DEVELOPMENT OF CELL-BASED ASSAY TO ASSESS THE BIOACTIVITY OF MACRO-ALGAE EXTRACTS

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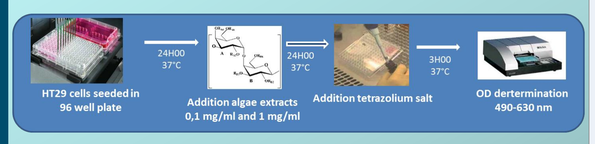
Abstract

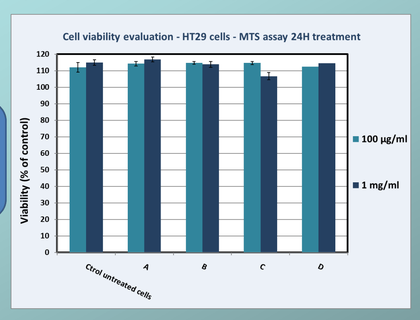
The algal biomass by its composition, compared to terrestrial plants and animals, constitutes an important source of molecules with nutritional and health properties. Within the ALGOLIFE consortium, our team has developed several tests to evaluate the bioactivity of macro-algae extracts using various *in vitro* cellular models. More specifically, we screened a total of 415 extracts and fractions in two concentrations on cell viability, oxidative stress, and inflammation tests. The purpose of this poster is to focus on three algae extracts from red (*Chondrus crispus*), green (*Ulva sp.*) and brown algae (*Saccharina latissima*) which gave different effects with our cellular models.

Cell viability and cytotoxicity evaluation

Cell viability evaluation on HT29 and THP-1 cells lines

- The CellTiter 96®Aqueous One Solution Cell Proliferation Assay (Promega) is a colorimetric method using the reduction of tetrazolium salt in to formazan for the determination of the number of proliferating cells at 490nm.

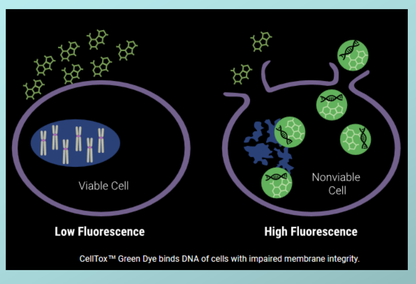


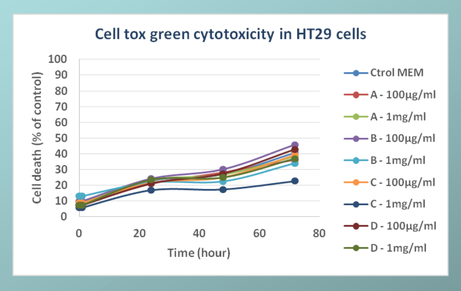


➢ The tested extracts didn't affect the cell viability of HT29 cell line.

Cytotoxicity assay

- The CellTox™ Green Cytotoxicity Assay (Promega) measures changes in membrane integrity that occur as a result of cell death by fluorescence (Ex: 485nm, Em: 520nm). The fluorescent signal produced by the dye binding to the dead-cell DNA is proportional to cytotoxicity. Lysis solution used as cell death control.



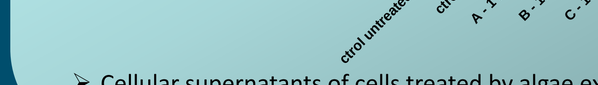


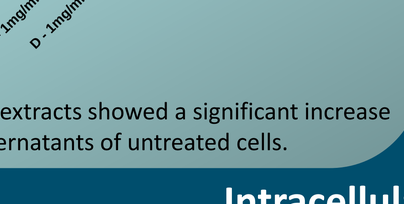
➢ None of teasted extracts showed a significant cytotoxicity on HT29 cells

Reactive Oxygen Species evaluation

H_2O_2 measurement

- The ROS-Glo™ H_2O_2 assay (Promega) is a sensitive luminescent assay that measures the level of hydrogen peroxide (H2O2), reflecting a general ROS level. In this test menadione and tocopherol are used as pro and anti-oxydant controls respectively. THP-1 cells (human monocytic cells) were treated with menadione during 15 min, then with extracts during 4h30.



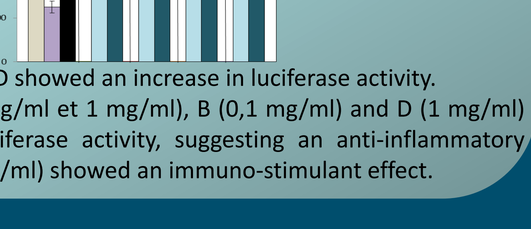


➢ Extracts A,B and C at 0,1 mg/ml and 1 mg/ml showed a significant anti-oxydant effect compared to menadione control.

➢ Extract D showed a significant pro-oxydant effect and potentialises the menadione effect.

Detection of intracellular Reactive Oxygen Species by fluorescence microscopy

- The cell-permeant 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) passively diffuses into cells. Upon oxydation by ROS is converted into the hightly fluorescent 2',7'-dichlorofluorescein (DCF) through cleavage by intracellular esterases.

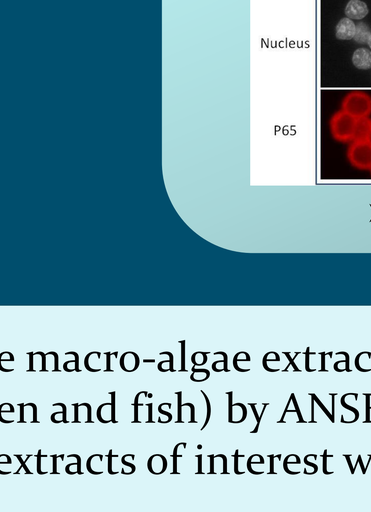


➢ Extract D gave a positive signal suggesting a pro-oxydant effect

Inflammation evaluation

TNF-α ELISA test on macrophage differentiated THP-1 cells

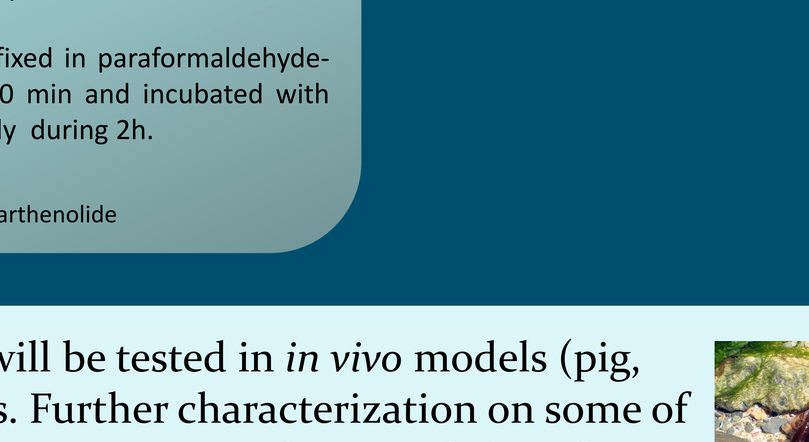
- The Human TNF-α ELISA kit (Peprotech) was used to determine the TNFα concentration in cellular supernatants after 24h treatment with macro-algae extracts at 1 mg/ml.



➢ Cellular supernatants of cells treated by algae extracts showed a significant increase of TNF-α production compared to cellular supernatants of untreated cells.

Immunomodulation assay based on luciferase activity on NF-κB Luc reporter - HT29 recombinant cell line

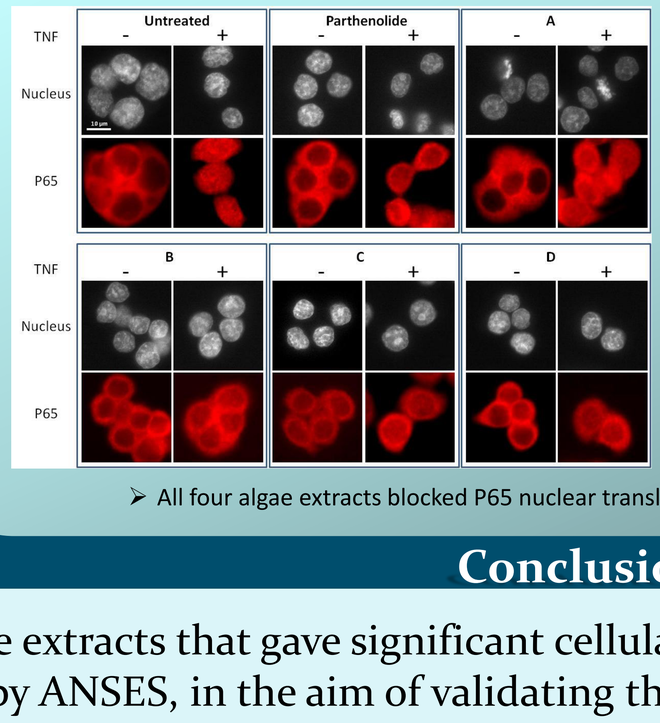
- Cells were treated with extracts for 1h. Then, was added or not TNF at 25 ng/ml during 24h. Parthenolide is used as an anti-inflammatory control..



➢ **[1]** Cells treated with extracts B, C and D showed an increase in luciferase activity.

➢ **[2]** Cells treated with extracts A (0,1 mg/ml et 1 mg/ml), B (0,1 mg/ml) and D (1 mg/ml) showed a significant decrease in luciferase activity, suggesting an anti-inflammatory effect. On the opposite, extract C (1 mg/ml) showed an immuno-stimulant effect.

Intracellular distribution of NF-kB P65



- HT29 pfireGreen cells were treated with extracts during 24h. Then was added TNF at 25 ng/ml or not for 3h. Parthenolide is used as an anti-inflammatory control.

- Cells were fixed in paraformaldehyde-triton for 20 min and incubated with P65 antibody during 2h.

➢ All four algae extracts blocked P65 nuclear translocation such as parthenolide

Conclusion

The macro-algae extracts that gave significant cellular effects will be tested in *in vivo* models (pig, chicken and fish) by ANSES, in the aim of validating these effects. Further characterization on some of our extracts of interest will be performed through bio-guided fractionation in the aim of identifying and purifying the active molecules.