Fitoterapia 126 (2018) 53–64

Contents lists available at ScienceDirect

Fitoterapia

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

A study of the chemical diversity of macroalgae from South Eastern Australia

Daniel Vuong[a], Matvi Kaplan[a], Heather J. Lacey[a], Andrew Crombie[a], Ernest Lacey[a,b], Andrew M. Piggott[b,*]

[a] Microbial Screening Technologies Pty. Ltd, Smithfield, NSW 2164, Australia
[b] Department of Chemistry and Biomolecular Sciences, Macquarie University, NSW 2109, Australia

ARTICLE INFO

Keywords:
Macroalgae
Chlorophyta
Rhodophyta
Phaeophyta
Southern Australia

ABSTRACT

Macroalgae are a rich source of biologically active chemical diversity for pharmaceutical and agrichemical discovery. However, the ability to understand the complexities of their chemical diversity will dictate whether these natural products have a place in modern discovery paradigms. In this study, we examined the relationship between secondary metabolite production and biological activity for a cohort of 127 macroalgae samples collected from various locations across South Eastern Australia. Approximately 20% of the macroalgae samples showed high levels of chemical diversity and productivity, which also correlated strongly with bioactivity. These "talented" species represent sustainable sources of metabolites that may be readily harvested for large-scale production. At a taxonomic level, significant differences in metabolite production and diversity were observed between Chlorophyta, Rhodophyta and Phaeophyta. For each talented species, the cometabolite pattern was unique to that species, with closely related species within the same genus displaying very different profiles. Despite over 50 years of investigation, we estimate that more than two-thirds of the chemical diversity of macroalgae remains unknown to science. By understanding the physicochemical properties and distribution patterns of metabolites, it is possible to make reasoned judgements about sustainable sourcing of macroalgae for biodiscovery.

1. Introduction

Since the early 1940s, the search for new drugs from both macroorganisms [1] and microorganisms [2] has prompted thousands of discovery campaigns that have seen over 250,000 natural products isolated, with hundreds of products launched as pharmaceuticals [3]. These discoveries have in turn spawned second, third, fourth or further generation products, with over 65% of pharmaceutical products directly or indirectly derived from natural products [4]. It is therefore surprising that our understanding of the chemical basis of the biodiversity that sparks these discoveries is very limited. There are relatively few publications that address the anatomy of chemical diversity in Nature, with the rationale for sourcing biological material, be it from exotic locations, ethnobotany, rare species, comprehensive sampling or targeted accumulation, generally not critically analysed [5]. Every successful discovery is dependent on the chemical diversity of its sources, yet paradoxically this is often the least understood aspect in the discovery process. Our failure to understand our source material is one of the root causes for the waning interest in natural products. Over the past 20 years, drug discovery has been dominated by high-throughput screening of large libraries of synthetic compounds and pure metabolites, where every structure is known and physicochemical properties can be easily calculated and modelled [6–7]. While this strategy promised to fill pipelines with hits and leads, in many therapeutic fields the launch of new therapeutics has stalled [8–9]. Perhaps in this discovery hiatus, the time has come to reconsider what natural products have to offer going forward into the new millennium.

Our knowledge of chemical biodiversity has traditionally been drawn from basic rather than applied research. While natural product databases, such as the Dictionary of Natural Products (DNP) [3], AntiBase [10] and MarinLit [11], represent important compilations of this knowledge, the records are themselves fragmentary, often describing only the first recorded discovery of a metabolite, and the associated taxonomic descriptions are often incomplete and become increasingly outdated over time. It is striking how little of our knowledge of the chemical complexity of unique species has been integrated into useful

* Corresponding author.
E-mail addresses: dvuong@microbialscreening.com (D. Vuong), mkaplan@microbialscreening.com (M. Kaplan), hlacey@microbialscreening.com (H.J. Lacey), acrombie@microbialscreening.com (A. Crombie), elacey@microbialscreening.com (E. Lacey), andrew.piggott@mq.edu.au (A.M. Piggott).

http://dx.doi.org/10.1016/j.fitote.2017.10.014
Received 4 September 2017; Received in revised form 10 October 2017; Accepted 20 October 2017
Available online 25 October 2017

tools for discovery [12].

Worldwide, there are over 15,000 species of macroalgae belonging to the phyla Chlorophyta (6379), Rhodophyta (7086) and Ochrophyta (4187), the latter phylum being dominated by the brown algal class Phaeophyceae (Phaeophyta) (2046) [13–14]. From these macroalgae, over 4000 metabolites are listed in the DNP database from the major taxonomic domains of macroalgae: Chlorophyta (633), Rhodophyta (1999) and Phaeophyta (1487) [3]. In Australia, basic research has generated over 40 papers on macroalgae from Australian waters since 1970 [15]. This research was driven by investment from government institutes, academic and commercial organisations, most notably Smith Kline Inc. in collaboration with CSIRO [1], the Australian Institute of Marine Science [5], the Roche Research Institute of Marine Pharmacology [16–17], AMRAD [18] and Griffith University in collaboration with Astra Zeneca [19], as well as the efforts of isolated researchers with regional priorities. In honouring Prof. Emilio Ghisalberti's outstanding contributions to the field of Australian natural products chemistry, it is noteworthy that he and co-workers investigated macroalgae from the Western Australian seaboard, contributing papers on the discovery of sesquiterpenes from *Caulerpa flexilis* var. *muelleri* [20], polyhalogenated monoterpenes from *Plocamium* sp. [21] and a dolastane diterpene from *Dictyota furcella* [22].

Chemotaxonomy provides the link between chemistry and biodiversity. When phenotyping is insufficient to identify a unique species, the secondary cometabolite profile, like the genotype, can provide an unequivocal fingerprint [23–24]. Indeed, the chemotype is becoming an integral tool for defining a species as unique [25]. In the present study, we have examined the relationship between the chemical diversity and taxonomy of a collection of macroalgae from South Eastern Australia. By understanding the distribution and patterns of secondary metabolites within the major classes of macroalgae, it is possible to explore the fundamental biodiversity criteria to better interface with modern discovery platforms.

2. Results and discussion

2.1. Biodiversity

One hundred and twenty-seven macroalgae samples were collected from marine and non-marine sources across South Eastern Australia between 1996 and 2001 (Fig. 1a). Voucher samples were submitted to the Tasmanian Herbarium and Melbourne Museum (Supplementary Data, Table S1) and were identified to the genus and species level (Fig. 1b). The algae comprised 26 Chlorophyta, 33 Rhodophyta and 68 Phaeophyta, representing the taxonomic domains of green, red and brown macroalgae, respectively. The Chlorophyta comprised 7 genera from 6 families, the Rhodophyta comprised 25 genera from 16 families and the Phaeophyta comprised 25 genera from 12 families (Tables 1–3).

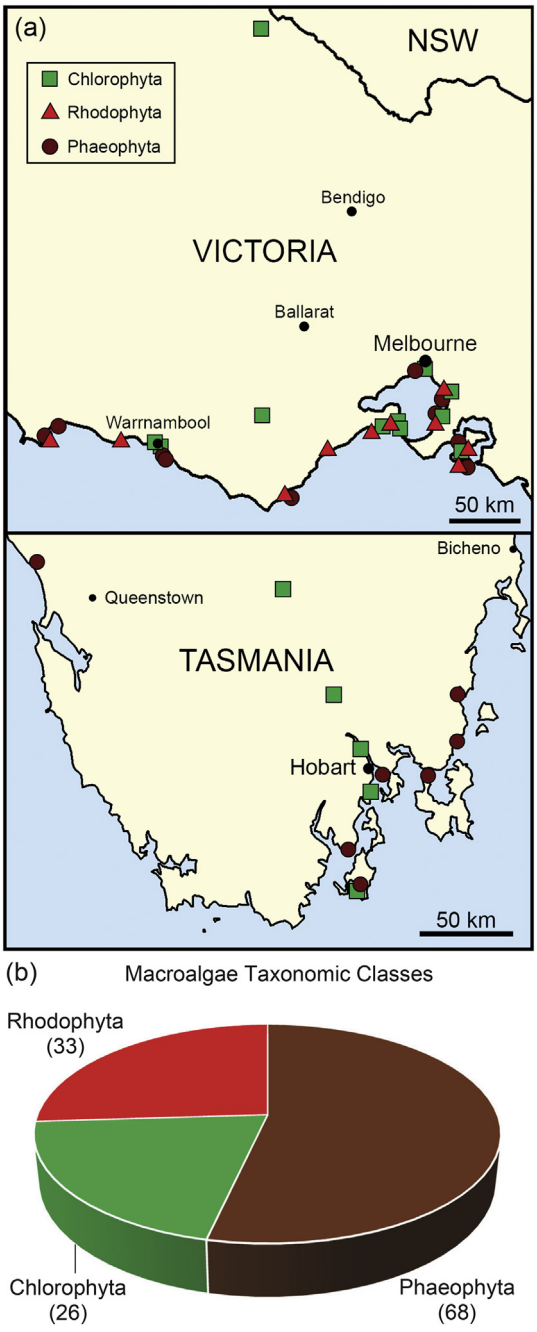

Fig. 1. (a) Macroalgae collection locations and (b) distribution of taxonomic classes.

2.2. Chemical diversity

The mass of material extracted from 1 g of each dried algal powder following maceration in 10 mL methanol (Fig. 2a and b) ranged from 2.0 to 94 mg (n = 125; mean = 27 ± 2 mg; median = 17 mg). Two outliers, 143-46 and 379-36, with masses of 372.8 and 281.2 mg respectively, contained appreciable levels of polar material that eluted with the HPLC solvent front, and were excluded from subsequent analyses. A Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks (H-test) revealed a statistically significant difference in median extracted mass (H = 7.01, p = 0.030) between Chlorophyta (n = 25; mean = 22 ± 4 mg; median = 14 mg), Rhodophyta (n = 32; mean = 22 ± 5 mg; median = 11 mg) and Phaeophyta (n = 68; mean = 30 ± 3 mg; median = 24 mg).

The crude methanolic extracts were subsampled and analysed by C_{18} HPLC with diode array (190–600 nm) and ESI(±)MS detection. A total of 2044 peaks were observed by diode array detection at 210 nm from the 127 samples, with a mean ± standard error (SEM) of 16 ± 1 peaks/sample, a median of 11 peaks/sample and a range of 1–62 peaks/sample (Fig. 2c). The mean total peak area (AUC) at 210 nm was 1390 ± 340 units/sample, with a median of 180 units/sample and a range of 20–29,000 units/sample (Fig. 2e). The wide data ranges with tight SEMs reflects an overdispersed distribution, with the majority of samples containing only a few metabolites in low abundance. As the crude extracts were standardised on volume (1 µL aliquot from 1 g of algal biomass macerated in 10 mL MeOH) and not on concentration of extracted material, the total AUCs provided a useful measure of metabolite abundance and therefore the metabolic productivity of each species. The following arbitrary productivity levels were defined based on total AUC: 0–50 = Very Low; 50–500 = Low; 500–5000 = Medium; 5000–10,000 = High; > 10,000 = Very High. Thirty-three of the macroalgae (26%) produced metabolites with a total