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Gelidium robustum agar: quality characteristics from exploited beds and seasonality from an unexploited bed at Southern Baja California, México

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Abstract

The yield and gel properties of agar from *Gelidium robustum*, harvested in Baja California for industrial production is affected by season of collection and epiphyte loading. The alga is epiphytized to various extents by the bryozoan *Membraniphora tuberculata* ('conchilla') and the resulting calcareous crust on the alga diminishes the price of the seaweed biomass. Classification of the algal biomass quality by the agar industry is based on the apparent 'conchilla' content from visual examination. The different quality classes can be categorized quantitatively into premium class (30–40% w/w of 'conchilla' load), 2nd class (~ 50% w/w) and 3rd class (> 60% w/w). For samples collected at two exploited beds, the biomass obtained from Bahía de Tortugas had a lower epiphytic coverage than that from Bahía Asunción. The agar yield from different quality classes of *G. robustum* was strongly affected by the bryozoan epiphytic coverage, while its gel characteristics were not. Algae collected at Punta Prieta, an unexploited bed not affected by 'conchilla', showed seasonality in agar yield. It ranged between 17.5 and 44.2% with two maximum values observed, one in summer and the other in winter. Gel strength ranged between 515 and 665 g cm^{-2}, reaching a maximum during autumn.

Introduction

Gelidium robustum (Gardner) Hollenberg & Abbott is the principal agar source in Mexico. This species is distributed in the cold up-welling waters of Baja California, from Punta Descanso (32° 16′ N, 117° 02′ W) to Isla Margarita (24° 26′ N, 111° 51′ W), growing from the intertidal to 15–20 m depth in areas of high water motion.

The Mexican agar industry started in 1956 when a private company got a concession to harvest *G. robustum*, gathering an average of 650 dry t annually, to produce between 40 to 75 t of agar. On the other hand, fishermen cooperatives harvest between 100–150 dry t annually, which are sold directly to the private company or exported to the U.S.A. (Robledo, 1998). *G. robustum* is harvested throughout the year, but the best period is between May and September due to optimum weather conditions (Zertuche-González, 1993). The biochemistry of agar are known to change in response to variations in environmental conditions (Mouradi-Givernaud et al., 1992; Freile-Pelegrín et al., 1995). This is especially important on the southern Baja California coast where environmental factors can be considerably affected by different phenomena (i.e. El Niño-Southern Oscillation). One problem associated with *G. robustum* beds is related with the epiphytic bryozoan, *Membraniphora tuberculata* Bosc. ('conchilla') which forms a calcareous covering on the alga and lowers its the commercial value.

Gelidium robustum is considered the second most important seaweed resource in México after *Macrocystis pyrifera* (L.) C. Agardh. Although the former is important for the Mexican agar industry, its agar content and physico-chemical properties have little been studied and no data are available on agar characteristics from more or less epiphytized algae. Espinoza & Rodríguez (1992) reported seasonal variations in yield and gel strength on *G. robustum* agar from the Mexican coast, however, the methods used in their study were not classical. On the other hand, two agar seasonality studies exist for *G. robustum* harvested in Northern California, U.S.A. (Cooper & Johnstone, 1944; Barilotti & Silverthorne, 1972) showing completely different seasonal patterns in the agar content. These contradictions may indicate that major differences can exist among geographically separated populations as well as between those in the subtidal and intertidal zones. The aim of this study was to compare the agar characteristics from three different quality classes of *G. robustum* in relation to their epiphytic covering by 'conchilla' and collected from two industrially exploited beds, Bahía Tortugas and Bahía Asunción. On the other hand, the agar characteristics from algae collected from an unexploited bed, Punta Prieta, are discussed in terms of seasonality.

Material and methods

Plant collection

The study area is located on the northwest coast of Southern Baja California. It is divided into six zones corresponding to different cooperatives (Figure 1). The local industry has classified three categories of *G. robustum* biomass based on minor to major epiphyte loading, referred to as 'conchilla': premium, second and third quality classes. Samples of the three quality classes were collected from the biomass harvested in August 1997 by fishermen cooperatives at two exploited beds, Bahía Tortugas (zone 3) and Bahía Asunción (zone 5). The harvest at both locations was done by diving between 5 to 10 m depth using an air compressor in the boat (Hooka equipment). Epiphyte loading for each quality class was expressed as a percentage of epiphyte weight present on the original sample.

The material for the agar seasonal study was collected from October 1995 to August 1996 by scuba diving at 3 m depth at the unexploited bed of Punta Prieta (zone 6). Harvested plants were transported to the laboratory, washed thoroughly with tap-water and dried in an oven at 60 °C for 48 h. Subsamples of fresh material (n = 4) were weighed, cleaned thoroughly of epiphytes and re-weighed in order to express agar yield as dry weight percent of epiphyte free seaweed.

Agar extraction

Dry samples (25 g) were soaked in a 0.5% w/v solution of Na_2CO_3 at 85–90 °C for 30 min prior to extraction. In order to eliminate excess alkali, the seaweeds were washed with running tap-water for 10 min. Agar extractions ($n = 3$) were carried out following the method described by Freile-Pelegrín et al. (1995).

Gel properties

Agar gel strength, melting and gelling temperatures, were measured in triplicate on 1.5% w/v solutions, made with ground dry agar (Tecator Cyclotec mill, 0.5 mm particle size) in deionized water. Gel strength (g cm^{-2}) was measured after standing overnight at room temperature, by measuring the load that causes a cylindrical plunger (1 cm^2 cross section) to break the gel within 20 s (Armisén & Galatas, 1987).

Gelling temperature was measured as follows: a hot agar solution (10 ml) was poured into a test tube containing a glass bead (5 mm diameter). The tube was tilted up and down in a water bath at room temperature until the glass bead ceased moving. The temperature in the gel was then immediately measured with a precision thermometer (0.1 °C divisions).

Melting temperature of the gel in a test tube was measured by placing a lead bead (9 mm diameter) on the gel surface. The test tube was clamped in a water bath and the temperature raised from 50 to 100 °C at 1 °C min^{-1}; the melting point was recorded with a precision thermometer when the bead sank into the solution.

Chemical characteristics

The weight percentage of sulphate was determined in triplicate by hydrolyzing 1 g of agar powder (previously dried at 105 °C) with 10 ml HNO_3 in 100 ml Kjeldahl flasks. The completely hydrolyzed ester sulphates were quantitatively precipitated with barium chloride, collected on ash-free gravimetric paper filters, dried, ignited at 600 °C for 3 h and precisely weighed. The weight of the obtained barium sulphate, multiplied by 0.4116, gave the weight equivalent sulphate.

Statistical analysis

Data were tested for normality (Kolmogorov-Smirnov), and subjected to the Bartlett's test for homogeneity of group variances using a statistical software package (Stasoft). Pearson's product moment correlation test was used to determine the linear correlation between epiphyte load, agar yield and physico-chemical properties. ANOVA comparison of means was done using a Tukey's HSD test to determine the seasonal-

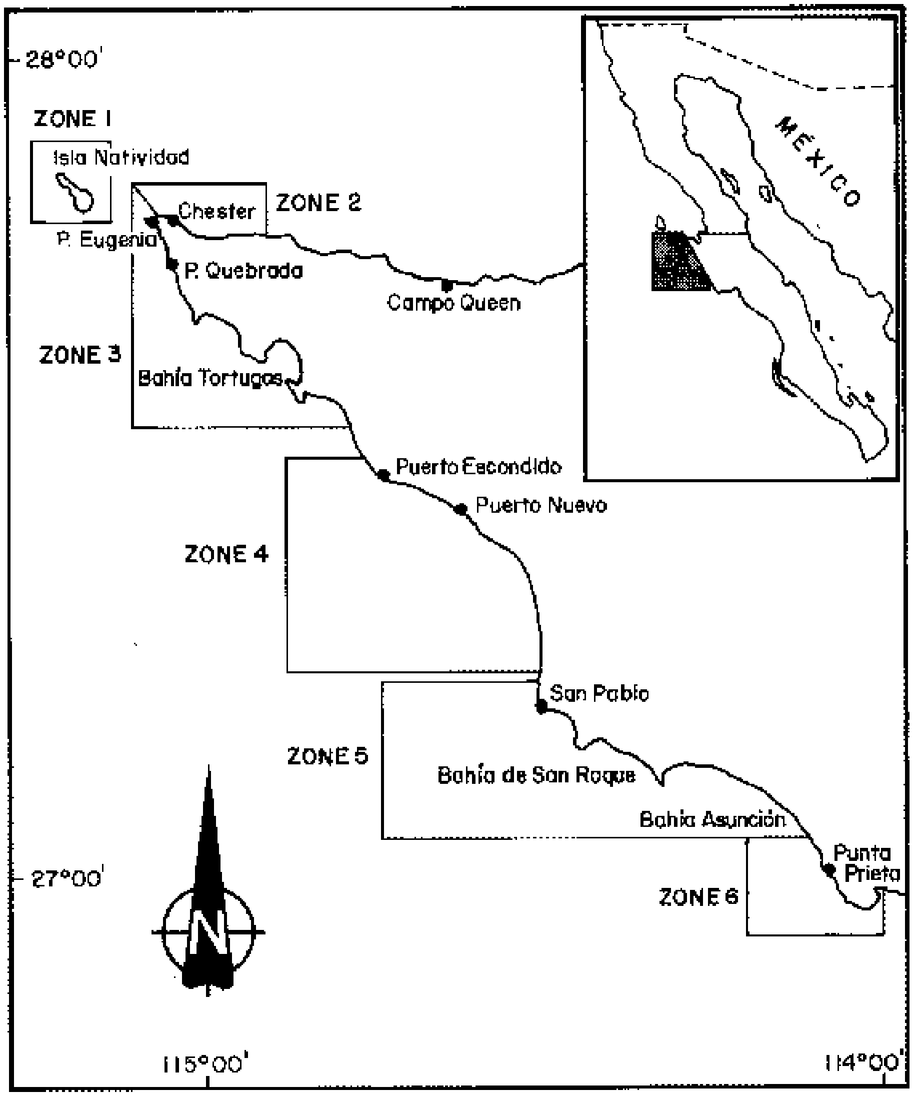

Figure 1. Map of Southern Baja California showing the distinct zones where *Gelidium robustum* is exploited.

ity and difference among the three quality classes. Groups with heterogeneous characters were tested using non-parametric Kruskal-Wallis one way analyses of variance.

Results

Quality classes at exploited beds

The epiphyte load of *G. robustum* from Bahía Tortugas and Bahía Asunción was mainly composed of the bryozoan epiphyte *Membraniphora tuberculata*. Samples collected at Bahía Tortugas showed an epiphyte content ('conchilla') varying between 31.2% w/w for premium class biomass, to 64.5% w/w for the 3rd quality class (Table 1). The epiphyte load of samples collected at Bahía Asunción was slightly higher for all quality classes, ranging from 44.5% w/w for the premium class, to 69.4% w/w for the 3rd class (Table 1).

The agar yield ranged between 21.1% for premium quality class to 13.9% for third class, from samples collected at Bahía Tortugas. The agar content in Bahía Asunción samples was slightly lower, and ranged between 19.2% for premium class to 10.5% for third class (Figure 2). ANOVA showed a significant dif-

Table 1. Epiphyte load ('conchilla') and physical properties of agar from three *G. robustum* quality classes at two exploited beds. Data are mean ± standard deviation.

	Epiphyte load (%)	Gel strength g cm^{-2}	Gelling temp. (°C)	Melting temp. (°C)
Bahía Tortugas				
1st class	31.2 ± 2.5	620 ± 14	35.0 ± 0.0	82.5 ± 0.7
2nd class	53.7 ± 2.5	623 ± 40	35.3 ± 0.2	89.2 ± 0.2
3rd class	64.5 ± 1.4	567 ± 22	34.4 ± 0.4	86.2 ± 1.0
Bahía Asunción				
1st class	44.5 ± 8.4	582 ± 27	34.7 ± 0.2	85.5 ± 0.0
2nd class	52.0 ± 3.1	650 ± 14	33.7 ± 0.5	86.2 ± 0.8
3rd class	69.4 ± 3.3	642 ± 41	35.0 ± 0.8	84.0 ± 0.7

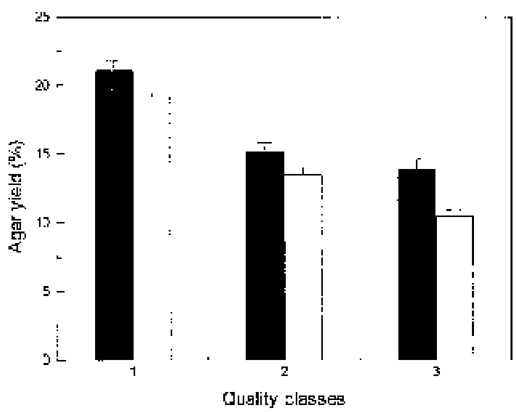

Figure 2. Agar yield of *G. robustum* from the three quality classes (premium, 2nd and 3rd) collected at Bahía Tortugas (black bar) and at Bahía Asunción (white bar). Bars represent standard deviation.

ference between the epiphyte load and the agar yield for the samples from the three quality classes at both localities ($p < 0.01$; Table 2). There was an inverse correlation between the epiphyte load and the agar yield for *G. robustum* from Bahía Tortugas ($r = -0.93$, $p < 0.01$) and from Bahía Asunción ($r = -0.97$, $p < 0.01$). However, no correlation was found between the epiphyte load and the agar gel strength, or between the former and the gelling and melting temperatures. The agar gel strength varied for all classes between 567 and 623 g cm^{-2} at Bahía Tortugas, and between 582 and 650 g cm^{-2} at Bahía Asunción, but there was no defined pattern between the two localities (Table 1). Gelling and melting temperatures of agar from the three quality classes of both localities are shown in Table 1. ANOVA showed no significant difference between the physical properties of agar from the three quality classes of both localities, except for the melting temperature at Bahía Tortugas (Table 2).

Seasonality of agar yield and characteristics from G. robustum *at unexploited bed*

The epiphyte load of *G. robustum* from Punta Prieta was mainly composed, throughout the year, of the red seaweeds *Laurencia* sp. and *Cryptopleura* spp. and by a very small amount of the bryozoan *Membraniphora tuberculata*.

The agar yield ranged between 17.5 and 44.2% reaching two maximum values, the highest in summer and another peak in winter months (Figure 3a). The agar gel strength ranged between 515 and 665 g cm^{-2}, with a maximum during autumn. In general, the gel strength decreased toward spring with a slight increase during the summer months (Figure 3b). Although the gel strength did not show any significant correlation with the agar content, the highest value of the former coincided with the minimum agar yield. The sulphate content ranged from 2.74 to 2.12% and was highest in autumn (Figure 3b). The agar gelling temperatures were higher in spring with a mean value of 35.7 °C, decreasing to a minimum in autumn (34.0 °C). The agar melting temperatures showed a similar seasonal pattern, ranging from 87.2 °C to 82.5 °C (Figure 3c). There was a positive correlation between the gelling and melting temperatures ($r = 0.75$, $p < 0.05$), and both were inversely correlated with the gel strength ($r = -0.78$, $p < 0.01$ for gelling temperature; $r = -0.85$, $p < 0.05$ for melting temperature). ANOVA showed a seasonal variation in agar content ($p < 0.01$)

Table 2. ANOVA test for significance differences among the three quality classes of *G. robustum* in epiphyte load and agar characteristics

Variable	df	F	p
Bahía Tortugas			
Epiphyte load (%)	2	182.73	0.0000[a]
Agar yield (%)	2	70.74	0.0000[a]
Gel strength (g cm^{-2})	2	1.70	NS
Gelling temp. (°C)[b]	2		NS
Melting temp. (°C)	2	45.59	0.0006[a]
Bahía Asunción			
Epiphyte load (%)	2	16.01	0.0011[a]
Agar yield (%)	2	180.85	0.0000[a]
Gel strength (g cm^{-2})	2	3.24	NS
Gelling temp. (°C)	2	2.62	NS
Melting temp. (°C)[b]	2	—	NS

[a]Highly significant (p <0.01); NS = no significant (p >0.05).
[b]Data with heterogeneous character.

and physico-chemical properties ($p < 0.01$) for the samples collected at Punta Prieta.

Discussion

Classification of algal biomass, made by fishermen cooperatives and the agar industry, is based on the apparent 'conchilla' content from visual examination. Based on our results, the different quality classes can be categorized quantitatively into premium class with a 'conchilla' content ranging from 30 to 40%, 2nd class with approximately 50%, and 3rd class above 60% w/w bryozoan epiphyte content. Bahía Tortugas is the zone with the highest *G. robustum* harvest values, corresponding to 60% of the total agar production from southern Baja California (Casas-Valdez & Fajardo, 1990). This may be related to the lower amount of bryozoan found for all *G. robustum* biomass classes collected at this site, compared with samples from Bahía Asunción.

In general, the price of seaweeds is related to quality class which depend on 'conchilla' load. This study has shown that agar content, but not its quality is dependent on epiphytic load. This is in agreement with recent studies on the agar from *Gelidium canariensis* (Grunow) Seoane-Camba (Freile-Pelegrín et al., 1995) and *Pterocladia capillacea* (Gmelin) Bornet et Thuret (Freile-Pelegrín et al., 1996), where different epiphytic coverings of two populations did not affect quality characteristics.

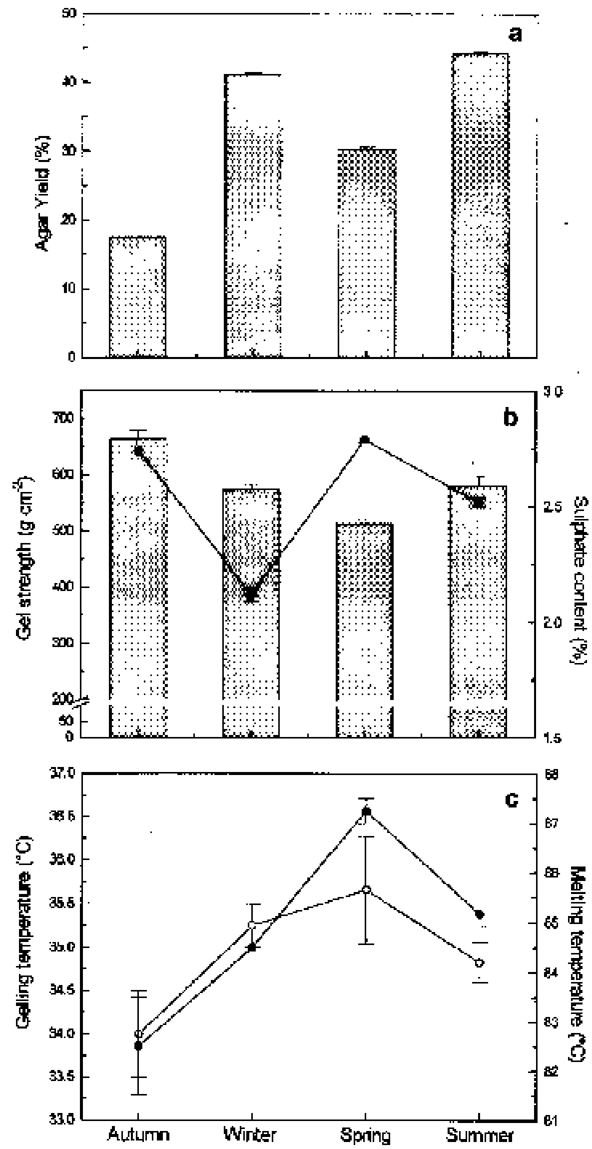

Figure 3. Seasonality of agar from *G. robustum* collected at Punta Prieta. (a) Agar yield expressed as percent of dry weight from cleaned seaweed. (b) Gel strength of 1.5% agar solution (bars) and sulphate content in agar samples (—•—). (c) Gelling (o) and melting (•) temperatures of 1.5% agar solution. Bars represent standard deviation.

On the other hand, reduction of the amount of light available for the algae can lower the agar yields (Santelices, 1988). Cancino et al. (1987) have shown that thalli of *Gelidium rex* Santelices et Abbot encrusted with *M. tuberculata* receive a lower irradiance and have a lower photosynthetic rate than non-encrusted thalli. According to Torres et al. (1991), maximal photosynthetic rates increase cell-wall polysaccharides synthesis in *Gelidium sesquipedale* (Clemente) Thuret. This may explain the differences found in *G. robustum* agar yield for the different quality classes.

In relation with the seasonal study, algae were collected at four periods over the year representing the most contrasting weather conditions in the region. A seasonal pattern for the agar yield and quality characteristics of *G. robustum* was shown. In general, the agar content increased towards the summer months, reaching values considered higher than commercial yields (17–25%). Similar results have been found for the same species (Barilotti & Silverthorne, 1972) and in *G. canariensis* collected at similar latitude (Freile-Pelegrín et al., 1995). The second peak may be related to slow growth rates influenced by the low seawater temperature during winter (Guzmán del Próo & De la Campa Guzmán, 1979). Under this condition, agar synthesis occurs at the expense of biomass production, as it has already been shown for other *Gelidium* species (Mouradi-Givernaud et al., 1992; Freile-Pelegrín et al., 1995).

During the spring months, when a decrease in temperature and high nutrient levels prevail due to up-welling conditions in the area, the lowest gel strength is found. Although nutrient enrichment has been proven to increase agar quality (Bird et al., 1981; Santelices, 1988), in *G. robustum*, the effect of high nutrient uptake is not reflected on the agar gel strength until summer when the plant reaches its highest growth (Barilotti & Silverthorne, 1972). The highest agar gel strength recorded for the autumn collected algae, could be related to an increase in water movement due to storms in this season, therefore, enhancing nutrient uptake and increasing the nitrogen status in *G. robustum*. The sulphate content of *G. robustum* agar is within the range required by industry (Armisén & Galatas, 1987), and there was no inverse correlation between this content and gel strength as other authors reported for other *Gelidium* species (Onraët & Robertson, 1987). The gelling and melting temperatures from *G. robustum* agar are similar to those of the commercially available agars obtained from *G. sesquipedale* (García, 1988). Based on our results, it is difficult to assess the optimum time for harvesting. In fact, industry collects this alga all year round, decreasing its harvest during autumn due to bad weather conditions. However, results showed that the best quality in terms of gel strength is found during these months when values are close to those of bacteriological agar (about 600 g cm^{-2}, McHugh, 1991).

G. robustum from Southern Baja California was shown to synthesize agar in quantity and quality suitable for industrial purposes. Agar yield is strongly affected by epiphytic covering, although values range within industrial requirements (17–25%). Agar gel strength is mainly affected by environmental conditions and this has to be taken into account when producing bacteriological grade agar from *G. robustum* biomass.

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