MASTS-SFC Saltire Emerging Researcher Scheme (MASTS-SERS)

Final Report

MASTS in association with the Scottish Funding Council supported the Saltire Emerging Researcher Scheme, which represented an important and exciting opportunity for Post Graduate Researchers (PGR) and Early Career Researchers (ECR) to engage in substantive collaboration with colleagues from Europe (EA, EEA and EFTA countries).

The scheme aimed to promote mobility between Scotland and European research partners with the aim of strengthening existing, and seeding future, research relationships. Participants are expected to demonstrate the impact of their exchange through the publication of novel research work, the formation of new collaborations and project/ funding submissions, and the dissemination of their results.

As your exchange has now come to a close, we ask that you reflect on the exchanges and provide a report by filling in the form below. The reports will need to demonstrate the potential benefits of the grant for both the recipient and their collaborators. Please return this within four weeks of completing your exchanges to **masts@st-andrews.ac.uk**. When you do so, you are agreeing that your answers may be used to promote the activities of MASTS, including being used on the website and social media channels.

Please note that MASTS may also contact you, the participants, and/or your supervisors to gather additional post-exchange impact information. This information must be provided on request.

Contact information

Participant name	Charlotte Lee
University & Department	University of Stirling (UoS) Division of Biological and Environmental Sciences Faculty of Natural Sciences Stirling FK9 4LA
Email address	chl1@stir.ac.uk
Host name	Professor Ruddy Wattiez
University or Institution & Department	University Of Mons, Department of Proteomics and Microbiology, Bd Dolez 31, 7000 Mons, Belgium
Email address	ruddy.wattiez@umons.ac.be

Scottish Funding Council
Comhairle Maoineachaidh na h-Alba

Scottish Government
Riaghaltas na h-Alba
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Exchange overview

Title	Comparative metaproteomics of microorganisms inhabiting plastic debris.
Start date	06/07/2022
End date	31/10/2022
Project location(s)	University of Mons, Department of Proteomics and Microbiology, Belgium

Abstract (max 300 words)

Provide a brief summary of the exchange using language accessible to a non-specialist. Describe what the exchange objectives were, the activities that were carried out, and the subsequent outcomes. This may be published on the MASTS website.

Marine plastic pollution threatens marine biodiversity and human health and is therefore of increasing concern globally. The plastisphere is the community of microorganisms which forms a biofilm on plastic. An understanding of the plastisphere's microbial composition is of critical and immediate importance for discerning the microbial dynamics of plastic degradation. Previous studies demonstrated that geographical location is the largest influence on plastisphere formation, while the influences of other chemicals on its microbial composition, including additives such as dyes and/or co-pollutants, are still unclear. Other emerging pollutants such as organic lipophilic UV-filters - found in personal care products (PCPs) - can become highly concentrated on plastic, and thus influence the plastisphere's activity.

In this project, Charlotte Lee (CL) – PhD student – studied the impact of dyes and common UV-filters on model marine bacteria and complex microbial communities inhabiting the surface of different plastics. CL used (meta)proteomics to investigate the effects of those chemicals on the microbial activity thus allowing to assess whether the regulation of key enzymes involved in plastic degradation were modified. CL analysed four sets of samples with high resolution mass spectrometry at UMons (Belgium):

(1) Proteins from the biofilms of known plastic-degrading bacteria exposed to dyes, and UV-filters.
(2) Proteins secreted by a marine bacterium (*Epibacterium mobile*) after exposure to UV-filters.
(3) Proteins from marine plastisphere communities exposed to a UV-filter.
(4) Proteins from the biofilms of microorganisms attached to coloured and uncoloured plastics in the ocean.

Data from sample set 1 were presented as a poster communication by CL at the well-known international conference in the field - ISME18 (International Society for Microbial Ecology, August 2022). Data from sample set 2, will lead to an original research article between both partnered universities. Quantitative metaproteomics on sample sets 3 and 4 require further investigation before publication.

Impact (max 600 words)
Please demonstrate the impact of your exchange from your perspective, and that of your exchange partner. Describe what the wider benefits of the exchange were to you as participant, your own and host institutions, and the wider community.

During my stay in Belgium, I worked with Alice Delacuvellerie (AD), who is a 4th year PhD student at UMons and has great expertise on 'the plastisphere', and associated topics. In this project, we built on the previous work recently published by AD and coworkers[1,2,3] and my preliminary results which also demonstrated that protein profiles on transparent, white, and colourful plastics are significantly different. This collaboration has been very beneficial for both of us, and for the progress of Plastisphere-related research projects led by both supervisors at their respective divisions. Two of the three sets of samples that we prepared together were very promising, while the fourth sample set is still to be processed. This exchange will lead undoubtedly to at least one joint publication for which I am the primary author (data analysis of 12 secretomes is currently in progress and the publication of those results is scheduled for the end of December 2022) and potentially will lead to one more publication.

This funding gave me the unique opportunity to use a high-resolution mass spectrometer for the first time at UMons, and learn each step of the proteomics workflow, instead of sending my samples to a sequencing platform. This method is highly transferrable in the field of molecular ecology and is particularly useful for future work in proteomics. This was also the first international exchange that I have undertaken during my PhD; in this way I have gained many insights from the experience. I also learned from how different research groups are run and organised, the different administrative duties and paperwork across countries. I gained organisational and adaptability skills.

Both supervisors in this project (at UoS and UMons) are currently leading research projects focusing on plastic pollution in different parts of the world (UK, Europe and SE Asia) and have great expertise in (meta)proteomics approaches, plastic pollution and ecotoxicology. This exchange project offered the unique opportunity to join the efforts of two ongoing research programmes on marine plastic pollution. The University of Stirling benefited from the access to advanced proteomics infrastructure, with high resolution mass spectrometers. The University of Mons, in Belgium benefited from the development of new experimental designs in ecotoxicology, and expertise in the study of new emerging pollutants such a s UV filters. Also, the University in Stirling developed of a new programme for metaproteomic (mPies) that is of interest for the University of Mons.

Returning from this exchange, I have a better understanding of the numerous applications that proteomics offer within ecology and environmental sciences. I was able to convey this through my poster presentation to the 2,000 attendees of the international ISME18 conference, which ultimately may encourage other research groups to use the same technique, so furthering scientific practice.

[1]**Delacuvellerie, A**., Ballerini, T., Frère, L., Matallana-Surget, S., Dumontet, B., & **Ruddy, W**. (2022). From rivers to marine environments: A constantly evolving microbial community within the plastisphere. Marine Pollution Bulletin, 179. https://doi.org/10.1016/j.marpolbul.2022.113660

[2]**Delacuvellerie, A**., Cyriaque, V., Gobert, S., Benali, S., & **Wattiez, R**. (2019). The plastisphere in marine ecosystem hosts potential specific microbial degraders including *Alcanivorax borkumensis* as a key player for the low-density polyethylene degradation. Journal of Hazardous Materials, 380, 120899. https://doi.org/https://doi.org/10.1016/j.jhazmat.2019.120899

[3]**Delacuvellerie, A**., Géron, A., Gobert, S., & **Wattiez, R**. (2022). New insights into the functioning and structure of the PE and PP plastispheres from the Mediterranean Sea. Environmental Pollution, 295, 118678. https://doi.org/https://doi.org/10.1016/j.envpol.2021.118678

Outputs (max 300 words)

Has this exchange resulted in clear outputs, such as the generation of a proposal, research results, or publication? Please provide brief details here. Do any of these outputs have relevance to larger programmes such as the UN SDGs, Blue Economy Action Plan etc?[1]

The first output of data from this exchange was from the analysis of proteins secreted by *Epibacterium mobile* – a marine bacterium [1] – after exposure to organic UV-filters. Its intracellular response to benzophenone-3 (BP3), a common marine pollutant, has been characterised in another study [2]. However, preliminary results (*Fig 1*.) show that *E. mobile* also secretes proteins in response to BP3.

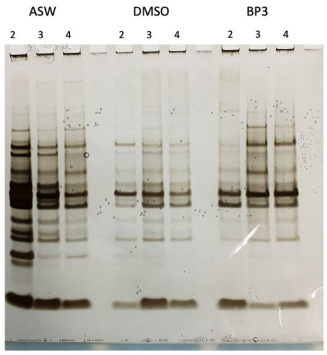

Figure 1. Silver stained *E. mobile* secretomes (i) ASW Control (ii) DMSO control (iii) BP3 – UV filters treatments .

My study therefore incubated *E. mobile* alone (negative control), with dimethyl sulphoxide (DMSO) – the solvent used to resuspend UV-filters – (DMSO control), with BP3 in DMSO, or with ethylhexyl methoxycinnamate (EHMC) – another UV-filter – in DMSO. The proteins secreted by these bacteria were then collected, processed, and analysed (UMons). The associated data is being analysed with ProteinPilot (Proteomics Software), to understand the bacterial response to UV-filters. The number of proteins retrieved are representative of a bacterial secretome (*Table 1*). Initial analysis also reveals a significant difference in protein expression when *E. mobile* is exposed to EHMC compared to any other treatments. These proteins will be characterised, and a chapter and research article will be published (2022 / 2023):

Lee, C, Messer, L, Wattiez, R., Delacuvellierie, A., Decroo, C. and Matallana-Surget, S., (In Preparation). The molecular effects of UV filters on the secretome of an environmentally relevant marine bacterium. ISME J Communications.

	Secretome				Proteome
	Control	Control DMSO	*DMSO-BP3*	*DMSO-EHMC*	*DMSO-EHMC*
Identified Proteins (99%)	61	84	48	78	909

Table 1. Number of secreted proteins of *E. mobile* (Secretome), intracellular proteins (Proteome) in the different treatments.

[1] All successful applicants will be expected to represent, promote and formally acknowledge the sponsors (MASTS, SFC & Scottish Government) during the course of their project and in any subsequent related outputs. All research outputs and any material used publicly must carry the funders' logos. The following acknowledgement should be used in all publications resulting from this funding. ["This work received funding from the Scottish Funding Council Saltire Emerging Researcher Scheme and the MASTS pooling initiative (The Marine Alliance for Science and Technology for Scotland) and their support is gratefully acknowledged. MASTS is funded by the Scottish Funding Council (grant reference HR09011) and contributing institutions"]

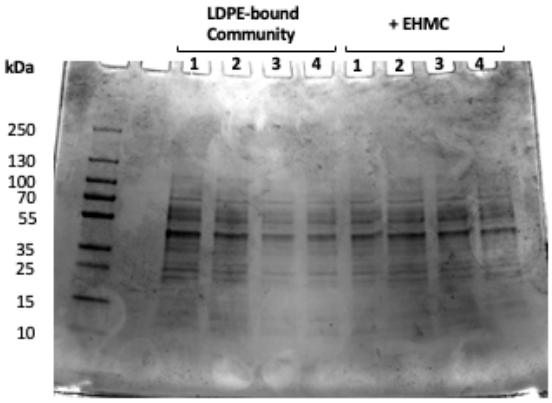

Figure 2. Coomassie stained LDPE-bound community proteomes.
Bands are clusters of proteins of the same molecular weight.

The second output will be from the metaproteomic analysis of proteins extracted from biofilms of natural plastisphere communities exposed to EHMC (*Fig 2.*). These samples were processed at UMons during my second stay in October, and an associated chapter and article will be published following data analysis:

Lee, C., Matallana-Surget, S., Wattiez, R., Delacuvellierie, A., Messer, L., & Decroo, C. (In Preparation). Marine plastisphere metaproteome response to the UV filter octinoxate. Microbiome.

Very few proteins were extracted from the biofilms of plastic-biodegrading bacteria exposed to pollutants, so ATR-FTIR at UoS was used to examine the surface chemistry of the plastics after incubation with the cultures and pollutants. Analysis of this data reveals that the inclusion of dyes and UV-filters before biofilm formation prevents colonisation (*Fig 3.*).

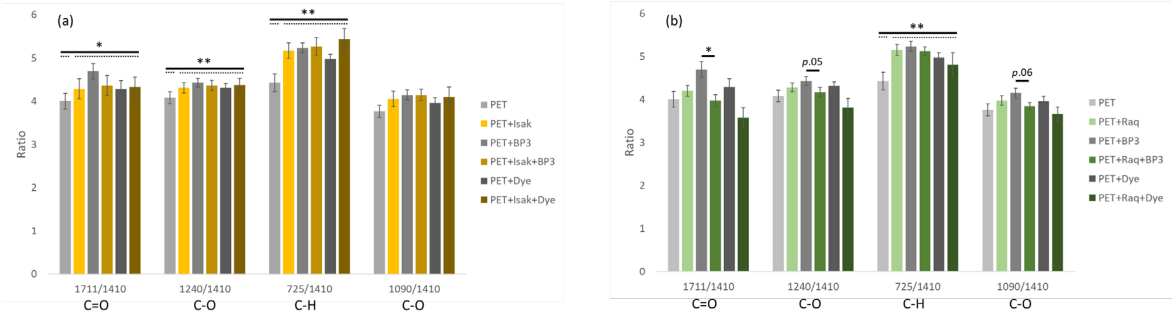

Figure 3. ATR-FTIR absorbance ratios of four PET-associated peaks when incubated with *I. sakaiensis* (a), and *R. aquimaris* (b). Peaks 1711 and 1240 – carboxylic acid (C=O/C-O) –, peak 1090 – ester bonds (C-O) –, and peak 1090 – aromatics (C-H) – were standardized using peak 1410cm-1. Increased absorbance ratio of peaks compared to the control is assumed to indicate oxidation. Bars represent 3 biological and 3 experimental replicates. 9 replicates total.

This data was presented at ISME18, while my second and third outputs relate to plastic bioremediation, contributing to sustainable development goals 12, and 14.

References

[1]Lozano, C., Matallana-Surget, S., Givens, J., Nouet, S., Arbuckle, L., Lambert, Z., & Lebaron, P. (2020). Toxicity of UV filters on marine bacteria: Combined effects with damaging solar radiation. Science of The Total Environment, 722, 137803. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.137803

[2]Lozano, C., Lee, C., Wattiez, R., Lebaron, P., & Matallana-Surget, S. (2021). Unraveling the molecular effects of oxybenzone on the proteome of an environmentally relevant marine bacterium. Science of The Total Environment, 793, 148431. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.148431

The Future (max 300 words)
How do you plan to ensure a sustainable collaboration in the longer-term and maximise opportunities and impact in the future? How will you carry forward the benefits now the exchange has been completed? Please outline five concrete plans for future collaboration as a result of your exchange.

This exchange is one of several collaborative projects between the two institutions. Before exchange commencement, samples and data had been exchanged for the progression of the NERC-SEAP Programme, and advice had been shared between research groups on how to approach our respective studies.

Sample set 4, which is still to be processed, may also be supplemented by samples collected by AD and colleagues in Southeast Asia. The results produced by sample analysis would then be written into a paper co-authored by myself, AD, SMS, RW and others. All parties would therefore be involved in all publications that would follow this, strengthening a lasting collaboration between institutions.

Plans for future collaboration and joint publications:

- Return trips to UMons for further mass spectrometry analysis.
- Joint publications on the secretome study of *E. mobile* – December 2022.
- 16S analysis and metaproteomics analysis of dataset gathered in November 2022
- Involvement of SMS / RW in the academic progression of PhD students working at either UMons and UoS (Note: SMS will be part of the examining committee of AD's viva at UMons on the 20th of December 2022).

Any further comments (max 500 words)
Please use this space to provide any additional comments. These may include, but are not limited to; what you would do differently if you could take the exchange again; what contingency measures you had to use (if any); details of any unexpected benefits or problems; any significant variations in costs;

This exchange was performed under significant time constraints due to a delay in the acceptance of our application (accepted 25/05/2022, instead of February as other MASTS funded projects). This meant that the biofilm colonisation experiment that had been running at the time (started 27/04/2022) was shortened so we could process those samples over the summer. However, note that other proteomics sample sets have also been included in the workflow. Despite this limitation, we successfully organised an exchange with our colleagues at the University of Mons, at a very short notice (Project awarded in May and exchange performed in July 2022 - 06/07/2022-29/07/2022). This exchange has been very beneficial to everyone (PhD student, supervisors, institutions, progress of larger projects), but of course, the project was not completed within the same allocated time than the other PhD students who were awarded the same funding.

In this way, SMS contacted MASTS to request for one month extension for the spending of the budget, which has been granted on the 2nd of September. Thanks to this granted extension, another stay in Belgium (2 weeks, 17/10/2022-28/10/2022) has allowed Charlotte to repeat more proteomics experiments which will lead to another joint publication.

We have provided below a forecast of the spending below.

Final expense report

Item Number	Date	Description and number of units	Total Amount (£)
1	16/06/2022	Edin-Brussesl flight	50.49
2	20/06/2022	1x 10kg DRYICE Pellets	36.80
3	28/06/2022	Brussels flight	42.35
4	02/07/2022	Accommodation	706.38
5	06/07/2022	1x Dryice shipment to Belguim	40.25
6	28/07/2022	1x EP0030108094-100EA	15.00
7	28/07/2022	1x B6916-500ML Bradford	113.64
8	28/07/2022	1x T6567-5X20UG Trypsin from	85.18
9	28/07/2022	1x 1153332500 Water for	8.28
10	28/07/2022	1x D8255-5G	180.00
11	28/07/2022	3x Pipette serological 25ml	203.40
12	28/07/2022	1x IODOACETAMIDE PROTEOMICS	161.10
13	28/07/2022	3x Pipette Tips GP UNV 1000ýL	101.52
14	28/07/2022	1x Shipping	30.00
15	28/07/2022	1x PET 0.1mm film	328.80
16	28/07/2022	1x Case3000 731175	74.51
17	29/07/2022	Consumables Microbiology	25.17
18	29/07/2022	1x Quantitative Colorimetric	422.94
19	29/07/2022	1x 4 to12%, Bis-Tris, 1.0û1.5	154.54
20	29/07/2022	1x 5L NuPAGEÖ MES SDS Running	529.60
21	29/07/2022	1x C18 Spin Tips & Columns	245.10
22	29/07/2022	1x 500g Dehydrated Culture	536.47
23	29/07/2022	1x 10ml NuPAGEÖ LDS Sample	17.19
24	29/07/2022	1x 10ml Sample Reducing Agent	69.49
25	29/07/2022	1x Pipetboy acu2 ocean dream	457.14
26	29/07/2022	4x Protein Concentrator PES,	974.59
27	08/08/2022	Mons Travel Expenses	37.96
28	26/08/2022	Travel to Conference Expenses	291.99
29	13/09/2022	Charlotte Lee - ISME18	15.00
30	13/09/2022	ISME Conference Exp	948.35
31	13/09/2022	Flight to Conference	484.96
32	15/09/2022	1x LDPE 0.1mm film	452.40
33	21/09/2022	Flight to Brussels	168.48
34	28/09/2022	Acommodation Mons	679.67

35		OneOmics Multiomics software. 1 Month Sub.	654.55
36	31/10/2022	Mons Expenses - CL	83.97
		Total of expenditure	**9,427.26**
		Awarded Budget from scheme	**9,477.00**
		(Remaining budget)	**49.74**
In-kind contributions		• Fund for Scientific Research (FRS-FNRS) FC 23347, RW PI • SPEM (*Stop plastics in Med Sea*) Project DEB19-405	**6,000**
In-cash contributions		• NERC/NRF project, SMS UK PI – SEAP Programme. Sources £5,000 impacts and solutions of plastics in SouthEast Asia • SUPER-DTP, CL	**5,000**
Grant Total (Expenditure on the scheme + in-kind+cash)			**20,427.26**