An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

15F698 – Seaweed-Microbe interactions to enhance bioactive yields for food applications

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

This project investigated the implications of seaweed-microbe interactions on the biochemical composition, and related bioactivity in commercially important Irish seaweed, as well as enzyme activities linked to associated bacteria. Cultivable and non-cultivable microbial communities isolated from different species of seaweed were characterized, and seasonal and spatial (including within-seaweed) variation in bacterial communities, chemical composition and bioactivity was established. Specific project objectives were to:

1) perform for the first-time a characterisation and isolation of bacteria associated with natural Irish seaweeds of food value,
2) evaluate the role of epiphytic bacteria in bioactive production by seaweeds and induced enhancement of bioactive yields,
3) assess the impact of storage and processing conditions on high value compounds in seaweed foodspecies, and
4) assess microbial enzymatic activity with potential applications in bioactive recovery by enzyme-assistant extraction.

The project impact relates specifically to the developed new scientific skills and protocols directly relevant to industry, new insights into the important of seaweed sourcing and pre-treatment prior to processing, and the growth in potential of use of novel marine enzyme in several industrial settings including the agri-food and cosmeceutical and homecare sectors.

KEYWORDS

Seaweed, Microbiome, Bioactivity

ACRONYM	SMI-BIO
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COLLABORATORS, INSTITUTION	Dr Dilip Rai, Teagasc Dr Alan Dobson, University College Cork
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Section 1 – Research Approach & Results

Start Date

01 November 2016

End Date

30 June 2022

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 1: Basic Principles Observed

NRPE Priority area

Food for Health

Total DAFM Award

€824,992.00

Total Project Expenditure

€706,566.16

Rationale for undertaking the Research

Significant recent interest globally and within Ireland has highlighted the potential of marine macrolagae (seaweeds) across multiple commercial sectors including agri/food and health. They contain several groups of chemical constituents which exhibit many bioactivities which are beneficial to human health. To fully implement the inclusion of seaweed-based materials into functional foods by Irish companies, a number of questions need to be addressed regarding product consistency, stability and bioactive bioavailability. These include a detailed characterisation of the biochemical composition of valuable seaweed species (i.e. improved profiling of bioactive components), linkage between bioactivity and chemical composition and natural variability in bioactive profiles which may be altered, positively or negatively, during storage and processing of algal biomass through a range of mechanisms.

In addition to processes that cause degradation of valuable bioactives such as oxidation processes, algal epibionts (microorganisms naturally associated with seaweed surfaces) can influence the production of secondary metabolites and commercially valuable bioactives. Here, the relationship between Irish seaweed biochemical composition and bioactivity, and naturally associated epiphytic bacteria were assessed. Additionally, bacterial strains from seaweed surfaces were isolated that are likely to exhibit novel enzyme activities, supporting the development of new bioactive extraction processes and thus improved yields.

This project provided I) detailed molecular characterisation of bacterial epiphytes on Irish food seaweeds, II) mechanisms by which bioactive production is influenced by preprocessing and III) improved extraction techniques based on natural bacterial enzymes isolated from Irish seaweeds.

Methodology

The methodologies used in this project included specialised phycological, biochemical, chemical and microbial methods, ranging from field sampling of natural seaweed and associated microbial populations, to molecular

characterisation of the interactions of seaweed holobiont, reflecting the complex multidisciplinary nature of this project. Advanced analytical procedures (HPLC, LC-MS) were applied to evaluate species-, site- and season-specific chemical profiles in relation to environmental parameters insitu as well as in laboratory settings. Seaweed species were challenged with 1) abiotic and 2) biotic stressors and changes in chemical composition and associated bioactivity was tested. Enzyme activities of isolated bacteria were characterized, and compared to commercial enzymes. Degradation of seaweed biomass was artificially induced under controlled conditions, and biochemical composition and microbial communities monitored. The effectiveness of seaweed pre-processing protocols relevant to industry were tested in relation to compound yield and effects on seaweed microbial loads. Novel enzymes derived from seaweed associated bacteria were screened and applied to seaweed biomass.

Project Results

To our knowledge this is the first time that the effect of combined environmental and biotic stressors has been applied to attempt to modify biochemical composition in algae with a range of food and agri/feed applications. Results highlight important species-specific responses to environmental stimuli/stressors that have led to differential profiles in biochemical composition and bioactivity. In particular the stimulation of algal biomass with external bacterial strains has revealed exciting new insights in the functioning, as well as the complexity, of the seaweed holobiont.

Project outputs form an important step towards realising and applying the concept of the holobiont of seaweed species that are currently utilised (either harvested or cultivated) commercially. New methodologies for the establishment of axenic algal cultures, and for the first time attempted to modify chemical and microbial profiles of commercial seaweeds.

Results have revealed interesting species-specific patterns with regard to physical biomass degradation in simulated commercial processing (informed by common practices applied in Ireland). Results further highlight the importance of methods and protocols of storage in particular under different seasonal scenarios which directly affect biomass composition and value (due to changes in bioactivity). Enzyme activities of isolates were screened and their performance compared to that of commercially available enzymes. A particular focus was on bacteria with cell wall degrading enzymes that were isolated from seaweed biomass following degradation. Results are highly relevant to various industries including food, cosmetics and health care industries where enzymes active at low temperatures are highly advantageous.

Section 2 - Research Outputs

Summary of Project Findings

This project successfully characterised seaweed-microbe interactions in a number of commercially valuable seaweed species. It highlighted the strong influence of place of harvesting/collection and cultivation methods on the yield and composition of bioactive compounds, and demonstrated dependency of species and habitat on microbial communities; some potentially pathogenic bacteria were associated with seaweed surfaces, and cultivation and processing protocols, relevant to industry, influences microbial flora in experimental setups. Novel enzyme activities of interest both to industry and the scientific community were detected, and controlled enzymatic degradation of seaweed biomass revealed valuable insights into the further biotechnological potential of bacteria associated with seaweeds. Methods were based on those currently applied by industry and results highlight important effects of seasonality and seaweed species which inturn affect the suitability of harvested or cultivated biomass post-storage, and thus project findings have direct implication for common practices of storage and processing for Irish industry. Results have already been disseminated at several national and international conferences, and outputs include recommendations for industry regarding the four target seaweed species. Further peer-reviewed publications are currently in preparation. The project further significantly

contributed to capacity development through the training of several postdocs, research assistance/technical officers and PhD students.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	0	2	2
Post Doctorates	2	3	5
Research Technicians/Assistants	0	2	2

Summary of Academic Outputs

Research Outputs	Total Number	Details
PhD Theses	2	1) Wada Maureen Ihua (2020, UCC): Thesis title 'Microbial populations associated with the Irish brown seaweeds, Ascophyllum nodosum and Laminaria digitata, and their biotechnological applications' Supervisor: Prof Alan Dobson. 2) Claudia Cara Ortega (2022, NUI Galway)'Influences of abiotic and biotic factors on biochemical lcomposition of selected temperate macroalgae'. Supervisor: Prof Dagmar Stengel
Publications in Peer Reviewed Scientific Journals	3	3 published to date, 9 more in progress/prep (see list in next section)
Training Courses	3	1) Dr Lawlor (NUI Galway): “Research Integrity – Natural and Physical Sciences” provided by Epigeum Online Course System (Oxford University Press). Completed during November/December 2020. 2) Research training workshop attended by PhD student Claudia Cara Ortega during conference ISAP2021 (online 1-3June 2021) “Springer Writing Seminar”: the journey of a scientific paper: from its writing to its dissemination. 3) Revealing algae biotechnological potentials to contribute to sustainable Blue Growth in the Mediterranean”, a course in applied phycology organised by INSTM (National Institute of Marine Sciences and Technologies) supported by GlobalSeaweedSTAR and ISAP. This was held virtually on 28th September 2021.
Other	4	1) Undergraduate theses: NUI Galway: 2022 The Role of Chemicals in Controlling Seaweed Bacteria Interactions’ (Lauren Davy)2021 “Assessing bacteria isolated from L. digitata thallus as a tool for estimating easily assimilable organic carbon(AOC) release by seaweeds in vitro” (Gillian Moran). 2) Teagasc: Mr. Samson Tillman: BSc (BioTech) 4th year project, NUI Maynooth was on placement at Ashtown and trained in relevant skills: Project

report on ‘Antioxidant activities of seaweeds' for 12 weeks, from Feb - March, 2022.

3) Prof Stengel contributed to an invited report for the Marine Institute on Blue Carbon in Irish coastal waters (‘Blue Carbon and Marine Carbon Sequestration in Irish Waters and Coastal Habitats’; Cott et al 2021; https://oar.marine.ie/handle/10793 /1685); this report was referred to in the Irish Governments Climate Action Plan2021. https://www.gov.ie/en/publication /6223e-climate-action-plan-2021/

4) Report of the Scientific Committee of the Food Safety Authority of Ireland. 2020. Safety Considerations of Seaweed and Seaweed-derived Foods Available on the Irish Market. https://www.fsai.ie

Intellectual Property

The work undertaken as part of this project was fundamental in nature and no patents have arisen (or were expected to arise).

Summary of Other Project Outcomes

Project Outputs	Details	Total No.
New Processes	Task 3 and 4 contributed to the development of processes for seaweed sterilisation and pre-drying protocols, respectively. 1) Sterilisation: Of the various sterilisation treatments that were tested (UV, chemical treatments with sodium hypochlorite or ethanol, antibiotics at different levels of intensity/concentration and duration), the most successful treatment was treatment with antibiotics mixture comprising penicillin G, streptomycin, and chloramphenicol. Sterilisation measures were only implemented for small seaweed pieces, and may not be appropriate at larger scale. Methods were also highly specific to seaweed species. It is unlikely that the protocols can be applied in a commercial situation at present but provided valuable insights into seaweed/microbe interactions that may be followed up in the future. 2) Pre-drying: Drying treatments were performed at two seasons under outdoor conditions which were similar to current industry practices of storing seaweeds prior to processing. Bioactive levels and composition depended on natural light, temperature and humidity and indicated that depending on seaweed species and bioactive compound of interest, simple measures such as storage at different temperatures and humidities, i.e., applying very simple and cheap measures, algal biomass composition can be influenced, both positively and negatively. Seasonality of biomass source was further key in determining final bioactive levels.	2

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	During the project period, Prof Stengel was a member of the BBI Scientific Committee
	Prof Stengel was appointed member of the NSAI Technical Committee TC454and CEN Committee on Algae and Algal Products
	Prof Stengel was appointed as a Member of the Scientific Committee of the Food Safety Authority of Ireland as of 26th February 2021.
Other	Report of the Scientific Committee of the Food Safety Authority of Ireland.2020. Safety Considerations of Seaweed and Seaweed-derived Foods Available on the Irish Market.
	Prof Stengel contributed to an invited report for the Marine Institute on Blue Carbon in Irish coastal waters ('Blue Carbon and Marine Carbon Sequestration in Irish Waters and Coastal Habitats'; Cott et al 2021;https://oar.marine.ie/handle/10793/1685); this report was referred to in the Irish Governments Climate Action Plan 2021.https://www.gov.ie/en/publication/6223e-climate-action-plan-20211/

Dissemination Activities

Activity	Details
Workshops at which results were presented	Conference presentations and session chairs: International Phycological Congress: Prof Stengel chaired and led discussion sustainable seaweed utilisation at the2021 International Phycological Congress (Chile/online), March 2021. International Society for Applied Phycology (online):KCL co-chaired the session "Oral Session 5 – Bioprocessing" and further contributed and oral presentation Haren, D., Walsh, D., Kirke, D., Stengel, D.B. and Rai, D. (2019). Anti-oxidant and anti-microbial activities of Irish seaweeds. 13th World Congress on Polyphenols Applications, Malta Sept/Oct 2019 (oral) Haren, D., Kirke, D.A., Stengel, D.B., and Rai, D.K (2019). Screening of Irish seaweeds for bioactivity using UV-Vis spectroscopic methods. European Conference on Spectroscopy of Biological Molecules (ECSBM 18), Dublin,August 2019 (poster). Ihua, M.W., Guihéneuf, F., Mohammed, H., Margassery L.M., Jackson, S.A., Stengel, D.B., Clarke, D.J. and Dobson, A.D.W. (2019). Experimental decay of Ascophyllum nodosum (Phaeophyceae) induces changes in associated microbial populations WITH applicability in enzyme-assisted extraction technologies 7th European Phycological Congress, Zagreb August 2019 (poster)https://www.tandfonline.com/doi/abs/10.1080/09670262.2019.1626628?journalCode=tejp20

Ihua, M., Dobson, A.D.W. and Stengel, D.B. (2019). Metagenomic analysis reveals significant seasonal variations in the epiphytic bacterial communities associated with different parts of the brown seaweed Laminaria digitatahttps://doi.org/10.1099/acmi.ac2019.po0177, Microbiology Society, Belfast, Apr2019 (poster)

Ihua, M.W., Guihéneuf, F., Mohammed, H., Margassery, L.M., Jackson, S.A., Stengel, D.B., Dow, M.J., Clarke, D.J. and Dobson, A.D.W. (2019) Decayin gAscophyllum nodosum as a source of algal cell wall degrading enzymes with potential utility in enzyme-assisted extraction technologies. https://doi.org/10.1099/acmi.ac2019.po0180. Microbiology Society, Belfast, Apr2019 (oral)

Aznar-Roca, R., Kirke, D.A., Stengel, D.B. and Rai, D. (2018). Hypocholesterolomic and atherogenic indices of Irish seaweeds. Irish Algal Researchers' Conference (iARC-2018) Sept 6-7, 2018, Galway, Ireland
(poster)

Haren, D., Kirke, D.A., Stengel, D.B., and Rai, D.K (2018). Total phenolic content and antioxidant potential of four Irish seaweed species. 47th Annual International Food Science and Technology Ireland Conference, University College Cork, December 2018 (poster). https://www.ucc.ie/en/fns/47thannualfoodresearchconference/

Cara-Ortega, C., Lawlor K., Stengel, DB. (2021). Biochemical changes upon seaweed-microbiome interaction. Conference: International Society for Applied Phycology (online, 14th May- 13th August). This poster won best poster award.

Lawlor K., Stengel, DB. (2021). Changes in high-value biomolecules and bacterial contamination during outdoor storage and degradation of four macroalgae. Conference: International Society for Applied Phycology (online,14th May- 13th August).

Lawlor K., Stengel, DB. (2022). Post-harvest stability of key biomolecules in seaweed biomass. Environ 2022, the 32nd Environmental Researchers Colloquium, 20-22nd June, Belfast.

Cara-Ortega, C., Lawlor K., Stengel, DB. (2022) Influences of natural andartificial abiotic factors on Ulva biochemical composition. Poster. SeaWheatCOST Action Conference "Ulva: from fundamental biology to aquaculture: stateof the art, bottlenecks and gaps" (COST Action CA20106, September2022).

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	Knowledge outputs relate to the development of new concepts of the seaweed holobiont applications by the seaweed industry and marine food processors. Specially, epipbiontic seaweed communities from Irish commercial seaweed populations have for the first time been characterised, which has direct implications for aquaculture and seaweed processors. Outputs highlight the presence of complex bacterial communities on

	natural seaweed materials, the effect of cultivation, drying and processing conditions on microbial communities and bioactive levels and finally, the potential to utilise seaweed-related microbial enzymes in commercial applications.
Identify any knowledge transfer activities executed within the project.	Amongst others, at European level, knowledge transfer has been implemented through participation in the BBI Scientific committee discussing strategy and plans for future funding opportunities for the European marine sector. In Ireland, the project results have directly impacted (guided) inputs into the FSAI report on Seaweed (2020). Additionally, international industry-relevant conferences have been used as a platform to disseminate project outputs, through conference presentations and sessions chairs (for details, see outputs). Further knowledge transfer has occurred through activities that have led to new projects in the bluebio sectors both within Ireland and at EU level where all project partners have been highly active and successful (for detail see funding).
List any impacts resulting from the knowledge transferred during the project.	Impacts directly relate to the successful interactions with relevant stakeholders including the NSAI and FSAI where project activities have been used to inform policy and industry recommendations. Scientific impacts have already been demonstrated through publications in excellent peer-reviewed scientific journals, and through active participation in key international conferences; this has enabled the dissemination of results and creating international awareness of SMI-BIO activities, and also enabled the team to linkup and network with international researchers. Impacts are also demonstrated through the successful funding of projects at EU levels, and the excellent interactions within the team(Galway, Teagasc/UCC) which have led to several joint project applications and collaborative initiatives (e.g. student supervision, GRCs etc). Further outputs (publications in prep) will have impacts for both scientific and industry audiences. Additionally, the project has allowed 2 students to successfully complete the PhDs, trained a number researchers at technician, undergraduate and postdoc level, thereby significantly contributing the bluebio research capacity building.

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Types of Funding Resource	Funding €	Summary
Exchequer National Funding	€1000.00	NUI Galway (national). SBIR SEawEed MAPping Solutions –SEEMAPS (Marine Institute and Enterprise Ireland). 2020-2021.Total €24,390.24 (ex. VAT)
Exchequer National Funding	€500,668.00	Teagasc (national) DAFM project ID 2021R616 – "Flavoromics of Grass-Fed Beef and Lamb" €500,668. Where Dr Dilip Rai is Task Leader on "Characterisation of Non-Volatile Dietary Biomarkers in Forage, Feed, Beef and Lamb". Forage includes seaweeds in this project.
EU R&I programmes	€572,976.00	NUI Galway (International) HORIZONMSCA-2021-DN-01 ('SEACHEM', Prof Stengel project partner)' Training a new generation of researchers in offshore seaweed aquaculture to produce high-value chemicals' €572,976.00
EU R&I programmes	€0.00	NUI Galway (International) Cost Action 20106 - TOMORROW'S 'WHEAT OF THE SEA': ULVA, A MODEL FOR AN INNOVATIVEMARICULTURE (Prof Stengel Project partner and Working Group leader); Bioactive chemicals and Ulva-associated microorganisms. No funding to Ireland directly, funding is linked to consortium activities. https://www.cost.eu/actions/CA20106/MoU - 046/21
Exchequer National Funding	€1,097,064.00	BlueBio-Cofund project (Prof Stengel Lead PI, Prof Dobson Collaborator). MINERVA. 36 months, 01/09/2020 - 31/08/2023.Marine Innovation using Novel Enzymes for waste Reduction and Valorisation of Algal biomass. EUR1.097.064
Exchequer National Funding	€72,000.00	NUI Galway Hardiman Scholarship 2022 awarded
EU R&I programmes	€606,505.00	FNR-2020 (MARBLES) (grant no. 101000392) 60 months 2020-2025. Marine Biodiversity as Sustainable Resource of Disease-Suppressive Microbes and Bioprotectants for Aquaculture and Crop Disease. (UCC)

Future Strategies

The project team are currently actively pursuing additional funding streams from within Ireland and theEU (Teagasc Walsh Fellowship, DAFM, Bluebio Partnership etc.).

Project Publications

List publications numerically.

1. Ihua MW, FitzGerald JA, Guihéneuf F, Jackson SA, Claesson MJ, Stengel DB, Dobson ADW. (2020) Diversityof bacteria populations associated with different thallus regions of the brown alga Laminaria digitata. PLoSONE 15(11): e0242675.
2. Ihua MW, Guihéneuf F, Mohammed H, Margassery LM, Jackson SA, Stengel DB, Clarke DJ, Dobson ADW.2019. Microbial population changes in decaying Ascophyllum nodosum produce macroalgalpolysaccharide-degrading bacteria with potential applicability in enzyme-assisted extraction technologies. Marine Drugs 17(4), 200; 10.3390/md17040200
3. Jackson SA, Duan M, Zhang P, Ihua MW, Stengel DB, Duan D and Dobson AW. (2022). Isolation, identification, and biochemical characterization of a novel bifunctional phosphomannomutase/phosphoglucomutase from the metagenome of the brown alga Laminaria digitata. Frontiers in Microbiology 13:1000634. doi: 10.3389/fmicb.2022.1000634

In preparation:

1. Lawlor, K.C., Rai, D. & Stengel, D.B. (In prep.). Degradation rates of high-value biomolecules during pre-drying storage of four industry relevant macroalgae species. Algal Research (journal to be confirmed). Draft available.
2. Lawlor, K.C., Stengel, D.B. (In prep.). Applying single image normalized difference vegetation index (SI-NDVI) as a non-invasive tool for in vitro fitness assessment of a selection of economically relevant macroalgal species. Sensors (journal to be confirmed). Draft available.
3. Jackson, S., Lawlor, K.C., Dobson, A.W., Stengel, D.B. (In prep.). Seasonal and spatial variation in the surface microbiome of wild-harvested Ulva sp.
4. Lawlor, K.C., Jackson, S., Dobson, A.W., Stengel, D.B. (Requiring further lab work, data analyses, in prep.). Changes in A. nodosum associated microbiome mediated by seasonal abiotic factors during post-harvest storage.
5. Cara Ortega, C., Lawlor KC, Stengel, D.B. (In prep.). Seasonal and spatial variation in chemical composition of four commercial seaweeds.
6. Cara Ortega, C., Lawlor KC, Stengel, D.B. (In prep.). Biochemical composition response of four Irish seaweeds to the effects of the abiotic environmental factors: light and temperature
7. Cara Ortega, C., Lawlor KC, Stengel, D.B. (In prep.). A comparative assessment of seaweed surface sterilization methods in vitro whilst maintaining seaweed health.
8. Cara Ortega, C., Lawlor KC, Stengel, D.B. (In prep.). Effect of foreign microbial strains infection on biochemical composition in seaweed in vitro culture.
9. Rai, D., O'Hara, Stengel, D.B. et al. (in prep.). Antioxidant and antimicrobial activities of Irish seaweeds. Marine Drugs (Journal to be confirmed).