

## Finding new bioactive products from algae: what for ?

- Establish a local **biorefinery** network based on macroalgae
- Develop enrichment of algae in trace elements by incubation
- Develop **new processes** of macroalgae valorisation
- Produce bioactive molecules, characterized in detail
- Target **complementary markets**:

### Health and nutrition for:

#### Human



#### Pet



#### Livestock



→ Prevention of antimicrobial resistance and health scandals

# Projects maturation and technology transfer

*The Algolife team: from the gene to the algal extract*



Gene cloning

Overexpression

Production by fermentation

Enzyme purification

**ENZYME**

Algal biomass hydrolysis

Fractionation

**ALGAL EXTRACT**

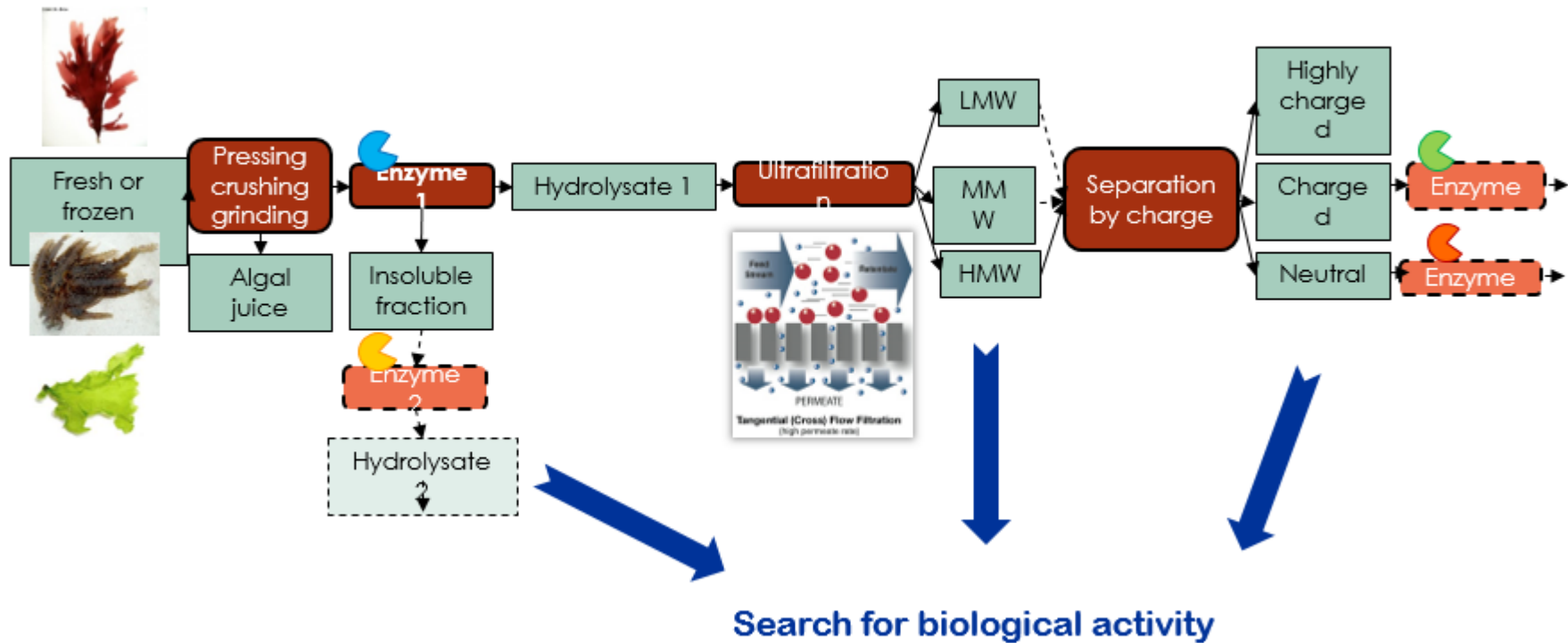
Analysis

All steps are optimized, and some are the object of technology transfer



Scale up is initiated in the lab, and continued by R&D services of our partners

## Preparation of algal extracts



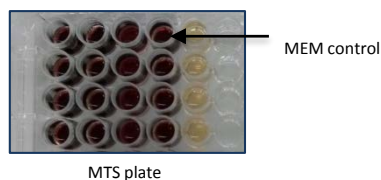
## Search for biological activity: *In vitro* tests

### I. Cell viability and cytotoxicity evaluation

#### Viability test: MTS

Cell line: HT29 (human colorectal cells), h-TERT RPE-1 (human retinal cells), THP-1 (human monocytic cells)

Method: reduction of MTS tetrazolium in formazan for determining the number of viable cells in proliferation by OD reading at 490nm



#### Cytotoxicity test: Cell Tox Green

Cell lines: THP-1, HT29, h-TERT RPE-1

Method: The CellTox™ Green Cytotoxicity Assay (Promega) measures changes in membrane integrity that occur as a result of cell death. Fluorescent signal produced by the dye binding to the dead cell DNA is proportional to cytotoxicity.

### II. Antioxidant activity: reactive oxygen species evaluation

#### Cellular test 1: ROS-Glo

Cell line: THP-1 (human monocytic cells)

Method: measures the level of H<sub>2</sub>O<sub>2</sub>, using menadione (pro-oxidant) and tocopherol (anti-oxidant) controls

#### Cellular test 2: GSH/GSSG

Cell line: THP-1

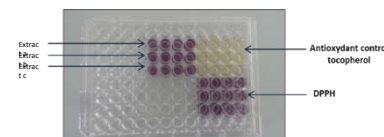
Method: luminescent-based system to detect and quantify total glutathione (GSH+GSSG), GSSG and GSH/GSSG ratios

#### Confirmation of ROS-GLO results

#### Chemical test: DPPH

Method: measures of the scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical

Sometimes in contradiction with ROS-GLO and GSH/GSSG tests



## Search for biological activity: *In vitro* tests

### III. Effect of algal extracts on inflammation

#### Immunomodulation assay

Cell line: HT29-pfireGreen NF- $\kappa$ B, human colorectal cells

Method: test based on luciferase activity, with or without prior treatment of cells with TNF- $\alpha$

#### Caspase-1 activity assay

Cell line: THP-1 monocytic cells differentiated into macrophages

Method: luminescence-based assay measuring the activity of caspase-1, an essential protease component of inflammasome

#### ELISA

Cell line: THP-1 differentiated into macrophages

Method: measures TNF- $\alpha$  concentration in cellular supernatants

### IV. Antibacterial activity

#### Growth monitoring in liquid medium

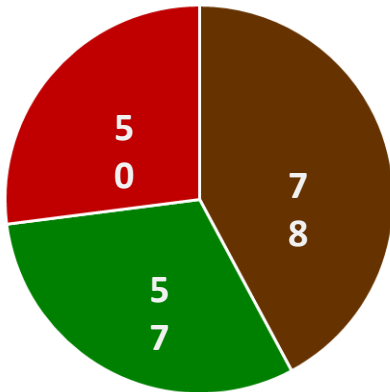
Bacteria strains tested: *E. coli* K12, *Vibrio* GV-544, *Bacillus megaterium*, *Staphylococcus epidermidis*

No anti-bacterial activity



## *In vitro* results

Samples tested:  
185



→ No effect on viability

→ 52 promising extracts:

- 28 antioxidants
- 18 immunostimulant
- 3 antioxidant and immunostimulant

→ 5 extracts selected for *in vivo* testing

## In vivo tests of algal extracts

### I. Harmlessness

Algal extracts are tested as ingredient in food

Following parameters are studied:

- mortality
- consumption/rejection
- growth
  
- blood analysis (including inflammation markers)
- organs sampling (search for antioxidant defenses)



### II. Challenges

Effects of algal extracts on animals challenged by a biological (infection) or chemical stress

- Campylobacter
- Salmonella
- Mycoplasma
- Circovirus
- Pendiméthaline (herbicide)

**First results seem to show harmlessness of the 5 extracts on fish, chicken and pork**

## What remains to be done



*In vivo* tests:

- Analysis of blood and organs samples
- Biological and chemical challenges
- Enzyme production: overexpression in GRAS organism
- Analytical chemistry: identification and characterization of active molecules:
  - Fine fractionation of active extracts (UF, chromatography) and activity screening
  - Chemical analysis of active fractions (MS, NMR, etc)
- *In vitro* tests: study of the mechanisms responsible for immunostimulant activity