Algal Research 24 (2017) 340–346

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

# Marine microalgae monosaccharide fluctuations as a stress response to nutrients inputs

Tomásia Fernandes[a], Igor Fernandes[a], Carlos A.P. Andrade[b,c], Artur Ferreira[d], Nereida Cordeiro[a,*]

[a] University of Madeira, Faculty of Sciences and Engineering, 9000-390 Funchal, Madeira, Portugal
[b] Mariculture Center of Calheta, Vila da Calheta, 9370-133 Calheta, Madeira, Portugal
[c] CIIMAR, Interdiciplinary Centre of Marine and Environmental Research, Rua dos Bragas 289, 4050-123 Porto, Portugal
[d] CICECO - Aveiro Institute of Materials, Escola Superior de Tecnologia e Gestão de Águeda, University of Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

*Keywords:*
Monosaccharides
Nutrient availability
*Nannochloropsis gaditana*
*Rhodomonas marina*
*Isochrysis* sp.

ABSTRACT

The monosaccharide patterns of the microalgae species *Nannochloropsis gaditana*, *Rhodomonas marina* and *Isochrysis* sp. were assessed, to verify if nutrients availability had an impact on these parameters. *Isochrysis* sp. and *Rhodomonas marina* monosaccharides content decreased 13–94% as the nutrients availability increased. The relative abundance of monosaccharides depicted that, at low nutrients availabilities, *Rhodomonas marina* and *Isochrysis* sp. had preference for glucans synthesis, accounting for up to 75% of the monosaccharides detected. Linking the monosaccharides trends with the phylogeny and glycolipid data, it was possible to establish which monosaccharides had a structural and/or storage role in the microalgae studied. Thermal analysis revealed that the microalgae submitted to low nutrient input treatments presented a reduced assimilation of the inorganic compounds. Nutrient concentrations affected microalgae monosaccharide patterns, highlighting their taxonomic differences.

## 1. Introduction

Microalgae are photosynthetic organisms known as potential sources of natural compounds which can be applied in therapeutics and biotechnological applications [33]. The ability of these microorganisms to readily adapt to growth fluctuating conditions, attributed to their metabolic flexibility, constitutes an advantage in modulating their biomass composition for commercial purposes [25,28]. In this regard, strategies like the manipulation of parameters such as media nutrient concentrations can be used in order to modify the nature, amount and composition of the products synthesized [24].

Carbohydrates are the main products of photosynthetically-fixed carbon which can be stored intracellularly in multiple forms, such as starch, or deposited into structural polysaccharides [8,10,37]. Monosaccharides are the building-blocks of the latest molecules and their composition can give an insight of polysaccharide predominance in microalgae [6,23,38]. This is a crucial factor when selecting species as feed sources in aquaculture and for biotechnological applications, since polysaccharides composition determines the microalgae breakage and digestibility, thus conditioning the extraction of cell wall coated valued products [2,6].

The polysaccharides synthesized by microalgae species can be applied in several areas [25]. For instance, starch and cellulose can be anaerobically converted into bioethanol, whereas β-D-glucans (*e.g.* laminarans) are under increasing attention due to their potential in therapeutic applications [8,25]. β-D-glucans are known for enhancing the host immune system by binding to β-glucan receptors of cells involved in immune responses, such as macrophages and neutrophils [14,33]. Sulphated polysaccharides have multiple biological activities, namely antiviral, antioxidant and anti-inflammatory [32]. In addition, polysaccharides can be used in food technology as emulsifiers and stabilizers in various food products [25].

To enhance microalgae compounds productivity it is essential to understand the metabolic pathways that lead to the partitioning of carbon precursors into the multiple forms of carbon storage [8,20,22]. Organizational differences within microalgae species might affect processes such as photosynthesis and carbon flux through metabolic networks [20]. The present study aimed to evaluate the impact of nutrient availability on the carbohydrate profile of three marine microalgae commonly used in aquaculture (*Nannochloropsis gaditana*, *Rhodomonas marina* and *Isochrysis* sp.) and attempt to link the monosaccharides composition with the origin based carbohydrate on phylogenetic data.

* Corresponding author.
*E-mail address:* ncordeiro@uma.pt (N. Cordeiro).

http://dx.doi.org/10.1016/j.algal.2017.04.023
Received 19 November 2016; Received in revised form 9 February 2017; Accepted 21 April 2017

**Table 1**
Components of the growth medium culture and respective concentrations used in the final growth media (mg $L^{-1}$).

| Component | Concentration in final growth medium (mg $L^{-1}$) | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 |
| $NaNO_3$ | 43 | 85 | 170 | 340 | 680 |
| $KH_2PO_4$ | 3 | 7 | 14 | 27 | 54 |
| EDTA | 2 | 4 | 8 | 15 | 31 |
| $FeCl_3.6H_2O$ | 1 | 3 | 5 | 11 | 22 |
| $ZnCl_2$ | 0.03 | 0.07 | 0.14 | 0.27 | 0.54 |
| $ZnSO_4$ | 0.07 | 0.14 | 0.29 | 0.57 | 1.15 |
| $MnCl_2.2H_2O$ | 0.04 | 0.08 | 0.16 | 0.32 | 0.65 |
| $Na_2MoO_4.2H_2O$ | 0.01 | 0.01 | 0.02 | 0.05 | 0.10 |
| $CoCl_2.6H2O$ | 0.01 | 0.01 | 0.02 | 0.05 | 0.10 |
| $CuSO_4.5H_2O$ | 0.01 | 0.01 | 0.03 | 0.05 | 0.10 |
| $MgSO_4.7H_2O$ | 0.12 | 0.25 | 0.49 | 0.98 | 1.97 |
| Vitamins | | | | | |
| Tiamine | 0.01 | 0.02 | 0.04 | 0.07 | 0.14 |
| Biotin | 0.001 | 0.003 | 0.005 | 0.010 | 0.020 |
| $B_{12}$ | 0.001 | 0.002 | 0.003 | 0.006 | 0.012 |

## 2. Materials and methods

### 2.1. Algal growth

The Eustigmatophyte *Nannochloropsis gaditana*, Cryptophyte *Rhodomonas marina* and Prymnesiophyte *Isochrysis* sp., were supplied by the Mariculture Center of Calheta (Madeira, Portugal). Each non-axenic microalgal strain was cultured in 500 mL of enriched seawater with commercial culture medium Nutribloom Plus (Necton, Portugal). The inoculation cell number was maintained at $2.6 \times 10^6$ cells $mL^{-1}$, for *Isochrysis* sp. and *N. gaditana*, and $1.4 \times 10^5$ cells $mL^{-1}$, for *R. marina*. Five different volumes: 250 (T1), 500 (T2), 1000 (T3), 2000 (T4) and 4000 (T5) μL of nutrient solution $L^{-1}$ of seawater (previously adjusted to a salinity of 25 g $L^{-1}$), were used for the preparation of the growth media. The nutrient concentrations in the final growth medium are presented in Table 1.

Aerated cultures (with compressed air – 125 mL $min.^{-1}$) were maintained at 23 °C, with a photoperiod of 18:6 h light/dark cycles, at a light intensity of 52 μmol $m^{-2}$ $s^{-1}$ provided by daylight fluorescent lamps as in Le Chevanton et al. [9]. The microalgae were harvested at stationary phase. More detailed information on the experimental design and algal growth is referred in Fernandes et al. [16].

### 2.2. Carbohydrate composition

#### 2.2.1. Acid hydrolysis and alditol acetate derivatization

Monosaccharides were analysed as alditol acetate according to modified Blakeney et al. [5]. Briefly, 10 mg of dried algal biomass were exposed to a two stage sulphuric acid hydrolysis (3 h at 20 °C in 72% sulfuric acid, followed by 2.5 h at 100 °C, after water addition, in an oil bath). After cooling to room temperature, 200 μL of internal standard (2–deoxyglucose – 20 mg $mL^{-1}$) was added to the hydrolysate. To 1 mL aliquot of hydrolysate mixture, 200 μL of 25% ammoniac were added and the reduction of monosaccharides to alditol was performed. The reduction procedure involved the addition of 100 μL of 3 M ammoniac solution containing 150 mg $mL^{-1}$ of sodium borohydride and the incubation at 30 °C for 1 h in a water bath. Then two additions of 50 μL of glacial acetic acid, followed by homogenization, were carried out. Alditol acetylation was performed by the addition of 0.45 mL of 1-methylimidazole and 3 mL of acetic anhydride to 0.3 mL of the previous mixture. Next, the solution was incubated at 30 °C for 30 min. in a water bath. The derivatized monosaccharides (alditol acetate) were extracted with dichloromethane being posteriorly washed several times with water. The solvent was evaporated under a nitrogen atmosphere. The standard solutions were also derivatized prior to GC–MS analysis. At least two replicates were made.

#### 2.2.2. GC–MS analysis

Monosaccharides alditol acetates were analysed by gas chromatography (Agilent HP 6890) equipped with a mass selective detector (Agilent 5973) and a capillary column DB-225 J & W (30 m × 0.25 mm inner diameter, 0.15 μm film thickness) from Agilent. The inlet temperature was 220 °C and the column temperature was held at 220 °C for 5 min., ramped at 10° $min.^{-1}$ to 230 °C and kept at this temperature for 6 min. The transfer line temperature was 280 °C, the split ratio was 1:30 and Helium was used as the carrier gas with a flow rate of 1.2 mL $min^{-1}$. The derivatized monosaccharides were identified by comparing the retention times and mass spectra fragmentation with that obtained through injection of the standards. The monosaccharides quantification was made through the calculated response factor of each standard towards the internal standard. The standards used were 2–deoxyglucose, L(+)arabinose, D(+)xylose, D(+)galactose, D(+)glucose, D(+)mannose, D(+)rhamnose, D(+)fucose purchased at Sigma-Aldrich (St. Louis, MO, USA). Four replicates were performed for each GC–MS analysis being the results presented as the mean value ± standard deviation (SD) of monosaccharides expressed in mg $g^{-1}$ of dry biomass weight (DW). All the reagents used had analytical grade.

### 2.3. Thermal analysis

Thermogravimetric analysis (TGA) was performed using a SETSYS Evolution 1750 thermogravimetric analyzer (Setaram) from room temperature up to 700 °C, at a heating rate of 20 °C $min.^{-1}$ and under oxygen flux (200 mL $min.^{-1}$).

### 2.4. Statistical analysis

Statistical analysis of the data was carried out using the software IBM SPSS Statistics 23. Differences between treatments were assessed by one-way analysis of variance (ANOVA) followed by a Tukey's Post-Hoc analysis; p-values $< 0.05$ were considered to be statistically significant. Principal component analysis (PCA) was applied to summarise the information in a reduced number of principal components. Varimax rotation was selected to represent the planar projection of the loadings (variables) for the two principal components.

## 3. Results and discussion

### 3.1. Monosaccharides profile

Monosaccharide compositional analysis can give an indication of the original polysaccharide structure, cell wall composition and storage products based on phylogeny information from the literature [8,39]. In this regard, the assessment of the nutrient concentration effect on microalgae is essential to give an insight of the structural and functional variations that lead to a successful adaptation of each species. Table 2 shows the variation in the monosaccharides detected among the three marine microalgae studied. The results indicate significant differences ($p < 0.05$) in the amount and composition of monosaccharides in the three microalgae grown under different nutrient availabilities.

Mannose, galactose and glucose were identified in *N. gaditana* and *Isochrysis* sp. in all treatments applied. However, the proportions of these monosaccharides have changed between and within these two marine microalgae species. The three monosaccharides are epimers – mannose at C2 position of glucose and galactose at C4 position [11]. Like glucose, mannose is a key precursor of the nucleotide-sugar interconversion pathway and can be derived from fructose 6-phosphate whereas galactose is known as a key structural component of chloroplast membranes [3,19,35].

*N. gaditana* displayed the more complex mixture of monosacchar-