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Reproductive sterility increases the capacity to exploit the green seaweed *Ulva rigida* for commercial applications

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ABSTRACT

Periodic biomass losses caused by reproduction have greatly limited the expansion of the land-based cultivation of the seaweed *Ulva*. In an attempt to resolve this, we obtained a sterile mutant (SM) of *U. rigida* by mutating a wild type (WT) strain using ultraviolet radiation (UVC). The SM grew five times faster than the WT with 40.0% and 30.9% higher nitrate and phosphate uptake rates. Crucially, the SM remained in the vegetative state throughout the 27-day trial, unlike the WT. The lipid content was more than double that of the WT with more monounsaturated, polyunsaturated, omega-3 and omega-6 fatty acids but with a 26.3% lower protein content. The swelling, water holding, and oil holding capacities were all lower than the WT. The rapidly growing SM showed distinct promise for application to carbon capture and wastewater bioremediation and for more conventional aquaculture practices. Despite some modified nutritional and functional properties, the SM still retained a desirable nutritional profile.

1. Introduction

The green seaweed *Ulva* is an increasingly important food [45], feed [1,13], and biofuel feedstock [6], as well playing a role in the delivery of crucial wastewater and CO_2 remediation services [2,11]. Interest in *Ulva* as a source of bioproducts and bioprocesses has driven concomitant efforts to optimize *Ulva* cultivation [5,7,27]. *Ulva* species should, in theory, be ideal cultivation candidates given their cosmopolitan distribution [31], very high growth rates [6], and broad environmental tolerances [35]. However, previous studies (for example, [3,8,14,46]) have experienced difficulty in maintaining *Ulva* species in the vegetative state. The formation and release of reproductive cells effectively terminates *Ulva* growth and leads to a disintegration of part or all of the thallus, dramatically reducing *Ulva* productivity [46].

There is thus an imperative to develop robust *Ulva* strains exhibiting traits of either reduced or absent reproduction. Several strain/trait optimization methods are currently available for seaweeds, the simplest being the selection of strains that preferentially express somatic growth over gamete production from the existing wild populations [57]. For example, fast growing clones of *Eucheuma* [16] and *Gigartina exasperata* [59] have been discovered and distributed to farmers. In terms of *Ulva* species, a sterile *U. pertusa* mutant was discovered at Omura Bay, Japan in 1973, which underwent vegetative development without sexual reproduction and could be maintained under axenic laboratory conditions [29,41,42]. The sterile strain also had a higher photosynthetic efficiency and growth rate, and also had a higher total free amino acid and total carbon and nitrogen content.

Although it is straightforward to look for robust strains from wild populations, the process can be time and labour intensive as it requires the collection of a wide range of ecotypes. Instead, mutagenesis and mutant strain screening can be used to avoid the process. Mutagenesis has successfully been employed for seaweeds [20,44], with physical mutagens such as ultraviolet light (UV) widely used [9]. These studies demonstrate that developing sterile, or at least low level reproductive strains is technically achievable and would be beneficial for long-term cultivation.

To date, sterile strains of *U. rigida* (a ubiquitous green seaweed implicated in green tide events) have not been reported. This study aimed to develop a sterile strain that would demonstrate high growth potential. To achieve that, varying doses of UV radiation were employed to mutate a wild strain of *U. rigida*. In addition, patterns in growth, nutrient uptake, and chemical composition were examined to shed light on whether the sterile strain can be used for bioremediation and food purposes.

2. Materials and methods

2.1. Plant collection

Vegetative *U. rigida* fronds of 50–60 mm in length were collected from the low intertidal zone of Cullercoats Bay, Tyne and Wear, UK

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(55.03°N, 1.43°W) after a spring tide in August 2013. The fronds were placed in a plastic bag and transported to the laboratory within 1 h where they were gently rinsed with 1 mm filtered natural seawater to remove any sediment, epiphytes or small grazers.

2.2. Mutation of Ulva gametes and isolation of sterile mutants

Gametes were obtained by inducing reproduction from 2.5 mm discs of *U. rigida* thallus [26]. One hundred discs were rinsed with autoclaved seawater and transferred to a flask containing 200 ml of autoclaved seawater. The flask was held at 4 °C in darkness for 6 h and then transferred to an 18 °C incubator with 80 μmol photons m^{-2} s^{-1} light intensity and 16L:8D photoperiod. Gametes were released three days later, attracted to a point source of white light (gametes being phototactic) and collected by pipette. Gametes were further concentrated by centrifugation (1000 *g*, 5 min) and then transferred to 85 mm diameter Petri dishes with the final density of 2×10^7 gametes per dish. Penicillin G and streptomycin sulfate (final concentrations of 1000 and 250 mg l^{-1} respectively) were added to restrict bacterial growth. Six dishes were exposed to UV lamps (40 W, 254 nm) for 18, 36, 54, 72, 90 and 108 s (one dish per treatment). These exposures were chosen as no gametes survived when exposed for >120 s in preliminary experiments (data not shown). Afterwards, the dishes were placed in darkness at 18 °C for 24 h to allow gamete settlement. Germlings from each dish were detached (112,486 individuals in total) and transferred to six tanks (13 l) when they reached the length of 1 cm. The cultures were aerated with a flow rate of 100 l min^{-1} to suspend the germlings. The seawater was enriched with 1000 μmol l^{-1} nitrate and 50 μmol l^{-1} phosphate and was exchanged every 3–4 days to avoid nutrient limitation. The culture temperature was 18 ± 1 °C and light intensity was 80 μmol photons m^{-2} s^{-1} with a 16L:8D photoperiod. Over the following four months any thalli showing reproductive features—the colour of thalli changing from green to yellowish—were removed and those that maintained the vegetative state were cultivated further. Thalli that failed to demonstrate any reproductive tendencies were identified as sterile. Two sterile individuals were obtained, one of which was used in this study.

2.3. Growth measurement

Five wild type (WT) and three sterile mutant (SM) thalli (one thallus per 500 ml conical flask) were grown at 18 ± 1 °C with a 16L:8D photoperiod and light intensity of 80 μmol photons m^{-2} s^{-1} using a combination of warm and cold fluorescent tubes. The temperature and light conditions used here were previously determined as optimum for *U. rigida* growth [21]. The cultures were aerated with a flow rate of 100 l min^{-1}. The seawater was enriched with 500 μmol l^{-1} nitrate and 25 μmol l^{-1} phosphate and was renewed every day to avoid nutrient limitation. The initial fresh weight for each thallus was ~0.3 g, equal to a stocking density of 0.6 g l^{-1}. The lower quantity of the SM thalli was due to the limited availability of the sterile material. Fresh masses of WT and SM thalli were recorded every three days for 18 or 27 days. The 27-day culture only applied to the SM strain as the WT thalli were lost after day 18 due to reproduction events. The SM was transferred to a 13 l tank on day 9 and then reduced to the stocking density of 1.8 g l^{-1} on day 18 to avoid/reduce self-shading. The stocking density for the WT strain was not reduced given the lower growth rate. The fresh mass was determined after gently blotting each thallus with tissue paper. Specific growth rates (SGR) were calculated using the formula: SGR (%) = [ln (M_2 / M_1)] / t × 100, where M_2 is the final mass, M_1 is the initial mass and t is the number of culture days. The mean SGR over 18 days was based on the initial biomass and the biomass reached by day 18.

2.4. Reproduction assessment

Discs of 7 mm diameter from WT and SM strains were placed in 500 ml flasks (three flasks for each strain, each flask containing 25 discs) and cultured under the same conditions as per the growth trial. This experiment was conducted for 27 days. Reproductive discs were recognized by their colour change; formation of reproductive cells in *Ulva* is accompanied by a change in colour from green (vegetative state) to yellowish (reproductive state) and then to white (after gamete release). This was verified microscopically. Sporulation formation in more than half of the disc area was considered equivalent to complete sporulation. Reproduction rate was expressed as the ratio of reproductive discs to all discs in a flask.

2.5. Nutrient uptake rate measurement

Fresh thalli of the WT and SM strains were cultivated in 13 l tanks with a stocking density of 0.5 g l^{-1} for 12 days. The culture conditions were the same as per the growth experiment. The thalli were cultured in seawater enriched with 500 μmol N and 25 μmol P for three days prior to the experiment. The change in nutrient levels in the seawater was monitored daily and the daily consumed quantities of nitrate and phosphate were replenished after the measurements. The seawater was renewed every three days. Nitrate and phosphate uptake rates by thalli on days 3, 6, 9, and 12 were estimated from the decrease of NO_3^- and PO_4^{3-} in the culture medium over a given time interval (24 h). Only the data on day 3 are presented as both strains were fully vegetative by day 3. Nitrate was measured by a rapid spectrophotometer method [12] and phosphate by the phosphomolybdenum blue colorimetry method [43].

2.6. Dry mass

The thalli in the cultures of Section 2.5 were harvested at the end of the experiment, and then oven dried at 50 °C until consistent mass was obtained (for 24 h). As shown in Fig. 1, the WT growth rate decreased with culture time and was close to zero by day 12, suggesting that biomass would not increase after day 12. To obtain a maximum biomass for biochemical composition analysis, we harvested the thalli on day 12. The tissues were ground to a powder, sieved and placed into tubes within a desiccator pending analyses of chemical composition and functional properties.

2.7. Biochemical composition

Triplicate samples were run for all biochemical and functional analyses for both strains.

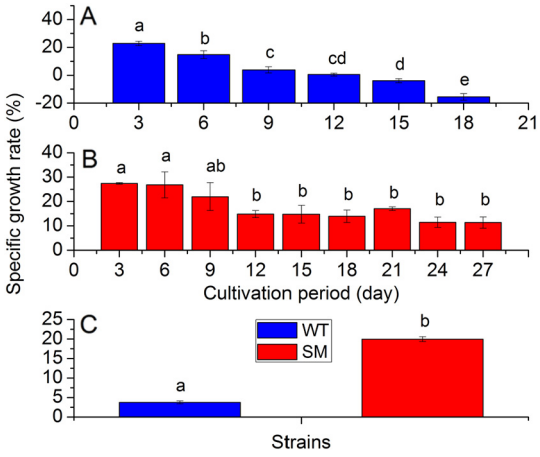

Fig. 1. The specific growth rates of wild type (WT) and sterile mutant (SM) strains of *Ulva rigida*: A) WT, B) SM, and C) mean specific growth rates of WT and SM over an 18-day cultivation. The error bars indicate the standard deviations (n = 3 or 5, 3 for the sterile strain and 5 for the wild strain). Bars sharing the same letter were not significantly different from each other over culture periods (A, B) or strains (C).