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A B S T R A C T

The properties of agar obtained from Gracilaria sp. collected at Pomquet Harbour, Nova Scotia have been investigated. Alkaline treatment increased the 3,6-anhydrogalactose content, and decreased the sulfate content, of the agar. Fractionation of the agar on DEAE Sephadex A50 indicated that the yield of gelling components is comparable to Difco Bacto agar. However the agar has a high gelling temperature, viscosity and elasticity.

INTRODUCTION

Agar, obtained from certain red seaweeds (Rhodophyceae), has been shown to consist of a spectrum of polysaccharides all of which contain repeating sequences of (1-4)-linked-α-L-galactose and (1-3)-linked-β - D-galactose residues, and which are substituted to a variable degree with charged groups. There are at least three extremes in structure: (1) essentially neutral agarose, (2) pyruvated agarose with little sulfation, and (3) highly sulfated galactans (Duckworth and Yaphe, 1971).

The properties of agar differ from species to species, reflecting differences in the proportions of these extremes in structure (Young, 1973). The best quality agar is obtained from species of Gelidium (Young et al, 1971).

Gracilaria sp. have also been used as a source of agar, chiefly for use in the food and pharmaceutical industries. Gracilaria is indigenous to the Maritime Provinces and, as there is an increasing demand for agar (Bissell, 1972), may have potential for industrial development.

The present study is an investigation of the yield and physical and chemical properties of agar from local Gracilaria.

MATERIALS AND METHODS

Collection of Gracilaria sp.

The samples were collected at Pomquet Harbour, Antigonish County, Nova Scotia (see map, Figure 1) through the summer of 1973. Water temperature and salinity were recorded on each occasion (Fig. 2). The collections were returned to the laboratory in an ice-chest.

Preparation of Polysaccharide Extracts

Extraneous algae, mussels, detritus, mud and other foreign material were removed by washing in tap-water and picking clean. The cleaned sample was air-dried for several days at room temperature, and finally oven-dried at 45°C.

10 - 20 gms. of dried Gracilaria were weighed, soaked for several hours in distilled water, and homogenized. The samples were extracted with 300 - 600 mls of distilled water for three hours at 120°C. (15 psi). Solids were removed by filtering through a pad of glass wool on a Whatman No. 1 filter paper with a layer of diatomite filter-aid. The retained solids were re-extracted twice.

The filtrates were allowed to gel at room temperature. The gel was cut into cubes and frozen to -10°C., then thawed and the excess water poured off. The polysaccharide was redissolved in distilled water, precipitated with 3 volumes of ethanol, and recovered by centrifugation. The precipitate was oven-dried at 45°C.

Those second and third extracts which did not gel were recovered by ethanol precipitation.

Duplicate samples of the Gracilaria were alkaline-treated. The weighed air-dried material was soaked in distilled water and subsequently treated with 3% sodium hydroxide for three hours at 85°C. The treated weed was washed overnight in running tapwater and residual alkali neutralized with dilute hydrochloric acid (pH adjusted to 6.0 - 7.0). Extractions were performed in the same manner as for untreated material.

Determinations of Physical Properties

1. Gelling temperature.

10 mls of a 1.0% solution were pipetted into a 20 X 125 mm test tube. The tube was allowed to cool at a rate of approximately 2°C. per minute with a thermometer immersed in the solution. Glass beads (2 mm in diameter) were introduced at surface at intervals and the gelling temperature taken as the temperature at which the bead just failed to sink. Using this method reproducibility is $\pm$ 0.25°C.

2. Viscosity

Viscosity of 1.0% solutions were determined using a 5 ml Ostwald capillary viscometer mounted in a water-jacket maintained at 65°C. 5 ml of the solution were introduced at a temperature above 65°C. and equilibrated at 65° before measuring the flow time.

The mean of three determinations was taken and expressed as specific viscosity using the flow time of distilled water at 65°C. as the reference.

Chemical Analyses

The sulphate content was determined using the method of Jones and Letham (1954), pyruvic acid using the lactate dehydrogenase method (Duckworth and Yaphe 1970), and 3,6-anhydrogalactose using the method of Yaphe and Arsenault (1965).

Fractionation of Polysaccharides

The agar was fractionated by column chromatography on DEAE Sephadex A-50 (Duckworth and Yaphe 1971).

1.0 gm. of agar was dissolved in 100 mls of distilled water, cooled to 65°C. and adsorbed on a DEAE Sephadex A-50 (Cl^-) jacketed column (4 x 30 cm) maintained at 65°C. The column was eluted successively with distilled water, 0.5M, 1.0M and 2.5M

sodium chloride solutions. Before eluting with the next higher concentration of NaCl, the eluate was checked to ensure it was free of polysaccharide by the phenolsulphuric acid test. (Hay, Lewis and Smith 1965).

Each fraction was dialyzed overnight, concentrated in a rotary evaporator, precipitated with three volumes of ethanol and removed by centrifugation. They were oven-dried at 45°C. to constant weight and the weight recorded.

RESULTS AND DISCUSSION

Pomquet Harbour

Pomquet Harbour (see map, Fig. 1) is a system of shallow saline embayments with access to George Bay via a single narrow channel. A number of streams and a small river (Pomquet River) discharge into it.

Because the waters are shallow and replaced slowly they reach high temperatures in the summer and freeze over in the winter. Maximum surface temperatures (24°C) were recorded at our collecting station in July (see Fig. 2).

Saline conditions are maintained by tidal exchange, but salinity is reduced by fresh water discharge. Salinity in May was 23 p.p.t. and thereafter gradually increased reaching levels (28-29 p.p.t.) approximating those in George Bay by October (Fig. 2).

Levels of dissolved orthophosphate and reactive nitrate recorded during the summer are (as µg-atom per litre):

	Phosphate	Nitrate
June 4/4	0.1	0.96
June 22/73	3.7	0.36
July 27/73	6.9	0.42
Sept 11/73	3.6	0.30
Oct 20/73	0.1	0.14

Gracilaria Populations

The Gracilaria in Pomquet Harbour is a loose-lying population associated with eel-grass (Zostera) beds on muddy bottom. The plants tend to form hemispherical clumps, occasionally held together by the byssus threads of mussels (Mytilus) the weight of which helps anchor the plants.

Dense populations were present when the first collections were made in June. Maximum densities were observed in September at which time the plants were lush in appearance, and very clean. Collections made earlier in the year included much more mud and detritus adhering to the plants.

The population was greatly reduced by October 26. Much of it appeared to have been cast up on the shore.

Associated algae include Enteromorpha, Monostroma and Ceramium. There were abundant large plants of Sphaerotrichia divaricata and Stilophora rhizoides in September. These latter sometimes grow as epiphytes on the Gracilaria but were not observed to be abundant.

Yield

The total yields of polysaccharide after three extractions at 121°C. are shown in Table 1. These results seem to indicate that the yield does not vary greatly through the summer months.

The decrease in yield after alkaline-treatment at 85°C. is minimal except in one sample (June 4) where only 14% yield was obtained. This low yield may be due to losses caused by homogenization before alkaline treatment: in the other cases the seaweeds were homogenized after treatment.

Yield after alkaline-treatment at 65°C. (3% sodium hydroxide, 6 hrs) was less than 50% of that after treatment at 85°C.

Viscosity

The Gracilaria extracts exhibit high viscosity ηsp = 14-76 compared to Difco Agar (Lot Number 580019. ηsp = 5). The viscosity also varied a great deal from sample to sample (Table 2).

Except for one sample (September 11), the viscosity increases after alkaline treatment. This may be attributed to the greater purity of alkaline-treated extracts.

Gelling Temperature

The gelling temperature of 45 - 51°C. is comparatively high (Table 3).

Agars with a high gelling temperature have been shown to have high methoxyl contents (Guiseley, 1970). However, agar obtained from Porphyra contains high 6-O-methyl but gels at a normal temperature (38°C) (Yaphe and Duckworth, 1972). It seems therefore that a high gelling temperature could be attributed to methoxyl content positioned other than at carbon-6 of D-galactose residues. Gracilaria agar has been shown to have a high methyl galactose content (Hong et al 1969). It would be interesting to determine the position of the methyl groups in the agar.

Pyruvic Acid Content

Although pyruvic acid has been shown to be present in agars from a number of agarophytes (Young et al 1971) as 4,6-O-(1-carboxyethylidene)-D-galactose residue, no detectable pyruvate was found in the Gracilaria agar.

Sulfate and 3,6-anhydrogalactose Content

Alkaline treatment increased the 3,6-anhydrogalactose content, whilst decreasing the sulphate content of the agar (see Table 1). It has previously been shown (Rees 1961) that the elimination of sulphate at C6 of L-galactose residues in polysaccharides by either chemical or enzymatic methods results in the

formation of 3,6-anhydro-L-galactose residues and in an increase in gel strength of the polysaccharide.

The total sulphate tends to decrease while the 3,6-anhydrogalactose tends to increase in the mid and late summer months.

Fractionation of the Agars on DEAE Sephadex A50 (Cl^-)

Fractionation of Agar on DEAE Sephadex A50 (Cl^-) yields information on the gelling properties of the unfractionated material since fractions eluted with sodium chloride higher than one molar are non-gelling. (Duckworth and Yaphe 1971, Young 1973). In general the yield of gelling components from Gracilaria agar is comparable to Difco agar.

The yields after fractionation of both alkaline-treated and untreated agars are shown in Fig. 3. The yields after alkaline-treatment clearly show that the spectrum of polysaccharides has been changed with a reduction of the highly charged fraction and an increase in the lower charged fractions, particularly the fraction eluted with distilled water. The latter consists essentially of neutral polysaccharide, i.e. agarose, which has the highest gelling capacity. Thus the gel strength of the alkaline-treated agar is expected to increase.

The 3,6-anhydrogalactose content of the fractions (Table 4) confirms the results of Duckworth and Yaphe (1971) and Young (1973). The 3,6-anhydrogalactose decreases as the ionic strength of the eluate increases.

The sulphate content of the fractions can be expected to increase with the strength of the elutant since the fractionation is based on ion exchange and pyruvate is extremely low or absent.

Conclusions

As mentioned above, the yield of gelling components from the Gracilaria agar is comparable to that from Difco agar. However, the product appears not to be suitable for microbiological and other biomedical uses unless its high viscosity, high gelling temperature and elasticity are lowered by chemical modification, fractionation or blending with other agar.

It could be useful in the food industry and other industries where high viscosity and elasticity are required.

Since the neutral fraction of this agar is relatively large after alkaline treatment it may be a potential source for the production of agarose.

For industrial use, the seaweed is best collected between July and September when the sulphate content is low and the 3,6-anhydragalactose is high thus yielding a better product. At this time of the year also the raw material is clean and most abundant.

Fig. 1

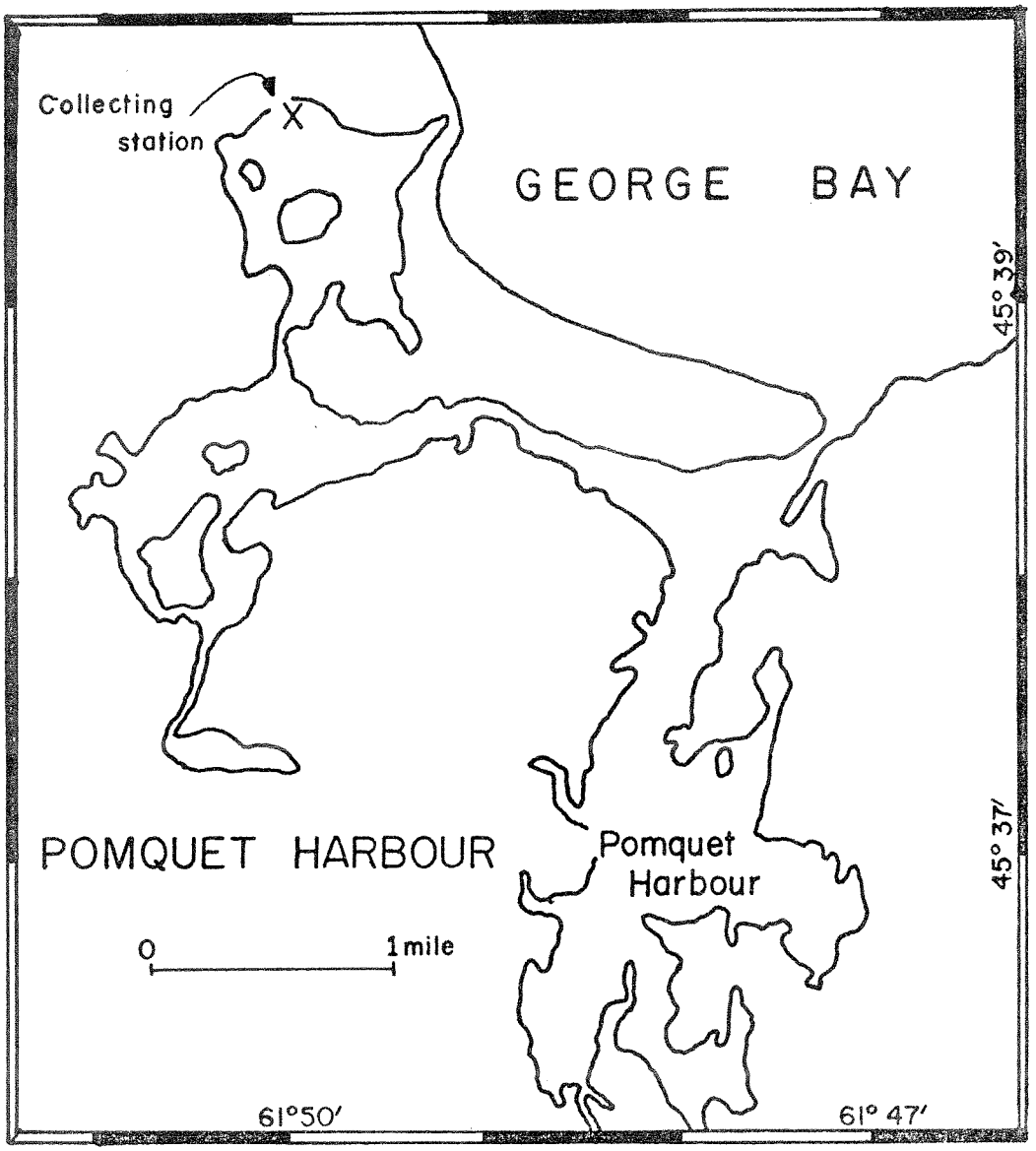

Fig. 2

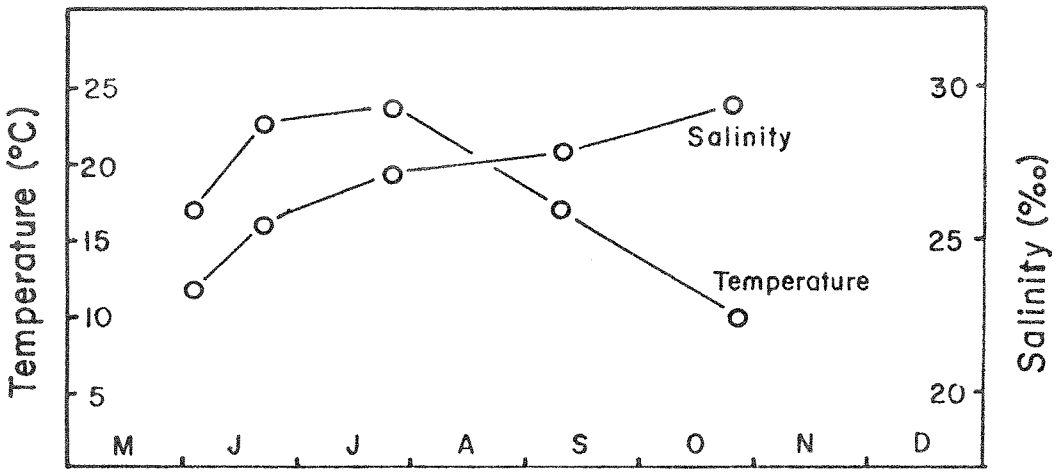

Fig. 1 Map of Pomquet Harbour. Fig. 2 Surface water temperature and salinity at collecting station, June to October 1973.

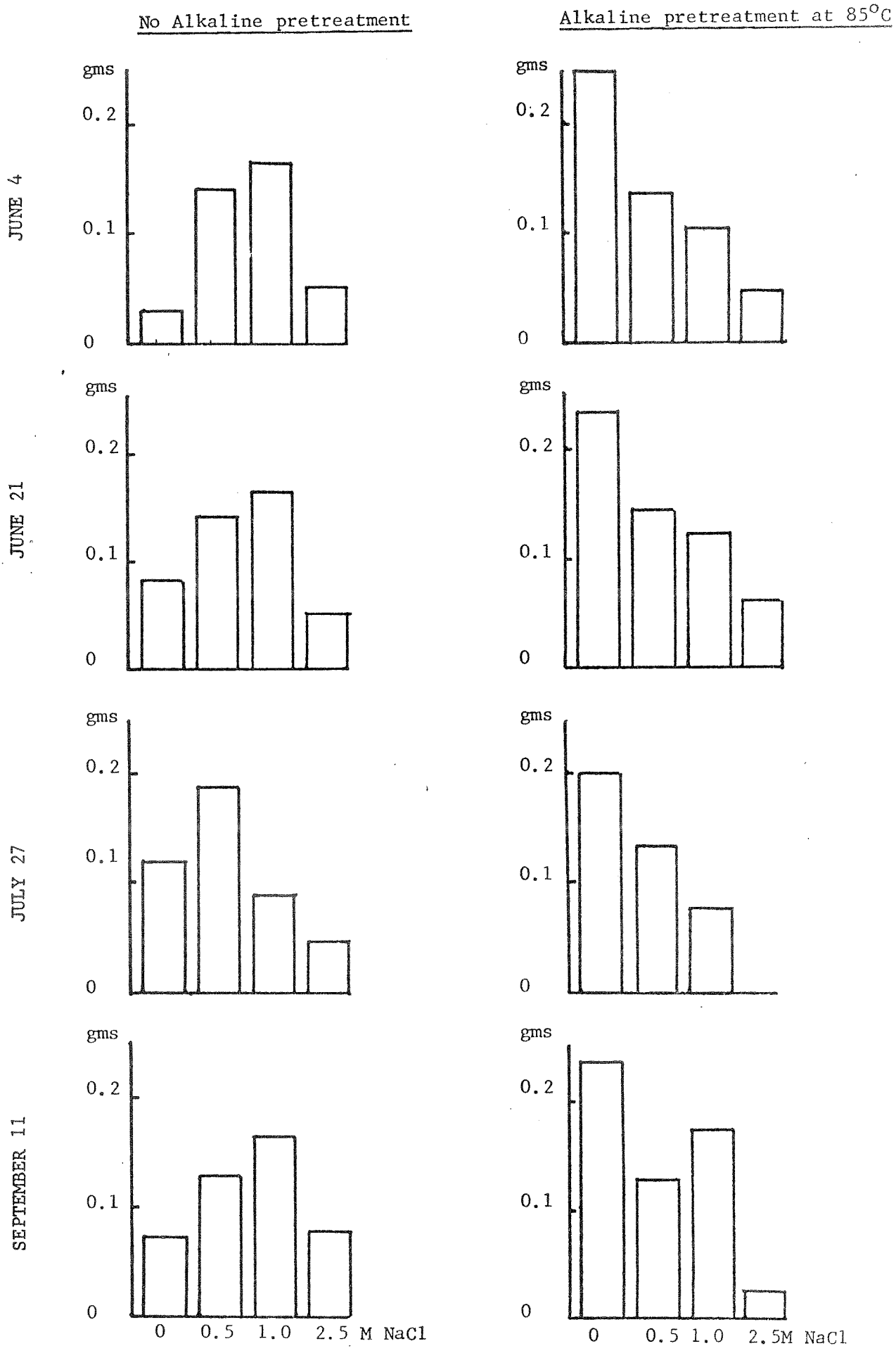

FIG. 3 - FRACTIONATION OF GRACILARIA EXTRACTS ON DEAE SEPHADEX A-50

TABLE I

YIELD, SULFATE AND 3,6-ANHYDROGALACTOSE CONTENT OF *GRACILARIA* SP.

	Yield of Extraction (%)		Sulfate (%)		3,6-anhydrogalactose %	
	-	+	-	+	-	+
June 4	27	14	4.00	2.50	23.14	23.46
June 21	24	28	3.40	1.45	19.09	26.22
July 27	29	20	2.75	1.85	26.54	32.74
September 11	29	23	2.80	1.85	28.87	32.40

\- = No Alkaline Treatment

\+ = Alkaline treatment at 85°C for 3 hrs. with 3% NaOH.

T A B L E 2

SPECIFIC VISCOSITY OF GRACILARIA EXTRACTS

	No Alkaline Treatment	Alkaline Pretreatment
June 4	12.94	76.21
June 21	31.29	42.32
July 27	15.53	24.66
September 11	66.99	21.63
Difco-Bacto Agar	4.90 (Lot No. 580019)	

T A B L E 3

GELLING TEMPERATURES OF GRACILARIA EXTRACTS

	No Alkaline Treatment (^{o}C)	Alkaline Treatment (^{o}C)
June 4	45.0	49.5
June 21	45.0	47.5
July 27	49.0	51.0
September 11	47.0	48.5
Difco-Bacto Agar	36.0 (Lot No. 580019)	

TABLE 4

3,6-ANHYDROGALACTOSE CONTENT OF DEAE SEPHADEX A-50 FRACTIONATED AGARS

		No Alkaline Treatment (%)	Alkaline Treatment (%)
June 4	Distilled Water	32.40	39.20
	0.5M NaCl	31.10	34.34
	1.0M NaCl	14.90	21.38
	2.5M NaCl	14.90	12.96
June 21	Distilled Water	37.91	38.56
	0.5M NaCl	29.81	23.33
	1.0M NaCl	19.44	15.23
	2.5M NaCl	17.17	10.37
July 21	Distilled Water	45.36	44.06
	0.5M NaCl	34.34	33.37
	1.0M NaCl	20.09	20.47
	2.5M NaCl	18.09	- -
Sept. 11	Distilled Water	47.63	45.04
	0.5M NaCl	29.19	26.89
	1.0M NaCl	19.44	18.79
	2.5M NaCl	14.58	18.14

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