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ORIGINAL RESEARCH

Growth rate and carrageenan analyses in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in the subtropical waters of São Paulo State, Brazil

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Abstract Growth rate, semi-refined and refined carrageenan yields, 3,6-anhydrogalactose and sulphate contents, and gel strength were investigated in four strains of *Kappaphycus alvarezii* (brown, green and red tetrasporophytic strains, and one strain derived from tetraspores progeny, called G11) farmed in subtropical waters of São Paulo State, Brazil. All studied strains showed higher growth rates from February to May, decreasing from July to December. The G11 strain exhibited lower growth rates, but had semi-refined and refined carrageenan yields significantly higher than the others and similar to a commercial sample. A negative correlation between growth rate and semi-refined carrageenan yield was verified for all strains. The brown strain had the lowest content of 3,6-anhydrogalactose, while G11 and green strains showed higher values. No clear pattern of variation and no relationship were observed for sulphate and gel strength. However, all strains presented gel strength values near the one obtained from commercial sample. These results show that the carrageenan extracted from brown, green, red, and G11 strains of *K. alvarezii* cultured in subtropical waters of São Paulo State, Brazil with commercial potentials.

Key words eucheumatoid species · exotic species · phycocolloids · seaweed

Dr. Edison Jośe de Paula passed away in 2003.

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Introduction

Kappaphycus alvarezii (Doty) Doty ex P. Silva is an important source of *kappa* carrageenan, a hydrocolloid that has been widely used in industry as gelling and thickening agent. This species has been farmed successfully in the Philippines since the 1970s, and derived strains have been introduced in more than 20 countries for mariculture purposes [(Trono 1993; Ask and Azanza 2002; Paula et al. 2002). About 120,000 dry tonnes year^{-1} of *K. alvarezii* are harvested mainly from the Philippines, Indonesia and Tanzania (Zanzibar), and it is responsible for 70% of worldwide processed raw seaweed for carrageenan production (Areces 1995; McHugh 2003).

There are two different commercial methods of producing carrageenan (McHugh 2003). In the first method, the carrageenan is never extracted from the seaweed. All soluble matter is washed out of the seaweed that will dissolve in alkali and water, leaving the carrageenan and other insoluble matter behind. This insoluble residue, consisting largely of carrageenan and cellulose, is then dried and sold as semi-refined carrageenan. In the second method, carrageenan is extracted from the seaweed into an aqueous solution, and the seaweed residue is removed by filtration. Then, it is recovered from the solution, eventually as a dry solid containing the refined carrageenan. This recovery process of refined carrageenan is difficult and expensive relative to the costs of the semi-refined carrageenan.

Carrageenan represents an annual global market over US$ 200 million, and in the last three decades it has been increased 5% year^{-1}, from 5,500 metric tonnes in 1970 to over 20,000 metric tonnes expected in 1995 (Bixler 1996). Brazil produces low amounts of carrageenan, approximately 10 tonnes year^{-1}, from natural harvests of *Hypnea musciformis* (Wulfen) Lamouroux (Oliveira 1997). This production is not enough to supply the national requirement, which is about 1,000 tonnes year^{-1}, it being necessary to import 2,000 tonnes of *K. alvarezii* from the Philippines (Furtado 1999).

The increase of Brazilian and worldwide consumption of carrageenan stimulated in 1995 an experimental introduction of the exotic species *K. alvarezii* in the subtropical waters of Brazil. Since then, several studies have been performed to evaluate the viability of commercial farms of this exotic species, from social, economical and environmental points of view (Paula and Pereira 1998; 2003; Paula et al. 1998a, b; 1999; 2001; 2002).

The present study has evaluated the growth rate, carrageenan yield and gel qualities of four strains of *K. alvarezii* farmed at Ubatuba Bay, São Paulo, Brazil, between February and December 2001.

Materials and methods

Brown, green and red tetrasporophytic clones strains of *Kappaphycus alvarezii* and one strain derived from the germination of tetraspores, called G11, were harvested from Mariculture Research Center (Núcleo de Pesquisa do Litoral Norte da Secretaria de Agricultura e Abastecimento do Estado de São Paulo) located at Ubatuba Bay, São Paulo State, Brazil (23° 26.9′S; 45°0.3′W) (Paula et al. 1999; 2002). The samples were collected from February to December 2001 at approximately 1-month intervals. Water temperature and salinity were measured early morning twice per month and represented as mean values.

Growth rate (GR) was calculated following the equation (Lignell and Pedersén 1989): GR (% day^{-1})= $[(Wt/Wi)^{1/n} - 1] \times 100$, where Wi = initial wet weight and Wt = wet weight after t days (approximately 30 days).

Two sorts of carrageenan (semi-refined and refined) were obtained from 20 to 50 g of dry seaweed after alkaline transformation. Two transformations were made: (a) “cold” alkali transformation (Sijan and Ping 1984), and (b) hot alkali transformation (Ohno et al. 1994). For semi-refined carrageenan, dry samples were soaked in 1 l of 6% KOH for 24 or 48 h in room temperature (ca. 25°C) (“cold” alkali transformation), washed with abundant water, sun bleached and dried at 60°C until they reached constant weight. Refined carrageenan was obtained by incubating “cold” alkali-transformed samples in 400 mL of 6% KOH at 80°C for 2 h (hot alkali transformation). The seaweeds were then washed and extracted in 400 mL of distilled water at 80°C for 2 h. After that, the solution was filtered in nylon tissue and the extract was gellified with 0.2% KCl, frozen and thawed at least twice, and dried at 60°C until it reached constant weight. For comparative analysis, the same procedure was performed on a commercial sample of dry *Kappaphycus* sp., imported from the Philippines and kindly donated by Griffith do Brasil S/A, a Brazilian company specialized in semi-refined carrageenan production.

3,6-anhydrogalactose and sulphate contents from refined carrageenan were quantified by spectrophotometric methods modified from Matsuhiro (1995) and Craigie and Leigh (1978) respectively. Gel strength of refined carrageenan was measured at 1.5% solution and gellified with 0.2% KCl at 20°C (Ohno et al. 1994) using a Stevens-LFRA Texture Analyzer (Montreal, QC, Canada). Gel strength was also quantified for the commercial sample.

Statistical analyses were determined by main factors Analysis of Variance (ANOVA), and the Newman–Keuls *a posteriori* test, if this was necessary. Coefficients of correlation were calculated using Pearson’s simple linear correlation. All statistical analyses were performed using the Statistica software package (Release 5.0) considering $p<0.05$.

Results

Growth rates and semi-refined and refined carrageenan yields from brown, green, red and G11 strains are shown in Figure 1a-c respectively. In addition, the semi-

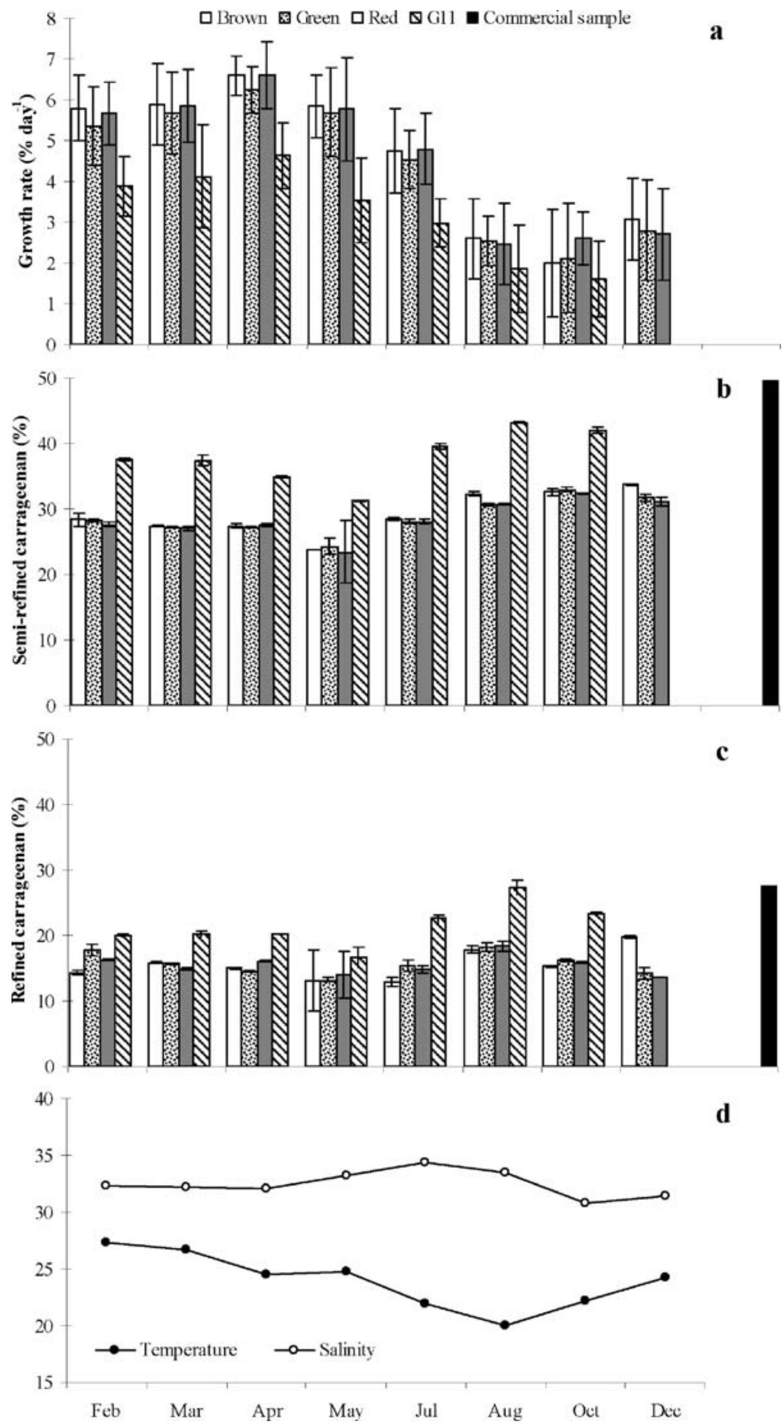

Figure 1 Seasonal growth rates and carrageenan yield of brown, green, red, and G11 strains of *Kappaphycus alvarezii* cultivated at Ubatuba Bay, São Paulo State, Brazil and a commercial sample. **a** Growth rate. **b** Semi-refined carrageenan yield. **c** Refined carrageenan yield. **d** Mean values of seawater temperature (°C) and salinity during the period of plant growth. *Vertical bars* represent standard deviations.

refined and refined carrageenan yields from a commercial sample of *Kappaphycus alvarezii* are presented (Figure 1b-c). Mean values of seawater temperature and salinity are also included (Figure 1d). For all studied strains, higher growth rates were observed from February to May, decreasing from July to December. During the experimental period, the growth rates of G11 strain were significantly lower (1.3% – 4.8%) than

Table 1 Coefficients of correlation (R) between temperature (°C), salinity, growth rate (% day^{-1}), semi-refined and refined carrageenan yield (%) for brown, green, red, and G11 strains of *Kappaphycus alvarezii* cultivated at Ubatuba Bay, São Paulo State, Brazil

Correlated parameters			Coefficient of correlations			
			Brown	Green	Red	G11
Temperature	x	Salinity	−0.2809	−0.2809	−0.2809	−0.2829
	x	Growth rate	0.7025	0.7016	0.6781	0.7977*
	x	Semi-refined	−0.4912	−0.4719	−0.5126	−0.6208
	x	Refined	−0.0847	−0.1863	−0.4283	−0.7651*
Salinity	x	Growth rate	0.2875	0.2565	0.2515	0.0603
	x	Semi-refined	−0.4314	−0.5247	−0.4752	−0.0876
	x	Refined	−0.2812	0.0053	0.1607	0.0790
Growth rate	x	Semi-refined	−0.8661*	−0.9003*	−0.8399*	−0.7897*
	x	Refined	−0.2970	−0.3024	−0.3397	−0.7390*

*represents significant differences at $p<0.05$

the other strains. Growth rates of brown, green and red strains were similar (2.5% – 6.6%) (Figure 1a).

The G11 strain had semi-refined carrageenan yields which were significantly higher (31% – 43%) than tetrasporophytic strains, and similar to those for the commercial sample (49%) for all months studied (Figure 1b). There were no significant differences among tetrasporophytic strains for these yields. All strains showed little variation of semi-refined carrageenan yields during the year, with significantly lower yields in May and higher from August to December (Figure 1b).

Refined carrageenan yield was significantly higher for the G11 strain than for the tetrasporophytic strains throughout the year, except in February (related to the green strain) and May (related to the brown and red strains) (Figure 1c). The values obtained from this strain (15% – 28%) are similar to those for the commercial sample (27%). Otherwise, differences were observed among tetrasporophytic strains, with significantly lower yields of brown strain in February and May, and significantly higher yields in December. Variation during the year was also verified, for all strains, except for the red one, which did not present significant differences during the experimental period.

Correlation coefficients among the parameters analyzed for *K. alvarezii* strains are shown in Table 1. Temperature showed significant correlation with growth rate (R^2=0.7977) and refined carrageenan (R^2=−0.7651) for the G11 strain. Negative coefficients were observed between growth rates and semi-refined carrageenan for all strains. No significant correlations between other parameters were regis-

Table 2 3,6-anhydrogalactose (3,6-AG) and sulphate contents, and gel strength of refined carrageenan of brown, green, red, and G11 strains of *Kappaphycus alvarezii* cultivated at Ubatuba Bay, São Paulo State, Brazil and of a commercial sample

Strain		3,6-AG (%)	Sulphate (%)	Gel strength (g cm^{-2})
Brown	May	8.69	32.86	688
	August	7.45	28.54	803
	October	7.48	27.24	795
Green	May	24.51	33.48	691
	August	22.42	27.35	818
	October	21.84	30.59	926
Red	May	12.80	25.63	803
	August	12.72	23.08	783
	October	11.81	24.86	859
G11	May	30.62	27.93	789
	August	26.06	30.06	728
	October	26.87	28.37	855
Commercial sample		nd	nd	842

nd=no data

Table 3 Comparative summary of 3,6-anhydrogalactose (3,6-AG) and sulphate contents, and gel strength from different species and from *Kappaphycus alvarezii* strains cultivated at Ubatuba Bay, São Paulo State, Brazil

Species	3,6-AG (%)	Sulphate (%)	Gel strength (g cm^{-2})	References
Eucheuma sp.	16.9–20.8	-	253	Dawes et al. (1977)
Eucheuma gelatinae	26.4–33.2	15.1–27.3	-	Maoxin & Zongcun (1988)
Eucheuma uncinatum	14.8–23.9	15.5–32.8	-	Zertuche-González et al. (1993)
Agardhiella subulata	15.7	37.7	-	Chopin et al. (1990)
Hypnea musciformis	25.2–31.4	17.6–20.1	-	Saito & Oliveira (1990)
Kappaphycus alvarezii	29	24	1158	Santos (1989)
	-	28.7–38.6	90–320	Azanza-Corrales & Sa-a (1990)
	-	-	25–65	Li et al. (1990)
	-	-	480–1960	Ohno et al. (1994)
	-	15.2–22.2	1–131	Hurtado-Ponce (1995)
	-	-	245–1712	Ohno et al. (1996)
	7.26–34.42	23.08–33.48	688–926	Present study
Commercial sample	-	-	842	Present study

tered. Salinity was almost constant during the experimental period (Figure 1d).

Table 2 shows 3,6-anhydrogalactose and sulphate contents, and gel strength of refined carrageenan from the four strains during May, August, and October. No differences were observed between the months for each strain. Maximal contents of 3,6-anhydrogalactose were registered for the G11 (30.62%) and green (24.51%) strains, while the brown strain showed the lowest values (7.45%). No clear pattern of variation was observed for sulphate content. The gel strength of each strain was close to the value obtained for the commercial sample.

Discussion

In this study, we have been analyzing the growth rate and carrageenan yield from brown, green, red and G11 *Kappaphycus alvarezii* strains farmed in Ubatuba Bay, São Paulo State, Brazil, to evaluate the potential and commercial feasibility of the experimental culture after the introduction program (Paula et al. 2002).

Growth rates and seawater temperature showed similar trends during the experimental period. Correlation of these parameters was significant for the G11 strain, agreeing with what has observed by Paula et al. (2002) for the brown strain. However, for tetrasporophytic strains, the same was not found. What is more, the G11 strain presented the lowest growth rates, while no significant differences were observed in tetrasporophytic strains (brown, green, and red), as registered by Muñoz et al. (2004).

The knowledge of seasonal growth patterns and carrageenan yield can help to plan a productive cycle, since low growth periods could be compensated with higher carrageenan yields. According to Ask and Azanza (2002), “the ability to mitigate or completely eliminate the negative effects of seasonal growth patterns so that year-round growth rates and carrageenan quality could be maintained at high levels, would be considered an advance in *K. alvarezii* culture”. In this work, semi-refined carrageenan yields were clearly related to growth rates in all strains, showing a negative correlation. This relationship was previously observed by Ohno et al. (1994) for carrageenans extracted from *K. alvarezii* farmed in Shikoku, Japan. Significant differences were not observed among tetrasporophytic strains during the year for semi-refined carrageenan yields. These results agree with those obtained by Trono and Lluisma (1992) for semi-refined carrageenan yields of the brown and green strains.

However, considering refined carrageenan yield, the tetrasporophytic strains did not show similar values. The brown strain presented significantly lower values in February and May, and significantly higher values in December. These results disagree with those obtained by Muñoz et al. (2004), who did not find significant differences for the same three colour strains cultured in Yucatán, México, and by Hurtado-Ponce (1995), who observed the highest yield for the red strain in *K. alvarezii* cultivated in two depths.

These differences could be attributed to the extraction methodology used in each study. In the literature, there are many reports about carrageenan yields, but quantitative and qualitative comparisons are difficult, since they vary depending on the extraction method, strain of the species and/or extracted raw material, semi-refined or refined carrageenan (Trono and Lluisma 1992; Zertuche-González et al. 1993; Ohno et al. 1994, 1996; Hurtado-Ponce 1995). In this study, the extraction of refined carrageenan could possibly damage the final yield, due to carrageenan lost in washes and excessive procedures of alkali transformation. Other studies must be performed to confirm this hypothesis.

All strains showed variable 3,6-anhydrogalactose content, while sulphate content was more constant. The gel strength also showed little variation. The relationship between 3,6-anhydrogalactose and sulphate contents and gel strength, as observed by Stanley (1987), was not verified by this study. These parameters vary considerably in different works (Table 3), probably due to the relative accuracy of different methodologies of chemical analysis (e.g., colorimetric, gravimetric), and different equipment and gel concentration used for analyses of gel strength (Table 3). Despite these differences, the gel-strength values obtained in this work are comparable to higher values showed in Table 3, and similar to those for the commercial sample.

The present study demonstrated that the three colour strains (brown, green and red) presented similar growth rates and carrageenan yields, while the G11 strain showed lower growth rates and higher yields. Although 3,6-anhydrogalactose content was variable, sulphate content was more constant in all strains. Gel strength, a parameter of great value commercially, presented similar values to that for the commercial sample. These results emphasize the findings by Paula et al. (1999): the potential of tetraspore progeny (G11 strain) for strain selection of *K. alvarezii*. Besides that, this work reinforces all favourable arguments for supporting the implementation of sustainable commercial culture of this specie in Ubatuba, São Paulo, Brazil: knowledge of seasonality, high growth rates, and carrageenan yield and quality comparable to commercial samples.

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