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Identifying a marine microalgae with high carbohydrate productivities under stress and potential for efficient flocculation

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ABSTRACT

Microalgal biomass represents a potential third generation feedstock that could be utilised as a source of carbohydrates for fermentative production of a range of platform biochemicals. Identifying microalgal strains with high biomass and carbohydrate productivities while also being amenable to downstream processes is key in improving the feasibility of these processes. Utilising marine microalgae capable of growing in seawater will decrease reliance on freshwater resources and improve the sustainability of production. This study screened several marine microalgae believed to accumulate carbohydrates to find new high performing strains. Four strains had high growth rates and accumulated carbohydrates > 35% DW under stress. The strain *Chlorella salina* demonstrated the highest biomass and carbohydrate productivity, and alkaline autoflocculation (4 mM NaOH) enabled biomass recoveries > 95% efficiency, resulting in an 8–10 × concentration of the culture. Under nutrient replete conditions, biomass productivity reached 0.6 $g\,L^{-1}\,d^{-1}$, significantly greater than that of nitrogen starved cultures. However, nitrogen starvation rapidly increased carbohydrate content to > 50% DW in 2 days, resulting in carbohydrate productivities > 0.20 $g\,L^{-1}\,d^{-1}$. *Chlorella salina* partitions the products of photosynthesis preferentially into carbohydrate synthesis under nitrogen starvation. A greater understanding of cellular physiology and carbon partitioning in response to nutrient stress will enable better control and optimisation of the bio-processes. This study has identified a potentially high performance marine microalga for carbohydrate production that is also amenable to low-cost harvesting.

1. Introduction

Microalgal biorefineries have the potential to produce bulk and fine chemicals for use in a range of markets, not least food, feed, cosmetics, as well as bioenergy. The production of biodiesel from algal lipids is the route to bioenergy production that has received the most attention [1], but this has perhaps been overtaken more recently by thermochemical processing routes to bio-oil production [2,3]. Another option is the utilisation of microalgal carbohydrates as a feedstock for microbial fermentation. This route not only allows for production of liquid biofuels in the form of bioethanol, but opens up the possibility of producing any of a range of higher value platform chemicals currently produced fermentatively by yeast or bacteria, such as amino acids or organic acids [4].

Terrestrial biomass sources of sugars are abundant and cheap. First generation crops such as sugar cane, corn and beets have long been used for bioethanol production, but issues regarding the competition for arable land for food production and increases in grain and meat prices, has resulted in greater incentives to develop more sustainable pathways. Second generation lignocellulosic feedstocks utilising agricultural and forestry residues, and crops that grow on marginal land such as miscanthus or switchgrass, have increasingly been acknowledged as a more sustainable feedstock for biofuel production [5]. However, many of these biomasses are recalcitrant and require complex processing under harsh conditions to liberate carbohydrates and enable biofuel production. In addition, the presence of lignin in many of these feedstocks means that an array of phenolics and compounds such as furfural, 5-hydroxymethylfurfural, acetic acid are components in the lignocellulosic hydrolysates, which results in lower productivities and yields relative to refined sugar streams [6,7].

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Microalgae have the advantages over terrestrial feedstocks in that they can be cultivated on non-arable land with comparable areal productivities to terrestrial crops [8,9], can accumulate > 50% of their dry weight as sugars [10], efficiently use nutrients [11], can potentially use marine or grey water for cultivation, and lack lignin. Additionally, microalgae are also highly amenable to biorefining because the biomass can be fractionated to valorise higher value products such as carotenoids and poly-unsaturated fatty acids prior to use of the bulk fraction of carbohydrates or proteins as feedstocks for lower value applications [4]. Higher value applications of the microalgal carbohydrate fraction include beta-glucans with therapeutic properties and food applications [12]. These benefits warrant consideration of microalgae as a potential third generation feedstock. However, microalgal biomass production is costly and can be highly resource intensive [13], and is still more expensive to produce than relatively cheap and abundant first and second generation feedstocks (sugars and lignocellulosic biomass, respectively) [5,14].

The key to improve the prospects of microalgae as a new feedstock for biorefining, is the identification of high performing, robust strains with high growth rates and a desirable biomass composition, i.e. high carbohydrates [15]. Furthermore, identifying strains that are able to grow in marine waters will aid in minimizing the water footprint of biomass production significantly, which is a critical issue if cultivation is sited in low latitude environments [16]. Our aims are to identify such strains and to conduct research to improve the cost and sustainability of biomass production. The amenability of strains to low cost and energy downstream processing is essential in improving the feasibility of biomass production, by decreasing the volumes of liquid requiring costly centrifugation. Lower-energy and cheap methods for inducing flocculation involve increasing the culture pH to induce autoflocculation and sedimentation by chemical precipitation or “sweeping” of calcium, magnesium or iron [17,18]. Strains that are promising in terms of growth and composition should also be examined at an early stage for the ability to autoflocculate to enable development of overall more efficient bio-processes.

This study aimed to identify marine microalgal strains with high biomass and carbohydrate contents, resulting in an overall high carbohydrate productivity. Cost-effective and low energy bio-processing such as alkaline flocculation induced harvesting should be possible with the target strain and was also assessed. Finally, for the most promising strain, the impacts of nutrient starvation (nitrogen and phosphorus) on growth, dynamic changes in biochemical composition and carbohydrate productivity were investigated. Identifying superior strains will decrease the costs of biomass production, downstream processing and potentially decrease the cost of carbohydrate production from microalgae.

2. Materials and methods

2.1. Experimental design

This study consists of three rounds of batch experiments, the first was a screening of six strains for biomass and carbohydrate productivity on ambient air (Exp.1). From this the most promising strains were carried forward to a second round of screening on CO_2-enriched air (Exp.2). The strain with the best performance regarding carbohydrate production was then evaluated for the impact of nutrient starvation (in comparison with nutrient replete conditions) with greater detail paid to changes in biochemical composition (Exp.3). These three experiments are hence referred to as Exp.1, Exp.2 and Exp.3. All cultivations were performed in triplicate for each strain or treatment.

2.2. Strain and stock maintenance

The following strains were obtained from the Culture Collection for Algae and Protozoa (CCAP, Oban, UK): *Chlorella stigmatophora* (CCAP 211/20; *C.stig*), *Chlorella salina* (CCAP 211/25; *C.sal*) and *Chlorella spaerckii* (CCAP 211/29A; *C.spa*). The following strains were obtained from the collection of the National Centre for Marine Algae and Microbiota (CCMP, Bigelow, USA): *Nannochloris atomus* (CCMP 509; *N.atom*), *Picochlorum oklahomensis* (CCMP 2329; *P.okla*) and *Schizochlamydella capsulata* (CCMP 245; *S.cap*). Cultures from CCAP were received as liquid cultures, while those from CCMP were received on solid media and upon arrival they were all transferred to solid Guillard's F/2 media agar plates, and thereafter transferred to liquid Guillard's F/2 media when colonies were observed [19]. Master cultures of each strain were maintained in F/2 media at 16 °C and sub-cultured every 4–5 weeks. For scaling up, cultures were sub-cultured first into 25 and then 75 mL tissue culture flasks with hydrophobic coating and sterile 0.2 μm air filter vented caps (Sarstedt AG & Co, Germany) and maintained as described in Materials and methods (Section 2.3). All stock cultures were maintained in natural seawater (salinity = 28 ± 2 ppt) based Guillard's F/2 media. The natural seawater was filtered (0.22 μm) into sterilised flasks prior to media preparation.

2.3. Media

For cultivation experiments, Artificial Seawater Media (ASWM) was used as the base to avoid seasonal variations in the nutrient content of natural seawater which could affect the experimental results [19]. The base of the media was 18.2 MΩ deionised water autoclaved (20 mins at 121 °C) prior to addition of salts (final salinity = ca. 37 ppt). To support high biomass concentrations, additional nitrogen (N) and phosphate (P) were added as sodium nitrate and disodium monohydrogen phosphate monohydrate, respectively. In Exps. 1 and 2, N and P were added at concentrations of 16.5 mg N L^{-1} and 5.16 mg P L^{-1} (7:1 N:P molar ratio) to match the concentrations present in Walne's media [19]. All other trace metals, salts and vitamins were added at the concentration specified in the ASWM recipe. All media were filter sterilised (0.22 μm) prior to use. To prevent large pH shifts of the media during cultivation experiment, 5 mM Tris-HCl was added and the pH was adjusted to 8.0 with the addition of 0.5 M NaOH at the beginning of the experiment. Subsequently, the pH was adjusted on a daily basis at the time of sampling by the addition of 0.5 M NaOH.

In the starvation experiments (Exp. 3), media concentrations of N and P were altered to simulate nutrient replete, nitrogen deplete and phosphorus deplete conditions. For nutrient replete cultures, the initial concentrations were 80 mg N-NO_3 L^{-1} and 25 mg P-PO_4 L^{-1}, maintaining the 7:1 N:P molar ratio. The N-deplete experiments were devoid of nitrogen and 25 mg P-PO_4 L^{-1}, and P-deplete were supplied with 80 mg N-NO_3 L^{-1} and no phosphorous. To avoid undesired nutrient limitation in these treatments, additional N and/or P were added on day 3 and day 5 of the experiment at the same concentrations as originally supplied.

2.4. Inocula

Inocula for the experiments were generated by repeated sub-culturing (3 times during approx. 3 weeks) in final volumes of 50 mL of ASWM media into 75 mL tissue culture-flasks placed on a shaker plate with illumination provided by cool-white LED (8WT8 4000 K, OSRAM GmbH) at an irradiance of ca. 125 μmol photons m^{-2} s^{-1}. To prepare the final inocula, cultures were transferred to bottles (400 mL, diameter = 0.09 m), which were stirred at 300 rpm with a magnetic stirrer (RO-5 5 position stirrer, IKA) and supplied with filtered ambient air (0.22 μm, VWR) at a rate of 0.2 L gas per L of culture per min (0.08 L min^{-1} $bottle^{-1}$). This allowed cultures to acclimate to the experimental conditions. Inocula were grown for 4–6 days to an adequate culture density for inoculation of the experimental cultures with a starting optical density of approximately 0.1 at 750 nm (OD_{750}). Cultures were inoculated at the beginning of the dark period.