Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA20106

Grantee name: Hermann Holbl

Details of the STSM

Title: Morphogen triggered changes in the transcriptome of Ulva mutabilis

Start and end date: 30/03/2025 to 19/04/2025 (three weeks)

Objectives

The Short-term Scientific Mission (STSM) was focused on the model system of *Ulva mutabilis* and its associated beneficial bacteria, *Maribacter* sp. strain MS6 and *Roseovarius* sp. strain MS2 (Blomme et al. 2023; Wichard 2023). *Ulva*'s cell differentiation is regulated by (−)-thallusin, an algal growth- and morphogenesis-promoting factor (AGMPF) produced by *Maribacter* (Alsufyani et al. 2020, Dhiman et al. 2022, Wienecke et al. 2024). In the absence of bacteria, *Ulva* lacks rhizoids, and deformed cell walls with protrusions are visible, emphasizing the crucial role of bacterial signalling in algal morphogenesis (Spoerner et al., 2012).

I have already demonstrated that *Ulva* calli inoculated with (−)-thallusin or *Maribacter* sp. MS6 exhibited rhizoid formation and significantly increased tissue growth with no cell wall protrusion compared to non-active treatments (**Fig. 1**). Experiments with treated axenic *U. mutabilis* calli were conducted before the STSM to investigate metabolic and transcriptomic changes caused by the presence or absence of thallusin. The goal of this STSM was to acquire bioinformatic workflows for transcriptome analyses and to understand best practices for *Ulva* data interpretation to analyze RNA sequencing data from the experiments with sampling times after 3 days and 28 days. In the framework of my PhD project, this data will be merged with endo-metabolome data for comprehensive analysis of molecular processes associated to thallusin signalling in *Ulva*.

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

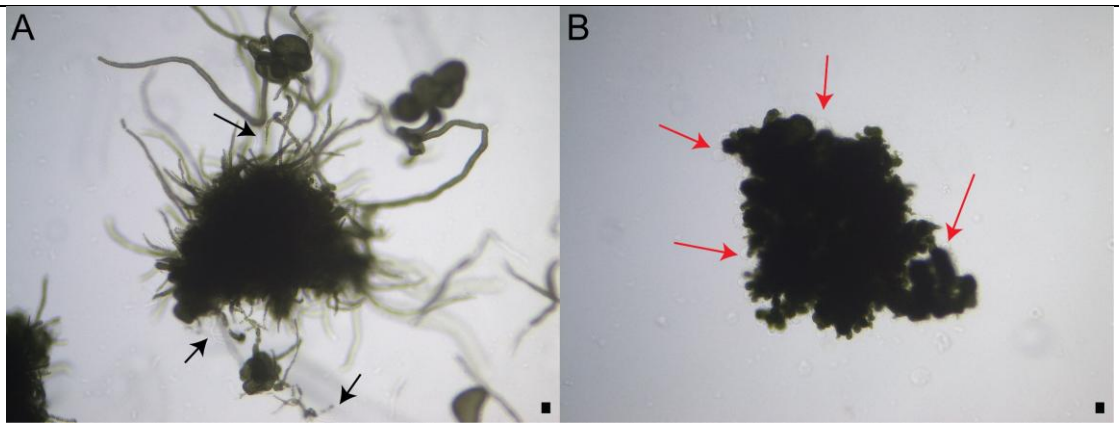

Figure 1*: (*A) Treatment - specimen of culture treated with *Roseovarius* sp. and exposed to **(−)-thallusin** for 28 days. This picture also represents cultures inoculated with *Maribacter* sp. instead of thallusin. **Black arrows** show rhizoid formation. (**B**) Control experiment - specimen of culture treated with *Roseovarius* sp. and the inactive **(+)-thallusin** and exposed for 28 days. This picture is representative of other treatments that were inactive regarding morphology changes. **Red arrows** show cell wall protrusions. Scales represent a length of 50 µm.

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

During the STSM at Ghent University in Prof. Olivier de Clerck´s research group, I learned extensive processing steps to analyze transcriptome data using the most up-to-date reference genome of *U. mutabilis*. Thus, I established a data processing workflow utilizing the high-performance computing (HPC) cluster of Ghent University to convert the paired-end sequencing reads into count data necessary for the analysis of differentially expressed genes (DEG).

This workflow is composed of the following steps:

- QC of reads
- Trimming of low-quality reads
- Mapping to the reference genome of *Ulva mutabilis* and receiving aligned reads
- Counting of aligned reads to the respective gene fit

This workflow can be easily adjusted for future analyses on the HPC cluster in Jena, as both utilize the same management system, Simple Linux Utility for Resource Management (SLURM).

Further, a workflow in the programming language R for the analysis of DEG was created during the STSM. Here we used “EdgeR” to normalize for sequencing depth and compare between different treatments sampled after 3 days of inoculation with thallusin, it’s derivatives *or Maribacter* sp. (MS6). A MDS plot of the dataset sampled at day 3 is shown in the description of the STSM main achievements (Fig. 3). To assess differences in the transcriptome due to the presence of the morphologically active (−)-thallusin, we searched for genes significantly up- or downregulated in treatments inoculated with (−)-thallusin as well as in *Maribacter* sp. but not in controls with (+)-thallusin and *Roseovarius* sp. only. In this comparison, we received 84 genes that were differentially expressed with a Benjamini-Hochberg corrected p-value below 0.05 and log base 2 transformed fold change of at least 1. Afterwards, gene enrichment analyses based on GO-Terms with the package “topGO” were conducted. With the list of regulated genes, we will now deduce further information about the molecular processes involved in (−)-

thallusin signalling, e.g., by blast search or genetic modification of genes of interest. An overview of the entire workflow is illustrated in Figure 2.

During the STSM, our primary focus was on developing scripts to analyze my data. Specifically, we concentrated on identifying core genes associated with the response to (−)-thallusin. In addition to the dysregulated genes, the following supplementary insights can be obtained:

- Mediated changes in the transcriptome by *Maribacter* sp. through cell-cell contact
- Impact of *Roseovarius* sp. on mRNA regulation
- Effect of the partially active derivative of thallusin on gene regulation
- Changes in regulation over time between 3 days and 28 days of exposure

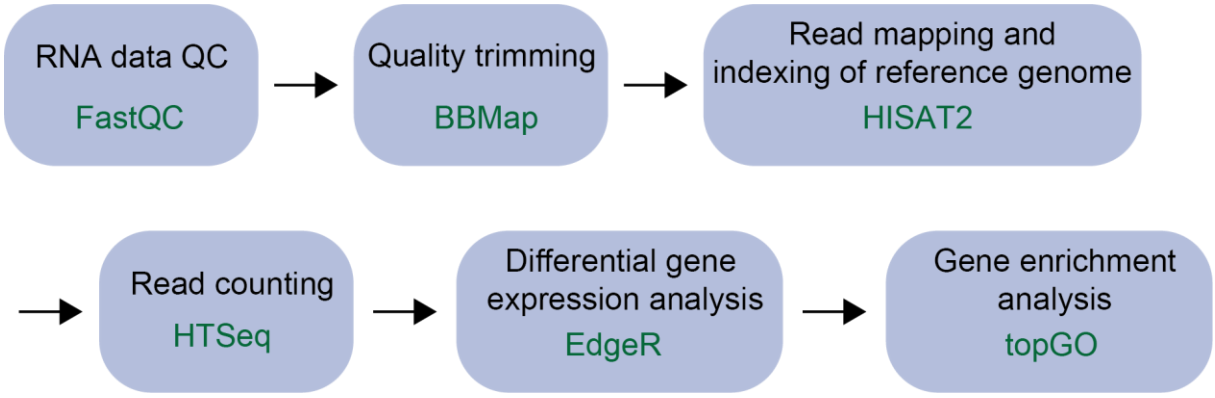

Figure 2: Overview of the processing and analysis steps performed during the STSM in Ghent. The software tools used for each step are highlighted in green.

Since the mRNA data is stored in the HPC cluster in Ghent, I can share it anytime for improvements of the current reference genome and *de novo* transcriptome assembly. The high sequencing depth (mostly above 30 million raw reads) and the very high mapping rate of ~95% to the recent annotated reference genome of *Ulva mutabilis* slender suggest that my transcriptome dataset is well-suited for this purpose.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

In total, the STSM at Ghent University was very successful. To demonstrate the productive STSM, a MDS plot based on normalized read counts representing sample variability is illustrated (Fig. 3). The referred samples were taken 3 days after inoculation. The plot shows much higher variability between treatments compared to variability between biological replicates, supporting the experimental design. Moreover, the morphologically active treatments ((−)-Thallusin and *Maribacter* sp.) cluster together on the positive side of dimension 1, whereas the negative treatments (negative control, *Roseovarius* sp. only, (+)-thallusin) are located at the negative side of dimension 1. Only the treatment with the partially morphologically positive derivative of thallusin, being more similar to the negative (+)-thallusin, does not align completely with our expectation. This can be explained by the fact that the derivative was tested on axenic gametes beforehand and not on axenic calli, describing the activity of this specific derivative.

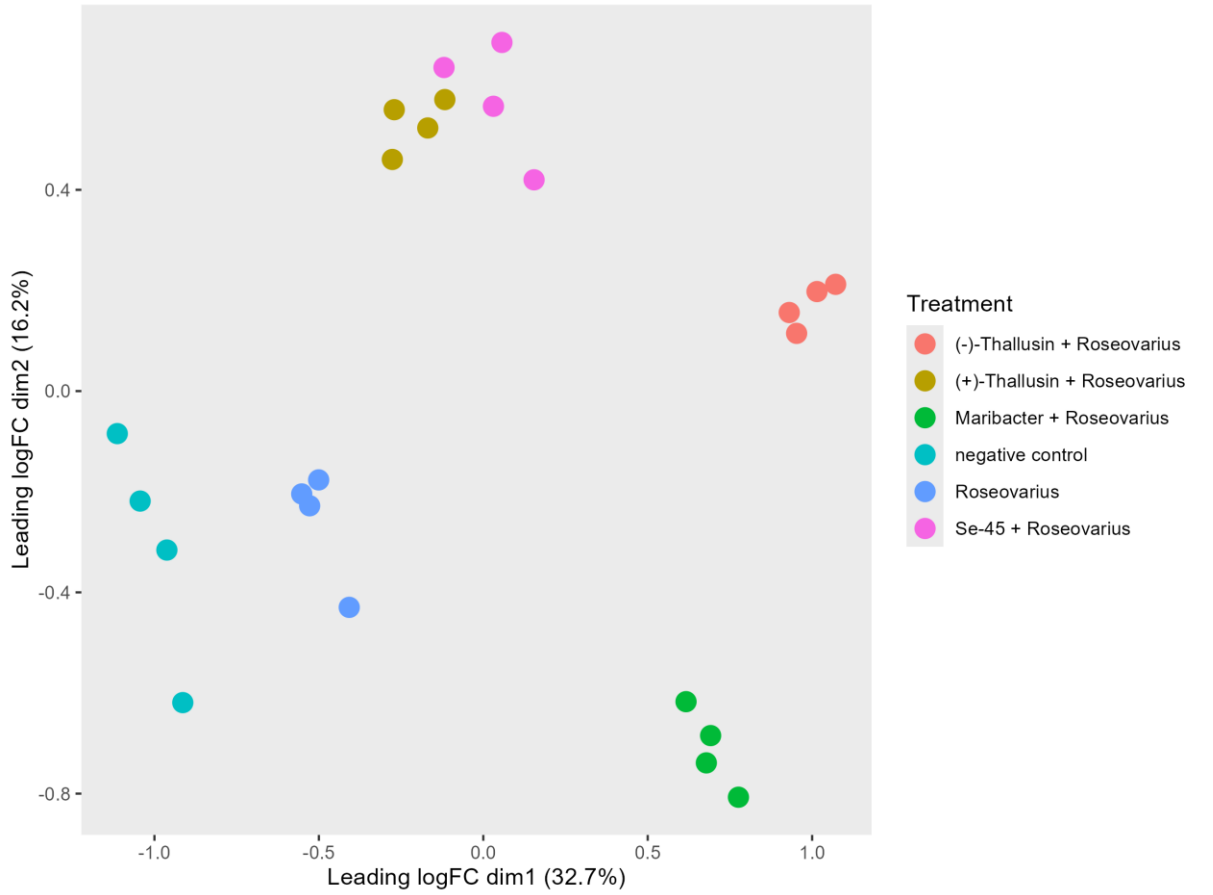

Figure 3: MDS plot of transcriptome data from calli with 3-day exposure time of thallusin, the partially active derivative of thallusin (Se-45, Dhiman et al. 2022) or *Maribacter* sp.

In detail, the following goals were achieved, and prospective activities are planned:

(1) With the support of Prof. Olivier de Clerck's research group, I created a transcriptome processing and analysis workflow that takes into account the properties of my sequencing data and experimental design. In the following weeks, this workflow will be amplified to extract more knowledge from the experiment (see description of work carried out) and appropriately visualize the data. Knowledge retrieved from my experiments is connected to bacterial–seawheat interactions on the molecular level **(COST Action Task 1.2 of WG1**).

(2) In a conservative approach, 84 genes were identified to be significantly regulated in treatments containing (−)-thallusin compared to the controls after a 3-day exposure time. These genes, regulated by (−)-thallusin, enable the targeting of specific metabolites crucial for the morphogenesis of *Ulva* (**COST Action Task 4.1 of WG2**). Additionally, we aim to evaluate the role of these genes on morphology by using gene knockout mutants in collaboration with the Phycology Department of Ghent University.

(3) Since my RNA-seq data is stored on the local HPC in Ghent, specific data can be easily shared for improvements on the *Ulva mutabilis* slender genome. This results in a more comprehensive version of the current reference genome. By improving the reference genome, the whole scientific community benefits working on *Ulva*.

We aim to publish our results in scientific journals. I highly appreciate the support of the Phycology research group at Ghent University during my STSM.

In summary:

The introduction to transcriptome analysis that I received during my STSM in Ghent has equipped me to expand the analysis workflow we developed and extract further insights from my experiments at the

University of Jena. We ensured the generation of high-quality data and successfully conducted differential gene expression analyses on a subset of the dataset to assess the effect of (−)-thallusin on gene regulation. Our ongoing goal is to integrate transcriptomic and metabolomic data to gain a more comprehensive understanding of thallusin signalling in *Ulva*. This STSM also strengthened the collaboration with Prof. Olivier De Clerck's research group, with future projects involving gene knockout mutants already in planning. The outcome of the STSM directly contributes to the **Delivery 1.1.b** of the COST Action entitled "*Ulva* microbiome, interactions with bacteria on and in the seaweed fronds, affecting *Ulva's* growth and development".

References

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DATE
13 May 2025

To Whom It May Concern,

I hereby confirm that Hermann Holbl, affiliated with the Friedrich Schiller University of Jena, successfully participated in a Short-Term Scientific Mission (STSM) at our institution, under my supervision.

The STSM titled “Morphogen triggered changes in the transcriptome of *Ulva mutabilis*” took place from 30.03.2025 to 19.04.2025 at Ghent University, within the framework of the COST Action “SeaWheat” (CA20106). During this period, Hermann Holbl actively contributed to the collaborative research activities, demonstrating a high level of scientific thinking and dedication.

The purpose of the STSM was mainly to create bioinformatic pipelines for transcriptome analysis for already obtained mRNA data and to acquire knowledge on best practices, specifically for *Ulva* transcriptome data. The work carried out has made a valuable contribution to our ongoing research efforts. We will stay in close contact for further collaborations. We appreciate Hermann Holbl’s efforts and commend the successful completion of the mission objectives.

Should you require any additional information, please do not hesitate to contact me.

Sincerely,

Prof. Olivier de Clerck
Head of Research Group Phycology
Ghent University

GHENT UNIVERSITY