

# FTIR spectroscopic investigations on the agars of *Gelidiella acerosa* and *Gracilaria millardetii*

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## Abstract

Polysaccharides obtained from summer and winter collections of *Gelidiella acerosa* and *Gracilaria millardetii* were subjected to FTIR spectral measurements and compared with that of commercial agar. The FTIR analysis of polysaccharides from *Gelidiella acerosa* and *Gracilaria millardetii* showed the presence of agar polymers. The intensity of absorption varied with species and season.

**Keywords** : agar, FTIR functional groups, polysaccharides, sea weeds, vibrational bands

## INTRODUCTION

Agar is a gelling polysaccharide obtained from red sea weeds. It is a commercially important and widely used algal polysaccharide. Agar is composed mainly of two sugar residues D-galactose and 3, 6 anhydrogalactose and with smaller proportion of other monosaccharides and substituents like methyl, pyruvate and sulphate esters. The degree and type of substitution which depends on the species influence the quality of agar. Infrared spectroscopy offers a suitable method to characterize agar with respect to the location of the sulphate group (Christiaen and Bodard, 1983; Whyte *et al.*, 1985 and Troung *et al.*, 1988). Fourier - transformed Infrared (FTIR) Spectroscopy has been widely used to provide information on vibrational frequencies of active functional groups including O-H, N-H, =C-H, -C-H, -CH<sub>2</sub> and >P=O) in biological materials (Mantsch and Chapman, 1996; Stuart, 1997). This paper describes FT-IR spectroscopic measurements on the vibrational bands of different types of polysaccharides from *Gelidiella acerosa* and *Gracilaria millardetii*.

## MATERIALS AND METHODS

The cell wall polysaccharides were extracted by hot water extraction method from the summer and winter collections of *Gelidiella acerosa* and *Gracilaria millardetii* and dried in dessicator. The dried polysaccharide was powdered, passed through 1mm IS sieve, transferred to air tight glass bottle and stored for qualitative analysis. One mg of the dry sample powder was mixed in a mortar with 99 mg of Potassium Bromide (KBr). A thin KBr disc was obtained by pressing the powder in French press. Fourier Transformed Infrared (FT-IR) spectra were recorded using Bruker Vector 20 FT-IR double beam Spectrophotometer in the range of 4000-600 cm<sup>-1</sup>. Spectra were taken under identical conditions and compared with the spectrum of commercial agar (Difco agar).

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## RESULTS AND DISCUSSION

### (a) Vibrational band assignments

The FTIR spectra of the samples of *Gelidiella acerosa* and *Gracilaria millardetii* are presented in Figures 1 & 2 and their probable assignments on the vibrational frequencies are summarized in table 1.

#### (i) O-H stretching vibrations

The characteristic bands are observed in the spectra of alcohol and phenols resulting from O-H stretching at 3500-3300 cm<sup>-1</sup>(UshaDesai and Gunasekaran, 1993; Stuart, 1997).

#### (ii) Methyl group vibrations

Methyl group has two types of stretching vibrations. In alkanes, CH symmetric stretch band at 2855cm<sup>-1</sup> and asymmetric C-H stretch band at 2925cm<sup>-1</sup> occur with strong intensity (Gunasekaran *et al.*, 1996). These vibrations occur around 3000cm<sup>-1</sup>. In the FTIR spectra of *Gelidiella acerosa* and *Gracilaria millardetii* weak bands were exhibited for methyl group asymmetric stretch at around 2920 cm<sup>-1</sup>. However there was a methyl group symmetric stretch at around 2840 cm<sup>-1</sup> in both summer and winter spectra of *Gracilaria millardetii*. The bands observed at around 1450cm<sup>-1</sup> and 1370cm<sup>-1</sup> are attributed to asymmetrical and symmetrical bending vibrations of CH<sub>3</sub>/CH<sub>2</sub> group, respectively (Mohaček-Grošev *et al.*, 2001; Sigeo *et al.*, 2002). In the present work, FTIR spectra of agars of different sources showed bands ranging from strong to weak intensity at or around 1450cm<sup>-1</sup> and 1380 cm<sup>-1</sup> for asymmetric and symmetric deformation of CH<sub>3</sub>/CH<sub>2</sub> respectively.

#### (iii) Ester-sulphate link vibrations

The ester-sulphate link vibrations are exhibited by the peaks at 1060, 1180, 1250, and 1370 cm<sup>-1</sup> (Cross,1964; Alkahane and Izumi, 1976). The position occupied in the chain by the sulphate is not clearly seen in agar due to the low content of the sulphate. In this investigation, the intensity of the absorption in different regions vary

from strong to weak or even very weak depending on the species and the seasons. The bands at 1060-1180  $\text{cm}^{-1}$  are due to symmetric stretching of S-O/C-O and asymmetric vibration at 1250-1380  $\text{cm}^{-1}$ . The sulphate ester in C-4 link vibration occurs at 850  $\text{cm}^{-1}$ . In the present study, this band was exhibited only in *Gracilaria millardetii*. This observations was in accordance with previous reports (Stancioff and Stanley, 1969; Miller, 2003).

The band at 890  $\text{cm}^{-1}$  in the spectra of agarophytes is due to non sulphated  $\beta$ -D galacto pyranose residues, typical of agar peak as described earlier (Truong *et al.*, 1988; Mollion, 1992)

#### (iv) Carbon-sulphur link vibrations

Earlier literature revealed that the carbon sulphur link vibration of polysaccharides occurs in the region 630-770  $\text{cm}^{-1}$  (Cross, 1964; Torres-Pombo, 1972). The spectra of present study showed the strong to weak band over the range 680-770  $\text{cm}^{-1}$  which might be attributed to the deformation of sulphate.

#### (v) 3,6 anhydro-galactose bridge vibrations

The strong to weak absorption bands occurring at 930 and 1070  $\text{cm}^{-1}$  in all the spectra of the present study are attributed to C-O ether bond of 3,6 - anhydro-galactose vibration as observed in previous works (Cross, 1964; Torres-Pombo, 1972). These two peaks are typical for phycocolloids.

#### (vi) Carbonyl vibrations

Carboxylic acids and carboxylic esters show strong C=O stretching bands in the region of 1700-1540  $\text{cm}^{-1}$  (Hill and Rendell, 1975). In the present work, very weak band was obtained at around 1740  $\text{cm}^{-1}$  in both the species studied. The strong absorption bands at 1640  $\text{cm}^{-1}$  was assigned to carbonyl vibration of carboxylic group (COO) due to asymmetric stretching (C=O). The medium to weak intensity of band at 1540  $\text{cm}^{-1}$  was assigned to symmetrical stretching of C=O. These assignments are in agreement with previous reports (Silverstein *et al.*, 1975).

#### (vii) $\text{NH}_2$ group vibrations

The presence of protein in polysaccharides shows carbonyl absorption as amide group. Primary and secondary amides display a band in the region 1650 and 1540  $\text{cm}^{-1}$  due to amine deformation vibrations and CO-NH peptide link vibrations, respectively. The spectra of the present investigation showed strong to medium band at 1640  $\text{cm}^{-1}$  and medium to weak band at 1540  $\text{cm}^{-1}$  thus confirming the earlier reports (Christiaen and Bodard, 1983; Truong *et al.*, 1988). A sharp peak centered at around 3500  $\text{cm}^{-1}$  may be due to NH stretching vibrations (Naumann *et al.*, 1996). These

bands are observed in the characteristic region in the spectra of the present study.

#### (viii) C-C stretching vibrations

The C-C vibrations are weak in the region of 1200-800  $\text{cm}^{-1}$  (Desai and Gunasekaran, 1993). In the present work the band observed in the region 1200-800  $\text{cm}^{-1}$  may be due to overlapping of C-C stretching and C-H bending modes.

#### (b) Qualitative analysis

Agar is known to be a lightly sulphated polysaccharide mixture consisting primarily of galactose and 3, 6 anhydro galactopyranosides. The FTIR spectra of Difco agar exhibited peaks at 668, 778, 890, 931, 1038, 1088, 1158, 1250, 1382, 1434, 1549, 1641, 1723, 2927, 3416 and 3470  $\text{cm}^{-1}$  and the results of FTIR spectra of samples of the present studies are given in Table 1. The intensity of bands varies with seasons and algal source. The spectra of these two samples, exhibited more or less common constant peaks both in summer and in winter seasons similar to those shown by Difco agar (Figures 1 & 2). Apart from these, additional peaks were exhibited in spectra of *G. millardetii*. A peak in the region 2850  $\text{cm}^{-1}$  characteristic of methyl group occurs in *G. millardetii*. This may be due to developmental and seasonal variations associated with enzymes involved in the synthesis of polysaccharides. An additional peak at around 850  $\text{cm}^{-1}$  was noticed in both the spectra of *Gracilaria millardetii* which is due to sulphate on C - 4 galactose.

Peaks at 670 and 770  $\text{cm}^{-1}$  are due to deformation of sulphates and peaks around 800 & 1200  $\text{cm}^{-1}$  are due to S-O stretching (sulphate). The spectra of both seasons obtained from all the samples show an intense peak at 890  $\text{cm}^{-1}$  characteristic of a non-sulphated  $\beta$ -D galacto pyranose residues as found in Difco agar (Truong *et al.*, 1988; Mollion, 1992). The peak at 930  $\text{cm}^{-1}$  is due to  $\text{SO}_3$  stretching symmetry. Most of the 4 - linked units are found as 3, 6-AG resulting in an intense peak at around 930  $\text{cm}^{-1}$ . It is the same in the two species studied in both the seasons; but absorbance varied from strong to very weak with species and seasons.

It is interesting to note that the band around 1240  $\text{cm}^{-1}$ , characteristic of sulphate esters appears in each of the spectra during both the seasons. There appears to be a variation in the intensity of this band with algal source. The peak is represented as a shoulder in the spectra of both seasons in *Gelidiella acerosa* as in Difco agar. A broad band is exhibited in *Gracilaria millardetii*. This indicates that the phycocolloids from latter mentioned species contains a greater amount of sulphate esters (Fig. 2). The peaks at 1030, 1070, 1160, 1240 and 1380  $\text{cm}^{-1}$  are also due to ester sulphate. The FTIR spectra of both samples are almost identical and similar to the spectrum of commercial agar.

**Table 1.** FTIR band assignments of standard agar (Difco agar) and agars of summer and winter collections of *Gelidiella acerosa* and *Gracilaria millardetii* (frequency cm<sup>-1</sup>)

Difco agar	<i>Gelidiella acerosa</i>		<i>Gracilaria millardetii</i>		Band Assignments
	Summer	Winter	Summer	Winter	
688( s)	668(vw)	669(ms)	668(mw)	669(mw)	C-S link vibration/S-O stretch/C-S deformation
778( s)	784(vw)	771(w)	770(w)	772(w)	C-S link vibration/S-O stretch /C-S deformation
-	-	-	852(vw)	854(vw)	Ester-sulphate in C-4 link vibration/C-C/C-O/ C-O-S stretch
890(s)	890(vw)	889(vw)	891(vw)	891(vw)	Non sulphated β-D galacto pyranose residues/C-C/C-O stretch
931(s)	931(vw)	932(w)	931(w)	931(w)	3,6 anhydro galactose vibration/C-C/C-O stretch
1038(vs)	1019(vw)	1030(ms)	1043(ms)	1045(m)	Ester-sulphate link vibration/C-C/C-O/ C-O-S stretch
1088(vs)	1076(s)	1072(ms)	1076(w)	1078(m)	C-O-S stretch
1158(s)	1131(vw)	1153(mw)	1166(mw)	1152(mw)	Ester-sulphate link vibration/C-C/C-O/ C-O-S stretch
1250(s)	1247(vw)	1257(w)	1261(mw)	1259(w)	Ester-sulphate link vibration/C-C/C-O/ C-O-S stretch
1382(s)	1385(vw)	1380(m)	1384(m)	1385(mw)	Methyl group vibration/S=O symmetric stretch
1434(ms)	1424(vw)	1436(m)	1420(mw)	1457(mw)	Methyl group vibration/S=O asymmetric stretch
1549(mw)	1560(vw)	1541(m)	1541 (w)	1541(m)	C=O symmetric stretch/ N-H deformation
1641(ms)	1620(ms)	1637(s)	1636(s)	1647(s)	C=O asymmetric stretch/ N-H deformation
1723(w)	1727(w)	1727(vw)	1727(vw)	1727(vw)	C=O stretch
-	-	-	2826(vw)	2848(vw)	Methyl group vibration/ C-H symmetric stretch
2927(w)	2924(vw)	2925(vw)	2937(s)	2945(vw)	Methyl group vibration/ C-H asymmetric stretch
3416(ms)	3417(vs)	3424(vs)	-	-	OH/NH stretch
3470 (ms)	3476(vs)	3462 (vs)	3455(vs)	3447(vs)	

(vs-very strong; s- strong; ms-medium strong; m-medium; mw-medium weak; w- weak; vw- very weak)

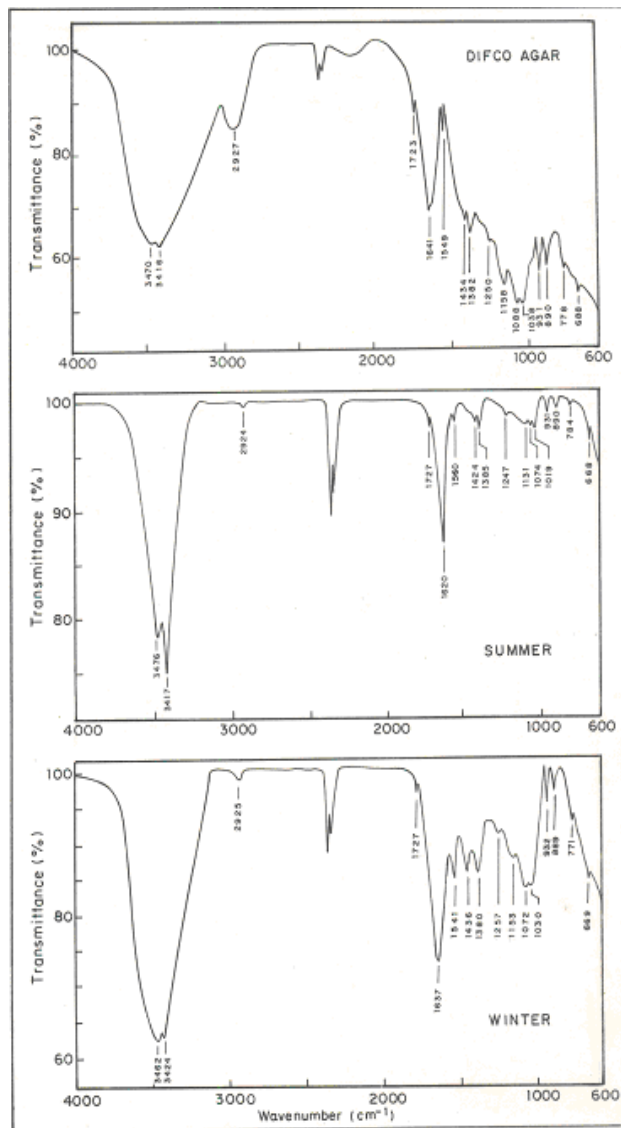


Figure 1. FTIR spectra of polysaccharides from winter and summer collections of *Gelidiella acerosa*, compared with Difco agar

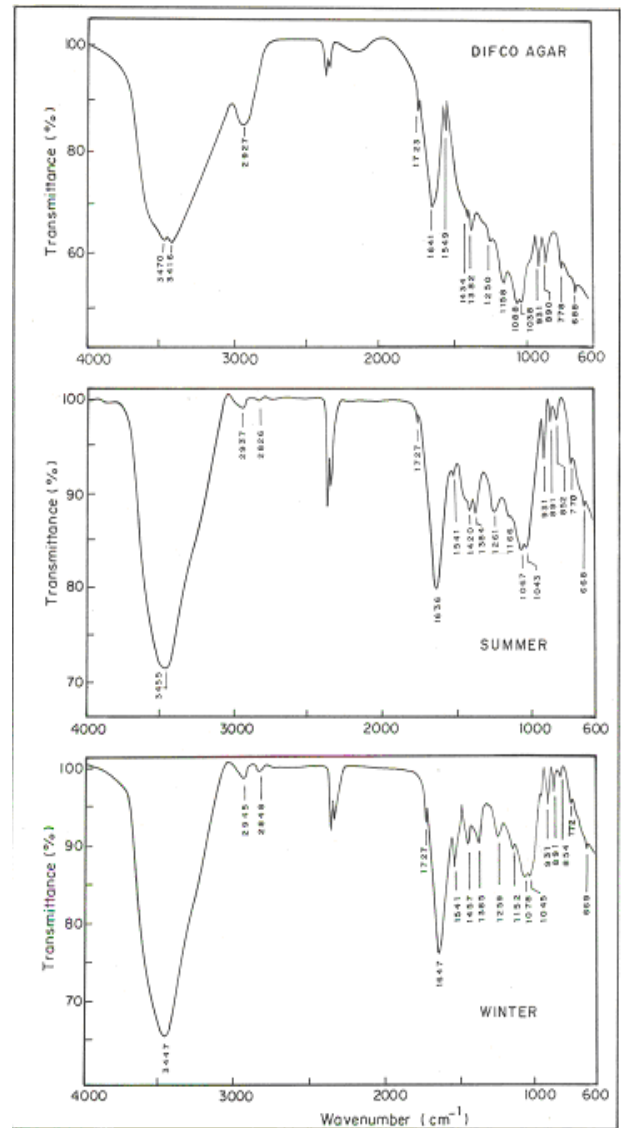


Figure 2. FTIR spectra of polysaccharide from winter and summer collections of *Gracilaria millardetii*, compared with Difco agar

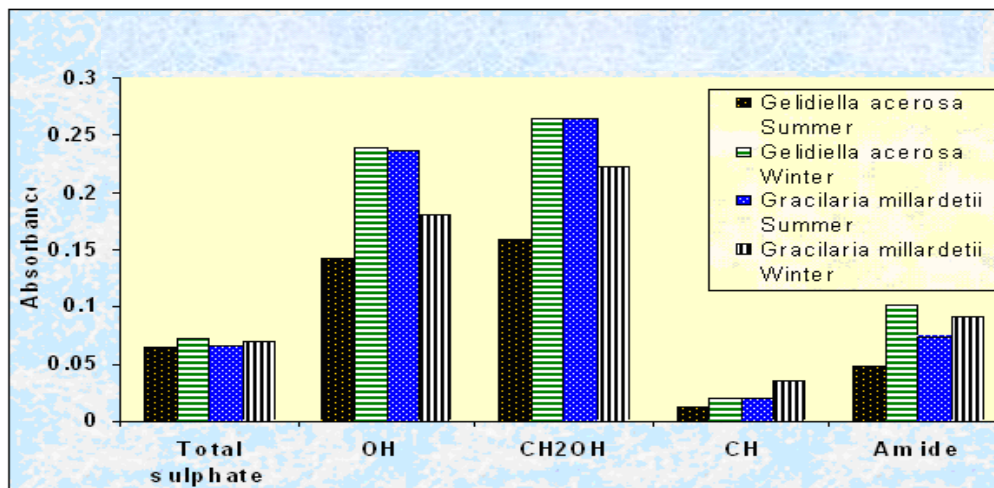


Figure 3. Absorbance value of different groups in agars of *Gelidiella acerosa* and *Gracilaria millardetii*

### c) Quantitative analysis

A comparative study was made for the samples from both the species of the present study to determine the variations in the levels of absorbance of major functional groups like hydroxyl, total sulphate, and CH<sub>2</sub>OH, C-H and amide groups. In each case optical absorbance showed considerable variations between summer and winter seasons (Fig. 3).

### CONCLUSION

The quality of agar is the reflection of degrees of substitution patterns, which differs in general with generic type. In the present study, agar obtained from *Gelidiella acerosa* is less sulphated than *G. millardetii*, and therefore gelling strongly, which could be useful for bacteriological and biomedical applications while *G. millardetii* affords soft gelling agars suitable for food industries.

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