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Agar from Vegetative and Tetrasporic *Gelidiella acerosa* (Gelidiales, Rhodophyta)

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Agar yield and properties were determined from vegetative and tetrasporic *Gelidiella acerosa* collected from two localities on the northwestern coast of Luzon Island, Philippines. Occurrence and percent abundance of the different life stages confirmed spatial and temporal variations in reproductive periodicity. Agar was extracted using acetic acid pretreatment and steam pressure. Vegetative plants yielded a higher agar content with a high gel strength which could be related to the biochemical changes in the wall matrix polysaccharide composition of the thalli relative to its physiological state. Agar yield did not vary significantly between sites, while gel strength varied significantly between sites and with life stage. No definite trend was observed in the gelling and melting temperatures. A relatively low percent sulfate content (range = 1.6–2.2%) was measured in all samples, while 3,6-anhydrogalactose contents were slightly higher in tetrasporic (range = 41.7–49.7%) compared to vegetative (range = 41.6–46.6%) plants. In this agarophyte, there seemed to be either another chemical variable that affected the physical gel properties or molecular variations (molecular weight and length) in the heterogenous polysaccharides that compose each specific life stage.

Introduction

Gelidiella acerosa (Forsskål) Feldmann *et* Hamel a red alga, is among the agarophytes that produce industrial quality agar (Murano *et al.* 1996, Roleda *et al.* 1997). In this species, only tetrasporic and vegetative thalli have been encountered in the field. The sexual thalli appear to be lacking in the natural population. A doubtful report of the haploid sexual phase requires confirmation (Santelices 1990). Circumstantial evidence of a triphasic life history is also provided by the haploid and diploid nuclear DNA levels obtained from presumptive gametophyte plants in culture derived from tetraspores (Kapraun *et al.* 1994).

A distinct variation in the biochemistry of phycocolloids from different life stages of certain species of carrageenophytes has been reported in members of the Gigartinaceae. For instance, haploid gametophytes produce kappa-carrageenan while diploid sporophytes produce lambda-carrageenan (McCandless *et al.* 1973, 1975, Pickmere *et al.* 1973). Studies on *Gracilaria* and *Gelidium* species have shown that agar yield and quality differ between life stages of the same species. *Gracilaria verrucosa* (Hudson) Papenfuss from Chile yields more agar of lower gel strength from the cystocarpic thalli than from the tetrasporic counterpart, suggesting a difference in the chemical structure of the agar in each life stage (Kim and Henriquez 1979). *Gracilaria* (*verrucosa* type) from British Columbia, likewise, showed variations in the yields, gel strengths, and gelation characteristics depending on the season and life stages. The cystocarpic, tetrasporic, vegetative and male gametophytes are ranked in decreasing order as a source of agar (Whyte *et al.* 1981). On the other hand, agar yield and gel strength from the tetrasporic, cystocarpic (female) and male gametophytic populations of *Gracilaria bursapastoris* (Gmelin) Silva and *G. coronopifolia* J. Ag. from Hawaii exhibited no significant differences (Hoyle 1978). Likewise, *G. tikvahiae* McLachlan [as *G. foliifera* (Forsskål) Børgesen] from New Hampshire showed little difference in agar yield between its tetrasporic, cystocarpic and vegetative populations (Penniman 1977). A representative from the Gelidiales, *Onikusa pristoides* (Turner) Akatsuka [= *Gelidium pristoides* (Turner) Kützing], showed no significant differences in agar yield, gel strength, percent sulphate and 3,6-anhydrogalactose contents between sporophytes and gametophytes (Onraët and Robertson 1987).

Not withstanding the relative importance of *Gelidiella acerosa* as a source of raw material for the world agar industry, there has been no published studies to date comparing agar yield and properties of vegetative and tetrasporic life stages of this species. Moreover, geographic distribution, which is also reported to affect agar content and quality of a particular agarophyte species (Santelices 1988), has not been studied in the Philippine *G. acerosa* population. This study aimed to determine variation in the yield and quality of agar from the vegetative and tetrasporic life phases of *G. acerosa*. Furthermore, variation in agar biochemistry was also determined in plants collected from different localities along the northwestern coast of Luzon Island, Philippines.

Material and Methods

Study site

Three sites were sampled along the northwestern coast of Luzon Island, Philippines:

(1) Dos Hermanos Island is situated north of Santiago Island, Bolinao, Pangasinan (lat. 16°25.71' N, long. 119°55.87' E). Sampling was done in the largest of a group of three islets where a tide flat extends 20–50 meters from the island which is primarily composed of sandy-rocky and coral rubble substrate. The intertidal flat is relatively sheltered from surf and exposed to wind and tide driven water movement.

(2) Patar is on the norwestern side of Bolinao, Pangasinan (lat. 16°18.6' N, long. 119°46.8' E), 21 km away from Dos Hermanos Is. An intertidal flat extends to about 20–100 meters offshore and ends with a sudden drop. The substrate is solid bedrock and constantly exposed to surf splash especially during emersion of the flat. Strong waves are observed during the soutwest monsoon.

(3) Bo. Pangil, Currimao, Ilocos Norte (lat. 18°23.7' N, long. 120°58.9' E) is situated in a fringing reef with an intertidal belt extending 50–140 meters offshore, characterized by a solid bedrock substrate with numerous tidepools of various shapes and sizes near the landward margin and canals near the seaward margin. The lower intertidal area is characterized by a substrate composed of bedrock and boulders. This is a surf area constantly battered by strong wind driven waves especially during the southwest monsoons.

Collection of samples

The sampling months were pre-selected based on the observed reproductive periodicity of *Gelidiella acerosa* (Ganzon-Fortes unpublished observations) with reference to the maximum and minimum occurrence of each life stage. Approximately 4 kg fresh weight of *Gelidiella acerosa* were haphazardly collected from the intertidal flat of Dos Hermanos Is. and the lower intertidal zone of the reef at Bo. Pangil in August 1995. Since no tetrasporic plants were encountered at Dos Hermanos, an additional site on the intertidal flat in Patar was sampled along with Bo. Pangil in November 1995.

Harvested plants were cleaned of epiphytes and foreign material and sorted. The tetrasporic thalli were distinguished from the vegetative thalli by having stichidia (swollen structures) formed at the apices of the ultimate branchlets. These were washed with tap water and air-dried. Percent abundance of vegetative and tetrasporic thalli (by weight) were estimated from the total dry weight of the raw material harvested.

Agar extraction and analyses

Seaweed samples were pre-treated with acetic acid and subjected to one hour steam pressure extraction at 15–20 psi using a portable 20 L pressure cooker vessel heated by an electric stove (Roleda *et al.* 1997), while agar properties in terms of gel strength, gelling and melting temperatures, percent sulfate and 3,6-anhydrogalactose were analyzed also as described in Roleda *et al.* (1997). Agar yield was determined in duplicate due to limited amount of raw material, while analyses of agar properties were done in triplicate.

Statistical analysis

The statistical significance of differences in agar yield and properties between life stages, sites, sampling months and their interaction were tested using analysis of variance (ANOVA $p = 0.05$) using SPSS software.

Results

Thalli collected at Dos Hermanos Island, Bolinao (August 1995) were 100% vegetative, while specimens from Patar, Bolinao (November 1995) were 60% vegetative and 40% tetrasporic. In Pangil, Currimao, abundance of tetrasporic thalli (57%) predominated over the vegetative thalli (43%) in August 1995. In November 1995, vegetative thalli (67%) were more abundant than the tetrasporic thalli (33%) (Table I).

Agar yield from vegetative plants was significantly higher than from tetrasporic plants. Agar content of each life stage, however, did not vary significantly between sites (Table II). Gel strength of agar from vegetative plants in Dos Hermanos was measured at 523 g cm^{-2}. The sample from Patar had significantly higher gel strength in its vegetative life stage compared to its tetrasporic life stage at 365 and 284 g cm^{-2}, respectively. Significantly higher gel strength was also recorded in the agar of the vegetative plants (272 g cm^{-2}) than in the tetrasporic plants (61 g cm^{-2}) from Pangil in August 1995. In November, however, there was no significant difference (T-test, $p = 0.349$) in the gel strength of agar from the vegetative (307 g cm^{-2}) compared to the tetrasporic (303 g cm^{-2}) plants. Analysis of variance showed gel strength varied significantly between life stages, sampling months, sites and the interaction between life stages and months, and life stages and sites (Table II).

No definite trend was observed in the gelling and melting temperatures (Table I). However, both agar properties were found to vary significantly between life stages and sampling months, and their interaction effect. Furthermore, melting temperature was also found to vary significantly between life stages and sites, and their interaction effect.

Table I. Percent abundance of vegetative and tetrasporic *Gelidiella acerosa* plants and their agar yield and properties collected from Dos Hermanos Is. and Patar, Bolinao and Pangil, Currimao.

Date	Site	Life stage	Percent abundance (%)	Agar Yield (%)	Gel strength (g cm^{-2})	Gelling Temp (°C)	Melting Temp (°C)	% SO4	% 3,6-AG
Aug 95	Dos Herm.	Vegetative	100	27.7 (1)	523 (5)	44 (0)	91 (2)	2.0 (0.3)	46.0 (0.6)
		Tetrasporic	0	–	–	–	–	–	–
	Pangil	Vegetative	42.9	33.8 (4)	272 (4)	42 (0.6)	87 (0.6)	1.6 (0.2)	43.2 (0.9)
		Tetrasporic	57.1	17.9 (4)	61 (6)	37 (0.6)	71 (2)	2.1 (0.2)	49.7 (0.8)
Nov 95	Patar	Vegetative	60	38.7 (0)	365 (6)	43 (0.6)	85 (1)	2.1 (0.2)	40.2 (0.7)
		Tetrasporic	40	36.1 (0)	284 (4)	43 (1)	86 (2)	1.7 (0.6)	42.8 (0.3)
	Pangil	Vegetative	67.3	39.9 (1)	307 (2)	43 (0)	88 (0.6)	2.2 (0)	41.6 (0.3)
		Tetrasporic	32.7	34.1 (3)	303 (6)	45 (0)	92 (1)	1.9 (0)	41.7 (0.1)

Values in parenthesis are standard deviations (SD).

A relatively low percent sulfate content was measured from vegetative (range = 1.6–2.2) and tetrasporic (range = 1.7–2.1) plants. This chemical variable was found to a very significantly within each life stage over time (Pangil, Ilocos Norte sample). In terms of 3,6-anhydrogalactose contents of agar, significantly higher amounts were measured in tetrasporic plants (range = 41.7–49.7%) compared to vegetative (range = 41.6–46.6%) plants. Analysis of variance showed that 3,6-anhydrogalactose contents of agar varied significantly between life stages, sampling months and sites, and the interaction between life stage and date, and life stage and site.

Discussion

The occurrence and percent abundance of the different life stages of *Gelidiella acerosa* at different sampling sites indicated temporal and spatial variations in the reproductive periodicity of this species. This observation concurred with previous studies on its reproductive periodicity (Umamaheswara Rao 1973, Thomas *et al.* 1975, Rama Rao *et al.* 1976). A previous study, conducted at a different site in Bolinao, Pangasinan (Bo. Lucero, 4 km away from Dos Hermanos) observed maximum occurrence (70%) of tetrasporic thalli in August and minimum (14%) in February. Conversely, 100% vegetative thalli were observed in March and April (Ganzon-Fortes unpublished observation). It has also been noted that tetraporic *G. acerosa* persists throughout the year in Bo. Pangil, Currimao, Ilocos Norte with a maximum of 98% in August and a minimum of 9% in November (Ganzon-Fortes, unpublished observation).

The significant differences in the agar yield and properties of vegetative and tetrasporic thalli suggest differences in the biochemical make-up of the different life history phases. Profound biochemical changes occur within the thalli of cystocarpic plants (female gametophytes) of other macroalgae where carbon allocation would be diverted into compounds necessary for sporulation and maintenance of reproductive processes, such as nutritive cells and gonimoblast development (Littler and Arnold 1980).

The high agar yield and gel strength observed in vegetative *Gelidiella acerosa*, compared to its tetrasporic stage, differed from *Gracilaria* (*verrucosa* type) where tetrasporophytes were ranked above the vegetative plants as an agar source (Whyte *et al.* 1981). Moreover, in *G. verrucosa* from Chile, female gametophytes yielded higher amounts of agar but with a lower gel strength than the tetrasporophytes (Kim and Henriquez 1978). On the other hand, no significant variation was observed in the agar yields of tetrasporic, cystocarpic and vegetative populations of *G. tikvahiae* (as *G. foliifera*) from New Hampshire (Penniman 1977). Likewise, the agar yield, gel strength, percent sulfate and 3,6-anhydrogalactose showed no significant variations between the sporophytic and gametophytic populations of *Onikusa pristoides* (= *Gelidium pristoides*) (Onraët and Robertson 1987).

Agar yields from vegetative and tetrasporic plants collected from wave-exposed sites (Pangil, Currimao vs. Patar, Bolinao) showed little variation. Similarly no difference was observed in agars of the vegetative plants collected from a wave exposed site (Pangil, Currimao) or a protected site (Dos Hermanos Is., Bolinao). In the related species, *Pterocladia capillacea* (Gmelin) Bornet *et* Thuret, a higher yield was obtained from plants inhabiting an exposed site (surf area) along the southwestern coast of Brazil, whereas low agar yield was obtained from plants collected from a sheltered site (Oliveira *et al.* 1996). Agar yields of vegetative and tetrasporic plants collected in Pangil, Currimao were observed to vary between the two sampling months. This confirms temporal variation in the agar yield reported in the *Gelidiella acerosa* population in Dos Hermanos Is., Bolinao (Roleda 1997).

Whereas the gel strength of agar from vegetative and tetrasporic *Gelidiella acerosa* plants varied with

Table II. Analysis of variance and significance values for main effects and interactions of life stages, sampling months and sites on the agar yield and properties of *Gelidiella acerosa*.

Sampling sites/ Life stages/ Sampling date	Variable	Source of variation	df	F	p-value
Pangil, Ilocos Norte Vegetative vs. Tetrasporic August 1995 vs. November 1995					
	Yield	Life stage (A)	1	24.0	0.008*
		Date (B)	1	25.0	0.007*
		A × B	1	5.3	0.082 ns
	Gel strength	Life stage (A)	1	1440.1	0.000*
		Date (B)	1	2380.5	0.000*
		A × B	1	1334.7	0.000*
	Gelling temp.	Life stage (A)	1	33.3	0.000*
		Date (B)	1	261.3	0.000*
		A × B	1	133.3	0.000*
	Melting temp.	Life stage (A)	1	88.9	0.000*
		Date (B)	1	315.1	0.000*
		A × B	1	258.8	0.000*
	Sulfate	Life stage (A)	1	1.0	0.344 ns
		Date (B)	1	9.1	0.017 ns
		A × B	1	36.8	0.000*
	3,6-AG	Life stage (A)	1	81.2	0.000*
		Date (B)	1	173.7	0.000*
		A × B	1	75.8	0.000*
Patar, Bolinao Vegetative vs. Tetrasporic					
	Yield		1	112.3	0.001*
	Gel strength		1	372.1	0.000*
	Gelling temp.		1	0.8	0.422 ns
	Melting temp.		1	0.3	0.609 ns
	Sulfate		1	1.2	0.336 ns
	3,6-AG		1	43.2	0.003*
November 1995 Vegetative vs. Tetrasporic Pangil vs. Patar					
	Yield	life stage (A)	1	15.8	0.017*
		Site (C)	1	0.1	0.725 ns
		A × C	1	2.3	0.201 ns
	Gel strength	Life stage (A)	1	227.6	0.000*
		Site (C)	1	50.0	0.000*
		A × C	1	186.9	0.000*
	Gelling temp.	Life stage (A)	1	8.2	0.021*
		Site (C)	1	4.2	0.076 ns
		A × C	1	1.5	0.256 ns
	Melting temp.	Life stage (A)	1	10.9	0.011*
		Site (C)	1	32.0	0.000*
		A × C	1	5.6	0.046*
	Sulfate	Life stage (A)	1	3.9	0.082 ns
		Site (C)	1	0.6	0.460 ns
		A × C	1	0.04	0.845 ns
	3,6-AG	Life stage (A)	1	38.6	0.000*
		Site (C)	1	0.3	0.624 ns
		A × C	1	32.6	0.000*
Vegetative August 1995 Dos Hermanos vs. Pangil					
	Yield		1	5.1	0.151 ns
	Gel strength		1	5049.1	0.000*
	Gelling temp.		1	49.0	0.002*
	Melting temp.		1	14.0	0.020*
	Sulfate		1	5.5	0.079 ns
	3,6-AG		1	27.5	0.006*

* significant; ns, not significant.

sampling sites and months and the interaction between the factors (life stage × date, lifestage × site), no significant difference was observed in the seasonal gel strength of agar from *Gelidium canariensis* (Grunow) Seoane-Camba collected from two study sites in Spain (Freile-Pelegrin *et al.* 1995). The physical properties of agar, such as gel strength, gelling and melting temperatures, are affected by the presence of sulfate, pyruvate and methoxyl residues in its structural unit (Craigie 1990). In this study, agar sulfate content of tetrasporic and vegetative thalli did not vary significantly, which implied that regardless of its life history, *Gelidiella acerosa* synthesized low L-galactose 6-sulfate or this compound underwent active enzymatic conversion to the anhydro form resulting in enhancement of its gel forming ability. The 3,6-anhydrogalactose content was found to be higher in tetrasporic plants than in vegetative plants but gave a low agar gel strength. In other studies, the increase in agar gel strength has been correlated with the increase in 3,6-AG and decrease in sulfate content (Lemus *et al.* 1991). Furthermore, no definite trend was observed in the gelling and melting temperatures of agar from vegetative and tetrasporic thalli in different sites. Most studies have directly related the increase in gelling temperature to methoxyl content, either by 6-O-methyl D-galactose or methyl groups on other secondary positions (Yaphe and Duckworth 1972, Cote and Hanisak 1986), as well as the molecular weight of the polysaccharide (Kapraun *et al.* 1994). In *G. acerosa*, there seemed to be either

1) another chemical variable that must have affected these physical gel properties, or

2) variation in the heterogenous composition (molecular weight and length) of polysaccharides that compose the agar during the specific life stage of this species.

To date, production of *G. acerosa* is dependent on the natural stocks available. Based on the result of this study, regulated harvesting of this agarophyte should be done during the vegetative life stage of the plant. Aside from obtaining optimum agar yield and quality from vegetative plants, this will allow the reproductive plants to shed spores for new recruits representing the missing sexual phase.

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