Effects of kinetin and nitrogen on growth rates, pigment and protein contents in wild and phycoerythrin-deficient strains of Hypnea musciformis (Rhodophyta)

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Abstract Hypnea musciformis (Wulfén in Jacqu.) J.V. Lamour. is the main source for carrageenan production in Brazil and strains with selected characteristics could improve the production of raw material. The effects of kinetin on growth rates, morphology, protein content, and concentrations of pigments (chlorophyll a, phycoerythrin, phycocyanin, and allophycocyanin) were assessed in the wild strain (brown phenotype) and in the phycoerythrin-deficient strain (green phenotype) of H. musciformis. Concentrations of kinetin ranging from 0 to 50 μM were tested in ASP 12-NTA synthetic medium with 10 μM nitrate (N-limited) and 100 μM nitrate (N-saturated). In N-limited condition, kinetin stimulated growth rates of the phycoerythrin-deficient strain and formation of lateral branches in both colour strains. Kinetin stimulated protein biosynthesis in both strains. However, differences between both nitrogen conditions were significant only in the phycoerythrin-deficient strain. In the wild strain, effects of kinetin on concentrations of phycobiliproteins were not significant in both nitrogen conditions, except for chlorophyll content. However, the phycoerythrin-deficient strain showed an opposite response, and kinetin stimulated the phycobiliprotein biosynthesis, with the highest concentrations of phycoerythrin in N-saturated medium, while the highest concentrations of allophycocyanin and phycocyanin were observed in N-limited medium. These results indicate that the effects of kinetin on growth, morphology, protein and phycobiliprotein contents are influenced by nitrogen availability, and the main nitrogen storage pools in phycoerythrin-deficient strain of H. musciformis submitted to N-limited conditions were phycocyanin and allophycocyanin, the biosynthesis of which was enhanced by kinetin.

Keywords Colour strains · Hypnea musciformis · Kinetin · Nitrogen · Pigments · Proteins

Introduction

The genus Hypnea (Gigartinales, Rhodophyta) includes 50 species distributed in warm waters (Masuda et al. 1997), many of them with economic importance in several countries (Critchley and Ohno 1998). In Brazil, Hypnea musciformis (Wulfén in Jacqu.) J.V. Lamour is the main raw material for carrageenan production (Oliveira 1998). Along the Brazilian coast, populations of Hypnea musciformis usually have a brown phenotype. However, a brown plant with only one green branch was collected in Espirito Santo State, Southeastern Brazil, and a phycoerythrin-deficient strain (green phenotype) was originated from this green branch (Yokoya et al. 2003). In the laboratory, brown and green specimens were isolated and cultured in different environmental conditions, such as irradiance levels, photoperiods, temperature and salinity, in order to check if the colour variation could be a response to environmental factors. The colour of these H. musciformis isolates is a stable characteristic, and is not a result of photoacclimation processes (Yokoya et al. 2003, 2006a).
The occurrence of color variants in Brazilian red algae was reported for Gracilaria species: G. birdiae Plastino and E. C. Oliveira, G. cornea J. Agardh, and G. domingensis (Kützing) Sonder ex Dickie (Plastino 2003). The first report on color variants of Hypnea musciformis was described by Yokoya et al. (2003), and the green strain had lower phycoerythrin concentration than the brown strain, characterizing a phycoerythrin-deficient strain.

The major nitrogen storage pools in seaweeds are amino acids and proteins, and in nitrogen-limited condition, phycoerythrin is very important as a source of nitrogen (Bird et al. 1982). Therefore, the phycoerythrin-deficient strain of H. musciformis could provide an interesting biological system to study nitrogen metabolism as well as other physiological processes. Moreover, there has been an increasing interest in the selection of seaweed strains not only for the improvement of cultivation for commercial purposes but also for their potential to produce different compounds with biological activities.

Cytokinins play an important role in a number of physiological processes, such as cell division and differentiation, morphogenesis, senescence and photosynthesis in different species belonging to seven algal divisions (Mooney and van Staden 1986). There are relatively few reports on the presence of hormones in seaweeds, but recently, these compounds have been studied more intensively. Stirk et al. (2003) identified 19 endogenous cytokinins, including the first report of aromatic cytokinins (benzyladenine and topolin derivatives) in 31 macroalgal species. Moreover, this study highlighted some differences on cytokinin composition in seaweeds in comparison with the higher plants. These differences include an abundance of isopentenyladenine (iP) and c-zeatin (cZ) conjugates in the seaweed samples and no dihydrozeatin (DHZ), while in higher plants, the most common cytokinin types are DHZ derivatives, and iP and cZ conjugates are not often detected (Stirk et al. 2003).

The wild and phycoerythrin-deficient strains of H. musciformis showed differences on cytokinin metabolism (Yokoya et al. 2006b). The phycoerythrin-deficient strain showed more cytokinin-like activities (measured by soybean callus biossay with kinetin standards) than the wild strain, with the highest activity co-chromatographing with zeatin (Z) and a low activity co-chromatographing with monophosphates. The zeatin-like activity was equivalent to 5 μg L⁻¹ kinetin in the phycoerythrin-deficient strain, while the wild strain presented a lower activity (equivalent to 1 μg L⁻¹ kinetin), in addition to a low iP-like activity.

Based on these reports, the aims of our study were to determine the effects of kinetin in both N-limited and N-saturated culture media on growth rates, morphogenesis, protein content and concentrations of pigments in the wild and in the phycoerythrin-deficient strains of H. musciformis.

Materials and methods

Phycoerythrin-deficient strain (green phenotype) of Hypnea musciformis was originated from only one green branch, which itself originated as a spontaneous mutation from a wild plant (brown phenotype) collected from Ponta da Baleia, Espirito Santo State, Brazil. In the laboratory, unialgal cultures of the brown and green strains were initiated by clonal propagation using segments (5-10 mm) of brown and green branches isolated from the same mother-plant. Culture medium was composed of sterilised seawater (salinity of 30±1 psu) enriched with von Stosch’s solution at half strength (VSES/2) following Edwards (1970), with vitamin concentrations reduced to 50%. Medium renewal was carried out every 2 weeks. Cultures were incubated under 24±2 °C, irradiances of 40.0-