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In-depth metabolic profiling of marine macroalgae confirms strong biochemical differences between brown, red and green algae

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ABSTRACT

In-depth metabolic profiling, also termed metabolomics, provides detailed information about the biochemical phenotype of an organism. Besides improving our understanding of biochemical processes, metabolomics is used for environmental monitoring, natural product discovery, or even chemotaxonomy, among others. However, for marine macroalgae, comparative large-scale metabolomics studies are lacking, even though seaweeds belong to the most important aquatic primary producers. In this study, we present via a broad scale systematic metabolomics survey 391 metabolites from 21 seaweeds species, representing brown, red and green algae. We demonstrate clear differences in metabolite composition of these seaweeds, reflecting their taxonomic classification. We highlight these differences for amino acid, amino acid derivative and peptide metabolites, energy and carbohydrate metabolites, for lipid, fatty acid and sterol metabolites and for secondary metabolites, including selected metabolic pathways such as the urea cycle, the citrate cycle and the glyconeogenesis/glycolysis, besides others. Additionally, we link selected seaweed biochemical properties to potential pharmaceutical and nutraceutical applications.

1. Introduction

Marine macroalgae, also known as seaweeds, are an important renewable resource of the ocean and comprise > 10,000 species worldwide [1]. They are morphologically and functionally diverse organisms commonly divided into three groups, according to their pigmentation: brown algae (Phaeophyceae, Heterokontophyta), red algae (Rhodophyta) and green algae (Chlorophyta). Seaweeds have emerged in the last five decades as a vast source of active metabolites used in such different fields as pharmaceutics, cosmetics, agriculture, bioenergy and nutrition [2]. > 3000 compounds have been reported for macroalgae [3], revealing the complexity of these organisms. This is thought to be due mainly to two factors: (a) unique evolutionary features and (b) life in a harsh environment. According to the endosymbiont theory, the red and green algae originated from a primary endosymbiosis of a prokaryotic photosynthetic cyanobacterium with a non-photosynthetic eukaryotic protist host [4,5]. In contrast, brown algae are a phylogenetically distant lineage from the red and green algae, as they developed from a secondary endosymbiosis event involving a non-photosynthetic eukaryote and a unicellular red alga [5]. Brown algae therefore hold several different morphological and metabolic features [6], for example, plastids which are surrounded by four membranes, a specific cell wall composition, derived metabolic pathways [7] and the ability to synthetize both plant-like (C18) and animal-like (C20) oxylipins [8]. Additionally, seaweeds inhabit often rather harsh environments and are therefore exposed to many biotic and abiotic stress factors such as herbivory, diseases, competition, nutrient depletion and, in the intertidal zone, desiccation, fast changing salinity, temperature and irradiation. The resulting defense strategies are contributing by a significant level to their structural–chemical diversity [2,9].

Some metabolites found in macroalgae are known to have health-promoting effects, including anti-inflammatory (sulfolipids and fucoidans), anti-microbial (fatty acids and phenolic compounds), anti-mutagenic (sulphated polysaccharides, sulfolipids and polyphenols), anti-diabetic (polyphenols) and anti-cancer properties (water-soluble polysaccharides and sulfolipids) [10]. Furthermore, seaweeds can be used as

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a resource for food and feed applications: depending on the species, marine macroalgae contain various amounts of protein, dietary fiber, polyunsaturated fatty acids (PUFAs), and a variety of minerals and vitamins [11–13].

In the last decade, metabolite profiling of algae was mostly focused on the identification of lipids and their derivatives [14–17] besides selected secondary metabolites involved in defense reactions, such as mycosporine-like amino acids, isoflavones and halogenated compounds [18–20]. However, broad large-scale comparative metabolomics studies are lacking for marine macroalgae. This is in sharp contrast to our knowledge about terrestrial plants, where abundant consolidated information on metabolites is available [21].

In this study, we present the to our knowledge the first systematic broad-scale metabolomics investigation of 21 species of seaweeds, representing three phyla and eight orders. Using liquid chromatography-mass spectrometry (LC-MS), a well-developed methodology to perform metabolite profiling [14,21–24], we detected 391 different seaweed metabolites and assigned these substances to metabolic pathways. We demonstrate not only major metabolic differences between the three algal groups but also highlight selected differences between close relatives within single genera.

2. Materials and methods

2.1. Macroalgae samples

A total of 21 species of seaweeds (seven red, three green and eleven brown algae) were collected in October 2014 in the vicinity of Bodø, Northern Norway, in the intertidal and upper subtidal zones (Table 1). Each sample consisted of pooled material of several to many individuals. The samples were packed in zip-lock bags and transported at ambient temperatures (0 °C to 7 °C) to the laboratory. Here, samples were quickly rinsed in cold freshwater, to remove any visible adhering contaminants, and frozen at − 20 °C. Initial morphological identifications were verified by DNA sequence comparisons (see [25] and references herein for details).

2.2. Metabolic profiling

Metabolic profiling was performed by Metabolon, Inc. (Durham, NC, USA, http://www.metabolon.com/). A detailed description of these platforms, including instruments, data acquisition and processing, compound identification and quantification was published previously [26]. Briefly, frozen algae samples were ground in liquid nitrogen using mortar and pestle and stored at − 30 °C before extraction in methanol containing several recovery standards, using an automated MicroLab STAR-system (Hamilton Company, http://www.hamiltoncompany.com). The resulting extracts were divided into four fractions: two for analysis by separate (Reverse Phase)/Ultra Performance Liquid Chromatography-tandem Mass-Spectrometry (RP)/UPLC-MS/MS with positive ion mode electrospray ionization (ESI), another for analysis by (RP)/UPLC-MS/MS with negative ion mode ESI, and the last one for analysis by hydrophilic interaction liquid chromatography (HILIC)-UPLC-MS/MS with negative ion mode ESI. Samples were placed briefly on a Turbo TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

2.2.1. Quality assurance/quality control (QA/QC)

Several types of controls were analyzed in concert with the experimental samples: a pooled matrix sample generated by taking a small volume of each experimental sample (or alternatively, use of a pool of well-characterized human plasma) served as a technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of QC standards that were carefully chosen not to interfere with the measurement of endogenous compounds were spiked into every analyzed sample, allowed instrument performance monitoring and aided chromatographic alignment. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled matrix samples. Experimental samples were randomized across the platform run with QC samples spaced evenly among the injections.

2.2.2. Ultrahigh performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS)

All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then

Table 1
Marine macroalgal species included in the study, with coordinates of the sampling location. (see Biancarosa et al., 2016 for European Nucleotide Archive (ENA)/GenBank accession numbers).

No	Class	Order	Species	GPS coordinates
1		Bangiales	*Porphyra dioica* J.Brodie & L.M.Irvine	67.323491, 14.478753
2			*Porphyra purpurea* (Roth) Agardh	67.323491, 14.478753
3			*Porphyra umbilicalis* Kützing	67.239783, 14.510323
4	Rhodophyta	Gigartinales	*Chondrus crispus* Stackhouse	67.412274, 14.621368
5			*Mastocarpus stellatus* (Stackhouse) Guiry	67.325565, 14.478626
6			*Furcellaria lumbricalis* (Hudson) J.V·Lamouroux	67.305987, 14.727638
7		Palmariales	*Palmaria palmata* (L.) Weber & Mohr	67.322567, 14.457314
8		Ulvales	*Ulva intestinalis* L.	67.323491, 14.478753
9	Chlorophyta		*Ulva lactuca* L.	67.323491, 14.478753
10		Cladophorales	*Cladophora rupestris* (L.) Kützing	67.305987, 14.727638
11		Fucales	*Fucus serratus* L.	67.323491, 14.478753
12			*Fucus vesiculosus* L.	67.240804, 14.712079
13			*Fucus spiralis* L.	67.305987, 14.727638
14			*Pelvetia canaliculata* (L.) Decaisne & Thuret	67.326911, 14.478223
15			*Halidrys siliquosa* (L.) Lyngbye	67.239783, 14.510323
16			*Himanthalia elongata* (L.) S.F.Gray	67.276063, 14.572370
17	Phaeophyceae (Heterokontophyta)		*Ascophyllum nodosum* (L.) Le Jolis	67.305987, 14.727638
18		Laminariales	*Saccharina latissima* (L.) C.E.Lane, C.Mayes, Druehl & G.W·Saunders	67.240804, 14.712079
19			*Laminaria digitata* (Hudson) J.V·Lamouroux	67.240804, 14.712079
20			*Alaria esculenta* (L.) Greville	67.276063, 14.572370
21		Ectocarpales	*Chordaria flagelliformis* (O.F.Müller) C·Agardh	67.239783, 14.510323