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PREVENTING SUN-INDUCED SKIN DAMAGE WITH NEW ZEALAND ALGAE-DERIVED COMPOUNDS: WORKFLOW FOR MOLECULAR TAXONOMIC IDENTIFICATION OF MACROALGAE

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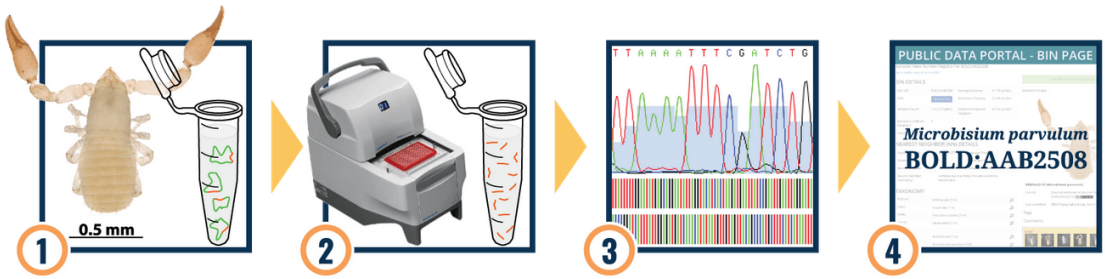
EXECUTIVE SUMMARY

Sunburn prevention and treatment is a large and growing worldwide market. Current options pose problems for both human health and the environment. Macroalgae are potential sources of novel, natural and ecologically friendly compounds that can be used for this purpose. Some of these sun-protective compounds have already been found in certain macroalgae. However, many others remain to be discovered with the screening of more macroalgae species. Macroalgae can be problematic to identify definitively by morphology due to a high degree of phenotypic plasticity, together with many species having morphological similarity. Identification of the source (i.e., the species) of useful compounds is critical to developing a commercial product.

Nucleic acid-based molecular taxonomic identification (DNA barcoding) can be used to complement morphological based identification of macroalgal species of interest (Figure 1).



Step 1: Isolate DNA from the sample
Step 2: Amplify the target DNA barcode region using PCR
Step 3: Sequence the PCR products
Step 4: Compare the resulting sequences against reference databases to find the matching species

Figure 1. Graphical overview of molecular taxonomy workflow (source: https://ibol.org/about/dna-barcoding/).

We report here the results of a literature review to identify appropriate genomic regions, primer sequences, and reaction conditions to enable molecular taxonomy of macroalgae.

1. INTRODUCTION

There is a substantial opportunity for new products in the sunburn prevention and treatment categories, and it is estimated the market will grow by over NZ$4 billion globally from 2022 to 2027 to reach NZ$27 billion (https://www.fortunebusinessinsights.com/sun-care-products-market-103821). Agents currently used to block or absorb ultraviolet radiation that causes sunburn are not 100% effective, making cumulative skin damage inevitable. Additionally, many sunscreens have damaging side-effects and are increasingly being banned due to their adverse environmental impacts (DiNardo & Downs 2018; Schneider & Lim 2019; Sen & Mallick 2021). Thus, there is considerable pressure to develop better sunscreen agents and treatments for sunburn (Pandika 2018).

One potential source of new sunscreen products is marine algae (Pandika 2018). The physical environment inhabited by algae (including seaweed and cyanobacteria) requires them to be masters of managing light exposure and its damaging effects (Sen & Mallick 2021). Algae are of interest as sources of novel sunscreen compounds as they have evolved finely-tuned ultraviolet radiation (UVR) control and damage-mitigation processes to ensure their survival. However, bioprospecting for natural products derived from wild sources is dependent on the correct identification of the source (Eisenman et al. 2012; Rosic 2021).

Macroalgae can be difficult to identify correctly from morphology alone (Andersen 1992; Robba et al. 2006). The morphology of macroalgae can vary during development, reproductive stages, seasonality and under different environmental conditions (Robba et al. 2006). A further complication is that many macroalgae species have complex life cycles that look very different at each stage and these differences are not well documented. For example, some species may be difficult to differentiate in the early stages of vegetative growth because of morphological similarities with other species. In other instances, the same species may have different morphologies depending on the environment in which it is growing (Robba et al. 2006). This remarkable degree of phenotypic plasticity poses immense challenges to definitive assignment of a specimen to species based on morphology alone. Identification based on DNA sequences offers a complementary approach to morphological-based identification and can help solve some of the issues associated with morphological taxonomy. We reviewed publications discussing DNA-based identification of macroalgae to identify reaction conditions and primers that would enable molecular-based approach to complement morphological identifications.

The overarching aim of this project is to search for moieties from algae (especially NZ endemic and native species) that can absorb UVR or otherwise be used for skin sun care. Our hypothesis is that the finely tuned ability of algae to protect themselves from damaging light, and the ability of some algal extracts to modulate human immune responses can be harnessed to deliver ecologically friendly products for the

prevention of sunburn damage, the mitigation of symptoms and the promotion of healing. The UVR absorbers identified will have the potential to be used in sun protection products as alternatives to currently available molecules with their issues around efficacy and environmental accumulation. They will provide consumers with new approaches and better products to ensure New Zealand no longer leads the world in skin cancer rates. To achieve these goals, robust identification of the algae of interest is required.

2. APPROACH / METHOD

Macroalgae of interest were collected as part of a seaweed biodiversity study on marine farms or were otherwise targeted and sourced. Samples were examined in the laboratory and assigned initially to species based on morphological characteristics. With the aid of textbooks (Adams 1994) and published papers, it was possible to identify most of the specimens using visual inspection and examination of fine structures with the aid of a stereomicroscope to further distinguish the morphological characteristics. Specimens of similar morphology were pooled, freeze-dried, and then finely milled to a powder. The powdered algae are used to make extracts that are screened for the presence of sun-damage prevention compounds. These same samples can be used to isolate DNA for molecular taxonomy if needed.

2.1. DNA extraction

Experience through other projects has shown that DNA can be extracted from dried specimens of *Porphyra* spp. and *Pyropia* spp. red seaweed using a simple method of heating to 90 ºC in Tris-EDTA buffer containing 5% Chelex resin beads (BioRad) for 20 min. However, many macroalgae contain high concentrations of metabolites that can contaminate extracted DNA and inhibit the PCR reaction. In this case, DNA can be extracted using the Qiagen DNeasy Plant mini kit (Qiagen, Hilden, Germany). Extracted DNA can be stored at -20 ºC and is stable for a number of years.

2.2. Polymerase chain reactions

Molecular taxonomy is the use of DNA sequence data from portions of the organism's genome (genes and/or non-coding regions of the genome) to identify species (Hebert et al. 2003). Different portions of the genome accumulate changes at different rates; thus, DNA data can be used to characterise taxonomic ranks from individuals to phyla depending on the region of the genome sequenced (Avise 1994). The portions of the genome sequenced are determined by the choice of primers used in the polymerase chain reactions (PCR) that amplify the region to yield sufficient DNA for sequencing.

Selection of appropriate regions for sequencing is guided by the length of the portion amplified, and the availability of regions flanking the region of interest that are conserved across the study group. Ideally, portions of the genome used to identify macroalgae species should be ~650 to 1,110 nucleotides long, as these lengths are tractable for Sanger sequencing (a cost-effective method of obtaining sequence data) (Crossley et al. 2020). They should be long enough and show enough genetic variation to assign individual specimens to their species.

A review of publications using DNA to identify species of macroalgae found that the internal transcribed spacer region (ITS) of the ribosomal DNA is suitable for barcoding algae specimens of interest in this project. Additionally, sequences obtained from the ITS region using primers such as the forward primers P1 and P2 and the reverse primers G4, HarvR4 and MEMR4 are likely able to discriminate between closely related species, and between more distantly related algal species (Saunders & Moore 2013). Then if required, primer sequences and amplification protocols for other regions of the nucleus such as the small and large ribosomal subunits, the ribulose-1 5 carboxylase large subunit (*rbc*L) gene, and also mitochondrial markers such as the cytochrome c oxidase subunit 1 (COI) and cytochrome b (COB) genes could be used to assign species identity to specimens (Saunders 2005; Saunders & McDevit 2012a; Saunders & McDevit 2012b; Saunders & Moore 2013).

3. SPECIES IDENTIFICATION FROM SEQUENCES

The sequences for amplified DNA fragments generated using PCR will be compared with sequences held in databases such as the Barcode of Life Data (BOLD) System (https://www.boldsystems.org/), an internationally curated, cloud-based archive dedicated to species identification. The BOLD database holds over 30,000 sequence records for red algae from 100 countries and will allow our sequences to be matched to published sequences. The BOLD database also allows metadata such as collectors, collection location, and specimen photos to be archived with the sequences (see https://www.boldsystems.org/index.php/Public_RecordView?processid=ABMMC1225-07 for an example of the meta- and sequence data held for a specimen). Information can be kept private (i.e., restricted to users that are granted access to the information by the project manager) for as long as necessary. DNA sequence data are also held in GenBank (a searchable database at https://blast.ncbi.nlm.nih.gov/Blast.cgi) providing another option for assigning specimens to a species.

These molecular taxonomy procedures will only be applied to collected samples that have potential as suitable sources of the sun-protective compounds. The same basic protocol described above for red macroalgae identification can be adapted and used for the identification of brown macroalgae or cyanobacteria.

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5. REFERENCES

Adams NM 1994. Seaweeds of New Zealand: an illustrated guide. Canterbury University Press, Christchurch. 360 p.

Andersen RA 1992. Diversity of eukaryotic algae. Biodiversity & Conservation 1(4): 267-292.

Avise JC 1994. Molecular Markers, Natural History and Evolution. New York, Chapman and Hall. 511 p.

Crossley BM, Bai J, Glaser A, Maes R, Porter E, Killian ML, Clement T, Toohey-Kurth K 2020. Guidelines for Sanger sequencing and molecular assay monitoring. Journal of Veterinary Diagnostic Investigation 32(6): 767-775.

DiNardo JC, Downs CA 2018. Dermatological and environmental toxicological impact of the sunscreen ingredient oxybenzone/benzophenone-3. Journal of Cosmetic Dermatology 17(1): 15-19.

Eisenman SW, Tucker AO, Struwe L 2012. Voucher specimens are essential for documenting source material used in medicinal plant investigations. Journal of Medicinally Active Plants 1(1): 30-43.

Hebert PD, Cywinska A, Ball SL, deWaard JR 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270(1512): 313-21.

Pandika M 2018. Looking to nature for new sunscreens. ACS Central Science 4(7): 788-790. doi:10.1021/acscentsci.8b00433.

Robba L, Russell SJ, Barker GL, Brodie J 2006. Assessing the use of the mitochondrial cox1 marker for use in DNA barcoding of red algae (Rhodophyta). American Journal of Botany 93(8): 1101-1108.

Rosic NN 2021. Recent advances in the discovery of novel marine natural products and mycosporine-like amino acid UV-absorbing compounds. Applied Microbiology and Biotechnology 105(19): 7053-7067.

Saunders G, McDevit DC 2012a. Acquiring DNA sequence data from dried archival red algae (Florideophyceae) for the purpose of applying available names to contemporary genetic species: a critical assessment. Botany 90(3): 191-203.

Saunders GW 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. Philosophical Transactions of the Royal Society B: Biological Sciences 360(1462): 1879-1888.

Saunders GW, McDevit DC 2012b. Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. In: Kress WJ, Erickson DL ed. DNA barcodes: methods and protocols. Totowa, NJ, Humana Press. Pp. 207-222.

Saunders GW, Moore TE 2013. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. Algae 28: 31-43.

Schneider SL, Lim HW 2019. Review of environmental effects of oxybenzone and other sunscreen active ingredients. Journal of the American Academy of Dermatology 80(1): 266-271.

Sen S, Mallick N 2021. Mycosporine-like amino acids: Algal metabolites shaping the safety and sustainability profiles of commercial sunscreens. Algal Research 58: 102425.