of chromerids: *Chromera velia* and *Vitrella brassicaformis*. Here, the biochemical composition of the *C. velia* cell wall was analyzed. Several polysaccharides adorn this structure, with glucose being the most abundant monosaccharide (approx. 80%) and predominantly 4-linked (approx. 60%), suggesting that the chromerids cell wall is mostly cellulosic. The presence of cellulose was cytochemically confirmed with calcofluor white staining of the algal cell. The remaining wall polysaccharides, assuming structures are similar to those of higher plants, are indicative of a mixture of galactans, xyloglucans, heteroxylans, and heteromannans. The present work provides, for the first time, insights into the outermost layers of the photosynthetic alveolate *C. velia*.

Key index words: **Alveolata; calcofluor white; cell wall; cellulose; *Chromera velia*; chromerids; monosaccharide linkage analysis**

***Abbreviations*: Ara, arabinose; CW, calcofluor white; Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; SEM, standard error of the mean; Xyl, xylose**

Chromerids are photosynthetic unicellular eukaryotes of the superphylum Alveolata (Moore et al. 2008, Cavalier-Smith 2018). Originally isolated from Australian scleractinian corals (Moore et al. 2008), the chromerids are also free-living and are now known to be globally distributed (Mathur et al. 2018). There are two described species of chromerids: *Chromera velia* isolated from the coral *Plesiastrea*

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versipora in Sydney Harbor (Moore et al. 2008), and *Vitrella brassicaformis* from the Great Barrier Reef coral *Leptastrea purpurea* (Oborník et al. 2012).

When first discovered (Moore et al. 2008), *Chromera* was hailed as a missing link that represents a transition form between symbiotic dinoflagellates (with a photosynthetic plastid) and parasitic apicomplexans (with a relict, non-photosynthetic plastid, referred to as an apicoplast; McFadden and Waller 1997, Okamoto and McFadden 2008, Janouškovec et al. 2010, Weatherby and Carter 2013). Indeed, analysis of *C. velia* and *Vitrella brassicaformis* plastid and nuclear genomes confirms a common origin of apicomplexan, chromerid, and dinoflagellate (peridinin) plastids from a complex red algal endosymbiont (Janouškovec et al. 2010, Oborník and Lukeš 2013, Woo et al. 2015). Chromerids, together with colpodellids, constitute the Apicomonada, an important major group of alveolate protists (Cavalier-Smith 2018), and they hold a key position in eukaryotic diversity.

Major groups of algae are characterized by signature morphologies (e.g., flagellar apparatuses and mitotic mechanisms), photosynthetic pigments (unique combinations of chlorophylls and accessory pigments), and their wall materials (e.g., cellulose, agar, carageenans, alginate, fucoidans, glycoproteins, calcium carbonate, silica, and others; Popper et al. 2011, Synytsya et al. 2015). Chromerids exhibit unique combinations of morphology (Oborník et al. 2011, 2016), pigments (Moore et al. 2008), and genome organizations (Janouškovec et al. 2010, Flegontov et al. 2015, Woo et al. 2015), but as yet there is no biochemical description of their cell walls. Here, we provide the first analysis of the chromerid cell walls with a monosaccharide linkage analysis, and cytochemical confirmation for the presence of a predominantly cellulosic wall.

To obtain a broad view of polysaccharides of cell wall preparations in *Chromeravelia*, we applied monosaccharide linkage analysis to isolated cell walls (Pettolino et al. 2012). *Chromera velia* cultures (CCMP2878) were grown in f/2 medium under constant temperature (26°C), 12:12 h light: dark photoperiod cycle, and 30–50 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} of light. Two replicates of 3×10^6 cells $\cdot$ mL^{-1} were used in the study to isolate cell walls. Cell aliquots were suspended in 0.6 M sorbitol, 20 mM Tris HCl pH 7.4, and 2 mM EDTA and processed (three cycles) in a French press at 35,000 psi. The homogenate was then centrifuged at 2,000*g*, and the supernatant spun at 17,000*g* on 10–50% Optiprep™ (Sigma-Aldrich, Australia) gradient for 10 min. Specimens for transmission electron microscopy (TEM) were prepared and visualized as in Moore et al. 2008, and purity of the *C. velia* cell walls was confirmed microscopically (Fig. 1, B and C). After washing twice with 80% ethanol, cell walls were analyzed by monosaccharide linkage analysis as described by Pettolino et al. (2012).

The cell wall comprised the following monosaccharides in decreasing abundance: glucose (Glc), galactose (Gal), mannose (Man), xylose (Xyl), fucose (Fuc), rhamnose (Rha), and arabinose (Ara; Table 1). Whereas glucose is overwhelmingly the dominant monosaccharide in the cell wall fraction ($\sim$80%), a total sugar analysis of the ethanol extract of whole cells (data not shown) revealed that

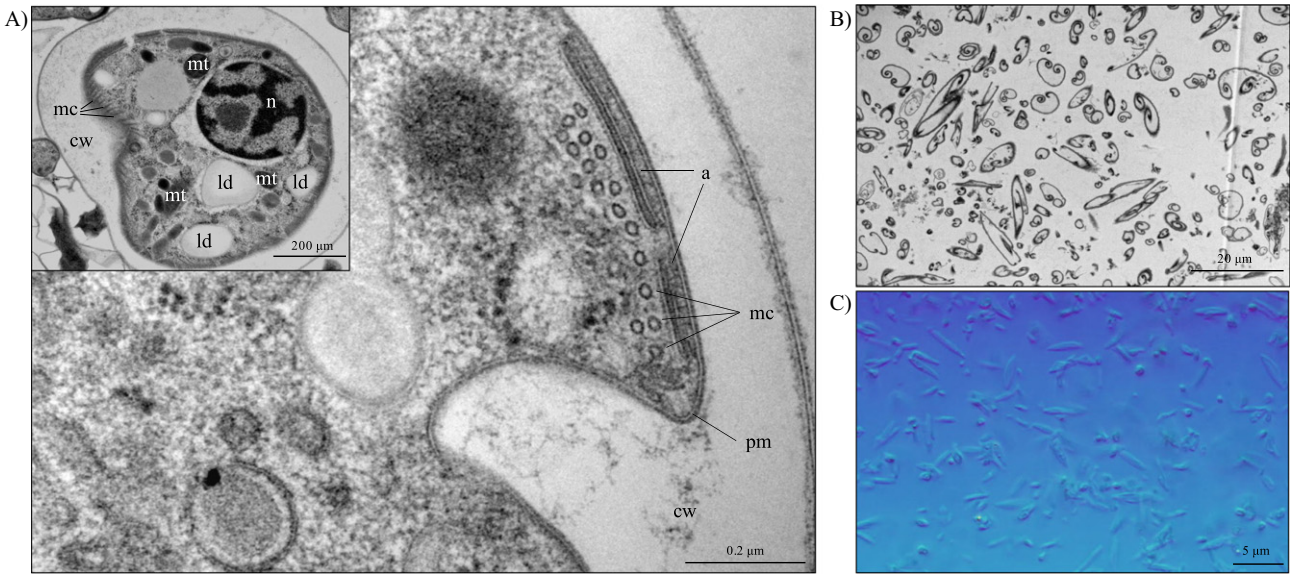

FIG. 1. (A) Transmission electron microscopy of a cross-section of *Chromera velia* coccoid stage and zoom in to show the thick cell wall surrounding the cell. a = alveoli; cw = cell wall; mc = microtubules; mt = mitochondrion; n = nucleus; pm = plasma membrane. B) Transmission electron microscopy and C) light microscopy of isolated *C. velia* cell wall residues. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1. Monosaccharide linkage composition (Mol %) of *Chromera velia* cell walls analyzed in duplicates.

Monosaccharide	Deduced linkage[a]	Mol%	SD
Ara*f*	5-	1.0	0.3
Fuc*p*	terminal	1.0	0.1
	2-	0.4	0
	4-	0.3	0
	Total	1.7	
Gal*p*	terminal	2.7	0.4
	2-	2.4	0.8
	4-	2.4	0.3
	6-	0.8	0.1
	Total	8.3	
Glc*p*	terminal	9.1	0.7
	2-	0.6	0.1
	3-	0.2	0.1
	4-	60.6	4.1
	2,3-	0.9	0
	2,4-	0.6	0.1
	3,4-	1.0	0.3
	4,6-	4.7	1.1
	3,4,6-	0.5	0.2
	2,3,4,6-	1.7	0.7
	Total	79.7	
Man*p*	terminal	0.9	0.3
	2-	0.5	0
	4-	0.5	0.5
	3,6-	0.5	0.4
	2,4,6-	0.7	0.4
	Total	3.0	
Rha*p*	2,4-	0.4	0.5
Xyl*p*	terminal	3.2	1.1
	4-	2.0	0
	2,4-	0.7	0.2
	Total	5.9	

SD = standard deviation.

[a]Linkages were deduced from 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methyl fucitol; 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methyl fucitol; 1,4,5-tri-*O*-acetyl-6-deoxy-2,3-di-*O*-methyl fucitol; 1,2,4,5-tri-*O*-acetyl-6-deoxy-3-*O*-methyl rhamnitol; 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl arabinitol; 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl xylitol; 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl xylitol; 1,2,4,5-tetra-*O*-acetyl-3-*O*-methyl xylitol; 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl mannitol; 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl mannitol; 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl mannitol; 1,3,5,6-tetra-*O*-acetyl- 2,4-di-*O*-methyl mannitol; 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl mannitol; 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl galactitol; 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl galactitol; 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl galactitol; 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl galactitol; 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol; 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl glucitol; 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl glucitol; 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl glucitol; 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methyl glucitol; 1,2,4,5-tetra-*O*-acetyl-3,6-di-*O*-methyl glucitol; 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl glucitol; 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl glucitol; 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl glucitol; and hexa-*O*-acetyl glucitol.

glucose constituted just 12% of *Chromeravelia* soluble total sugars. This suggests that polymeric glucose is preferentially accumulated in the *C. velia* cell wall.

The monosaccharide linkage composition of *Chromeravelia* cell wall, deduced from the linkage analysis, is reported in Table 1, with 4-Glc being the predominant linkage type (61% of 80% total), which is indicative of a cell wall of *C. velia* is composed predominantly of cellulose, a β-1,4-linked-Glc polymer that is also the predominant fibrillar polysaccharide of all plant species and some bacteria and fungi. To further confirm the cellulosic nature of *C. velia* cell wall, we used calcofluor white, a fluorescent stain indicative for β-1,4 linkages, to probe the cell walls of intact cells. Chromerid cells were visualized with a Nikon A1R confocal laser scanning microscope (Nikon, Tokyo, Japan) with a 489 nm laser to detect chlorophyll autofluorescence, and a 409 nm laser to detect calcofluor white stained cell walls. The calcofluor white positive material surrounds the entire chromerid cell (Fig. 2), further suggesting the presence of cellulose in the *C. velia* wall, which is consistent with the thick cell wall shown by electron microscopy (Fig. 1A; Oborník et al. 2011). No cellulose synthase genes are currently annotated in the *C. velia* genome (www.cryptodb.org), but a thorough phylogenetic analysis of the genome for members of the cellulose synthesis machinery should be undertaken based on our biochemical evidence for a cellulosic cell wall.

Cellulose is the main component of so-called armored (walled) dinoflagellates, so identification of cellulose in the related *Chromera velia* is not surprising. Dinoflagellate cellulose is deposited as ornate thecal plates (Fritz and Triemer 1985) within the alveolar sacs (variously known as amphiesma or alveolae or inner membrane complex) that are a defining feature of the superphylum Alveolata (Gould et al. 2008) and also characteristic of chromerids (Oborník et al. 2012). However, the thick cell wall of *C. velia* is clearly extracellular and not within its alveolae (Fig. 1), so clear differences in cell wall architecture and location are already apparent between dinoflagellates and *C. velia*. It will now be interesting to analyze the cell surface structures of the other chromerid *V. brassicaformis* and determine how cellulose has been utilized as a cell wall material in this branch of the eukaryotic tree.

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AUTHOR CONTRIBUTIONS

G. Tortorelli: Data curation (equal); Methodology (equal); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead). **F. Pettolino:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (lead); Writing-review & editing (equal). **D.H. Lai:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing-review & editing (equal). **A. Tomčala:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal).

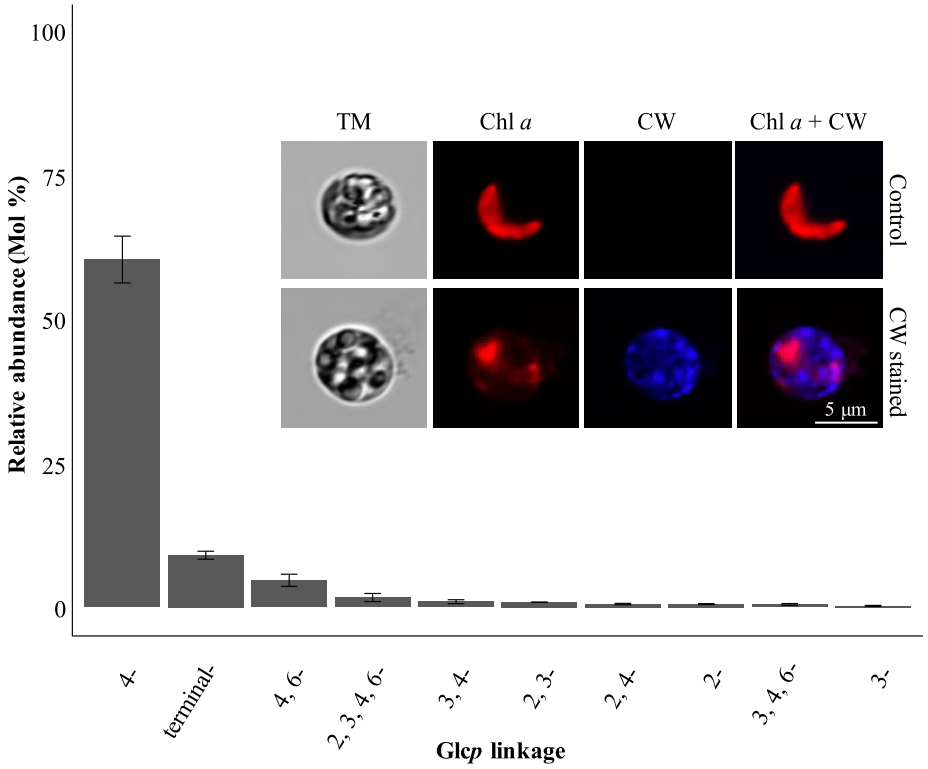

FIG. 2. Bar plot of the glucose linkages (Glc*p*) in the cell wall of *Chromera velia*. Each bar represents the relative abundance molar percentage (Mol%) ± SEM of a glucosyl linkage. Microscopy images of control and calcofluor white (CW) staining of *C. velia* cell wall. TM = transmitted light microscopy. Chl *a* = algal chlorophyll autofluorescence in red. CW stain of the cellulosic *C. velia* wall is in blue. Chl *a* + CW = merged channels. [Color figure can be viewed at wileyonlinelibrary.com]

A. Bacic: Conceptualization (equal); Funding acquisition (equal); Investigation (lead); Methodology (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal). **M. Oborník:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (lead); Methodology (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal). **J. Lukeš:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (lead); Methodology (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal). **G.I. McFadden:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (lead); Methodology (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal).

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