Optimizing the protoplast isolation from different *Ulva* strains

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Objectives and Research Questions

The aim of this Short Term Scientific Mission was to optimize the existing protocol for the isolation of protoplasts in *Ulva* spp.. Protoplasts could be an easy way to overcome some of the existing limitations in the production of *Ulva*, particularly in species where the life cycle cannot be controlled. I hypothesized that protoplast isolation could be optimized by using abiotic stressors, such as light and salinity, as pre-treatments to reduce the cell wall thickness, thereby improving the efficiency of the enzyme mixture for protoplast isolation. The experimental design is shown below (Fig. 1).

I attempted to answer the following three research questions:

1) Are the existing protocols for the isolation of protoplasts reliable and easy to replicate?

2) Do salinity and light reduce the cell wall thickness of *Ulva* sp. cells?

3) Can these abiotic factors be used as pre-treatments to optimize the isolation of protoplasts in *Ulva* sp.?

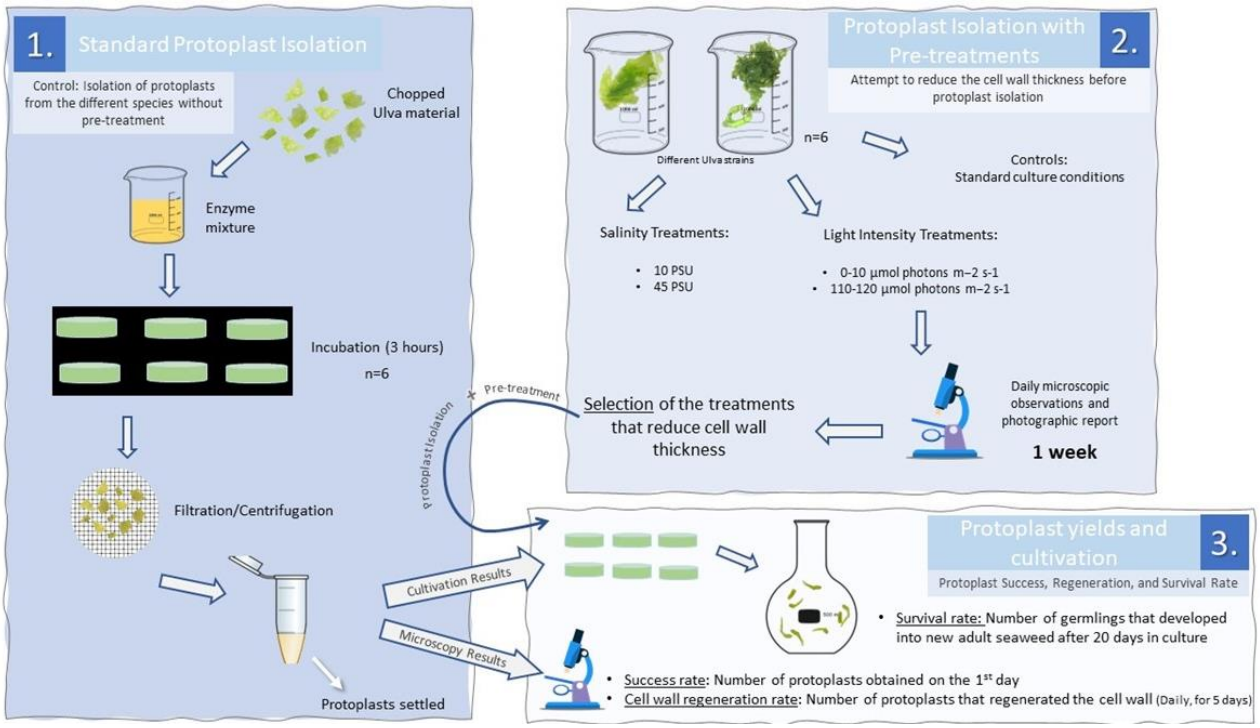

Figure 1 – Experimental design developed for the STSM. 1) Determine the standard protoplast isolation protocol; 2) Determine the salinity and light treatments that were more effective to reduce the cell wall thickness; 2+1) Adding successful treatments from (2) as "pre-treatments" to the standard protoplast isolation protocol (1); 3) Cultivation of protoplasts and evaluation of the success, survival and regeneration rate of the protoplasts isolated with the standard protocol alone (1) and with pre-treatment (2+1).

The standard protocol for protoplast isolation was created by compiling different variations of protocols already published. When isolation was achieved, protoplasts were counted by an automatic cell counter and a fluorescent dye was used to confirm the absence of the cell wall (characteristic of protoplasts) (Fig. 2).

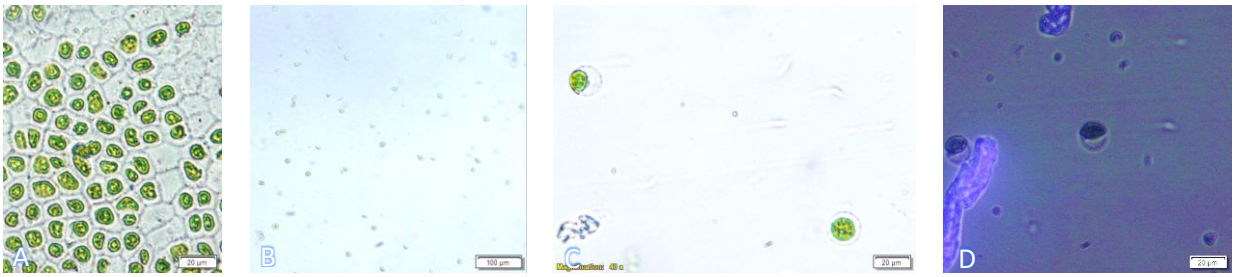

Figure 2 - Microscopic observations of protoplast formation: changes in the thalli before protoplast detachment (A). Protoplasts after detachment from the thalli (B and C). Protoplasts dyed with calcofluor-white to confirm the absence of cell wall (cell wall would appear in fluorescent blue) (D).

The impact of light and salinity on the cell wall thickness was measured by setting up two short experiments (5 days) where *Ulva* discs from two species were placed under different conditions of salinity and light intensity (separately). Under the microscope, I measured the cell wall thickness of the samples collected each day to see how the cell wall was adapting to the different conditions and to assess on which day the cell wall thickness was reduced the most (Fig. 3). To eliminate the possibility of human error during the measuring of the cell wall and to be sure about how this treatment would be reflected in the protoplast yields when used as a pre-treatment, a new light experiment was designed for both species (8 days).

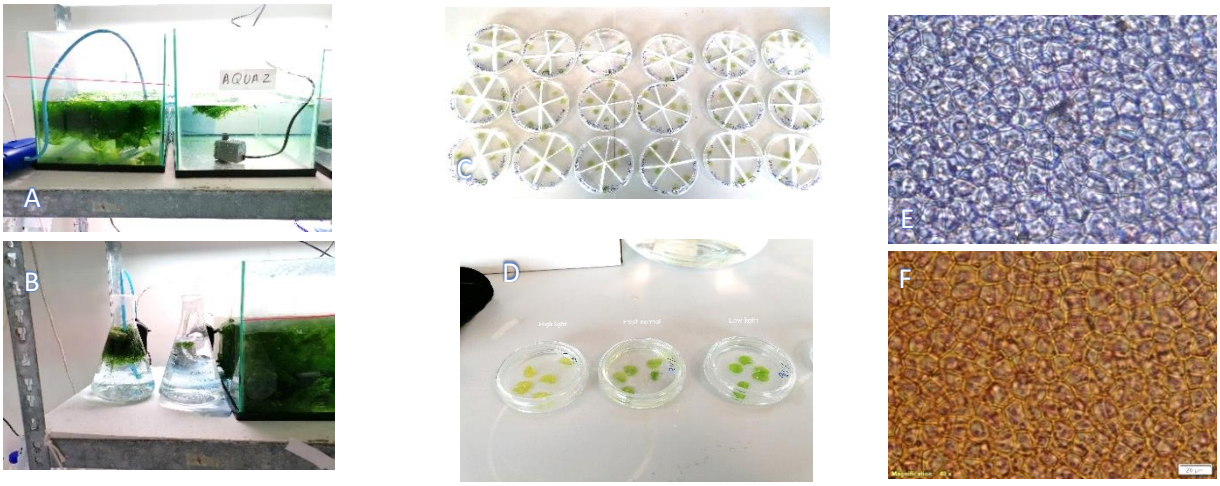

Figure 3 - From initial cultures to microscope observations: cultures of U. lacinulata and U. compressa (A and B). Experimental set-up to test the effects of the pre-treatments (Salinity and Light) on cell wall thickness (C). Visible changes on the thalli appearance after exposed for 5 days to the light pre-treatment (D). Microscopy images of the seaweed thalli with cell well highlighted (E and F).

In the pre-treatments experiment, results showed that the salinity and light treatments did not reduce the cell wall thickness and, as for the isolation of protoplasts, the initial success of this process was low, and many attempts on correcting the original protocol needed to be made before protoplasts were obtained.

In the end, although the protoplast yields proved to be lower than the results already published in the literature, protoplasts were isolated from both species, adaptations to the available protocols were made and the conclusion was made that both salinity and light intensity do not reduce the cell wall thickness or increase the protoplast yield.

In conclusion, I was able to:

1) test two pre-treatments for their impact on the thickness of the cell wall and their potential to facilitate protoplast isolation,
2) test a new method for measuring the cell wall thickness,
3) test several protocols on protoplast isolation (based on the existing literature),
4) isolate protoplasts from *U. lacinulata* without a pre-treatment,
5) isolate protoplasts from both species (*U. lacinulata* and *U. compressa*) when a pre-treatment was added to the original protocol.

In future collaborations, I intend to try the isolation of protoplasts from different *Ulva* species as well as working with cultured strains instead of wild material and, in this way, try to improve the success of the protoplast isolation and their survival rates.

This STSM allowed me to learn new techniques and meet extraordinary people with the same questions as me. The exchange of knowledge between the two institutes made the success of the Mission possible and I am looking forward to the future collaborations we have planned.