Phycological Research 1998; **46**: 63–73

The cell wall chemistry of *Bangia atropurpurea* (Bangiales, Rhodophyta) and *Bostrychia moritziana* (Ceramiales, Rhodophyta) from marine and freshwater environments

Heather L. Youngs,[1] **Michael R. Gretz,**[1] **John A. West**[2]* **and Milton R. Sommerfeld**[3]

[1]Department of Biological Sciences, Michigan Technological University, Houghton, Michigan 49931, USA, [2]School of Botany, University of Melbourne, Parkville 3052, Australia and [3]Department of Botany, Arizona State University, Tempe Arizona 85287, USA

SUMMARY

The cell wall polysaccharides of two species of red algae, which are adapted to both freshwater and marine environments, were analysed to determine the effect of these widely different environments on their commercially important agarocolloids and to investigate the possible role of the cell wall in environmental adaptation. Cell wall polymers of freshwater isolates of *Bangia atropurpurea* (Roth) C. Agardh and cultured freshwater and marine *Bostrychia moritziana* (Sonder *ex* Kützing) J. Agardh were isolated and the polysaccharides chemically fractionated and characterized. Wall polysaccharides of freshwater *B. atropurpurea* were similar to those previously reported for marine isolates with repeating disaccharide units of agarose and porphyran predominant in the hot water extracts. In the insoluble residues, 3-linked galactosyl and 4-linked mannosyl residues were predominant. *Bostrychia moritziana* wall polysaccharides included agarocolloids with various patterns of methyl ether substitution similar to those previously described for other Ceramiales. Differences in the position of methyl ether substituents were detected in the hot water extracts of the freshwater and marine specimens. Polymers of freshwater *B. moritziana* cultures were composed of a complex mixture of repeating disaccharide units including 2′-*O*-methyl agarose, 6-*O*-methyl agarose and 2′-O-methyl porphyran. Polymers of marine isolates of *B. moritziana* differ in that they contain only trace amounts of 2-*O*-methyl saccharides and increased amounts of 6-*O*-methyl saccharides. The hot water insoluble residues of both freshwater and marine isolates of *B. moritziana* contain a mixture of 3-linked galactosyl and 4-linked glucosyl residues. These results indicate that the adaptive response of *B. moritziana* to changing osmotic and ionic conditions may include changes in cell wall chemistry: notably, the pattern of methyl ether substitution.

Key words: agarocolloid, *Bangia*, Bangiales, *Bostrychia*, cell wall, Ceramiales, freshwater algae, native methylation, osmoacclimation, polysaccharides, Rhodophyta.

INTRODUCTION

Bangia atropurpurea (Roth) C. Agardh (Bangiales) and *Bostrychia moritziana* (Sonder *ex* Kützing) J. Agardh (Ceramiales) are two species of red algae that tolerate a wide range of salinities and occupy both freshwater and marine environments (Sheath and Cole 1980, 1984; Sheath *et al.* 1993). *Bangia atropurpurea* is distributed worldwide. North American marine habitats range from Newfoundland to Georgia along the east coast and include the entire west coast including Alaska and Hawaii. North American freshwater habitats include the St Lawrence Seaway and four Laurentian Great Lakes: Ontario, Erie, Huron and Michigan (Sheath and Cole 1980). The alga occurs in a wide zone at or above the water line (Sheath and Cole 1984). The distribution pattern of *B. atropurpurea* (Sheath and Cole 1984) and culture studies (Geesink 1973) indicate that it may only be able to make the transition between marine and freshwater environments through a spore stage. In contrast, culture studies and geographical distribution of *B. moritziana* indicate that this alga is quite tolerant of wide salinity ranges (Karsten *et al.* 1993). It occupies coastal waters in south-western Asia including Australia and New Zealand, South Africa and ranges from tropical North America to Brazil (King and Puttock 1989; Karsten *et al.* 1993; Sheath *et al.* 1993). It is an epiphyte in freshwater streams, mangroves and salt marshes (Sheath *et al.* 1993). Both species also are subjected to frequent exposure to air and desiccation and provide useful models to examine the effects of freshwater and marine environments on cell wall chemistry.

The production of agarocolloids, which are widely

*To whom correspondence should be addressed.
Communicating editor: K. Okuda
Received 31 July 1997; accepted 11 November 1997

used industrially, is known to be affected by seasonal changes as well as nutrient levels (Frei and Preston 1961). An increase in the native 4-*O*-methyl and 6-sulphate substituents and a decrease in the 3,6-anhydrogalactose content in *Gracilaria* occur when nitrogen levels are decreased (Craigie *et al.* 1984). Similarly, levels of 3-*O*-methyl pentoses and 2,4-*O*-methyl hexoses in *Porphyridium* sp. increase with nitrate and sulphate deprivation (Ucko *et al.* 1994). Methylation patterns have been correlated to tissue age; agarocolloids of mature tissues of *Gracilaria tikvahiae* McLachlan contain higher amounts of 4-*O*-methyl substituents than younger tissues (Craigie and Wen 1984; Craigie and Jurgens 1989). Ekman and Pedersen (1990) were able to increase agar production in *Gracilaria sordida* W. Nelson and *Gracilaria verrucosa* (Hudson) Papenfuss by increasing photon irradiance, independent of tissue age. It has also been suggested that increased temperatures result in agar with low gel strength and increased sulphate and *O*-methyl substituents (Craigie and Wen 1984; Ekman and Pedersen 1990). Factors such as growth rate and tissue age make an interpretation of these results difficult (Ekman and Pedersen 1990).

The effects of salinity changes on cell wall composition in red algae have not been studied despite the known influence of small cations on the gelling properties of red algal polysaccharides (Rees *et al.* 1982; Durairatnam *et al.* 1990). The activities of enzymes speculated to affect carbon flow to and from red algal cell walls, such as α-galactosidase, have also been correlated with changes in salinity (Ekman and Pedersen 1991). The activity of α-galactosidase in *G. sordida* and *Gracilaria tenuistipitata* Zhang *et* Zia was reduced in hypersaline medium (Yu and Pedersen 1990). Several environmental parameters, including hydrodynamic stress (Hackney *et al.* 1993), temperature, pH, irradiance and nutrient availability, also affect properties of red algal wall polymers such as the agarocolloids and carrageenans (see Craigie 1990 for a brief review); however, a relationship between wall composition and osmotic conditions remains unclear.

The majority of red algal cell wall research has focused on macroscopic marine species which are easily collected or cultivated and from which large quantities of polymer may be extracted. Fewer studies have involved the characterization of cell walls of the freshwater rhodophytes (Iriki and Tsuchiya 1963; Iriki and Takashima 1970; Craigie 1990 and citations therein; Gretz *et al.* 1991). We present previously unreported chemical characterizations of the cell wall polymers of marine and freshwater cultures of *B. moritziana* and freshwater isolates of *B. atropurpurea*. Variations in the composition of extracellular matrix polymers associated with freshwater and marine environments and the plasticity of wall characteristics as applied to phylogenetic considerations are examined. The mechanisms through which cell wall composition is altered in response to osmotic and/or ionic conditions remain unknown.

MATERIALS AND METHODS

Source of algal material

Bangia atropurpurea was collected in October 1991 from rocks in Lake Michigan at Tawas City, Michigan. In April 1991, thalli of *B. moritziana* were collected from the freshwater Rio El Pilar and marine environments near Punta Uricaro in Edo Sucre, Venezuela. Both the freshwater and marine isolates of *Bostrychia* were subsequently cultured as previously described (Karsten *et al.* 1993) and only cultured plants were used for analysis (referred to as freshwater *B. moritziana* and marine *B. moritziana*).

Isolation of cell wall polymers

Thalli of *B. atropurpurea* were washed with distilled water and microscopically analysed for epiphytic and epizoic contaminants. Holdfasts were removed and the thalli were air-dried at room temperature (approximately 20°C). Thalli of *B. moritziana* were rinsed with distilled water and air-dried. Hydrated material of both *B. moritziana* and *B. atropurpurea* was frozen with liquid nitrogen and ground with a mortar and pestle. The powder was then defatted with acetone for 18 h, dried, extracted with boiling 80% ethanol and dried under vacuum in the presence of P_2O_5. The insoluble residue was fractionated by three successive extractions with 0.5 mol/L $NaHCO_3$ at 85–90°C for 2 h. The $NaHCO_3$ soluble (water-soluble) fraction of marine cultures was further fractionated by precipitation with cetylpyridinium chloride (CPC) as in Gretz *et al.* (1991). Polymers from algae grown in freshwater did not precipitate with CPC, therefore the water-soluble material was precipitated with ethanol. Defatted material (215 mg) of freshwater *B. atropurpurea* was fractionated to yield water-insoluble material (136 mg) and water-soluble, ethanol-insoluble material (68 mg). Defatted (acetone/ethanol extracted) material (162 mg) of freshwater *B. moritziana* was fractionated to yield water-insoluble material (31 mg) and water-soluble, ethanol-insoluble material (116 mg). Defatted material (117 mg) of marine *B. moritziana* was fractionated to yield water-insoluble material (43 mg) and water-soluble material (67 mg). The latter was further fractionated by CPC precipitation (44 mg). Cetylpyridinium chloride-soluble material was precipitated with ethanol (22 mg). Most of this CPC-soluble, ethanol-insoluble material (21 mg) was removed with chloroform:methanol (3:1) extraction.

Monosaccharide analysis

Samples were pretreated with fuming HCl at 5°C for 45 min in sealed vials. Samples were hydrolysed with 3N trifluoroacetic acid (TFA) for 3 h at 121°C in sealed vials

and subsequently reduced with sodium borodeuteride as in Blakeney *et al.* (1983) or simultaneously hydrolysed and reduced with 4-methylmorpholine-borane (MMB) to preserve 3,6-anhydrogalactosyl residues as in Stevenson and Furneaux (1991). Alditol acetates were formed as in Blakeney *et al.* (1983) and detected with GC/MS (Gretz *et al.* 1991) with a 15 m Supelco SP-2330 capillary column (0.25 mm ID, 0.20 μm film thickness) using a Finnigan/MAT Magnum gas chromatograph/mass spectrometer (GC/MS) with helium as the carrier gas. Column temperature was maintained at 200°C for 3 min, ramped from 200°C to 240°C at the rate of 5°C·min^{-1} and maintained at 240°C for 5 min. Column linear flow rate was approximately 39.5 cm·s^{-1}. Native methyl constituents were identified in monosaccharide preparations by GC/MS using a 30 m SP-2330 column under instrument parameters used in the following methylation analyses. Identification of derivatives from relative retention times and characteristic ions was as in Jansson *et al.* (1976) and Waeghe *et al.* (1983). In this procedure, no methyl ethers were chemically introduced; all were native to the polysaccharide. Therefore, a 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-galactosyl unit was identified as representing a 2-*O*-methyl galactosyl residue in the native polymer. Linkages for such residues could not be assigned through our GC/MS techniques and were thus determined from nuclear magnetic resonance (NMR) results.

Methylation analysis

Prior to per-*O*-methylation, polymers were pretreated with Dowex 50W-X12 (H^+ form) as in Waeghe *et al.* (1983) to protonate uronic acid residues, to ion-exchange sulphate groups in preparation for conversion to pyridinium salts (Barker *et al.* 1984) and to enhance solubility in dimethyl sulfoxide (DMSO) (Stevenson and Furneaux 1991). Polymers were then treated with per-*O*-methyl with butyl lithium in DMSO and methyl iodide (Paz Parente *et al.* 1985; Kvernheim 1987). Polymers were purified as in Waeghe *et al.* (1983) with the exception that polymers containing sulphate were dialysed against running distilled water for 24 h. Samples pretreated to preserve uronic acids were prereduced with 1 mol/L lithium triethyl borodeuteride as in York *et al.* (1985). Hydrolysis with 2 N TFA and reduction of hydrolysates was performed by procedures in Waeghe *et al.* (1983). Acetylation of reduced hydrolysates was as in Harris *et al.* (1984). Reductive hydrolysis with MMB and 3 N TFA to preserve 3,6-anhydrogalactosyl residues was performed as in Stevenson and Furneaux (1991). Detection with GC/MS was as performed in Gretz *et al.* (1991) using a 30 m SP-2330 capillary column (0.25 mm ID, 0.20 μm film thickness) with helium as the carrier gas. Column temperature was ramped from 150°C to 245°C at a rate of 4°C·min^{-1} and then maintained at 245°C for 16 min. Glycosyl linkages were determined according to characteristic ions reported by Jansson *et al.* (1976) and Waeghe *et al.* (1983). In this procedure, methyl groups are introduced at all available hydroxyl sites in the unhydrolysed polymer. The polymer is then hydrolysed and undergoes further derivatization such that any remaining carbons which would be involved in linkages or substitution are acetylated. Therefore, a 1,3,4,5,6-penta-*O*-acetyl-2-*O*-methyl-galactosyl residue is the derivative formed from a 1,3,4,6-substituted galactosyl unit in the polymer (carbon 5 is involved in the pyranosyl ring structure and is also acetylated).

^{13}C nuclear magnetic resonance spectroscopy

Proton decoupled, ^{13}C-NMR spectra were recorded with a Varian XL-200 spectrometer (50.3 MHz – ^{13}C) at approximately 15 mg mL^{-1} in D_2O. Analyses were performed at 80°C, with a spectral width of 11 kHz, a 90° pulse angle (7.5 s), a 1.0 s acquisition time, and typically 50 000–60 000 transients. Chemical shifts were measured relative to internal dimethyl sulfoxide (DMSO 39.45 p.p.m.).

Additional analyses

Sulphate was determined by the method of Craigie *et al.* (1984) to represent 3% of the dry weight for freshwater *B. moritziana* and *B. atropurpurea* polymers and 3.5% of polymers of marine isolates of *B. moritziana*. Uronic acids were not detected in any samples using the carbazole method of Bitter and Muir (1962).

RESULTS

The results of monosaccharide and methylation analyses are summarized in Tables 1 and 2. The predominant saccharides in water-soluble polymers of *B. atropurpurea* and *B. moritziana* were galactose and 3,6-anhydrogalactose (Table 1).

The water-soluble fraction of freshwater *B. atropurpurea* yielded primarily 3- and 4-linked galactosyl and 4-linked 3,6-anhydrogalactosyl residues (Table 2). Small amounts of 4,6-substituted galactosyl, 4-linked glucosyl (presumably starch) and 4-linked mannosyl residues were detected. Native 6-*O*-methyl galactosyl residues were detected in moderate amounts (Table 1). Linkages of the saccharides containing native methyl ethers could not be assigned by our GC/MS procedure. The ^{13}C-NMR signals corresponding to a 6-*O*-methyl substituent which would indicate linkage were not resolved, therefore the 6-*O*-methyl galactose could be either 3-linked or 4-linked. The water-insoluble fraction of *B. atropurpurea* contained a mixture of 3-linked galactosyl and 4-linked mannosyl residues. Trace amounts of terminal xylosyl residues were present in both the water-soluble and water-insoluble fractions. The ^{13}C-NMR spectrum of the water-soluble extract contained two pairs of anomeric signals at 103.8, 101.2, 102.5

Table 1. Monosaccharide composition of freshwater (FW) and marine *Bostrychia mortiziana* and freshwater *Bangia atropurpurea* as determined by gas chromatography/mass spectrometry

	Gal*	Glc*	Man*	Xyl*	Angal†	6-*O*-Me-Gal†	2-*O*-Me-Gal†	2-*O*-Me-Angal†
Bangia								
Soluble‡	60	20	15	5	+++	+		
Insoluble	40	5	50	5				
Bostrychia FW								
Soluble	69	28		3	++	+	++	++
Insoluble	60	37		3				
Bostrychia Marine								
Soluble	80	10		10	+++	++	−	−
Insoluble	45	50		5				

Gal, galactose (includes sulfated gal); Glc, glucose; Man, mannose; Xyl, xylose; Angal, 3,6 anhydrogalactose; 6-*O*-Me-Gal, 6-*O*-methylgalactose; 2-*O*-Me-Gal, 2-*O*-methylgalactose; 2-*O*-Me-Angal, 2-*O*-methyl-3,6-anhydrogalactose.

*Monosaccharide percentages are expressed as weight percentages of unsubstituted neutral sugar detected (excluding natively methyl and 3,6-anhydro sugars). † Relative amounts of natively methyl and 3,6-anhydro sugars: +++, most abundant; ++, major component; +, minor component; −, trace. ‡ Soluble/insoluble in 0.5 mol/L, $NaHCO_3$, at 80°C.

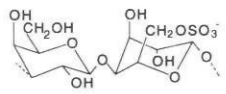
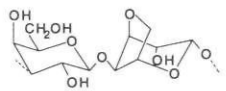
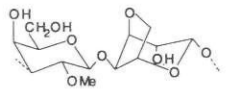
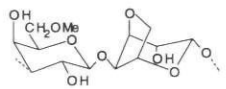
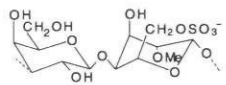
Table 2. Glycosyl linkages/substitutions of saccharide residues in cell wall polymers of freshwater (FW) and marine *Bostrychia moritziana* and freshwater *Bangia atropurpurea* as determined by methylation analysis and expressed as the positions of substitution in addition to carbon 1 (e.g. 4-Angal represents a 4-linked 3,6-anhydrogalactosyl residue, 3,6-Gal represents a galactosyl residue with linkages or substitutions, other than *O*-methyl, at carbons 1, 3, and 6, and t-Xyl designates a terminal xylose with linkages at carbon 1 only

	Bostrychia moritziana				*Bangia atropurpurea*	
	Soluble FW	Polymers Marine	Insoluble FW	Polymers Marine	Soluble FW	Insoluble FW
4-Angal*	++	++			++	
3-Gal†	+++	+++	+++	++	+++	+++
4-Gal†	++	++			++	
3,6-Gal‡	+	+		+	−	
4,6-Gal‡	+	+		+	+	
3,4-Gal‡		−		+		
t-Xyl		+				
4-Glc	++	−	+++	+++	+	
4,6-Glc		−		−		
4-Man					+	+++

Some residues may contain natively occurring methyl ethers on carbon *2, carbon †6 or either carbons ‡2 or 6. All substitution sites listed designate non-methyl substituents. +++, most abundant; ++, major component; +, minor component, −, trace.

and 98.3 p.p.m. (Table 3). These signals have been previously assigned to the anomeric carbons (C-1) of the repeating disaccharide units of porphyran (*O*-β-D-galactopyranosyl [1→4] *O*-α-L-galactopyranosyl-6-*O*-sulphate [1→3]) and agarose (*O*-β-D-galactopyranosyl [1→4] 0–3,6-anhydro-α-L-galactopyranosyl [1→3]) by Usov *et al.* (1983). Signals at 61.7 and 61.5 p.p.m. represent the chemical shifts of carbon six (C-6) of the 3-linked galactosyl residues of porphyran and agarose, respectively. In addition to data in Table 3, a signal at 61.2 p.p.m. was detected. This signal most probably represents C-6 of a 4-linked galactosyl unit of what has been termed 'desulphated porphyran' (O-β-D-galactopyranosyl [1→4] O-α-L-galactopyranosyl [1→4]) (Lahaye and Yaphe 1985). The anomeric shifts of this compound, which were determined to be 103.1 and 101.3 p.p.m. by Lahaye and Yaphe (1985), could be detected as splitting of the anomeric signals at 103.8 and 101.2 p.p.m. The presence of native *O*-methyl compounds is evidenced by a small signal at 59.0 p.p.m. (Usov *et al.* 1980, 1983) which represents the 6-*O*-methylgalactosyl residue detected by monosaccharide analysis. However, no ^{13}C-NMR shifts assignable to a repeating methyl agarose from freshwater *Bangia* were detected. This indicates that disaccharide units containing this substituent either do not repeat and/or the repeating units occur only in small amounts in the native polymer.

Table 3. ^{13}C-NMR resonances of disaccharide repeating units of water-soluble polymers of marine and freshwater *Bostrychia moritziana* and freshwater *Bangia atropurpurea*

	3-Linked							4-Linked						
	X							Y						
	C-1	C-2	C-3	C-4	C-5	C-6	*O*-Me	C-1	C-2	C-3	C-4	C-5	C-6	Repeating unit
Bangia	**103.8**	**70.3**	**81.1**	**69.2**	**75.7**	**61.7**		**101.2**	**69.9**	**71.1**	**79.1**	**67.7**	**67.6**	Agarobiose precursor
Usov *et al.* (1983)	*103.7*	*70.5*	*81.1*	*69.1*	*75.9*	*61.8*		*101.2*	*69.8*	*71.0*	*79.0*	*67.7*	*67.5*	
Bangia	**102.5**	**70.2**	**82.3**	**68.5**	**75.4**	**61.5**		**98.3**	**69.9**	**80.2**	**77.4**	**75.6**	**69.2**	Agarobiose
Bostrychia marine	**102.4**	**70.2**	**82.2**	**68.4**	**75.6**	**61.4**		**98.3**	**69.9**	**80.1**	**77.5**	**75.7**	**69.3**	
Usov *et al.* (1983)	*102.4*	*70.1*	*82.2*	*68.4*	*73.3*	*61.4*		*98.4*	*69.8*	*80.2*	*77.4*	*75.6*	*69.3*	
Bostrychia fresh	**102.6**	**70.2**	**82.7**	**68.7**	**75.7**	**61.5**	**59.1**	**98.7**	**78.8**	**78.5**	**77.6**	**75.3**	**69.8**	2-*O*-methyl-agarobiose
Usov *et al.* (1980)	*102.6*	*70.2*	*82.7*	*68.7*	*75.6*	*61.4*	*59.1*	*98.7*	*78.8*	*78.4*	*77.6*	*75.3*	*69.8*	
Bostrychia fresh	**102.4**	**70.1**	**82.1**	**68.7**	**73.7**	**71.8**	**59.1**	**98.2**	**69.8**	**80.1**	**77.4**	**75.6**	**69.3**	6-*O*-methyl agarobiose
Usov *et al.* (1980)	*102.4*	*70.0*	*82.0*	*67.2*	*73.2*	*70.0*	*59.0*	*98.3*	*70.0*	*80.0*	*77.4*	*75.6*	*69.0*	
Bostrychia fresh	**103.6**	**69.8**	**80.6**	**68.9**	**75.6**	**61.3**	**57.9**	**100.1**	**78.0**	**68.1**	**78.8**	**70.1**	**67.4**	2-*O*-methyl porphyran
Lahaye *et al.* (1986)	*103.7*	*69.8*	*81.1*	*69.0*	*75.8*	*61.7*	*58.0*	*100**	*79**	*69**	*78.9*	*70.1*	*67.7*	

Our values appear in boldface type, values cited in previous literature appear in italics. 'X' and 'Y' represent the 3-linked and 4-linked glycosyl residues of the disaccharide repeating unit. 'C-1', 'C-2', etc. designate carbons one through six of the glycosyl residue and '*O*-Me' designates a methyl subtituent on either of the glycosyl residues as indicated by the structure in the far right column.

*As predicted by Usov *et al.* (1980).

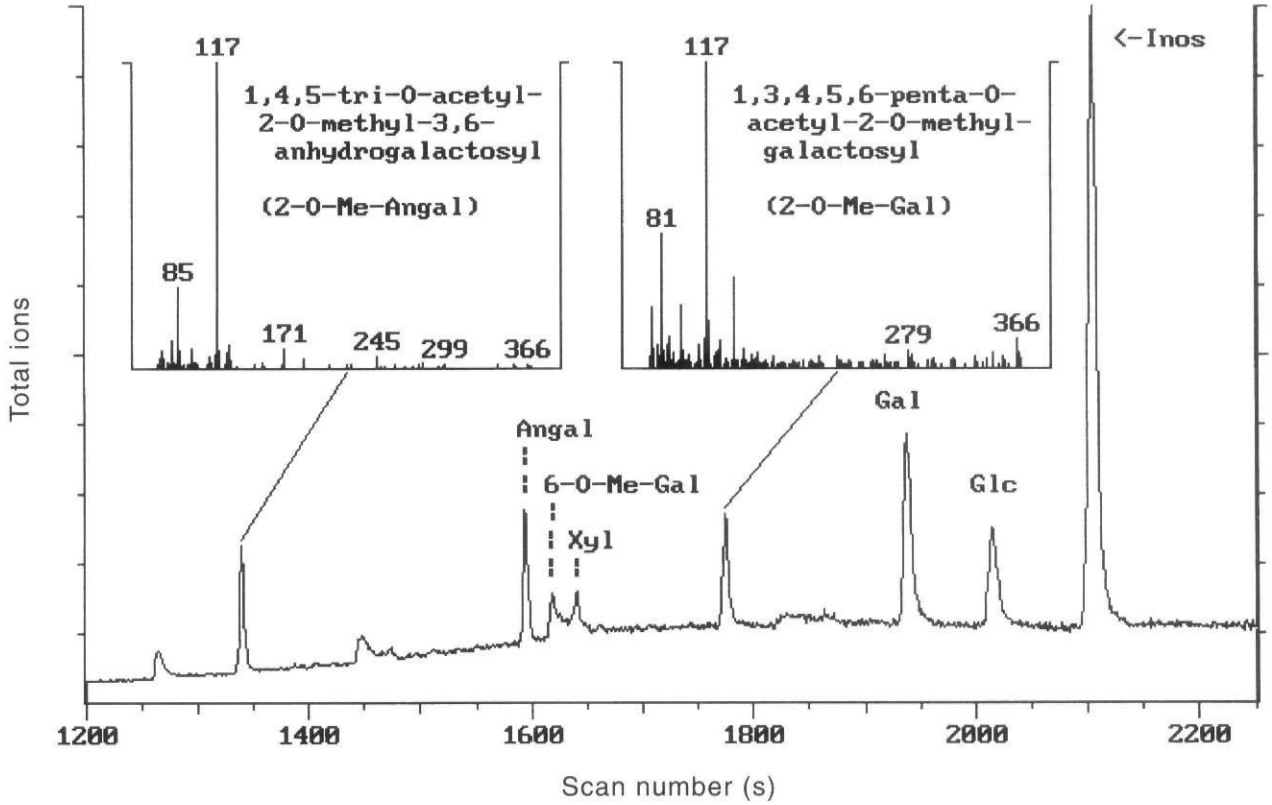


Fig. 1. Gas chromatogram (mass spectra insert) of monosaccharide analysis with 4-methylmorpholine-borane (MMB) hydrolysis of freshwater *Bostrychia moritziana*. Inositol was added as an internal standard. Mass spectra indicate derivatives formed from native methyl saccharides.

Notable differences were observed in the cell wall polymers of *B. moritziana* cultured from marine and freshwater environments (Tables 1 and 2). Monosaccharide and methylation analyses of the water-soluble polymers of freshwater *B. moritziana* indicated 3-linked galactosyl, 4-linked 3,6-anhydrogalactosyl, 2-*O*-methyl-3,6-anhydrogalactosyl and 2-*O*-methylgalactosyl residues as major components. Minor components included 4-linked glucosyl (presumably starch), 3,6-substituted and 4,6-substituted galactosyl and 6-*O*-methyl galactosyl residues were present as minor components. Trace amounts of terminal xylosyl residues were also detected. The distribution of methyl residues in marine *Bostrychia* was markedly different than that for fresh water *Bostrychia*. Water-soluble polymers of marine *Bostrychia* contained greater amounts of 6-*O*-methylgalactosyl and terminal xylosyl residues, decreased *2-O*-methylgalactosyl and *2-O*-methyl-3,6-anhydrogalactosyl residues as well as trace amounts of 3,4-linked galactosyl residues and reduced sulphate levels. The water-insoluble fractions of both freshwater and marine *B. moritziana* were found to contain predominantly 3-linked galactosyl and 4-linked glucosyl residues.

Three sets of repeating units were identified by ^{13}C-NMR in freshwater *B. moritziana* water-soluble polymers (Table 2, Fig. 1). Anomeric shifts at 102.4 and 98.2 p.p.m. were assigned to 6-O-methyl agarose (6-*O*-methyl-β-D-galactopyranosyl [1→4] 3,6-anhydro-*O*-galactopyranosyl [1→3]) and those at 102.6 and 98.7 p.p.m. to 2′-*O*-methyl agarose (*O*-β-D-galactopyranosyl [1→4] 2-*O*-methyl-3,6-anhydro-*O*-α-L-galactopyranosyl [1→3]) based on Usov *et al.* (1983). We identified shifts at 103.6 and 100.1 p.p.m. as the anomeric carbons of 2′-*O*-methyl porphyran [*O*-β-D-galactopyranosyl [1→4] 2-*O*-methyl-6-*O*-sulphate-*O*-α-L-galactopyranosyl [1→3]) based on the assignments of Lahaye *et al.* (1986) and predicted by Usov *et al.* (1980). Strong signals at 61.3 and 61.5 p.p.m. represent the C-6 chemical shifts of methyl porphyran and 2′-*O*-methyl agarose, respectively (Usov *et al.* 1980, 1983). The chemical shift at 59.1 p.p.m. is attributed to the methyl groups of both the 2-*O*- and 2′-*O*-methyl agaroses (Usov *et al.* 1980, 1983). Finally, the signal at 57.9 p.p.m. can be assigned to the methyl group of 6-*O*-methyl porphyran as discussed by Lahaye *et al.* (1986). Whether the three sets of repeating units form separate polymers or occur in the same polymer is not known.

^{13}C-NMR revealed only the repeating units of agarose in marine *Bostrychia* water-soluble polysaccharides, with anomeric signals at 102.4 and 98.3 p.p.m. Although methyl residues were detected by GC/MS in marine *Bostrychia* water-soluble polymers, these methyl groups were not detectable by ^{13}C-NMR, indicating their presence in quantities less than approximately 10%.

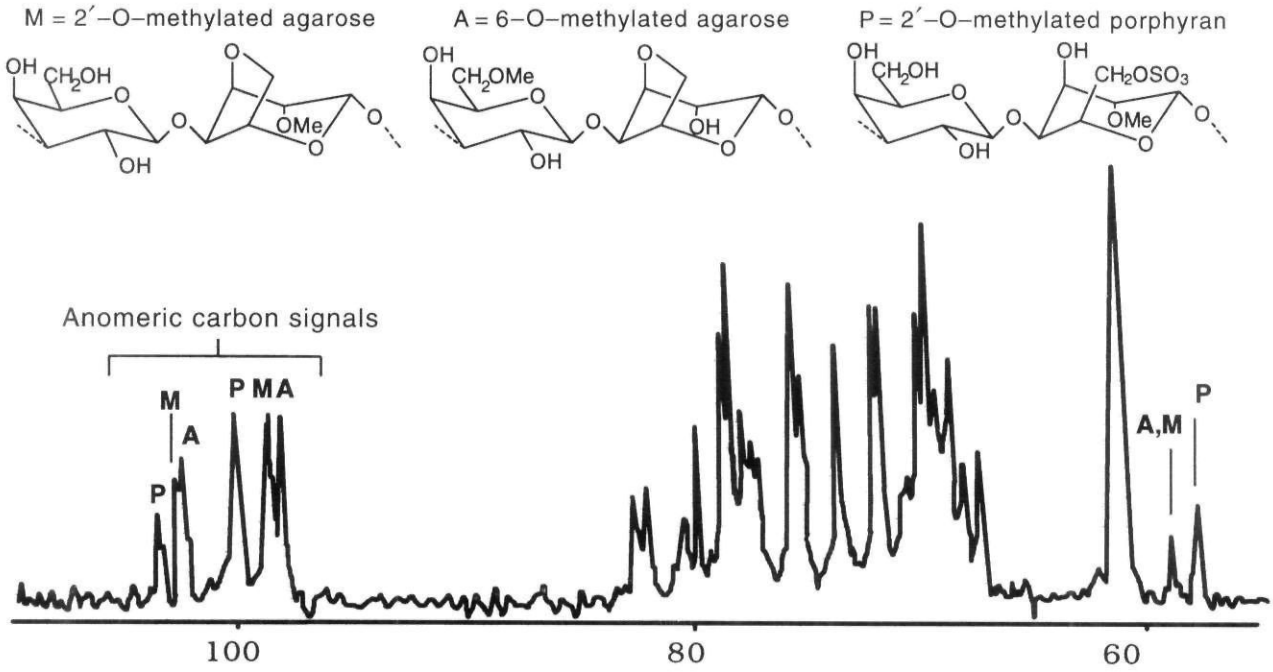


Fig. 2. ^{13}C-NMR spectrum of the water-soluble polymers of freshwater *Bostrychia moritziana*. 2′-O-methylated agarose; A = 6-O-methylated agarose; P = 2′-O-methylated porphyran.

DISCUSSION

The carbohydrate components of the cell walls of freshwater isolates of *B. atropurpurea* correspond directly with previously determined polymers for generic phase marine isolates analysed with similar techniques (Shashkov *et al.* 1978; Usov *et al.* 1978). Marine isolates were found to contain hot water-soluble agarocolloids, including a porphyran-type polymer, and hot water-insoluble β-1,4-mannans (Usov *et al.* 1978; Gretz *et al.* 1982). Shashkov *et al.* (1978) also reported the presence of trace amounts of methyl agarocolloids in marine isolates of *B. atropurpurea*. It is notable that water-soluble polymers of freshwater *B.atropurpurea* did not precipitate with CPC. Water-soluble polymers of the marine form are known to precipitate rather easily in CPC (Gretz *et al.* 1982). This may represent a difference in the ionic characteristics of the polymers associated with arrangement of polymers or repeating disaccharide units within polymers. In view of the higher plant literature demonstrating cell wall salinity-adaptation (Zhong and Lauchli 1988; Iraki *et al.* 1989a,b,c; 1993), it is surprising that *B. atropurpurea* grown under very different osmotic and ionic conditions shows no apparent change in cell wall chemistry. However, it must also be noted that this alga has successfully been cross-cultured between freshwater and marine media only through neutral spores (Geesink 1973). It is possible that the failure of generic phase *B. atropurpurea* to adapt readily to salinity stress is connected with a lack of ability to rapidly modify wall composition. Nevertheless, the absence of any detectable differences in the wall chemistry of marine and freshwater isolates of *B. atropurpurea* provides further support of the assessment of Geesink (1973) that these plants are, in fact, the same species, although the marine populations were previously classified as *Bangia fuscopurpurea*.

Similarly, the cell wall polysaccharides of both the marine and freshwater isolates of *B. moritziana* correspond to those found in other ceramialean algae. All ceramialean algae investigated thus far have been found to produce agarocolloids which contain a higher degree of sulphate ester and methyl ether than is found in polysaccharides of other agarophytes. Agarocolloid composition for several species was summarized by Craigie (1990), including polymers of *Ceramium boydenii* Gepp, *Ceramium rubrum* (Hudson) C. Agardh, *Congregatocarpus pacificus* (Yamada) Mikami (as *Delesseria crassifolia*), *Bryothamnion triquetum* (S.G. Gmelin) Howe, *Digenea simplex* (Wulfen) C. Agardh, *Osmundea pinnatifida* (Hudson) Stackhouse (as *Laurencia pinnatifida*), *Neorhodomela larix* (Turner) Masuda (as *Rhodomela* larix), *Odonthalia corymbifera* (S.G. Gmelin) Greville and *Polysiphonia lanosa* (Linneaeus) Tandy. Polymers of *Lenormandia chauvinii* Harvey, *Lenormandia angustifolia* Harvey (J.Agardh), *Bryocladia ericoides* (Harvey) Schmitz, *Osmundaria colensoi* (Hooker f. *et* Harvey) R.E. Norris and *Laurencia thyrisifera* J. Agardh were reported by Miller *et al.* (1993); those of *Chondria macrocarpa* Harvey by Furneaux and Stevenson (1990), and *Delesseria sanguinea* (Hudson) Lamouroux by Potin *et al.* (1992). Miller and Furneaux (1997) reported methyl ether and sulphate ester substitutions at the primary hydroxyl sites of the agarocolloids of *Euptilota formosissima* (Montagne) Kützing,

Streblocladia glomerulata (Montagne) Papenfuss, *Polysiphonia abscissoides* Womersley, and *Polysiphonia strictissima* Hooker f. *et* Harvey. A wide range of sulphate and methyl substitution patterns occurs throughout the order. Methylation at C-6 of the 3-linked galactosyl residue is the most common substituent and has been reported for all of the above taxa except *Delesseria*, *Rhodomela*, *L. thyrisifera* and *L. angustifolia*. Methylation at C-2 of the 4-linked galactosyl residue or C-2 of the 4-linked 3,6-anhydrogalactosyl residue and sulphation at C-6 of the 3-linked and/or 4-linked galactosyl residues and/or C-4 or C-2 of the 3-linked residues appear to be more common in this order than other orders containing agarophytes (e.g. Bangiales).

Taylor-Wood (1997) investigated the physiology, structure and chemistry of the cell walls in *Bostrychia tenella* ssp. *flagellifera* (Post) R. King *et* C. Puttock. The cell wall is composed of three regions: inner matrix with sulphated polysaccharides, outer matrix with neutral polysaccharides and multilayered outer region with protein and various polysaccharides. Chemical analyses of the water-soluble polysaccharides revealed 18.3% sulphate (w/w). The sulphate values are five times greater in *B. tenella* ssp. *flagellifera* than in either freshwater or marine *B. moritiziana*. On a mole percentage basis, the monosaccharide composition is 59% galactose, 9% 3,6 anhydrogalactose, 9.5% 6-*O*-methylgalactose, 8.5% 2-*O*-methylgalactose, 2.5% 4-*O*-methylgalactose, 6% xylose, 4% glucose (possibly starch) and 1.5% mannose. The galactose levels range between 59 and 80% in both species. Methyl galactose levels appear higher in *B. tenella* than in either *B. moritziana* strain, although this was not quantified in the latter. Anhydrogalactose values also appear higher in *B. moritziana*. Xylose levels differ in both species: 3% in freshwater *B. moritziana* and 10% in the marine isolate while in *B. tenella* it is 6%. The position of xylose in these polysaccharides is not precisely known, although it is thought be terminal in other Rhodomelaceae (Furneaux and Stevenson 1990). Although mannose is present in the extracellular polysaccharides of *B. tenella* (Taylor-Wood 1997), it appears to be absent in other Ceramiales (Furneaux and Stevenson 1990; Miller *et al.* 1993) including *B. moritziana*.

The usefulness of red algal polysaccharides as taxonomic indicators is directly related to the limited plasticity in the backbone composition of red algal polysaccharides (even under environmental extremes) and the types and frequencies of modifications when they do occur. The introduction or removal of methyl substituents to change polymer conformations and physical characteristics without altering basic polymer composition in response to environmental stimuli is a basic theme in macromolecular biochemistry. It is perhaps not surprising that this simple modification may also occur in red algal cell walls in response to changing environmental factors. The facility to make such modifications may be an environmentally induced genetic characteristic.

There is evidence that changing osmotic and ionic conditions can affect the physical properties and chemical composition of cell walls in higher plants. Zhong and Lauchli (1988) reported that cellulose biosynthesis in cotton seedlings was inhibited by hypersalinity. However, the inhibition was apparently negated by the addition of calcium ions (Zhong and Lauchli 1993). Calcium-independent decreases in cell wall tensile strength and primary cell wall crystalline cellulose content were reported in tobacco cells adapted to hyperosmotic conditions (Iraki *et al.* 1989a). Alterations in wall composition such as the introduction of highly substituted rhamnogalacturonans, increased polygalacturonic acid content, reduced hydrolysis of hemicellulosic xyloglucan and reduction of the cell wall protein extension accompanied the adaptation which resulted in a rigidification of the cell wall in salt adapted cells (Iraki *et al.* 1989b,c).

A relationship between the gel strength and substitution patterns in agarocolloids has been suggested. Guiseley (1970) reported that in 47 out of 50 samples tested, the gelling temperature was proportional to methoxyl content. Craigie *et al.* (1984) found that in several species of *Gracilaria,* the gel strength was inversely proportional to the degree of sulphate and 4-*O*-methyl substituents. The degree of substitution was also found to change according to tissue age and a correlation with temperature was suggested. Miller *et al.* (1993) studied several marine ceramialean algae including *L. chauvinii*, *L. angustifolia*, *B. ericoides*, *O. colensoi* and *L. thyrsifera* and found agar-type polymers containing 2-*O*-methylgalactosyl, 2-*O*-methyl-3,6-anhydrogalactosyl, 6-*O*-methylgalactosyl, and xylosyl residues in various combinations and percentages. However, it was observed that the methyl polymers had 'very low gelling properties'. These observations correlate with the controlled experiments of Garnier *et al.* 1993 in which citrus-derived pectic polysaccharides were sequentially methyl and a marked decrease in gel strength was associated with the introduction of methyl ethers. It has been suggested that the interruption of the repeating disaccharide backbone of agarocolloids by substituents may interfere with interactions with small divalent cations and hydrogen bonding in wall polymers as well as altering water structure and activity in the cell wall. Such changes affect the characteristics of the three dimensional matrix formed during gelation (Rees *et al.* 1982).

The difference in cell wall chemistry of marine and freshwater forms of *B. moritziana* is most likely attributable to an osmotic or ionic adaptation. Cultured plants were maintained under the same irradiance, photoperiod and temperature in enriched freshwater and marine media with ample nutrients. Elucidation of the exact parameter inducing the changes in the polysac-

charide substitution patterns we observed will require well-controlled culture experiments. Growth rate and tissue age will also need to be considered in the analysis of such experiments. However, some general speculations can be made regarding the possible adaptive advantages of changes in wall polysaccharide substitution patterns.

The freshwater adapted alga that is subjected to sudden increases in salinity is at risk from incipient plasmolysis. Reed (1980) proposed that cells with walls that were flexible enough to accommodate slight volume changes were more tolerant to sudden increases in salinity by avoiding damage due to cell membrane/wall separation. A decrease in the gel strength of wall polymers due to increased methyl ether substitution could result in a 'softening' of the cell walls of freshwater *B. moritziana*. This could represent an adaptation to prevent cell damage. Conversely, a marine alga which is subjected to sudden decreases in salinity is at risk due to cell wall failure caused by increased turgor pressure. In this case, rigidification of the cell wall seems the most appropriate response for the cell. Indeed, marine *B. moritziana* may produce more rigid cell walls by removing or redistributing methyl groups, thus increasing gel strength of the colloids. No 'wall hardening' was noted in *B. tenella* when subjected to short-term (20 min) hypo- and hyper-saline conditions (0–200‰), although there is a 3.0–11.0 μm increase in wall thickness (Taylor-Wood 1997). Longer-term experiments (to 20 h) in hypo- and hyper-saline conditions with *Calloglossa leprieurii* (Montagne) J. Agardh from freshwater (<1 p.p.t.) and marine (35 p.p.t.) habitats revealed the following: in hypersalinity cell wall surface area of the marine population increased from 5.7 to 38.2% and in the freshwater population from 14.2 to 47.9% (Mostaert and King 1993). Ion compartmentation appears to be a major factor in these changes of wall volume with K and Na binding to the sulphated polysaccharides (Mostaert *et al.* 1996).

Additionally, the degree of hydration of the cell wall may be affected by the changes in methyl ether substitution. Increased methyl ether substitutions may exclude water from the cell wall, whereas decreased methyl ether substitutions could increase wall hydration which would influence water partitioning from the protoplast to the wall and could promote the use of the wall as a buffer against changes in water levels in the protoplast. However, the degree of sulphation is most likely the main chemical property associated with water binding of these polymers. Another consideration is that complex cell wall polysaccharides may serve as protection against pathogens that lack the enzyme systems to degrade the walls. These pathogens are quite likely to be very different in freshwater and marine habitats.

It should be pointed out that after extensive observations and collection of mangrove algae from around the world there is no evidence that either pathogens or grazers constitute a problem in the biology of *Bostrychia* (West, unpublished).

A study of the physical properties of the cell walls of *B. moritziana* at differing salinities may provide further indication of the role of red algal agarocolloid methyl substituents as a method of wall adaptation to osmotic or ionic stress. Modifications in gel properties of the agarocolloids through environmental conditions could also be of industrial interest.

ACKNOWLEDGEMENTS

We gratefully acknowledge Y. Wang and J. Lutz for assistance with NMR analyses. HLY gratefully thanks R. Joly and J. Morris for helpful comments. Dr James Craigie offered many very thoughtful insights in his review of the manuscript. This work was partially funded by NSF INT-902691 awarded to MRG and NSF BSR8919009 awarded to MRG and MRS.

REFERENCES

Barker, S. A., Hurst, R. E., Settine, J., Fish, F. P. and Settine, R. L. 1984. Structural analysis of heparin by methylation and GLC-MS: Preliminary results. *Carbohydr. Res.* **125**: 291–300.

Bitter, T. and Muir, H. M. 1962. A modified uronic acid carbazole reaction. *Anal. Biochem.* **4**: 330–4.

Blakeney, A. B., Harris, P. J., Henry, R. J. and Stone, B. A. 1983. A simple and rapid preparation of alditol. acetates for monosaccharide analysis. *Carbohydr. Res.* **113**: 291–9.

Craigie, J. S. 1990. Cell walls. *In* Cole, K. M. and Sheath, R. G. (Eds). *Biology of the Red Algae.* New York, Cambridge University of Press, pp. 221–57.

Craigie, J. S. and Jurgens, A. 1989. Structure of agars from *Gracilaria tikvahiae* Rhodophyta: Location of 4-O-methyl-L-galactose and sulphate. *Carbohydr. Polym.* **11**: 265–78.

Craigie, J. S. and Wen, Z. C. 1984. Effects of temperature and tissue age on gel strength and composition of agar from *Gracilaria tikvahiae* (Rhodophyceae). *Can. J. Bot.* **62**: 1665–70.

Craigie, J. S., Wen, Z. C. and van der Meer, J. P. 1984. Interspecific, intraspecific and nutritionally determined variations in the composition of agars from *Gracilaria* spp. *Bot. Mar.* **27**: 55–61.

Darvill, A., McNeill, M., Albersheim, P. and Delmer, D. 1980. The primary cell walls of flowering plants. *In* Tolbert, N. E. (Ed.). *The Biochemistry of Plants,* Vol. I. New York, Academic Press, pp. 91–162.

Durairatnam, M., De Brito Medeiros, T. M. and De Sena, A. M. 1990. Studies of the yield and gel strength of agar from *Gracilaria domingensis* Sonder *ex* Kützing (Gracilariales, Rhodophyta) following the addition of calcium. *Hydrobiologia* **204/205**: 551–4.

Ekman, P. and Pedersen, M. 1990. The influence of photon irradiance, day length, dark treatment, temperature, and growth rate on the agar composition of *Gracilaria sordida*

W. Nelson and *Gracilaria verrucosa* (Hudson) Papenfuss (Gigartinales, Rhodophyta). *Bot. Mar.* **33**: 483–95.

Ekman, P. and Pedersen, M. 1991. Effects of altered salinity, darkness and algal nutrient status on floridoside and starch content, α-galactosidase activity and agar yield of cultivated *Gracilaria sordida*. *Br. Phycol. J.* **26**: 123–31.

Frei, E. and Preston, R. D. 1961. Variants in the structural polysaccharides of algal cell walls. *Nature (Lond.)* **192**: 939–42.

Furneaux, R. H. and Stevenson, T. T. 1990. The xylogalactan sulfate from *Chondria macrocarpa* (Ceramiales, Rhodophyta). *Hydrobiologia* **204/205**: 615–20.

Garnier, C., Axelos, M. A. V. and Thibault, J. F. 1993. Phase diagrams of pectin-calcium systems-Influence of pH, ionic strength, and temperature on the gelation of pectins with different degrees of methylation. *Carbohydr. Res.* **240**: 219–32.

Geesink, R. 1973. Experimental investigations on marine and freshwater *Bangia* (Rhodophyta) from the Netherlands. *J. Exp. Biol. Ecol.* **11**: 239–47.

Gretz, M. R., McCandless, E. L., Aronson, J. M. and Sommerfeld, M. R. 1983. The galactan sulfates of the conchocelis phases of *Porphyra leucosticta* and *Bangia atropurpurea* (Rhodophyta). *J. Exp. Bot.* **34**: 705–11.

Gretz, M. R., Sommerfeld, M. R. and Aronson, J. M. 1982. Cell wall composition of the generic phase of *Bangia atropurpurea* (Rhodophyta). *Bot. Mar.* **25**: 529–35.

Gretz, M. R., Sommerfeld, M. R., Athey, P. V. and Aronson, J. M. 1991. Chemical composition of the cell walls of the freshwater red alga *Lemanea annulata* (Batrachospermales). *J. Phycol.* **27**: 232–40.

Guiseley, K. B. 1970. The relationship between methoxyl content and gelling temperature of agarose. *Carbohydr. Res.* **13**: 247–56.

Hackney, J. M., Kraemer, G. P., Atalla, R. H., VanderHart, D. L. and Chapman, D. J. 1993. Influence of hydrodynamic environment on composition and macromolecular organization of structural polysaccharides in *Egregia menziesii* cell walls. *Planta (Berl.)* **192**: 461–72.

Harris, P. J., Henry, R. J., Blakeney, A. B. and Stone, B. A. 1984. An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydr. Res.* **127**: 59–73.

Iraki, N. M., Bressan, R. A., Hasegawa, P. M. and Carpita, N. C. 1989a. Alteration of the physical and chemical structure of the primary cell wall of growth-limited plant cells adapted to osmotic stress. *Plant Physiol.* **91**: 39–47.

Iraki, N. M., Singh, N., Bressan, R. A. and Carpita, N. C. 1989b. Cell walls of tobacco cells and changes in composition associated with reduced growth upon adaptation to water and saline stress. *Plant Physiol.* **91**: 48–53.

Iraki, N. M., Bressan, R. A. and Carpita, N. C. 1989c. Extracellular polysaccharides and proteins of tobacco cell cultures and changes in composition associated with growth-limited adaptation to water and salinity stress. *Plant Physiol.* **91**: 54–61.

Iriki, Y. and Takashima, S. 1970. Cell wall component of *Compsopogon oishii* sugars in the hydrolyzate of the water-soluble polysaccharide. *Bull. Inst. Natural Educ. Shiga Heights (Shinzu University)* **9**: 65–8.

Iriki, Y. and Tsuchiya, Y. 1963. Constituents of the cell wall of *Batrachospermum virgatum* from Shiga Heights. *Contrib. Shigakogen Biol. Lab. Shinshu University* **2**: 1–8.

Jansson, P.-E., Kenne, L., Leidgren, H., Lindberg, B. and Lonngren, J. 1976. A practical guide to the methylation analysis of carbohydrates. *Department of Organic Chem. Stockholm University Commun. no.* **8**, 1–75a.

Karsten, U., West, J. A. and Ganesan, E. K. 1993. Comparative physiological ecology of *Bostrychia moritziana* (Ceramiales, Rhodophyta) from freshwater and marine habitats. *Phycologia* **32**: 401–9.

King, R. J. and Puttock, C. F. 1989. Morphology and taxonomy of *Bostrychia* and *Stictosiphonia* (Rhodomelaceae/Rhodophyta). *Aust. Syst. Bot.* **2**: 1–73.

Kvernheim, A. L. 1987. Methylation analysis of polysaccharides with butyllithium in dimethyl sulfoxide. *Acta Chem. Scand.* B**41**: 150–2.

Lahaye, M., Rochas, C. and Yaphe, W. 1986. A new procedure for determining the heterogeneity of agar polymers in the cell walls of *Gracilaria* spp. (Gracilariaceae, Rhodophyta). *Can. J. Bot.* **64**: 579–85.

Lahaye, M. and Yaphe, W. 1985. ^{13}C-NMR-spectral analysis of sulfated and desulfated polysaccharides of the agar type. *Carbohydr. Res.* **143**: 240–5.

Miller, I. J., Falshaw, R. and Furneaux, R. H. 1993. The chemical structures of polysaccharides from New Zealand members of the Rhodomelaceae. *Bot. Mar.* **36**: 203–8.

Miller, I. J. and Furneaux, R. H. 1997. The structural determination of the agaroid polysaccharides from four New Zealand algae in the order Ceramiales by means of 13C NMR spectroscopy. *Bot. Mar.* **40**: 333–9.

Mostaert, A. S. and King, R. J. 1993. The cell wall of the halotolerant red alga *Caloglossa leprieurii* (Montagne) J. Agardh (Ceramiales, Rhodophyta) from freshwater and marine habitats: effect of changing salinity. *Cryptogamic Bot.* **4**: 40–6.

Mostaert, A. S., Orlovich, D. A. and King, R. J. 1996. Ion compartmentation in the red alga *Caloglossa leprieurii* in response to salinity changes: freeze substitution and X-ray microanalysis. *New Phytol.* **132**: 513–19.

Paz Parente, J., Cardon, P., Leroy, Y., Montreuil, J. and Fournet, B. 1985. A convenient method for methylation of glycoprotein glycans in small amounts by using lithium methylsulfinyl carbanion. *Carbohydr. Res.* **141**: 41–7.

Potin, P., Patier, P., Floc'h, J.-Y., Yvin, J.-C., Rochas, C. and Kloareg, B. 1992. Chemical characterization of cell-wall polysaccharides from tank-cultivated and wild plants of *Delesseria sanguinea* (Hudson) Lamouroux (Ceramiales, Delesseriaceae): culture patterns and potent anticoagulant activity. *J. Appl. Phycol.* **4**: 119–28.

Reed, R. H. 1980. Physiological responses of *Porphyra* under steady state and fluctuating salinity regimes. *Proc. Int. Seaweed Symp.* **10**: 509–13.

Rees, D. A., Morris, E. R., Thom, D. and Madden, J. K. 1982.

Shapes and interactions of carbohydrate chains. *In* Brant, D. A. (Ed.). *The Polysaccharides*, pp. 367–78.

Shashkov, A. S., Usov, A. I., Yarotski, S. V. and Rabovski, A. B. 1978. Synthesis of ^{13}CNMR spectra of mono- and dimethyl ethers of methyl-β-D-galactopyranoside. *Bioorg. Khim.* **4**: 1489–94.

Sheath, R. G. and Cole, K. M. 1980. Distribution and salinity adaptations of *Bangia atropurpurea* (Rhodophyta) in North America. *J. Phycol.* **16**: 412–20.

Sheath, R. G. and Cole, K. M. 1984. Systematics of *Bangia* (Rhodophyta) in North America. I. Biogeographic trends in morphology. *Phycologia* **23**: 383–96.

Sheath, R. G., Vis, M. L. and Cole, K. M. 1993. Distribution and systematics of freshwater Ceramiales (Rhodophyta) in North America. *J. Phycol.* **29**: 108–17.

Stevenson, T. T. and Furneaux, R. H. 1991. Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydr. Res.* **210**: 277–98.

Taylor-Wood, E. 1997. The extracellular material of *Bostrychia tenella* ssp. *flagellifera*. PhD thesis, University of New South Wales. Sydney, Australia, 206pp.

Ucko, M., Garesh, S., Simon-Berkovitch, B. and Arad (Malis), S. 1994. Predation by a dinoflagellate on a red microalga with a cell wall modified by sulfate and nitrate starvation. *Mar. Ecol. Prog. Ser.* **104**: 293–8.

Usov, A. I., Ivanova, E. G. and Shashkov, A. S. 1983. Polysaccharides of algae. 33. Isolation and ^{13}C-NMR spectral study of some new gel-forming polysaccharides from Japan Sea red seaweeds. *Bot. Mar.* **26**: 285–94.

Usov, A. I., Yarotsky, S. V. and Esteves, M. L. 1978. Polysaccharides of algae. 23. Polysaccharides of the red alga *Bangia fuscopurpurea* (Dillw.) Lyngb. *Bioorg. Khim.* **7**: 1261–70.

Usov, A. I., Yarotsky, S. V. and Shashkov, A. S. 1980. ^{13}C-NMR spectroscopy of red algal galactans. *Biopolymers* **19**: 977–90.

Waeghe, T. J., Darvill, A. G., McNeil, M. and Albersheim, P. 1983. Determination, by methylation analysis, of the glycosyl-linkage compositions of microgram quantities of complex carbohydrates. *Carbohydr. Res.* **123**: 281–304.

York, W. S., Darvill, A. G., Stevenson, T. T. and Albersheim, P. 1985. Isolation and characterization of plant cell walls and cell wall components. *In* Weissbach, A. and Weissbach, H. (Eds). *Methods in Enzymology*, Vol. 118. New York, Academic Press, pp. 3–40.

Yu, S. and Pedersen, M. 1990. The effect of salinity changes on the activity of α-galactosidase of the red algae *Gracilaria sordida* and *G. tenuistipitata*. *Bot. Mar.* **33**: 385–91.

Zhong, H. and Lauchli, A. 1988. Incorporation of [^{14}C] glucose into cell wall polysaccharides of cotton roots: effects of NaCl and $CaCl_2$. *Plant Physiol.* **88**: 511–14.

Zhong, H. and Lauchli, A. 1993. Changes of cell wall composition and polymer size in primary roots of cotton seedlings under high salinity. *J. Exp. Bot.* **44**: 773–8.