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SHORT COMMUNICATION

Does salinity affect growth and carrageenan yield of *Kappaphycus alvarezii* (Gigartinales/Rhodophyta)?

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Introduction

Kappaphycus alvarezii (Doty) Doty ex. P. C. Silva is one of the most important sources of raw material for the carrageenan industry (Ask & Azanza 2002). In Brazil, only *Hypnea musciformis* (Wulfen) J.V. Lamouroux is used as raw material and its natural stocks are not sufficient to supply the Brazilian demand (Bulboa & Paula 2005; Reis, Yoneshigue-valentin & Santos 2008). This was one of the reasons why, in 1995, *K. alvarezii* was introduced at the Brazilian Southeastern coast, in São Paulo State (Bulboa & Paula 2005).

The carrageenan yield (CY) can change in accordance to environmental parameters as a mechanism of prevention against stressful situations like salinity fluctuations (Hayashi, Oliveira, Bleicher-lhonneur, Boulenguer, Pereira, Von Seckendorff, Shimoda, Leflamand, Vallée & Critchley 2007; Reis *et al.* 2008).

Few works discuss the salinity effects on daily growth rate (DGR) and CY of eucheumatoids in spite of its importance (Ask & Azanza 2002). This information could help the identification of good sites for cultivation and would help mitigation activities (Ask, Batibasaga, Zertuche-gonzález & De San 2003). Thus, the aim of this study was to analyse the effect of the salinity on DGR and CY values of *K. alvarezii in vitro*.

Materials and methods

Green, brown and red variants of *K. alvarezii* were acquired at a farming located at Praia Grande, Itacuruçá Island, north of Sepetiba Bay, Rio de Janeiro State, Brazil (22°57′02″S and 43°54′22″W). *Kappaphycus alvarezii* voucher material was included in the Herbarium of the Botanical Garden of Rio de Janeiro (RB 425.507).

The variants were cleaned of epiphytes, using clamps and paper towel and acclimated for 30 days in 5 L glass tanks (water temperature – 22 ± 2 °C, water surface irradiance – 130 ± 20 μmoL m^{-2} s^{-1} photoperiod – 12-h day, salinity (PSS) – 35 ± 2). After 35 days, the samples were weighted and the CY and quality were measured considering that a production cycle rates from 30 to 60 days (Neish 2006).

The tested salinities (15, 25, 35 and 45) were obtained by freezing and defreezing seawater (Oliveira, Paula, Plastino & Petti 1995) and seawater (35) was used as control. Six replicates of each salinity were tested. Apical portions of each variant of *K. alvarezii* (brown, red and green) with 5 cm, making a final weight of 5 g, were placed in Erlenmeyer flasks with 200 mL of seawater under aeration for 2 weeks. The seawater was filtered in an ester cellulose membrane (0.45 μm, Millipore Corporate Headquarters, Billerica, MA, USA) and 10 mL L^{-1} of enriched seawater – ES/2 were added (Starr & Zeikus 1993) and weekly changed.

Growth was estimated as the mean DGR over a 35-day period, calculated according to the formula $DGR = \ln(W_f/W_o)/t \times 100$, where W_o stands for initial dry weight, W_f for final and t for time of cultivation (Hurtado, Agbayani, Sanares & Castro-mallare 2001). The initial dry biomass was established with 5 g of algal wet mass dried in an oven until constant weight (60 °C).

The semi-refined carrageenan extraction was obtained with hot alkali transformation in 0.2% KCl

and 6% KOH solution followed by successive water showering to remove any alkaline residues. Carrageenan yield results were expressed as the percentage of carrageenan from a sample of the individual dry mass (Reis *et al.* 2008) according to the formula: Yield = $(W_c/W_s) \times 100$, where W_c is the extracted carrageenan dry weight and W_s is the dry seaweed weight used in the extraction.

The normality (Shapiro Wilk's test) and homogeneity (Cochran test) assumptions of the variances were tested. Carrageenan yield was transformed to its arcsine because it is recommended to transform percentages (Zar 1996). DGR were transformed using the equation x = square root(x)+square root(x+1). To verify the accuracy of the different tested salinities, obtained by the freezing and defreezing method, and the interaction of salinity and the variants of *K. alvarezii* on DGR values and in the CY of the samples, the two-way analysis of variance (ANOVA) was used. The *post hoc* Fisher's LSD test was used to separate these differences. The salinity dependence on DGR and CY values was tested by polynomial regression tests. STATISTICA 6.0 (Statsoft South America, São Paulo, Brazil) was used on all analysis. Tests were carried out at $P = 0.05$ level of statistical significance and data are expressed as mean ± standard deviation.

Results and discussion

The accuracy of freeze–defreeze seawater method was attained because no difference was obtained between the DGR (Two-way ANOVA, $F = 3.40$, $P = 0.07$) and CY (Two-way ANOVA, $F = 0.01$, $P = 0.90$) values on the variants of *K. alvarezii* – brown, red and green (Fig. 1 a–f). An interactive effect of the variants and different the salinities on DGR values (Two-way ANOVA, $F = 6.41$, $P < 0.001$, Fig. 1) was observed. After 35 days *in vitro*, the variants presented higher DGR values in salinities of 25, 35 and 45. The lowest DGR of all variants occurred in salinity 15; however, the brown variant (Fig. 1a) showed higher DGR results in this salinity level when compared with the other variants (red – Fig. 1b, green – Fig 1c). In other studies, *K. alvarezii* presented lower DGR values in periods of rainfall at Brazilian southeastern coast (Paula, Per-

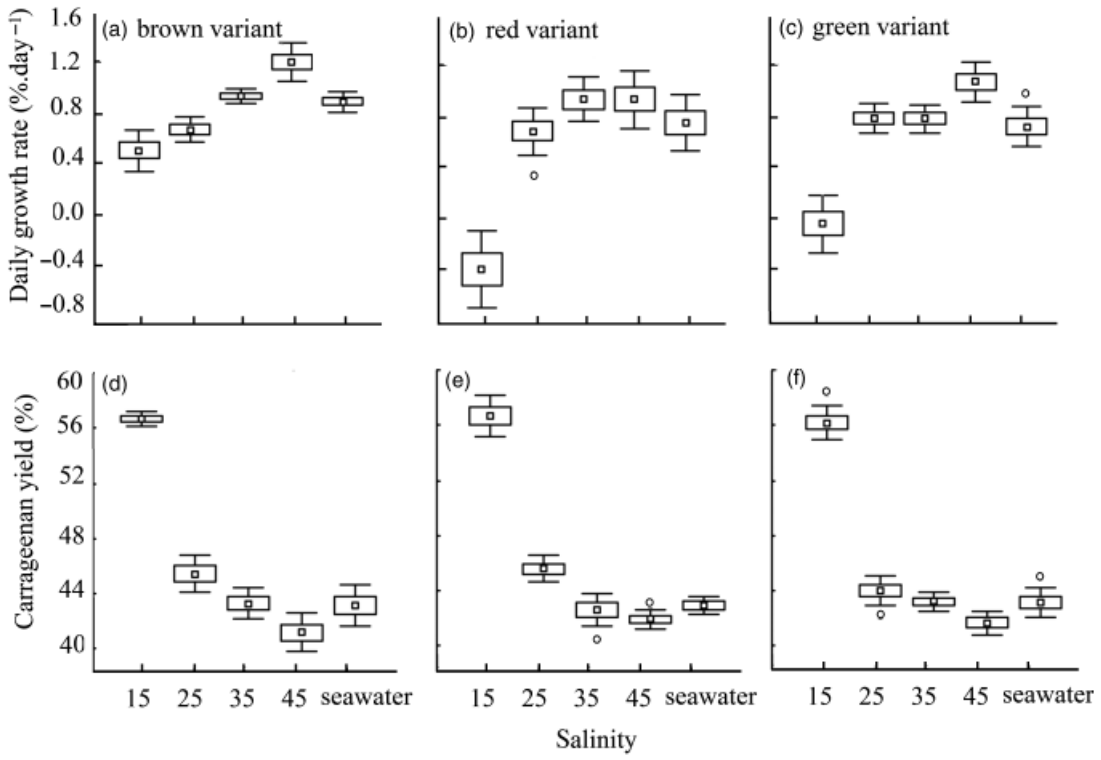

Figure 1 Daily growth rate (% day $^{-1}$) of the brown (a), red (b) and green (c) variants of *Kappaphycus alvarezii* and carrageenan yield (%) extracted from the brown (d), red (e) and green (f) variants of this species. Square: median; rectangle: standard error; vertical bar: standard deviation and circle: outliers.

eira & Ohno 2002; Bulboa & Paula 2005). The DGRs of the brown and the green variants in salinity 45 were higher than the red variant ($P < 0.001$, LSD test, Fig. 1a).

There is no clear reason why the brown variant obtained higher DGR values at this given salinities (15 and 45 PSS) when compared with the green and red variants. Santelices 1999 relates seaweed growth to a complex interaction between irradiation, water motion, temperature and nutrients. Muñoz, Freile-pelegrín and Robledo 2004 correlate these factors explaining that when a major decline of one particular factor occurs it can be compensated by another, thus regulating the growth of the affected individual. The brown variant could show higher DGR values in lower salinity levels due to its more effective ion sorbing capabilities when compared with other variants (Kumar, Ganesan & Rao 2007) as a direct response to this particular stress. No difference was observed between the variants in salinity 35. Higher DGR values were obtained by the brown variant in farmings at Kenya (Wakibia, Bolton, Keats & Raitt 2006) and by the green variant at the Philippines (Hurtado *et al.* 2001).

There is not an interactive effect of the variants and the different salinities on the CY (Two-way ANOVA, $F = 1.2$, $P = 0.33$). All the variants produced higher CY values at salinity 15 ($F = 536.6$, $P < 0.001$, Fig. 1d–f); on the other hand, the DGR values of *K. alvarezii* ($r^2 = 0.68$, $P < 0.001$, Fig. 2a) and its CY ($r^2 = 0.94$, $P < 0.001$, Fig. 2b) had a high dependence on salinity. This environmental factor is one of the most important influences on the CY of *K. alvarezii* (Hurtado *et al.* 2001) and *Hypnea musciformis* (Reis *et al.* 2008). It is a natural defensive response to stressful conditions for most red algae in particular *Gracilaria* sp. and carragenophytes like *Chondrus* sp. and *Solieria* sp., to increase the cell-wall contents of such components in order to rapidly mitigate any harmful consequences caused by either salinity, light or water motion (Goulard, Diouris, Quere, Deslandes & Flocapos 2001; Freile-pelegrín, Robledo, Pedersén, Bruno & Rönnqvist 2002; Villanueva, Hilliou & Sousa-pinto 2009). They directly contribute to the osmotic equilibrium of the cell (Percival 1979) and it is important to the survival of the algae in sites with fluctuating salinity conditions (Percival & McDowell 1967).

It is difficult to compare the CY results obtained in this work with others studies due to the different extraction techniques applied (Muñoz *et al.* 2004; Hayashi, Oliveira *et al.* 2007). The CY of *K. alvarezii* from Sepetiba Bay *in vitro* in salinity 35 was higher than

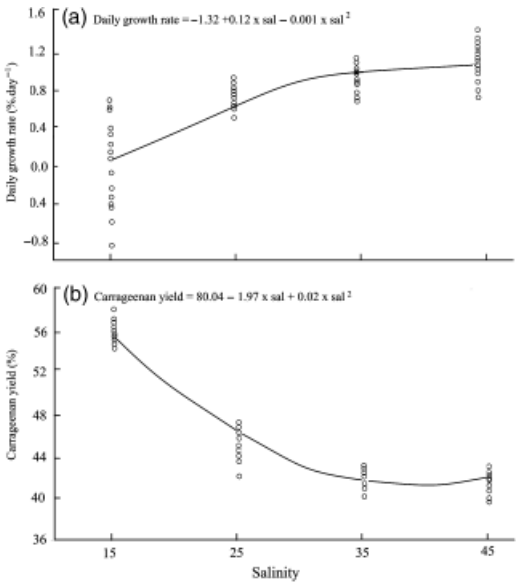

Figure 2 Linear regression of daily growth rate (a) and carrageenan yield (b) of *Kappaphycus alvarezii* under different salinities.

the semi-refined carrageenan (Hayashi, Paula & Chow 2007) and refined carrageenan extracted from seedlings cultivated in the Brazilian Southeastern (Hayashi, Oliveira *et al.* 2007) from Mexico (Muñoz *et al.* 2004) and lower than the ones cultivated at Philippines (Trono & Lluisma 1992). Because Estuaries and coastal lagoons are common in southern Brazil and these environments represent 15% of coastal areas (Barnes 1989), the information found in this study could be important to consider the expansion of *Kappaphycus* cultivation.

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References

Ask E.I. & Azanza R.V. (2002) Advances in cultivation technology of commercial eucheumatoid species: a review

with suggestions for the future research. *Aquaculture* **206**, 257–277.

Ask E.I., Batibasaga A., Zertuche-González J.A. & de San M. (2003) Three decades of *Kappaphycus alvarezii* (Rhodophyta) introduction to non-endemic locations. In: *Proceedings of the 17th International Seaweed Symposium* (ed. by A.R.O. Chapman, R.J. Anderson, V.J. Vreeland & I.R. Davison), pp. 49–57. Oxford University Press, Oxford, UK.

Barnes R.S.K. (1989) *Coastal Lagoons – The Natural History of a Nagleted Habitat*. Cambridge University Press, Cambridge, UK.

Bulboa C.R. & Paula E.J. (2005) Introduction of non-native species of *Kappaphycus* (Rhodophyta, Gigartinales) in subtropical waters: Comparative analysis of growth rates of *Kappaphycus alvarezii* and *Kappaphycus striatum in vitro* and in the sea in south-eastern Brazil. *Phycological Research* **53**, 183–188.

Freile-Pelegrín Y., Robledo D., Pedersén M., Bruno E. & Rönnqvist J. (2002) Effect of dark and salinity treatment in the yield and quality of agar from *Gracilaria cornea* (Rhodophyceae). *Ciencias Marinas* **28**, 289–296.

Goulard F., Diouris M., Quere G., Deslandes E. & Flocapos J-Y. (2001) Salinity effects on NDP-sugars, floridoside, starch, and carrageenan yield and UDP-glucose pyrophosphorylase and –epimerase activities of cultivated *Solieria chordalis*. *Journal of Plant Physiology* **158**, 1387–1394.

Hayashi L., Oliveira E.C., Bleicher-Lhonneur G., Boulenguer P., Pereira R.T.L., von Seckendorff R., Shimoda V.T., Leflamand A., Vallée P. & Critchley A.T. (2007) The effects of selected cultivation conditions of carrageenan characteristics of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in Ubatuba Bay, São Paulo, Brazil. *Journal of Applied Phycology* **19**, 505–511.

Hayashi L., Paula E.J. & Chow F. (2007) Growth rate and carrageenan analyses in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in subtropical waters of São Paulo State, Brazil. *Journal of Applied Phycology* **19**, 393–399.

Hurtado A.Q., Agbayani R.F., Sanares R. & Castro-Mallare M.T.R. (2001) The seasonality and economic feasibility of cultivating *Kappaphycus alvarezii* in Panagatan Cays, Caluya, Antique, Phillipines. *Aquaculture* **199**, 295–310.

Muñoz J., Freile-Pelegrín Y. & Robledo D. (2004) Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatán, México. *Aquaculture* **239**, 161–177.

Neish I.C. (2006) *Eucheuma* seaplant agronomy, biology and commerce. In: *World Seaweed Resources – An Authoritative Reference System. DVD-ROM* (ed. by A.T. Critchley, M. Ohno & D.B. Largo), ETI BioInformatics, Amsterdam, the Netherlands.

Oliveira E.C., Paula E.J., Plastino E.M. & Petti R. (1995) Metodologias para o cultivo axênico de macroalgas marinas *in vitro*. In: *Manual de métodos ficológicos* (ed. by K. Aveal, M.E. Ferrario, E.C. Oliveira & E. Sar), pp. 429–455. Universidad de Concepción, Concepci ó n, Chile.

Paula E.J., Pereira R.T.L. & Ohno M. (2002) Growth rate of the carrageenophyte *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) introduced in subtropical waters of São Paulo State, Brazil. *Phycological Research* **50**, 1–2.

Percival E. (1979) The polysaccharides of green, red and brown seaweeds: their basic structure, biosynthesis and function. *British Phycological Journal* **4**, 103–117.

Percival E. & McDowell R.H. (1967) *Chemistry and enzymology of marine algal polysaccharides*. Academy Press, London, UK.

Reis R.P., Yoneshigue-Valentin Y. & Santos C.P. (2008) Spatial and temporal variation of *Hypnea musciformis* carrageenan (Rhodophyta – Gigartinales) from natural beds in Rio de Janeiro State, Brazil. *Journal of Applied Phycology* **20**, 1–8.

Santelices B. (1999) A conceptual framework for marine agronomy. *Hydrobiologia* **398/399**, 15–23.

Starr R.C. & Zeikus J.A. (1993) Utex-the culture collection of algae at the University of Texas at Austin. *Journal of Phycology* **29**, 92–93.

Kumar K.S., Ganesan K. & Rao P.V.S. (2007) Phycoremediation of heavy metals by three-color forms of *Kappaphycus alvarezii*. *Journal of Hazardous Materials* **143**, 590–592.

Trono G.C. & Lluisma A.O. (1992) Differences in biomass production and carrageenan yields among four strains of farmed carrageenophytes in Northern Bohol, Philippines. *Hydrobiologia* **247**, 223–227.

Villanueva R.D., Hilliou L. & Sousa-Pinto I. (2009) Postharvest culture in the dark: Na eco-friendly alternative to alkali treatment for enhancing the gel quality of κ/é- hybrid carrageenan *Chondrus crispus* (Gigartinales, Rhodophyta). *Bioresource Technology* **100**, 2633–2638.

Wakibia J.G., Bolton J.J., Keats D.W. & Raitt L.M. (2006) Factors influencing the growth rates of three commercial eucheumoids at coastal sites in southern Kenya. *Journal of Applied Phycology* **18**, 565–573.

Zar J.H. (1996) *Biostatistical Analysis*. Prentice-Hall International Editions, NJ, USA.

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