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Partial Purification and Monosaccharide Composition of Polysaccharides of Hypnea Musciformis by GC Analysis

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Abstract

Dried chopped plant of *Hypnea musciformis* (red algae) was extracted with hot water and cold dilute acid. Yield of High Molecular Weight Crude Polysaccharide 'HMWCP' obtained by ethanol precipitation and lyophilization were in the range of 15-20 %. Anion exchange chromatography of dialyzed material provided a homogenous fraction. Acid hydrolysis and monosaccharide composition were determined as a function of time. Separation achieved by paper chromatography and GC analysis as an alditol acetates showed galactose as a predominant sugar of the polysaccharides. A significant amount of glucose was found in crude sample. Xylose/arabinose and some fucoses were also detected in various proportions in crude and partially purified fractions.

Introduction

Hypnea musciformis of class Rhodophyceae is widely distributed at Karachi coast of Arabian sea. Carageenan/agar agar are water extractable polysaccharides from *H. musciformis* and differ in their chemical composition and also structure varies among the species and with extraction procedures.[1,2] These polysaccharides are range of economically important sulphated galactans, extensively used as gels and thickening agents.[3,4] Agglutinin from *H. musciformis* exhibited antifungal properties.[5] Series of papers have been published for evaluating seaweed poly saccharide as an elicitor of plant disease resistance responses in treated tissues of chickpea.[6-8]

Karachi, Pakistan coastline is rich in both its abundance and diversity of red algae, however not much work has been reported on their phycocolloids. Work presented here includes the isolation and partial purification of cell wall polysaccharide of *H. musciformis* and identification of monosaccharide compositions.

Materials and Methods

H. musciformis (red algae) was collected from Capmonz, coastal area of Karachi Pakistan in the month of February and November. Plant was extracted with hot water and dilute (0.1N) HCl, ethanol precipi-

tation and lyophilization provided HMW crude polysaccharides. The chemical composition and their elicitor activity in terms of induced browning in treated tissues of chickpea were established previously.[9]

Crude polysaccharides were dissolved in water with constant stirring, dialyzed through dialysis bags (cutoff value of Mr, 12000 dalton), Solid particles settled down in dialysis bags, collected as dialyzed insoluble fraction, substance present in the bag in solution was lyophilized and called as dialyzed soluble fraction.

Dialyzed (5 mg) fraction was dissolved in 1 mL of 10mM tris-HCl buffer pH 8.0 (buffer A) and applied to a column (3.5 x 7.5 cm) of DEAE-Cellulose, which had been equilibrated in tris buffer. The column was first eluted with buffer A, followed by 1M NaC1 in the same buffer at a flow rate of 0.5 mL/min. 20 fractions each of 2 mL were collected and carbohydrate content was determined by pheno1/H_2SO_4 Method.[10]

Acid hydrolysis and paper chromatography

100 µL solution (1 mg/mL) of HMW crude polysaccharide obtained by acid extraction was taken into air tight tubes and dried over KOH pellets in a vacuum desicator. 100 µL of 90 % formic acid was added and flushed with nitrogen and heated at 100^{O} C for various lengths of time i.e. 0.5, 1.5, 3 and 5 hours and neutralized with 0.1N NaOH. Hydrolysates dried over P_2O_5, suspended into 50 µL of water and applied on Whatman paper no. 1 and run by the well shaken top layer of BuOH: Acetic acid: water (4:1:5) for 18 hours and developed with silver nitrate reagent.[11]

Monosaccharide composition as an alditol acetates, analyzed by GC

Alditols from the polysaccharide preparations were generated by reductive hydrolysis, used myoinositol as internal standard and acetylated as described by Alvin Fox.[12] The acetate derivatives were separated by gas chromatography.

GC conditions

GC was performed on Perkin Elmer gas chromatograph, separation was achieved on a wide bore GC column of SGE, BP x 70 (cyanopropyl polysiloxane) of size 25 m x 0.53 mm id. The injector and detector temperatures were 280^{O} C; oven temperature was 250^{O} C - 280^{O} C (with increment of 10^{O} C/ min). Helium was used as a carrier gas.

Results and Discussion

Yield of HMW crude polysaccharide isolated from hot water and cold HCl (dilute) extract of *H. musciformis* was 17 % and 20 % respectively. Differences were observed in physical appearance such as polysaccharide from acid extract appeared as flocculent precipitate where as aqueous extract provided a thick jell like material.[3] Polysaccharides obtained from acid and aqueous extraction

were partially purified by dialysis. After dialysis combine recoveries of dialyzed soluble and insoluble fractions were high in aqueous extracts 77.1 % and about 54 % in HCl extract, Table I. This suggest that content of low molecular weight material were high in acid extract which were subsequently dialyzed.

homogeneity in construction of these polysaccharides where as the polysaccharides obtained from hot aqueous extract was resolved into several components of different ionic charges. The aqueous extract was polydisperse in nature and indicate heterogeneity in composition. Column recoveries in Table I after anion exchange chromatography were

Table I. Recoveries of partially purified HMW polysaccharides of *H. musciformis* after dialysis and anion exchange chromatography

Extracts	Hot H_2O	Cold HCL
Dialysis (g % w/w)		
Dialysed soluble farction	68.0	39.8
Dialysed insoluble farction	9.1	13.9
Total recovery	77.1	53.7
Anion exchange chromatography (g % w/v)		
Fraction-A	57.6	42.5
Fraction-B	17.6	53.1
Total recovery	75.2	95.6

The presence of sulphate group in these polysaccharides provides charge, which can be exploited in the separation of sugar sulphates from each other and also from other carbohydrates.[13,14] Dialyzed soluble fractions were further purified by anion exchange chromatography on DEAE-Cellulose. Initially applied material was eluted with tris buffer; no sugar was detected upto 20-30 mL (column volume was 27 mL). The acidic fractions were eluted with 1M NaCl solution and two fractions were obtained for two extracts, fraction A and B. Cold acidic extract was eluted quickly as sharp peaks indicating the maximum for acidic fraction (95.6 %). Aqueous fraction showed recovery of 75.2 % this suggest that some sugars from aqueous extracts were irreversibly bound to the column and not eluted.

Polysaccharide (crude) obtained from acid extract was subjected to acid hydrolysis and hydrolyzates were analyzed as a function of time by paper chromatography. Galactose appeared as major sugar component and released as early as 0.5 hour of hydrolysis, regular increases were observed and maximized at 3 hour of hydrolysis. The degenera-

tion of some components was noticed at 5 hour of hydrolysis, as the intensity of the spots were slightly redused. Xylose, arabinose and glucose were found in substantial amount and some fucoses were also detected. Literature review suggested that structural variation exist in different species of the same class as well as in the different extracts of same species.[15]

To obtain more and clear information the depolymerized polysaccharides were analyzed as alditol acetates (Fig. 1-A and B). Results indicated that galactose was the dom

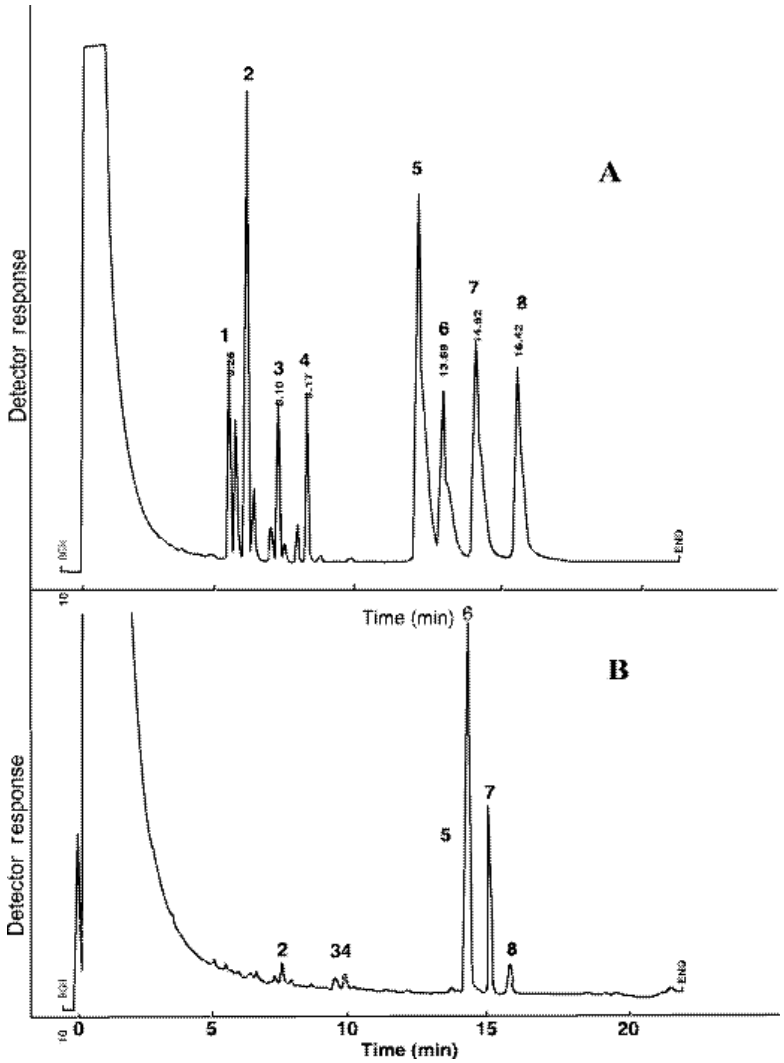

Fig. 1. Gas liquid chromatogram of alditol acetate derivatives. A) Mixture of neutral standard sugars. Peaks : I = Rhamnose. 2 = Fucose. 3 = Arabinose. 4 = Xylose. 5 = Mannose. 6 = Galactose

inating sugar of these preparations; this confirmed our previous results obtained by paper chromatography. Glucose/xylose were found in crude as well as in partially purified fractions, especially a significant amount of glucose (966 mg) was present in crude prepa rations released at 1.5 hour of hydrolysis, Table II. It is reported in literature that glucose is likely to originate from co-precipitation of floridean starch.[16] Normally xylose in red algal polysaccharides has been treated as sulphated galactan polymer.[17,18] A consider able amount of arabinose was detected in crude and dialyzed insoluble fractions and significantly reduced to low content in crude sample as the heating prolong.[19] Fucose was also present in some samples. We were unable to find any remarkable difference in elicitor activity in terms of induced browning in the chickpea tissues treated with crude and partially purified polysaccharides (results are not included here).

Table II. Monosaccharide composition of neutral sugars of *H. musciformis* (red algae), determined by GLC. as an alditol acetate derivatives

Algal plant extracts	Hydrolysis time (h)	Ret. time (min) of monosaccharide components						
		Glu	Gal	Man	Xyl	Ara	Fuc	Rham
		14.92	13.69	12.80	9.17	8.10	6.93	6.26
Polysaccharides (cold HCL) µg (w/w)								
Crude sample	1.5	966	526	-	29.6	104	9.5	-
	3	298	2207	-	8.4	8.4	11.9	-
Dialysed sample								
Insoluble dialysed fraction	1.5	21.1	56.4	-	2.13	2.4	-	-
	3	61.8	148.1	4.4	12.4	4	-	-
soluble dialysed fraction	1.5	36	385.7	-	12.7	-	6.8	-
	3	49.8	377.4	-	13.5	-	7.2	-
Partially purified sample (after anion exchange chromatography)								
Fraction A	1.5	42.0	420.5	-	15.4	-	-	-
	3	51.2	410.2	-	16.8	-	-	-
Fraction B	1.5	31.5	365.1	-	9.8	-	4.5	-
	3	20.1	332.5	-	5.9	-	4.0	-

a contaminant by many phycologists. However recently fine structural studies have identified xylose residues as substituent covalently attached to the backbone of the

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