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Title	Localisation and substrate specificities of transglycanases in charophyte algae relate to development and morphology
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Publication date	2018
Original Citation	Herburger, K., Ryan, L. M., Popper, Z. A. and Holzinger, A. (2018) 'Localisation and substrate specificities of transglycanases in charophyte algae relate to development and morphology', Journal of Cell Science, 131, jcs203208 (10pp). doi: 10.1242/jcs.203208
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://jcs.biologists.org/content/131/2/jcs203208 - 10.1242/jcs.203208
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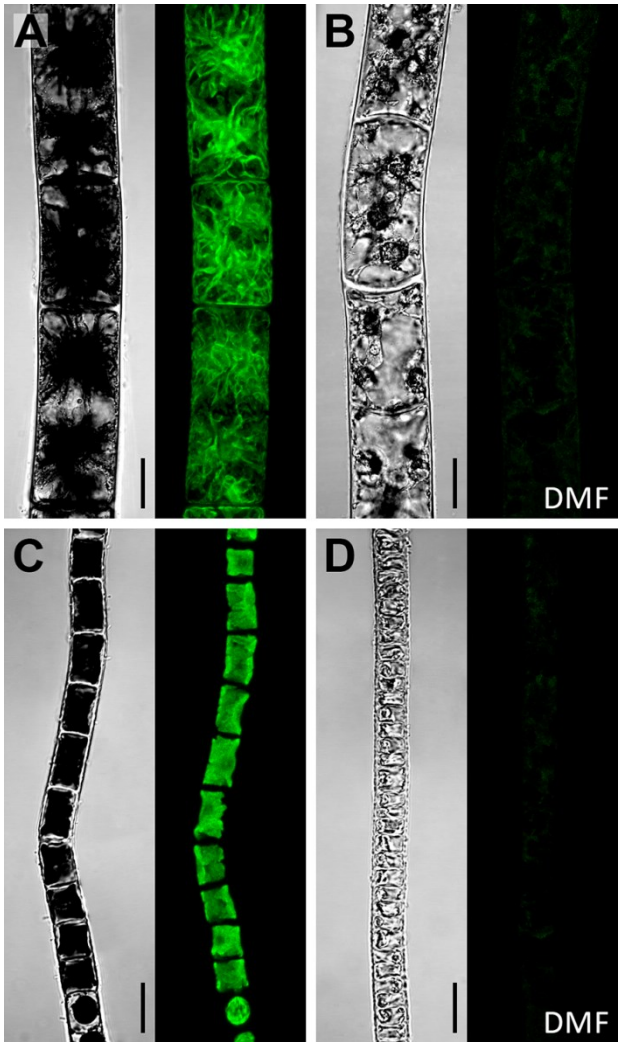


Fig. S1. Effect of dimethylformamide (DMF) on chloroplast auto-fluorescence. CLSM micrographs of *Zygnema* S (A, B) and *Klebsormidium crenulatum* (C, D) filaments before (A, C) and after (B, D) incubation in DMF. Bars = 10 µm.

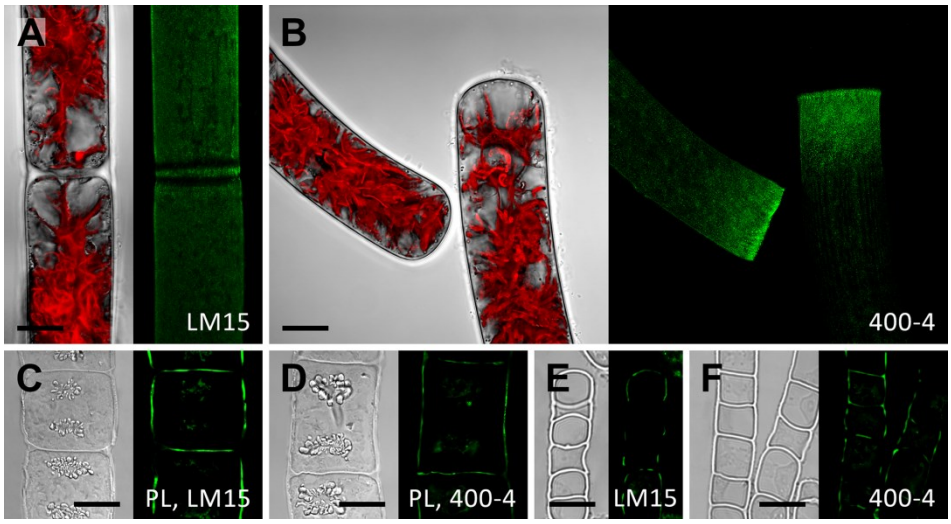


Fig. S2. Immunolabelling of whole cells and semithin sections (CLSM micrographs). *Zygnema* S (A-D) and *Klebsormidium crenulatum* (E, F) filaments labelled with the monoclonal antibodies LM15 or 400-4. Whole cell labelling (A, B), labelling of semithin sections (C-F). Red autofluorescence is shown. (A) Weak fluorescence in outer and cross cell walls. (B) Recently fragmented filament with fluorescence close to terminal cross cell walls. (C-F) Labelling in outer and cross cell walls. Bars 10 = µm

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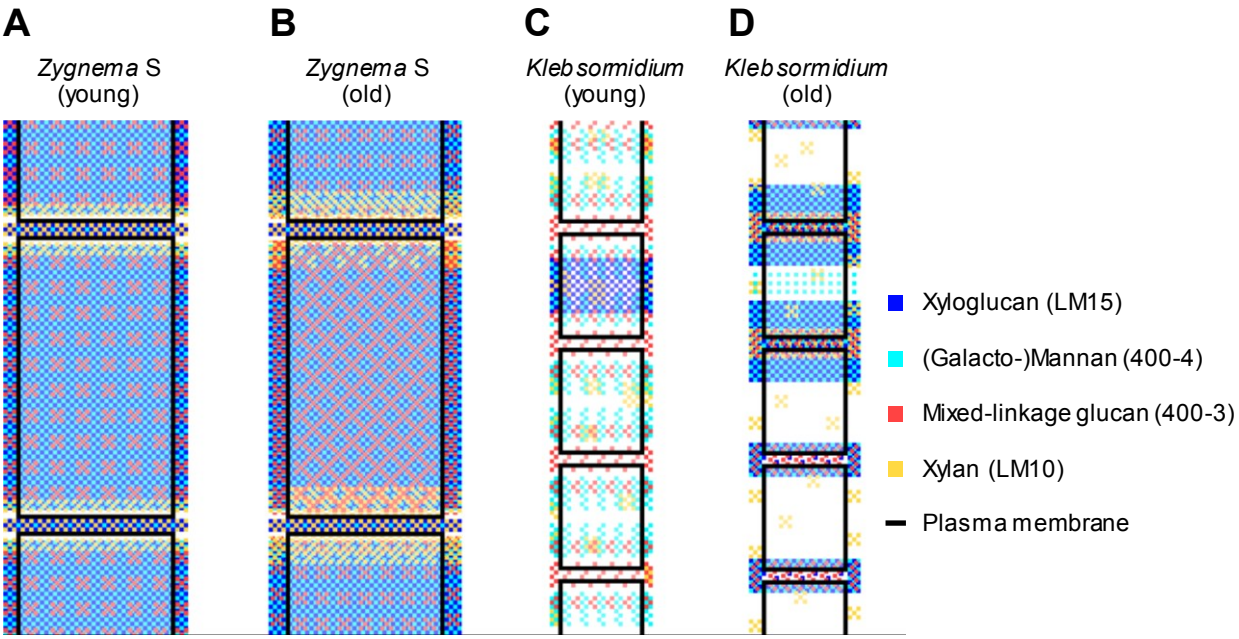


Fig. S3. Hemicellulose distribution in filamentous charophytes. Schemes are based on immuno-localisations of hemicellulose epitopes (antibody names in brackets) in young (1 month) and old (12 months) filaments of (A, B) *Zygnema* S and (C, D) *Klebsormidium crenulatum*.

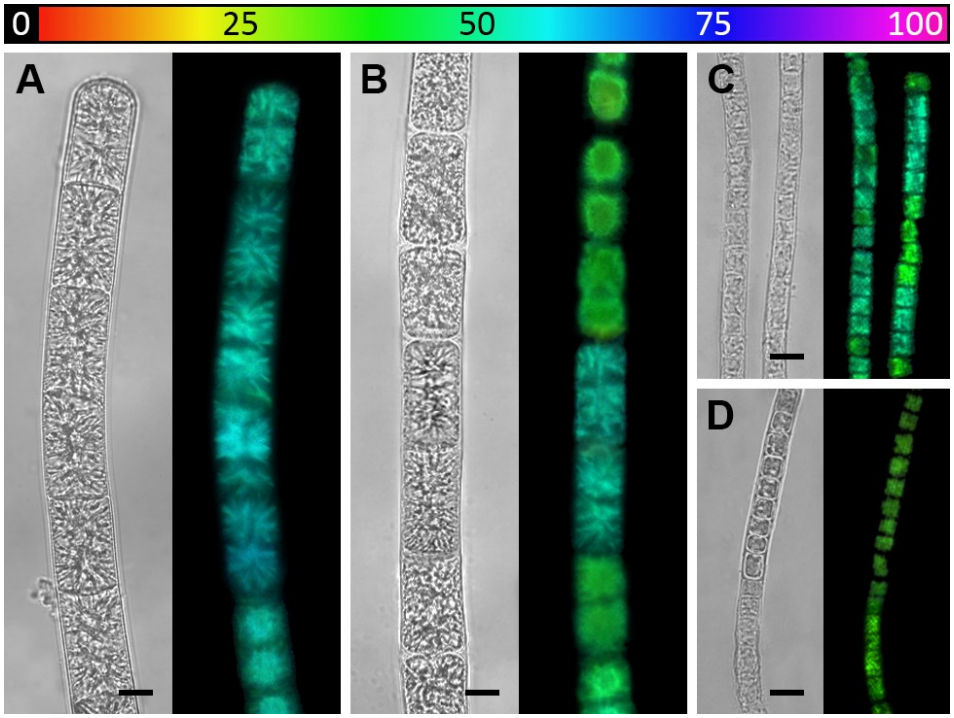


Fig. S4. Photosynthetic performance of *Zygnema* S and *Klebsormidium crenulatum*. NIR and corresponding Y(II) images (false colour) of young (A, C) and old (C, D) filaments of *Zygnema* S (A, B) and *Klebsormidium* (C, D). The colour bar at the top indicates the relative Y(II) as a percentage. (A) Vacuolated cells containing two stellate chloroplasts and high Y(II) values (~0.64). (B) Akinete cells dominate old filaments and exhibit a lower Y(II) (i.e. ~0.41) than younger (i.e. vacuolated, thin-walled, growing) cells within the same filament (~0.6). (C) Cells of young filaments with a higher Y(II) (~0.48-0.59) than cells of old filaments (D) (~0.3). Bars = 10 µm.

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Tab. S1 Evidence for major cell wall polysaccharides in Charophytes.

Cell wall component	Klebsormidiophyceae	Charaphyceae	Zygnematophyceae
Cellulose	A[1,5]B[7]C[6]	A[5]B[8,7]	A[5]B[3,5,7]
Callose	A[5]B[3,5]C[6]	A[5]B[5,8]	A[5]B[3]
Pectins			
HG	A[1,5*]B[5]C[6]	A[5,7]B[8]	A[5,7]B[4,9,10]
RGI/RGII	-	A[5,7†]	A[7†]
Hemicelluloses			
Xyloglucan	B[5]C[6]	A[5]B[5,8]	B[5,8]
MLG	B[5]	B[5,8]	A[4,5]B[4,5]
Mannans	B[5]	B[5,8]	B[5]
Xylans	B[5]	B[5,8]	B[5]
(Glyco)Proteins			
Arabinogalactan proteins	-	B[5]	B[5]
Expansins	C[6]	C[11]	C[11]
Extensins	C[12]	-	B[5]

Key: A biochemical, B immunological/histochemical, C genetically (genomic/transcriptomic data), '-' no evidence.
*Biochemical evidence for HG in Klebsormidiophyceae is ambiguous. While Domozych et al. (1980) and Sorensen et al. (2011) detected galacturonic acid in *K. flaccidum*, O'Rourke et al. 2015 suggested its absence in *K. flaccidum* and *K. subtile*.
†Suggested by the co-occurrence of 1→5-Ara *f*, 1→4-Gal *p* and 2→4-Rha *p*.
[1]Domozych et al. 1980, [2]Popper and Fry 2003, [3]Herburger and Holzinger 2015, [4]Eder et al. 2008, [5]Sørensen et al. 2011, [6]Hori et al. 2011, [7]O'Rourke et al. 2015, [8]Domozych et al. 2009, [9]Domozych et al. 2006, [10]Eder and Lütz-Meindl 2010, [11]Vannerum et al. 2011, [12]Liu et al. 2016

Referneces not in the main document:

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