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The effect of salinity on the biomass productivity, protein and lipid composition of a freshwater macroalga

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ABSTRACT

A critical knowledge gap in the production of macroalgae for protein (animal feed) and lipid (bioenergy) is the ability of target species to grow in saline groundwater and thereby avoid competition with traditional crops. We assessed the effect of increased salinity (0.11 ppt–3 ppt) on the growth of 5 strains of the freshwater macroalga *Oedogonium* in laboratory cultures and subsequently on the productivity and biochemical composition in outdoor cultures under ambient conditions. Growth and biomass productivity decreased with increasing salinity in both experiments across all strains. However, in contrast to biomass productivity, protein content increased with increasing salinity and consequently, protein productivity (0.2–0.6 g DW m^{-2} day^{-1}) did not decrease markedly as salinity increased. Salinity had inconsistent effects on the lipid content among the strains, with the content of 2 strains increasing 3 to 4-fold under the 3 ppt treatment compared to 0.11 ppt. However, lipid productivity decreased with increasing salinity for 4 of the 5 strains. Similarly, biomass energy values increased with increasing salinity across all strains while bioenergy productivity decreased. These findings demonstrate that *Oedogonium* grown in salinities of up to 3 ppt maintains its productivity as a source of protein, potentially for animal feed, but not for bioenergy.

1. Introduction

Salinization of soils and groundwater is a significant global problem, with more than 800 million hectares of land, or more than 6% of the worlds' total land area, affected by salinity [32]. Nearly 20% (45 million hectares) of all irrigated land and 2% (32 million hectares) of dryland agricultural land are salt affected [32], and more than 50% of all arable lands are expected to be affected by salinity by the year 2050 [51]. Although some commercially important plants and crops such as barley, cotton and wheat can tolerate high salinity, a potential use of this salt affected land and groundwater is to cultivate macroalgae for biomass applications.

The use of saline groundwater to cultivate algae has been a focus of research and development for biomass applications [12,40,49,50]. However, successful cultivation of algae in these areas is dependent on the tolerance of strains to higher salinities. Most research on this topic has focused on selecting salt-tolerant strains of microalgae for biofuel production [40,50]. In a different but analogous approach, research has also been conducted on the cultivation of the marine macroalga *Gracilaria chilensis* for hydrocolloid production in dryland salinity areas where the salinity of the water in evaporation basins (20–40 parts per thousand (ppt)) is close to that of seawater (35 ppt) [12]. This is possible because many marine macroalgae can tolerate a wide range of salinities [25]. For example, *Porphyra umbilicalis* grows well in salinities ranging from 7 to 52 ppt [25], while *Chaetomorpha indica* and *Ulva ohnoi* can grow in salinities ranging from 5 to 45 ppt [13]. However, saline groundwater supplies often have lower salinities than those tolerated by marine macroalgae (e.g. <5 ppt, [50]). Consequently, the only macroalgae suitable for cultivation in these waters are salt-tolerant freshwater species that, similar to microalgae, are capable of tolerating or adapting to the changes in salinity that would occur in open culture systems due to evaporation and rainfall.

The freshwater macroalgal genus *Oedogonium* has recently been identified as a target for biomass applications due to its high productivity, favorable biochemical composition, cosmopolitan distribution and competitive dominance over other algal species in open culture systems [9,27,34,55]. *Oedogonium* has been cultivated in water sources with very different chemical compositions. Successful production has been achieved in water rich in heavy metals and metalloids [41,42] and water with high alkalinity [10]. Moreover, growth rates among 11 *Oedogonium* strains differ under a range of temperature treatments with some having better tolerance to lower temperatures [28]. The broad environmental distribution of *Oedogonium*, its ability to grow across water sources with a range of chemical compositions and its among-strain variability in response to temperature support the potential for strains to vary in their tolerance to other environmental parameters such as salinity. However, the salinity tolerance of *Oedogonium*, and more generally the tolerance of freshwater macroalgae, is not well understood. Identification of salt-tolerant strains of *Oedogonium*

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would enable production of this alga utilizing saline groundwater across a broad range of sites, including those unsuitable for agriculture.

Two key factors determine the suitability of algae for biomass applications, areal productivity (the amount of dried ash-free biomass per unit area (m^2) per unit time (day)) [17,36] and biochemical composition. The proportion of protein in the biomass is a key parameter for animal feed applications [6] and the proportion of lipids in the biomass is a key parameter for the thermochemical production of biocrude, a promising pathway to biofuels from macroalgae [15,43,44]. The energy potential of the biomass is important for both applications. However, the effect of increased salinity on the biochemical composition of freshwater macroalgae is fundamentally unknown. Therefore, the objective of this study was to assess the salinity tolerance of multiple *Oedogonium* strains and determine the effect of increased salinity on the productivity and biochemical composition of the biomass. To achieve this objective we assessed the effect of increased salinity over a period of 3 weeks on the growth of 5 strains of *Oedogonium* in small-scale laboratory cultures, and subsequently on the productivity and biochemical composition of the same 5 strains of *Oedogonium* in outdoor cultures under ambient conditions.

2. Methods

2.1. Sample collection and isolation

Tolerance to salinity was assessed in 5 genetically distinct strains of *Oedogonium* — Tar1, Tar3, Tsv1, Tsv2 and Riv6 [28,29]. *Oedogonium* is a cosmopolitan genus of filamentous freshwater green macroalgae that is a common component of freshwater ecosystems. It is a genus of unbranched, uniseriate algae made up of small cylindrical cells. Strains were originally isolated from samples of freshwater macroalgae collected from naturally occurring water bodies, irrigation channels and wetland areas in 3 distinct geographic regions of Australia–Riverina (35°S, 145°E: "Riv"), Tarong (26°S, 151°E: "Tar") and Townsville (19°S, 146°E: "Tsv"). Detailed collection information and methods for species identification are provided in Lawton et al. [28] for strains Tar1, Tar4, Tsv1, Tsv2 and Lawton et al. [29] for Riv6. Following isolation, strains were maintained in nutrient-enriched autoclaved freshwater (MAF growth medium, Manutech Pty Ltd, 13.4% N, 1.4% P; 0.05 g L^{-1}) in a temperature controlled laboratory under low light (12:12 light:dark cycle, 50 μmol photons PAR m^{-2} s^{-1}, 23 °C) for at least 1 year and were well acclimated to culture conditions. All strains are maintained in culture collections at James Cook University, Townsville, Australia.

2.2. Laboratory salinity tolerance experiment

Laboratory growth trials were conducted to determine the salinity tolerance of 5 strains of *Oedogonium*. Laboratory trials enabled us to test a greater number of treatments than outdoor experiments, providing greater resolution on the point of salinity tolerance. These trials were conducted on each strain under eleven salinity treatments plus a freshwater control. Thirty-six filaments of each strain were cut to a standardized length of 6 mm. Three filaments from each strain were then grown using each of eleven different salinity treatments ranging from 0.5 ppt (parts per thousand, equal to percentage/10) to 3 ppt increasing in 0.25 ppt increments (Fig. 1) for a period of 7 days. This upper limit of 3 ppt was chosen based on the results of a pilot trial (Appendix 1) and falls within the range of saline groundwater (up to 5 ppt; [50]). These values equate to approximately 0.3% to 8.6% of seawater salinity and many groundwaters are commonly up to 10% of the salinity of seawater (35 ppt, 3.5%, 3500 mg L^{-1} or ppm). Nutrient enriched (MAF growth medium, Manutech Pty Ltd., 0.05 g L^{-1}) dechlorinated freshwater was used as a control and had a salinity of 0.11 ppt. Salinity treatments were created by adding NaCl to the dechlorinated nutrient enriched freshwater until desired salinities were reached. Each individual filament was maintained in a sterile 60 mm Petri dish in culture cabinets at 24.5 °C with 12 hour light: 12 hour dark cycles and a light level of 50 μmol photons PAR m^{-2} s^{-1}. These conditions correspond to the middle temperature treatment used in a previous growth experiment with these strains [28] and are comparable to ambient summer conditions in the majority of regions where samples were originally collected. Each replicate was photographed under a dissecting microscope (Olympus model SZ61) at the start and end of the 7 day period and the 2-dimensional surface area of filaments was determined using ImageJ [47]. Specific growth rates (SGR) were calculated for each individual replicate of each strain under each treatment using the equation SGR (% day^{-1}) = $\mathrm{Ln}(B_f/B_i)/T * 100$, where B_f and B_i are the final and initial surface areas (mm^2) and T is the number of days in culture. This entire protocol was repeated a further 2 times to give a total of 3 replicate weeks of growth data. In the second and third week of the experiment, new filaments were cut from the biomass grown in each replicate during the previous week of the experiment and then placed into new, independent Petri dishes. Permutational analysis of variance (PERMANOVA) was used to analyze the effects of strain, salinity (fixed factors) and week (random factor) on the specific growth rate of isolates. Analyses were conducted in Primer v6 (Primer-E Ltd., UK) using Bray–Curtis dissimilarities on fourth root transformed data and 9999 unrestricted permutations of raw data [1].

2.3. Outdoor salinity tolerance experiment

To determine the salinity tolerance of each strain under intensive cultivation conditions, outdoor growth trials were conducted on all 5 strains under 3 salinity treatments — 1 ppt, 2 ppt, 3 ppt and a control treatment of nutrient enriched (MAF growth medium, Manutech Pty Ltd., 0.05 g L^{-1}, 0.11 ppt) dechlorinated freshwater. Salinity treatments were created by adding NaCl to the nutrient enriched dechlorinated freshwater until desired salinities were reached. Stock cultures of each strain were grown in the control treatment of nutrient-enriched dechlorinated freshwater in 5 L plastic buckets in a greenhouse with ambient natural light at the Marine and Aquaculture Research Facility Unit, James Cook University. Buckets were placed in a water bath with continuous flow to minimize large temperature fluctuations. Average water temperature was 24.2 °C (±2.5 S.D.) and cultures received an average photosynthetically active radiation of 97.4 mol photons m^{-2} $week^{-1}$ (±24.5 S.D.). Cultures were provided with aeration by a continuous stream of air entering the cultures through multiple inlets around the base of the buckets. All experimental replicates were maintained under identical conditions. Stock cultures were maintained in the experimental culture system for a period of at least 3 weeks prior to the start of each experiment to allow acclimation to the culture system and ensure that all strains were pre-exposed to identical conditions. Biomass was transferred from stock cultures into the relevant salinity treatments. Four replicate cultures of each strain were grown under each treatment. Cultures were stocked at a rate of 0.5 g fresh weight (FW) L^{-1} and harvested and weighed after 7 days. Following harvesting, the same biomass was restocked into each replicate and stocking density was reset back to 0.5 g FW L^{-1} by removing excess biomass in each culture. The experiment was run for a total of 3 weeks, providing for 3 harvests with the final week 3 samples used for biochemical analyses (see below).

At each harvest point a sample was taken from the excess biomass of each replicate, spun to remove excess water and weighed to determine the FW. Samples were then dried in an oven at 65 °C for at least 24 h and then reweighed to determine the fresh weight:dry weight (FW:DW) ratio for each individual replicate for each week of growth. The ash content of each replicate was quantified by combusting a 500 mg subsample of dried biomass at 550 °C in a muffle furnace until constant weight was reached. Ash-free dry weight (AFDW) productivity (g AFDW m^{-2} day^{-1}) was calculated for each replicate using the