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Quantification and characterization of nuclear genomes in commercial red seaweeds (Gracilariales) from the Philippines

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Abstract

Eight species of Gracilariaceae from the Philippines, representing the genera *Gracilaria*, *Gracilariopsis* and *Hydropuntia*, were investigated to quantify and characterize their nuclear genomes. DNA reassociation kinetics were used to determine nuclear genome organization and complexity in six of these species. Results indicate the presence of three second order components corresponding to fast, intermediate and slow fractions. Repetitive sequences varied from 13–74% and unique DNA ranged from 26–84%. Microspectrophotometry with the DNA-localizing fluorochrome DAPI was used to quantify nuclear DNA contents. Comparisons of mean nuclear DNA (I_f) values to chicken erythrocytes (RBC) resulted in an estimate of 0.38–0.43 pg/2 C genomes for seven of the species investigated. Preliminary analyses of agar content and quality confirm the economic potential of *Gracilaria firma*, *Gracilaria* sp. 2 from Sorsogon and *Gracilariopsis bailinae*. Nuclear genome profiles developed from data for genome size, organization and complexity are compared with data for agar quantity and quality. Gel quality and quantity do not appear to be correlated with either large repetitive fraction DNA or a high degree of genome complexity.

Introduction

The seaweed *Gracilaria* is the most economically important source of agar in the Philippines (Hurtado-Ponce & Umezaki, 1988). Both species which produce hard, brittle gels required for bacteriological agars, and the soft, elastic gels preferred for food industry applications have been identified (Hurtado-Ponce, 1992b). In the Philippines, *Gracilaria* production relies on harvest of wild populations (Guanzon & de Castro, 1992). A general decline in production in 1987 has been attributed to depletion of stocks due to unregulated harvesting practices (Hurtado-Ponce et al., 1992b). Inevitably, stabilization of production capacity and control of agar quality will require a shift from the harvest of mixed-species wild populations to a reliance on mariculture of carefully selected strains (Hurtado-Ponce & Umezaki, 1987, 1988).

The present paper reviews our continuing effort to quantify and characterize nuclear genomes in commercially important agarophytes (Kapraun et al., 1994; Lopez-Bautista & Kapraun, 1995), and to relate the observed genome profiles to agar quality and quantity (Kapraun et al., 1993b). It is hoped that this information will provide a basis for cultivar selection and, eventually, the genetic improvement of fast-growing strains. Preliminary data are included for agar quantity and quality to relate genome profiles to agar biosynthesis (Bird & Hinson, 1992). However, detailed agar analyses will be published elsewhere.

Materials and methods

Source of specimens

Collection sites for the eight species of the Gracilariaceae included in the present study are listed in Table 1. Algal samples were fixed in Carnoy's solution and stored in 70% ethanol (Kapraun & Bailey, 1992). Algal material for cytophotometry was fixed and prepared for examination on subbed slides as described previously (Kapraun et al., 1992).

Coverslips with attached medullary cells were stained with DAPI (0.5 μg ml^{-1} 4′,6′-diamidino-2-phenylindole) (Goff & Coleman, 1985, 1990). Detailed procedures for DNA quantification of red algae with DAPI have been specified previously (Kapraun et al., 1992). Nuclear DNA contents for algal samples were estimated by comparison of fluorescence intensity (I_f) values for algal nuclei and chicken erythrocytes (RBC): RBC I_f/algal I_f = 2.4 pg/× pg (Clowes et al., 1983). The number of slides (specimen samples) and nuclei examined, and the estimated nuclear genome sizes are recorded in Table 2.

DNA isolation and characterization

Detailed procedures for nuclear DNA isolation and purification (Dutcher et al., 1990) and requirements for efficient, reproducible reassociation kinetics have been specified previously (Britten & Kohne, 1970). In the present study, DNA reassociation kinetics were carried out under moderately stringent conditions ($T_m - 25$°C, 0.18 M Na^+). Reassociation data were analyzed with a computer program generating a best-fit non-linear regression representing three second-order components (Kapraun et al., 1992). DNA of *Escherichia coli* (4.2×10^6 bp, C_0t 1/2 pure 2.93) was used as a standard to convert C_0t values into nucleotide base pairs or complexity of each component for algal samples (Lewin, 1990).

Agar analysis

Sun dried samples of the Gracilariaceae from the Philippines were ground to a powder in a blender and analyzed for agar yield (Lemus et al., 1991). Native agar analyses were performed on samples (3 g per replicate) held for 48 h at 40°C in a forced air herbarium plant drier and then extracted directly in distilled water. Alkali treatment involved pretreatment with 1 N NaOH at 80°C for 1 h, followed by neutralization and extraction in water. Agars were filtered through Celite 545 (Fisher Scientific, Pitsburgh, PA 15219) and a 10 μm polypropylene filter using pressure filtration with N_2 gas (Lemus et al., 1991). Gel strengths were determined for 1.5% gels in plastic containers with a 1 cm^{-2} plunger at the top of the gels, and a Marine Colloids (FMC Bioproducts, Rockland, ME 04841) gelometer. Dynamic gelling temperature was recorded with a thermometer, and melting temperature using small metal beads (Bird & Hinson, 1992). All determinatins were made in triplicate.

Results

Microspectrophotometry

DAPI staining with the protocol modified after Goff & Coleman (1985, 1990) resulted in reproducible, stable fluorescence of medullary nuclei without appreciable cytoplasmic interference. Comparison of I_f values for algal samples and RBC permitted extrapolation of algal DNA contents (Table 2). For species in which both gametophyte and carposporophyte specimens were examined, variations in nuclear DNA levels associated with ploidy level differences were observed (e.g. see data for *Gracilaria salicornia* (C. Agardh) Dawson in Table 2). In such cases, estimated DNA contents for G_2-phase haploid gametophyte nuclei and for G_1-phase diploid carposporophyte nuclei were essentially identical (Table 2). In addition, DNA levels for 2 C nuclei closely approximate 50% of the 4 C values. Extrapolated nuclear DNA contents reveal a 2 C genome size of 0.38–0.42 pg for five species of *Gracilaria* (*G. arcuata* Zanardini, *G. eucheumoides* Harvey, *G. firma* Zhang *et* Xia, *G. salicornia* (C. Agardh) Dawson and *Gracilaria* sp. #2). *Gracilariopsis bailinae* Zhang *et* Xia (= *Gracilariopsis heteroclada* Zhang *et* Xia, 1991) and *Hydropuntia fastigiata* (Zhang *et* Xia) Wynne had similar genome sizes of 0.43 and 0.38 pg, respectively (Table 2).

Reassociation kinetics

Computer analysis of DNA reassociation kinetics for five species of *Gracilaria*, and for *Hydropuntia fastigiata* indicate the presence of three second-order components (Table 3): a fast fraction representing highly repetitive sequences, an intermediate fraction of mid-repetitive sequences and a slow fraction of unique or single-copy sequences (Figure 2). Repeated sequences

Table 1. Source of specimens

Species	Location	References
Gracilaria		
G. arcuata Zanardini	Bolinao (Pangasinan)	Abbott 1994
G. eucheumoides Harvey	Bolinao (Pangasinan)	Abbott 1994
G. firma Zhang *et* Xia	Bolinao (Pangasinan)	Abbott 1994
G. salicornia (C. Agardh) Dawson	Mactan Is. (Cebu)	Abbott 1994
Gracilaria sp. 1	Baguey, Cagayan (northern Luzon)	Trono et al. 1983
Gracilaria sp. 2	Sorsogon	Taw 1993
Gracilariopsis		
G. bailinae	Mactan Is. (Cebu)	Abbott et al. 1991
Hydropuntia		
H. fastigiata (Zhang *et* Xia) Wynne	Bolinao (Pangasinan)	Wynne 1986, Abbott 1991

Table 2. Nuclear genome size (pg) in species of the Gracilariales

Species	Number of slides	Number of nuclei	Nuclear genome size (pg) 1 C	2 C	4 C
Gracilaria					
G. arcuata	3	81		0.38 ± 0.02	
	1	24			0.84 ± 0.25
G. eucheumoides	4	142		0.42 ± 0.01	
G. firma	1	18		0.38 ± 0.08	
G. salicornia	3	124	0.22 ± 0.01		
	4	79		0.39 ± 0.01	
	1	15			0.78 ± 0.05
Gracilaria sp. 2	3	67		0.38 ± 0.02	
Gracilariopsis					
G. bailinae	6	227	0.21 ± 0.02		
	5	157		0.43 ± 0.01	
	1	13			0.79 ± 0.04
Hydropuntia					
H. fastigiata	3	80		0.38 ± 0.02	

varied from 11–74% (Table 3). Use of dilute concentrations (50–60 μg ml^{-1}) required by optical (UV spectrophotometry) measurement prevented a determination of slow component C_0t and, thus, of genome size. This information was obtained by cytophotometry (Table 2).

Agar characterization

Preliminary data for agar yields, quality and chemistry for four species of the Philippine Gracilariaceae are summarized in Table 4. Additional data for these and other species will be published separately. Agar yields following alkali pretreatment ranged from 10–24% (Table 4). Gel strengths of modified agars varied greatly, from 188–905 g cm^{-2}. Highest values of 876 and 905 g cm^{-2} were found in agars from *Gracilaria*

Table 3. Kinetic analysis of DNA reassociation for species of *Gracilaria* and *Hydropuntia*

Isolate & kinetic component	Fraction (%)	Complexity (base pairs)	Repetition frequency
G. eucheumoides			
Fast	11	–	–
Intermediate	63	–[1]	–
Slow	26	3.16×10^8	1
G. firma			
Fast	3	–	–
Intermediate	22	2.5×10^6	38
Slow	75	3.16×10^8	1
G. salicornia			
Fast	3	–	–
Intermediate	13	2.86×10^6	17
Slow	84	3.16×10^8	1
Gracilaria sp. 1			
Fast	11	–	–
Intermediate	63	2.6×10^6	7
Slow	26	0.07×10^8	1
Gracilaria sp. 2			
Fast	3	–	–
Intermediate	22	1.74×10^6	46
Slow	75	2.77×10^3	1
H. fastigiata			
Fast	1	–	–
Intermediate	12	3.2×10^6	14
Slow	87	3.35×10^8	1

[1] *E. coli* standard reached 44% (50% required) preventing calculation of intermediate complexity values.

firma and *Gracilariopsis bailinae*, respectively. Agars from these two species had both high gelling (> 42°C) and high melting (> 90°C) temperatures.

Discussion

Absence of taxonomic stability is often a significant impediment to the efficient management and utilization of seaweed resources (Abbott, 1988). Recently, molecular characteristics have been shown to be powerful tools to augment traditional morphological features in delimiting taxa in the Gracilariales (Goff et al., 1994). Recognition of the genus *Gracilariopsis* circumscribed by Fredericq and Hommersand (1989) and amended by Steentoft et al. (1995) is increasingly supported by molecular studies using both plastid DNA restriction patterns (Bird & Rice, 1990; Rice & Bird, 1990; Goff, 1993; Bird et al., 1994) and molecular sequences of the nuclear genes encoding small-subunit ribosomal RNAs (18S rRNAs) (Scholfield et al., 1991; Bird et al., 1992; Ragan et al., 1994; Goff et al., 1994). In the present study, species have been referred to three of the seven genera currently recognized in the family Gracilariaceae: *Gracilaria, Gracilariopsis* and *Hydropuntia* (Bird & Rice, 1990). The generic name *Hydropuntia* (including *Polycavernosa* Zhang *et* Xia, 1963) is retained as proposed by Wynne (1986) even though the relationship of this entity to *Gracilaria* remains uncertain (Abbott et al., 1991; Bird et al., 1992; Gargiulo et al., 1992; Bird, 1995).

Microspectrophotometry

Present data for Philippine specimens support the generalization that the Gracilariaceae are characterized by a narrow range of small genome sizes. Estimates of 0.38–0.43 pg are well within the range of 0.33–0.47 pg previously reported in this family (Kapraun, 1993; Kapraun et al., 1993a; Lopez-Bautista & Kapraun, 1995).

Reassociation kinetics

Six species of the Gracilariaceae in this study exhibited a range of 26–87% unique DNA sequences. To date, reassociation kinetics data for 14 species representing three genera (*Gracilaria, Gracilariopsis* and *Hydropuntia*) indicate a range of 5–87% unique DNA sequences (Dutcher et al., 1990; Kapraun et al., 1993a; Lopez-Bautista & Kapraun, 1995).

Comparison of the percentage of unique to repetitive sequences (U/R ratio) (Dutcher et al., 1990) reveals wide variations among the 14 species for which reassociation kinetics data are available: U/R = 0.05–6.7 (Dutcher et al., 1990; Lopez-Bautista & Kapraun, 1995). Present results are consistent with our previous speculation that speciation may have been accompanied by changes in the ratio of unique to repetitive segments rather than by segment multiplication which typically results in genome size increase (Wenzel & Hemleben, 1982). It has been suggested previously that these unique sequences may represent diverged repeated sequences (or fossil repeats) of little-transformed isochores (non-coding nucleotide sequences) (Kapraun et al., 1993b).

Table 4. Characterization of 1 N NaOH alkali modified agars from *Gracilaria*, *Gracilariopsis* and *Hydropuntia*. Gel strengths, gelling and melting temperatures were determined using 1.5% w/v agar samples. All determinations were made in tripliate

Species	Agar yield (% dry weight)	Gelling temp. (°C)	Melting temp. (°C)	[2] Gel strength G cm^{-2})
Gracilaria firma	20	42	94.6	876
Gracilaria sp. 2	[1]24	38	92	670
Gracilariopsis bailinae	21	45	90	905
Hydropuntia fastigiata	10	44	86.6	188

[1] Data from analyses performed at the University of the Philippines
[2] A Difco agar sample gave gel strengths of 367 g cm^{-2}, and a commercial *Gracilaria* agar (TIC Gums) was determined to be 703 g cm^{-2}.

Agar characterization

Although data included in this study are preliminary, they confirm the commercial potential of *Gracilaria firma* and *Gracilariopsis bailinae*, with gel strengths of 876 and 905 g cm^{-2}, respectively. Philippine cultivars of *Gracilariopsis bailinae* have been characterized previously as producing ideal commercial gels which are strong, elastic, non-rigid and flexible (Hurtado-Ponce, 1992a, 1992b; Luhan, 1992).

We have speculated that genomes with large repetitive fractions have an abundance of gene loci for enzymes associated with polymerization of galactan subunits (Dutcher et al., 1990). In the present study, as well as in a recent parallel investigation of Mexican agarophytes, large repetitive fraction DNA and a high degree of complexity (number of base pairs) were correlated with species of the Gracilariaceae having both poor and good gel quality (Lopez-Bautista & Kaparaun, 1995). Consequently, it now seems unlikely that target gene loci associated with enzymes for polymerization of galactan subunits are restricted to particular DNA kinetic components.

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