The effect of salinity on growth, photosynthesis and respiration in the estuarine red alga *Bostrychia radicans* Mont.

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ABSTRACT: The estuarine red alga, *Bostrychia radicans*, was subjected to osmotic stresses ranging from hypo-osmotic (9.9%) to hyperosmotic conditions (37.4%). The growth rate decreased with increasing salinities and showed a maximum in a mesohaline medium, while the photosynthetic rate and the chlorophylla content increased under hyper-osmotic conditions. The rate of respiration remained constant over the salinity range tested. *B. radicans* revealed typical characteristics of "shade plants" having a low light compensation point at $3-4 \,\mu\text{Em}^{-2}\text{s}^{-1}$ correlated with a low photon flux density of 70–100 $\mu\text{Em}^{-2}\text{s}^{-1}$ for saturation of photosynthesis. These physiological properties may explain the success of *B. radicans* in estuarine habitats.

INTRODUCTION

The red alga *Bostrychia radicans* Mont. (Ceramiales, Rhodomelaceae) is abundant in habitats with different salinities, i.e. in lagoons, in mangrove swamps, in freshwater and in many estuaries (Post, 1963a).

The algae, living in the upper eulittoral zone of estuaries are subjected to daily and seasonal changes of salinity due to the mixing of freshwater from the river with seawater, and the rise and fall of the tides (for review see Knox, 1986). For example, in the Mullica River estuary (New Jersey, USA) *B. radicans* tolerates a range of salinities between 5 and 30 ‰ (Yarish et al., 1979).

Based on its ability not only to survive but to grow and reproduce in various salinities (5-35 %), *B. radicans* was characterized as a "euryhaline" alga (Yarish et al., 1979). Photosynthesis of another species, *B. binderi*, showed little effect in a broad range of salinities between 10 and 40 ‰ (Dawes et al., 1978). In the same algae the effect of Ca²⁺, HCO₃⁻, NO₃⁻ and PO₄⁻³ on the photosynthetic rate under hyposaline conditions was tested (Dawes & McIntosh, 1981). These nutrients elevated and stabilized the photosynthesis of *B. binderi* in extreme low salinities between 0 and 5 ‰.

It is obvious that *Bostrychia* must have physiological properties supporting the organism's salinity tolerance. The study presented here provides further and new data on growth, photosynthesis and respiration of *B. radicans* under various external salinities.

MATERIAL AND METHODS

Plant material and cultivation

The estuarine red alga *Bostrychia radicans* Mont. obtained from the Göttingen Culture Centre, F.R.G., was cultivated in 500-ml beakers in aerated, artificial brackish water (19.4 ‰; prepared from synthetic sea-salt used for fish tanks; WIMEX[®], Wiegandt, Krefeld, F.R.G.) at 22 °C illuminated with 30 μ E m⁻² s⁻¹ (two 18 W daylight, Sylvana) and a light: dark cycle of 16 h: 8 h. The media were enriched with 5.5 mM NaNO₃ and 1 mM NaHCO₃. For all experimental procedures, algae were equilibrated to a range of hypo-and hypersaline conditions (9.9, 19.4, 28.9, 37.4 ‰). The brackish water with the salinity of 19.4 ‰ will be defined as steady-state medium. Salinity was changed by increasing the NaCl concentration or dilution with distilled water. The final NaHCO₃-concentration of the growth media was 3.5 mM. The plants were adapted step wise to the different salinities (5.1–6.8 ‰ per day) and media were changed weekly over a 4-week period.

Physiological experiments

Growth responses of the algae were determined weekly by fresh weight measurements estimated after standardized careful blotting with several layers of paper tissue. The relative growth rate μ was calculated from the initial (W_o) and the final (W_t) fresh weights of the algae using the equation:

$$\mu = 100 (\log_e W_t - \log_e W_o)/t (n = 4 \text{ for each salinity}).$$

Photosynthetic and respiratory rates were determined employing a Clark-O₂-probe (YSI 53) in a temperature-controlled (24 °C) glass cuvette. Photon flux density (Schott quartz fibre with halogen lamp) was varied by adjusting the distance between cuvette and light source accordingly. Three replicates of each irradiance were used. The experimental media contained 4 mM NaHCO₃; the temperature was 24 °C and the pH 8.1–8.3. To obtain saturated photosynthesis a photon flux density of 250 μ E m⁻² s⁻¹ was applied. The O₂ content of the different media was calculated according to Truesdale et al. (1955). Chlorophyll was extracted and concentrations of the algae were estimated according to Inskeep & Bloom (1985).

RESULTS

An increase in salinity of the media was accompanied by a decrease in the relative growth rate μ (Fig. 1). Towards hyposaline conditions the growth rate increased linearly: there was a 95% decrease in growth between plants cultured in 9.9% to 37.4%. Maximum growth of *Bostrichia radicans* at given conditions occurred at 9.9% representing a mesohaline medium.

B. radicans exhibited a low light compensation point at 3–4 μ E m⁻² s⁻¹ and a saturation of photosynthesis between 70 and 100 μ E m⁻² s⁻¹ (Fig. 2).

The respiratory and net photosynthetic rates of *B. radicans* cultivated under different salinities were measured after 4 weeks adaptation under conditions of light saturation (250 μ E m⁻² s⁻¹) (Fig. 3). Photosynthesis changed with different levels of salinity. Under steady-state conditions (= 19.4 ‰), a rate of photosynthesis of about 40 μ mol O₂ mg⁻¹ chlorophyll a h⁻¹ was observed for *B. radicans*. The photosynthetic rate decreased with



Fig. 1. Relative growth rate μ of *Bostrychia radicans* as a function of salinity. Data are expressed as mean \pm standard error (n = 4)



Fig. 2. Photosynthetic oxygen evolution of *Bostrychia radicans* grown at 19.4 ∞ as a function of photon flux density. Data are expressed as mean \pm standard error (n = 3)



Fig. 3. Net photosynthesis and respiration rates of *Bostrychia radicans* grown under different salinities; measurement after 4 weeks adaptation. Data are expressed as mean \pm standard error (n = 3)

decreasing salinity $(9.9\% :\approx 30 \ \mu\text{mol}\ O_2\ \text{mg}^{-1}$ chlorophylla h⁻¹), and was stimulated under hypersaline conditions, resulting in about 50 μ mol O₂ mg⁻¹ chlorophylla h⁻¹ at 28.9‰. However, in the highest salinity tested (37.4‰) no further increase of the photosynthetic rate was observed. In contrast, the rate of respiration remained constant over the salinity range tested.

The chlorophyll a content of *B. radicans* was, under steady-state conditions $(19.4 \ \infty)$, $631 \pm 31 \ \mu g \ g^{-1}$ fresh weight. Under hyposaline conditions $(9.9 \ \infty)$ the chlorophyll a concentration decreased slightly to $590 \pm 10 \ \mu g \ g^{-1}$ fresh weight, while under hypersaline conditions, a linear increase in chlorophyll a content occurred. However, the increase was much more pronounced between $28.9 \ and \ 37.4 \ \infty$ ($826 \pm 83 \ \mu g$ chlorophyll a g^{-1} fresh weight) compared with the results between $19.4 \ and \ 28.9 \ \infty$ ($694 \pm 32 \ \mu g$ chlorophyll a g^{-1} fresh weight). A rise in chlorophyll a content of about $30 \ \infty$ was observed over the experimental range of salinities.

DISCUSSION

Under hypersaline conditions the growth response (Fig. 1) was in agreement with the results of Yarish et al. (1979), who investigated ecotypes of *Bostrychia radicans*, their growth pattern being correlated with the salinity regime in their natural habitat. One isolate of *B. radicans* showed optimal growth at 15 ‰, the others between 15 and 25 ‰. In contrast, algae investigated in this study exhibited maximum growth under hyposaline conditions in a mesohaline medium (9.9 %). Therefore, it is assumed that this isolate represents a separate ecotype.

The low light compensation point (3-4 μ E m⁻² s⁻¹) together with a low photon flux

density for saturation of photosynthesis (70–100 μ E m⁻² s⁻¹) (Fig. 2) characterized *B.* radicans as a typical "shade-plant" (Raven et al., 1979). This agrees with the usual conditions in their natural habitat: Post (1963b) described the alga as a plant preferring shaded and protected sites on rocks or banks such as crevices and cavities. When exposed to higher photon flux densities (>100 μ E m⁻² s⁻¹), *B. radicans* became green indicating photo damage, and the growth was inhibited.

The increase in chlorophyll a content with increasing salinity is well documented for microalgae (Al-Hassan et al., 1987), but a satisfactory explanation is still lacking.

The rhodophytes are equipped with typical accessory pigments including the phycobilins (Lüning, 1985; Wilhelm et al., 1987), which represent an efficient light-harvesting system, and which may explain the low light compensation point of *B. radicans*. In the red alga Griffithsia monilis Harvey the phycobilins are the major light-harvesting pigments under low light intensities, whereas with increasing photon flux density, carotenoids and chlorophylla contribute proportionally more (Larkum & Weyrauch, 1977). B. radicans is very likely to respond in a similar manner as G. monilis. Ogata & Matsui (1965a, b) investigated the photosynthesis of different Japanese marine algae. In nearly all plants, the maximum photosynthetic rate was found in normal marine habitat, and photosynthetic depressions were observed both in diluted and in concentrated sea water. In contrast, the photosynthetic rates of the red alga Porphyra purpurea (Roth) C. AG. subjected to quasi-estuarine salinity fluctuations was shown to decrease and increase in a synchronous manner (Reed et al., 1980). The increase of the photosynthetic rates of B. radicans under hypersaline conditions (28.9 and 37.4 ‰), and the decrease of the photosynthetic rate under hyposaline conditions (9.9 ‰) were similar. The enhanced photosynthetic rate under high salinities after 4 weeks of culture can partly be explained by the increase in the chlorophyll a content. It seems that the photosynthetic process of B. radicans under these conditions was functioning more effectively. However, in most algae investigated photosynthesis was inhibited under short-term as well as long-term hyperosmotic stresses (Kirst, 1981; Reed, 1983; Wright & Reed, 1985; Kirst & Wichmann, 1987). The inhibition of the photosynthesis is most likely due to changes in the fine structure of the chloroplasts (Wiencke, 1982) causing a disruption of energy transfer between the two photosystems.

Gessner & Schramm (1971) emphasized the ecological importance of the time span of exposure to reduced or increased salinities and the speed with which a given change – here photosynthesis – is effected.

B. radicans subjected to a sudden hyperosmotic shock responded with only a little inhibition of photosynthesis and respiration (data not shown) compared to other macroalgae such as *G. monilis* (Kirst, 1981). After severe changes in salinities from 19.4 to 45.9 %, photosynthetic rates were inhibited to about 24 %, the respiratory rates to about 2 % only. In contrast, Ogata & Takada (1968) reported considerably decreased intensities of respiration in the red alga *Ceramium* sp. in very low and very high salinities, whereas Wiencke & Davenport (1987) showed a stable respiration in *Cladophora rupestris* (L.) Kütz. under fluctuating salinity regimes. As observed in other species of macroalgae, respiration of *B. radicans* was much less affected compared to photosynthesis e.g. *Lamprothamnium papulosum* (Wallr.) J. Gr. (Kirst & Wichmann, 1987). In conclusion, photosynthesis and respiration of *B. radicans* under long-term as well as short-term hypersaline conditions appear to be relatively insensitive to osmotic stresses. This, and the broad growth pattern may assure survival in the estuarine habitats as described in the introduction.

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