MarineBiotech

# Mar3Bio

## Biorefinery and biotechnological exploitation of marine biomasses

Coordinator:
SINTEF
Partners: NTNU, KTH, NIC, MATIS, UNITS, DuPont

ERA-MBT 1st Transnational Joint Call: Biorefinery processes

21st November 2017

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**December 2013 - November 2017**

# THE CONSORTIUM

| PRINCIPAL INVESTIGATOR | INSTITUTION | COUNTRY |
|---|---|---|
| Håvard Sletta | SINTEF | NO |
| Finn L. Aachmann | Norwegian University of Science and Technology (NTNU) | NO |
| Vincent Bulone | Royal Institute of Technology (KTH) | SE |
| Gudmundur Hreggvidsson | MATIS | IS |
| Blaž Likozar | National Institute of Chemistry (NIC) | SI |
| Ivan Donati | University of Trieste (UNITS) | IT |
| Olav Gåserød | DuPont Nutrition & Health | NO |

**Project period:** June 2016 to May 2019

# Objectives

- To increase quality and yield of high-value products from marine biomass
- Expand product spectrum from algal biomass (fucoidan, laminaran)
- Chemoenzymatic modification of polysaccharides to create new high-value products
- Reduce the use of energy, water and harsh/toxic chemicals in biomass processing
- Increased recovery from waste streams

# Why this project and partnership?

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*Laminara hyperborea stipe*

- Alginate
- Fucoidan
- Laminaran
- Polyphenols & Pigments
- Mannitol
- Cellulose
- Proteins
- Minerals

# Why this project and partnership?

Chitin/Chitosan

Proteins

Minerals

Pigments

# Why this project and partnership?

## needs/gaps

- Current technology for marine biomass processing → not useful for cost efficient separation/recovery of multiple products
- The knowledge about structure and composition of marine biomass not good enough to suggest good enzyme assisted strategies for treatment and fractionation
- The enzyme toolbox for processing of marine biomass is not yet developed for industrial utilization
- Products are poorly characterised
- Some of the products lack good applications

# Fresh raw materials

## Why this project and partnership?

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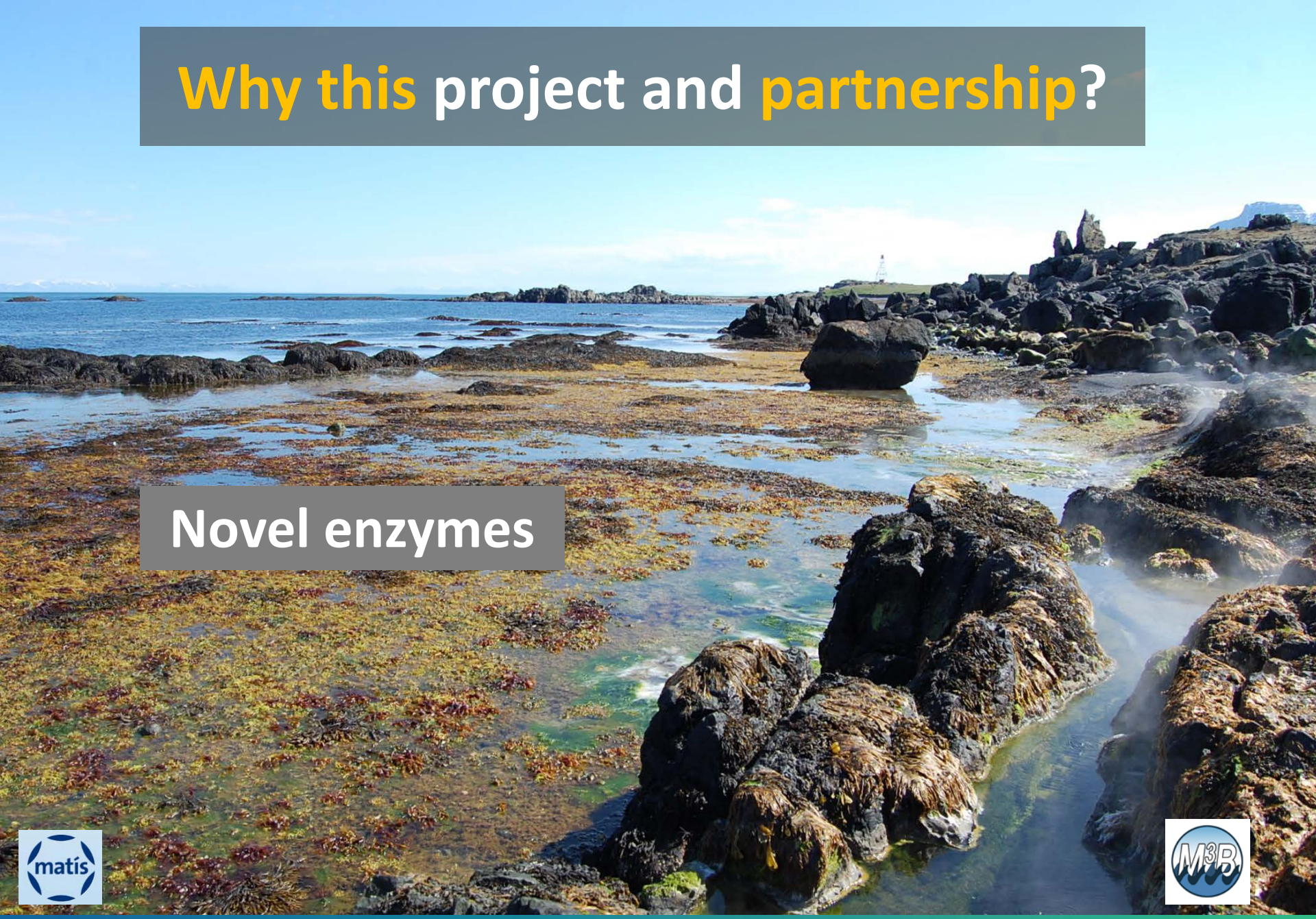
**Access to samples and waste streams from large scale macroalga processing plant**

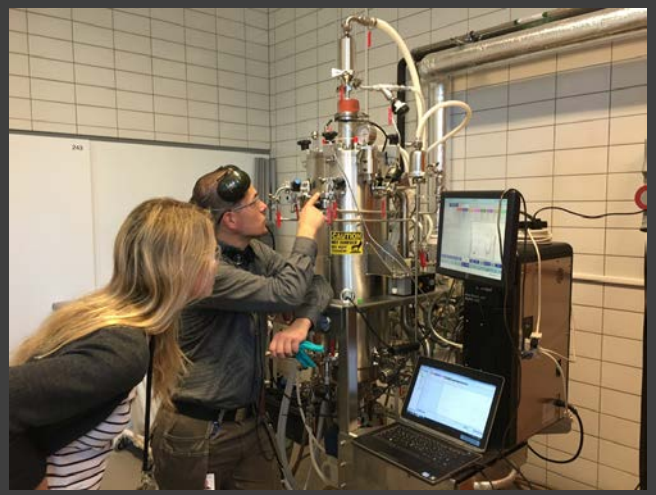
# Why this project and partnership?



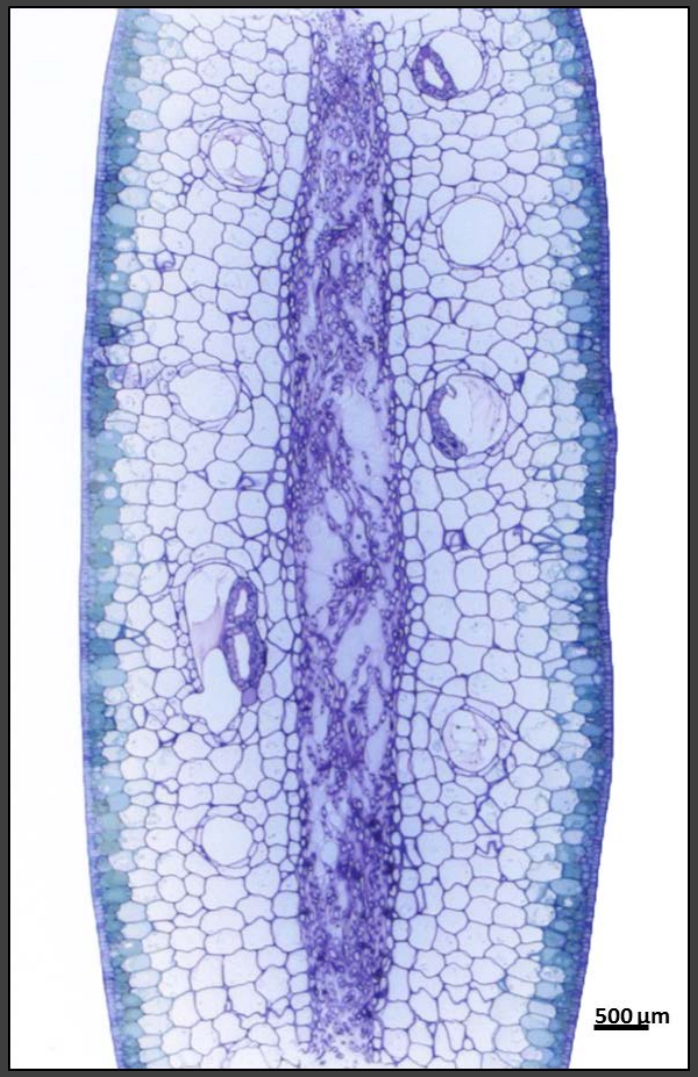
## Novel enzymes

# Why this project and partnership?



# Progress to date - examples




*L. Hyperborea* leaf

**WP 1 – Upstream biorefinery of marine biomass (DuPont)**

**WP 2 – Characterization of biomass process streams and products (NTNU)**

**WP 4 – Enzyme and substrate production (KTH)**

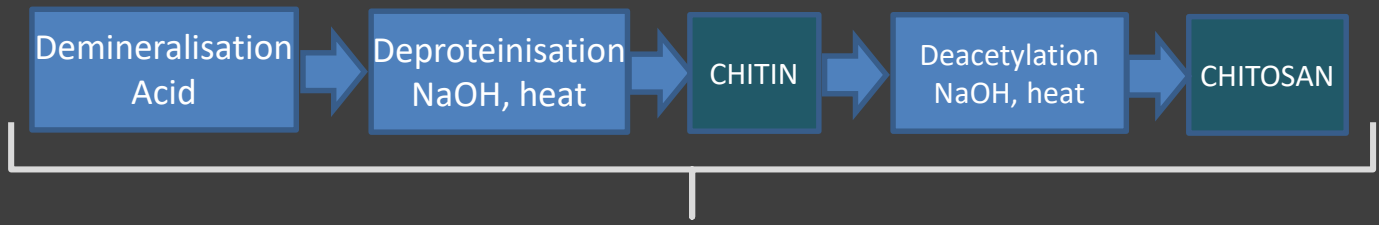
**WP 3 – Production of polysaccharide products targeting multisectorial applications (UNITS)**

**WP5 Management, coordination and training (SINTEF)**

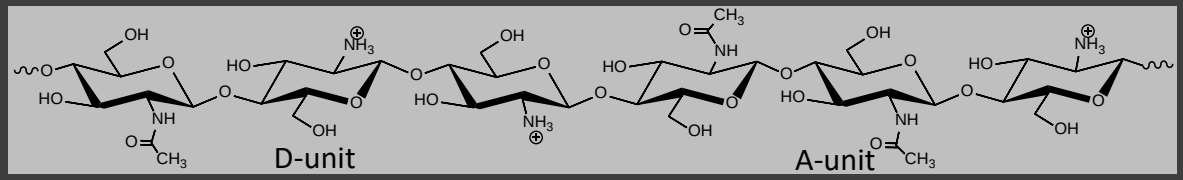
# WP 1 – Upstream biorefinery of marine biomass



Chitin/Chitosan production



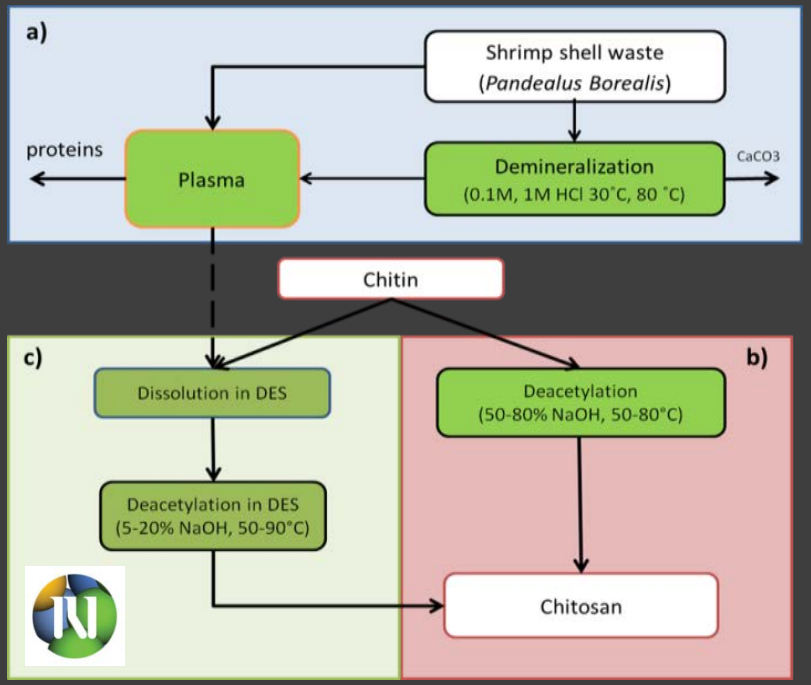

- New Chemical pre-treatment methods
- Enzymes for deproteination



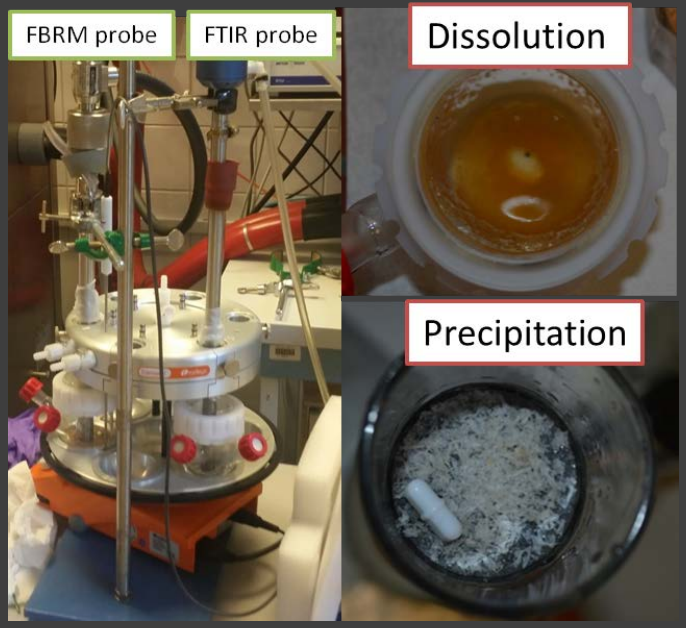
# WP 1 – Upstream biorefinery of marine biomass

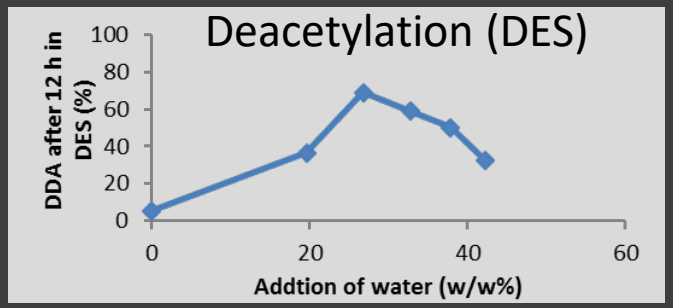
Non-thermal plasma

## New Chemical pre-treatment metodes for chitosan production




Use of deep eutectic solvents (DES)



# Enzyme assisted chitosan manufacturing

Demineralisation Acid → Deproteinisation NaOH, heat → CHITIN → Deacetylation NaOH, heat → CHITOSAN

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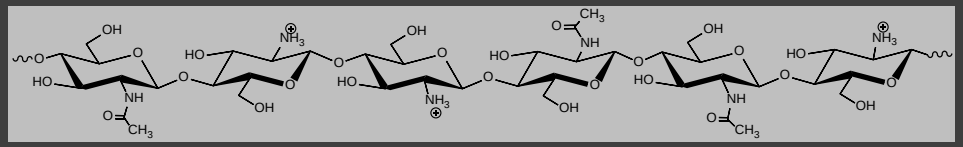
One step chemoenzymatic procedure → CHITIN → Enzymatic deactylation depolymerisation → CHITOSAN

One step chemoenzymatic procedure → PEPTIDES → Enzymatic deactylation depolymerisation → CHITO-OLIGOMERS



# WP 2 – Characterisation of biomass process streams and products

Processing

Isolation/purification

**Biomass anlayses**

Structure
Composition
Distribution
Interactions

**Fractions Waste streams**

Composition
Quantification

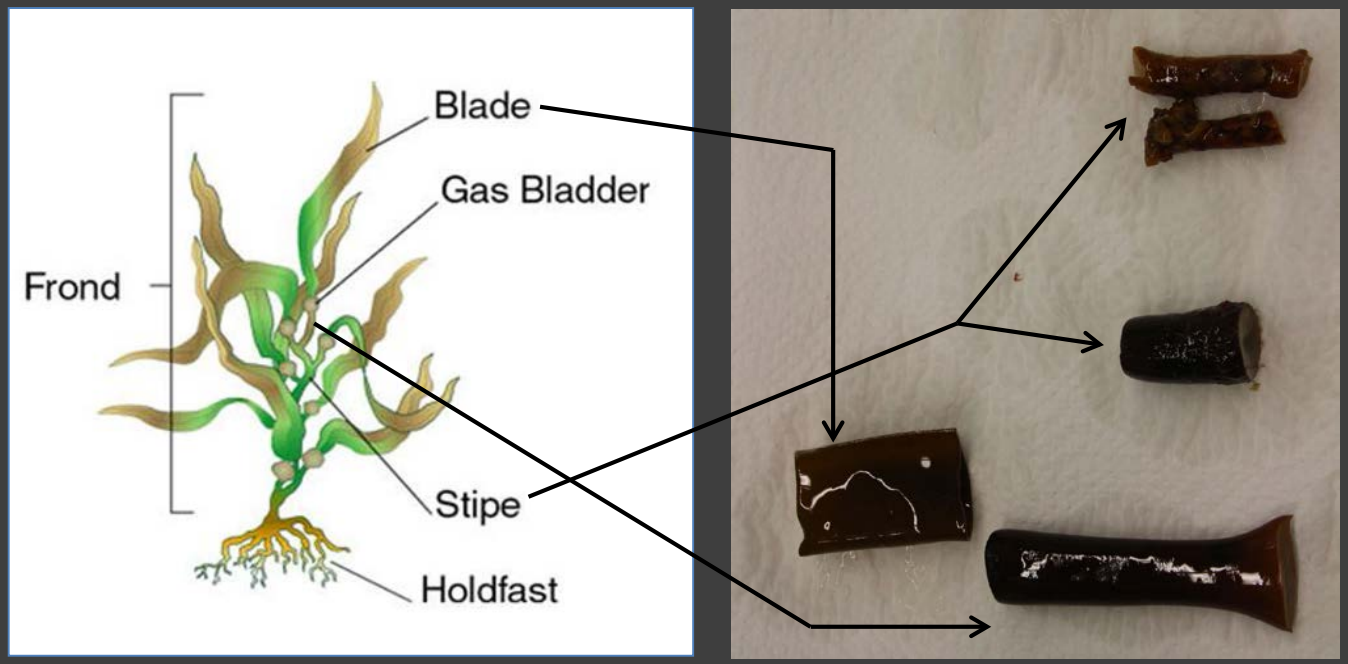
**Products**

Purity
Structure (linkages)
True biological effects

**A wide range of analytical tools and analytical infrastructure necessary**
**Lack of good standards is a challenge**

# WP 2 – Characterization of biomass process streams and products

Strategies for chemoenzymatic biomass fractionation and enzymatic modifications based on detailed understanding of structure/composition of the biomass and derived products



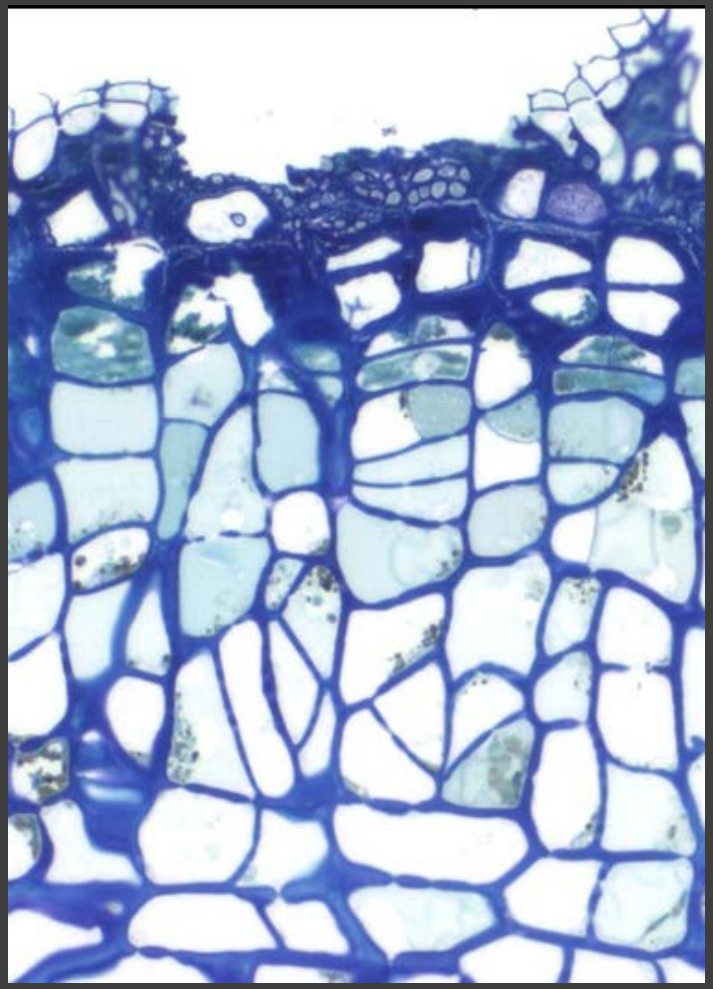
# WP 2 – Characterization of biomass process streams and products

Epidermis

Meristoderm

Outer Cortex

Inner Cortex



*Laminaria hyperborea stipe*

# WP 2 – Characterization of biomass process streams and products

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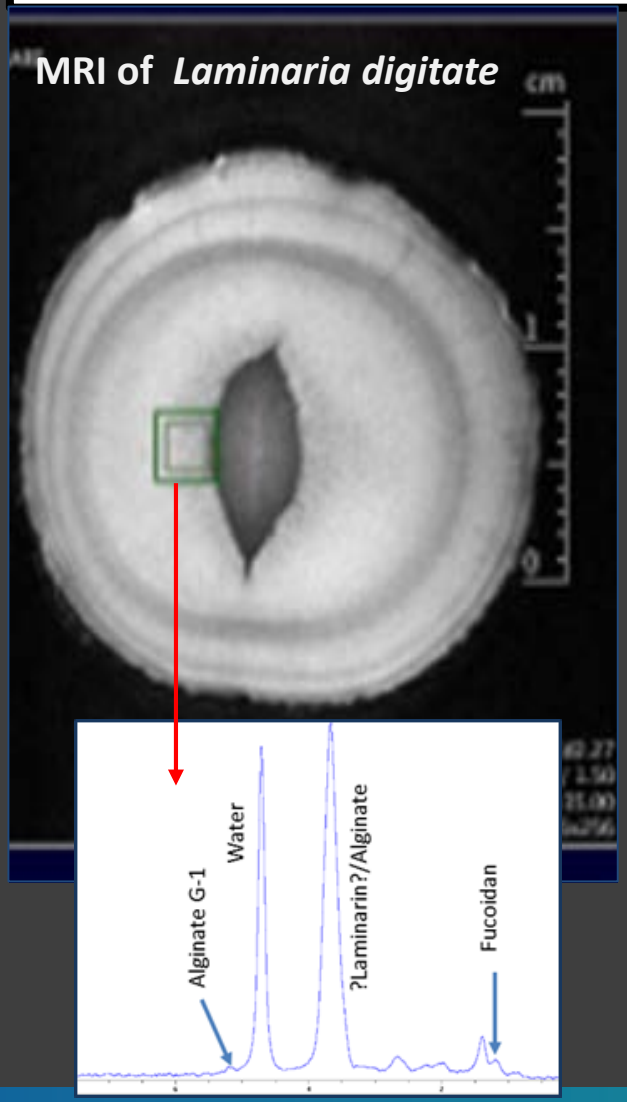
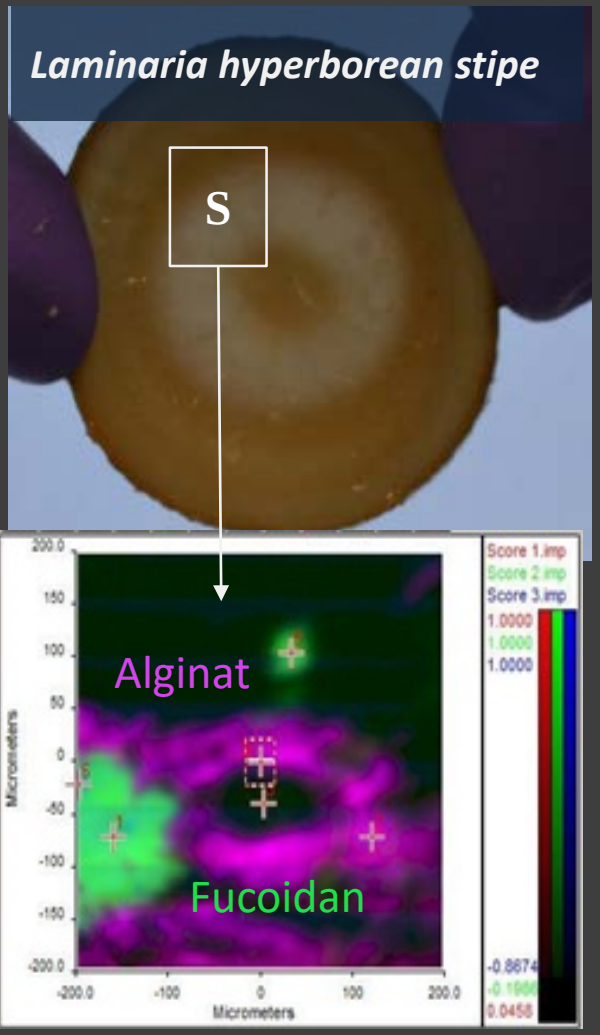
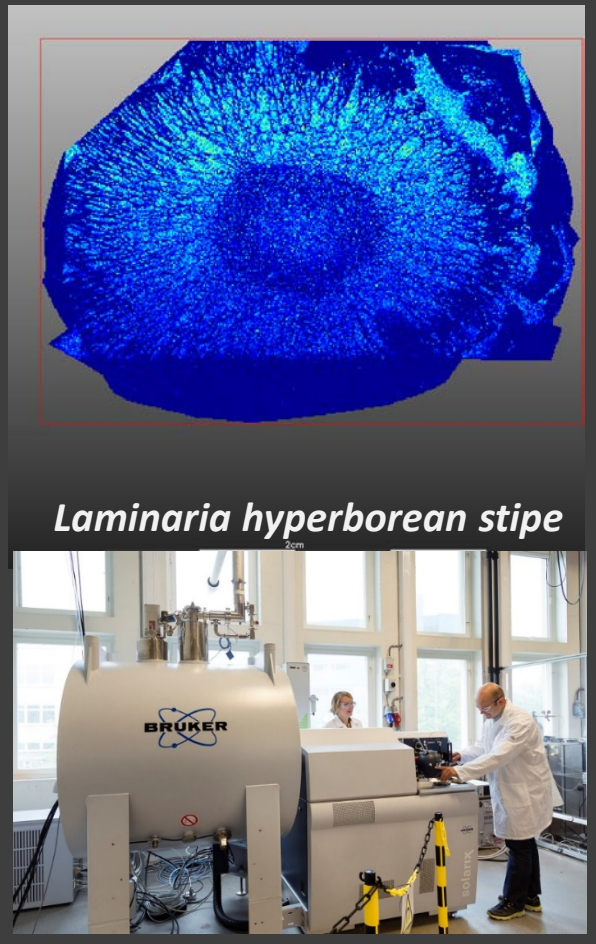


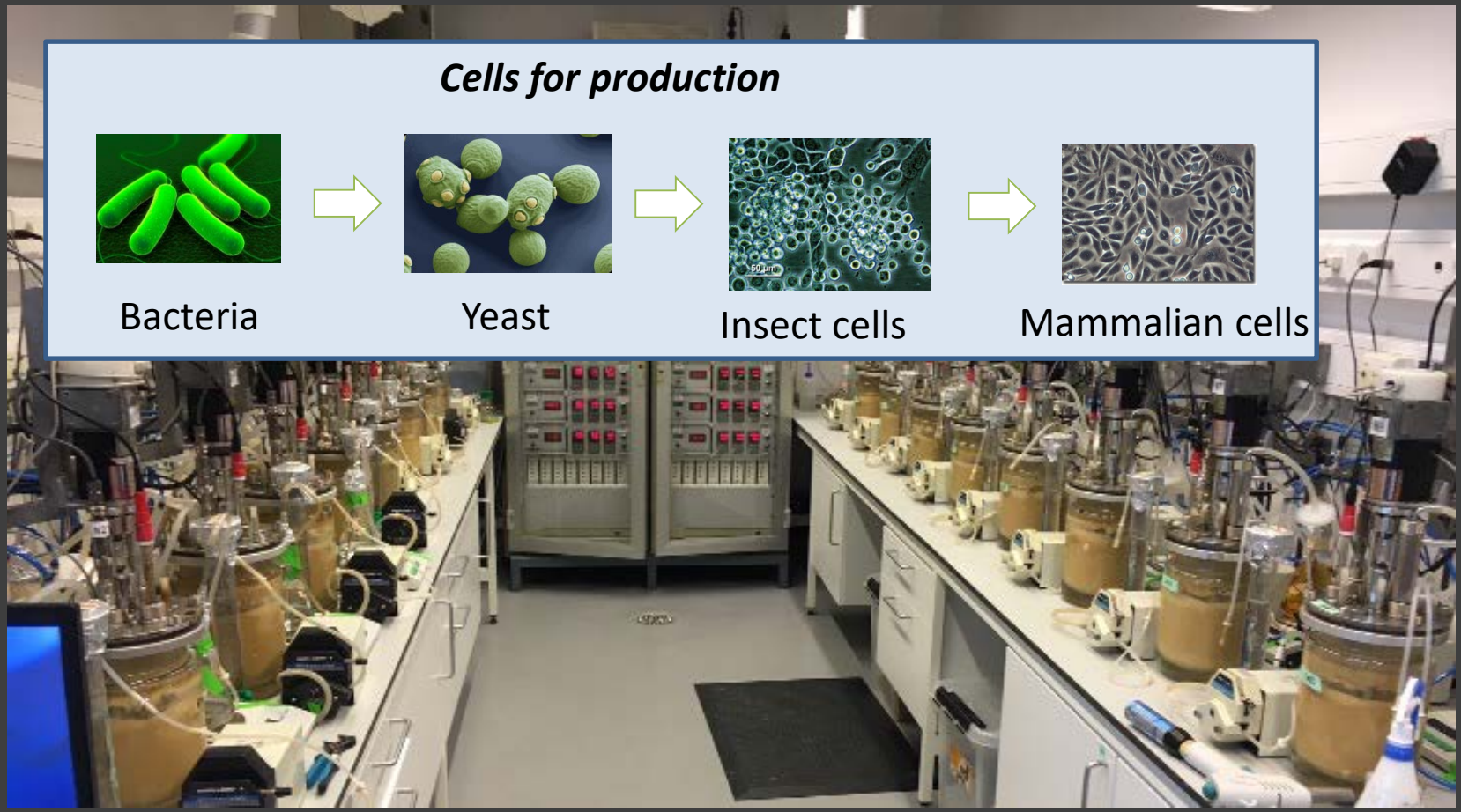

FT-ICR-MS ultra high resolution MS-identification.

# WP 4 – Enzyme and substrate production

## Thermostable enzymes in Mar3Bio

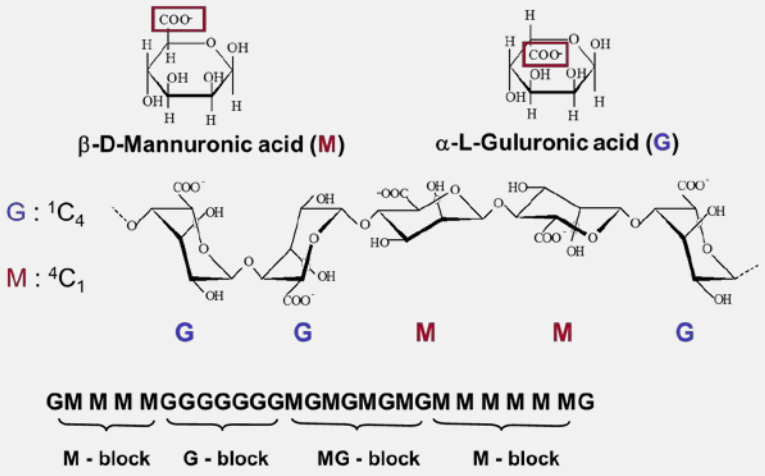
| Biomass | Application | Enzyme 1 | Enzyme 2 | Enzyme 3 |
|---|---|---|---|---|
|  | **Laminarin degradation** | β-1,3 endoglucanase (Amo176) | β-1,3 exoglucanase (Amo305) | β-1,6 endoglucanase (Cel136) |
|  | **Laminarin branching and degradation** | Beta-transglucosidase Glt20 (GH17) | Beta-transglucosidase Glt20 (GH17) | |
|  | **Fucoidan degradation** | α-L-fucanases (aFuc) | Novel putative endo-fucosidases | |
|  | **Fucose desulfation** | Sulfatase (SulA) | | |
|  | **Alginate degradation** | Endo alginate lyases AlyRm1, 2 and 3 (PL6 and unknown) | Exo alginate lyase AlyRm4 (PL17) | |
|  | **Alginate extraction** | Putative laccases | | |
|   | **Protein degradation** | Aqualysin (AQUI) | | |
|  | **Chitin degradation** | Chitinases ChiM6 and two domain ChiM7 (GH18) | | |

# Available expression systems



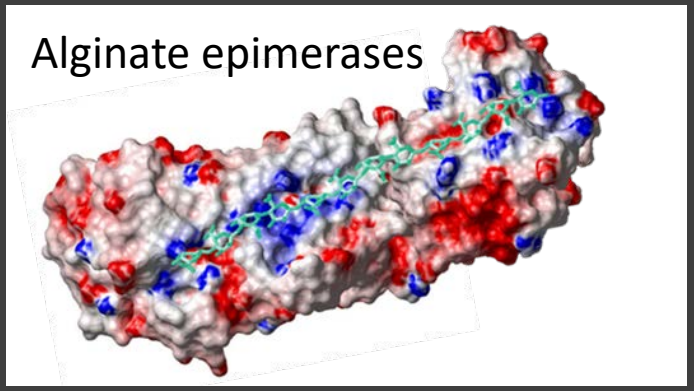
# WP 3 – Production of polysaccharide products targeting multisectorial applications
# WP 4 – Enzyme and substrate production

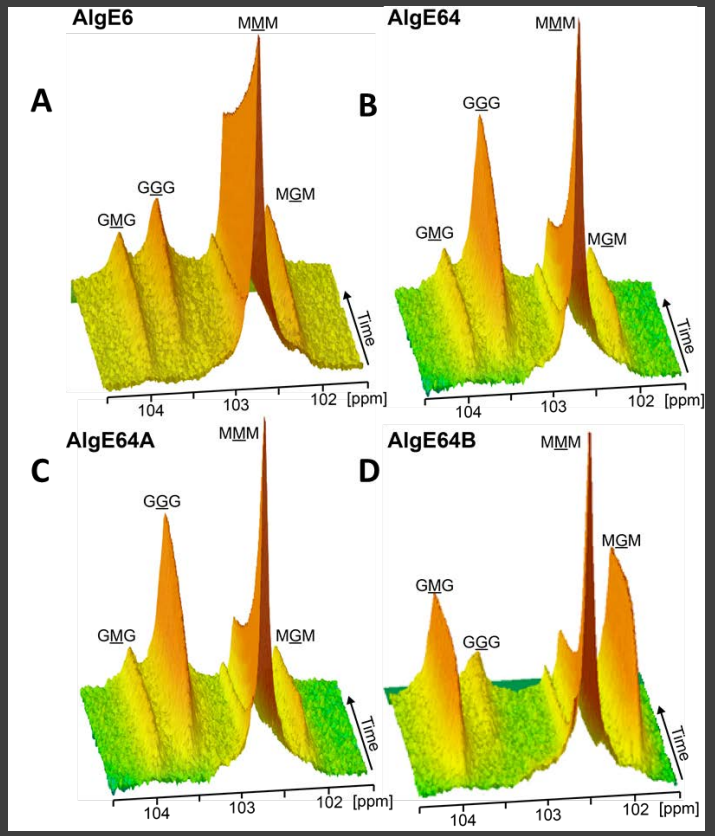
## Alginate composition




(Fisher and Dörfel 1955; Atkins et al. 1970; Haug et al 1964-1967)

Alginate epimerases






Time-resolved NMR

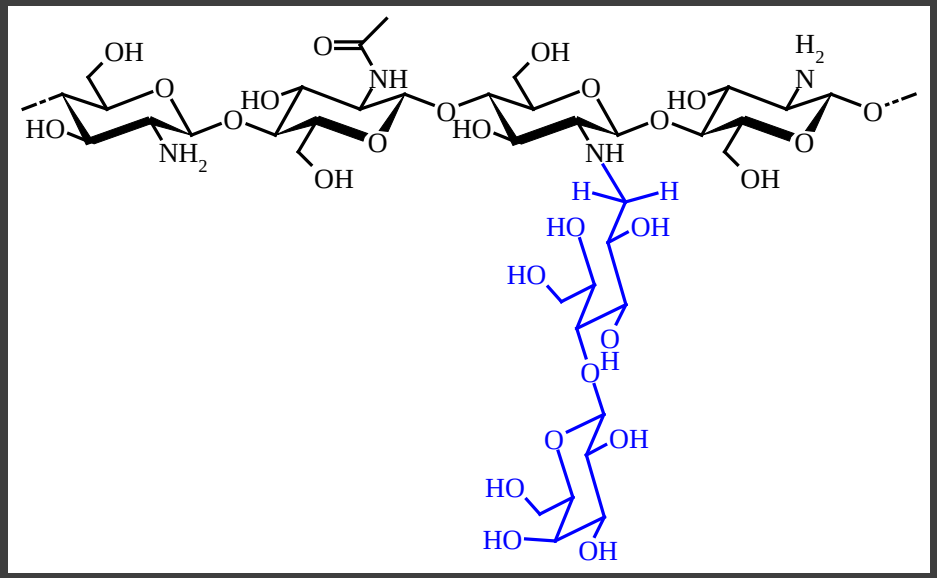
# TAILORED ALGINATES

# WP 3 – Production of polysaccharide products targeting multisectorial applications

## High added value modified polysaccharides from chitosan

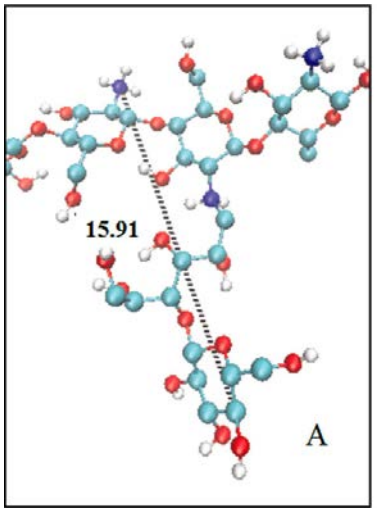
### Chitlac

Chitosan

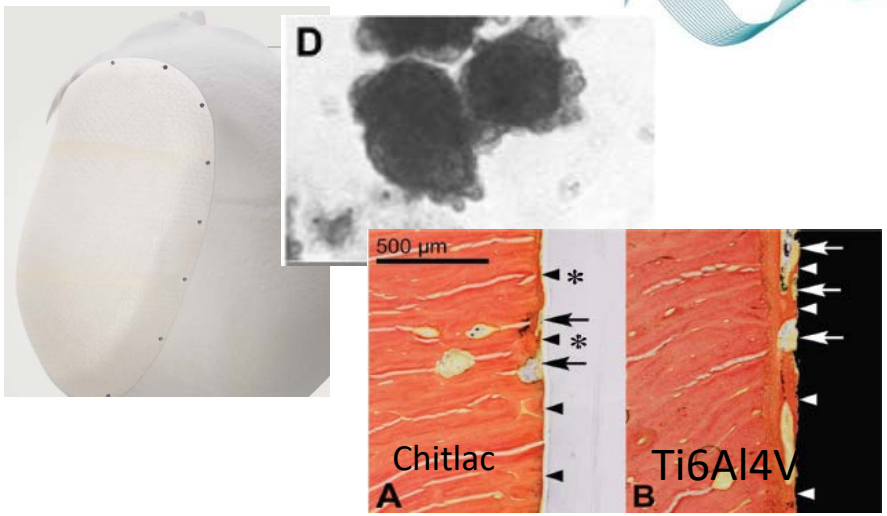


Lactose

# Chitlac. A bioactive molecule with applications in biomaterials




Coatings for hard tissue applications

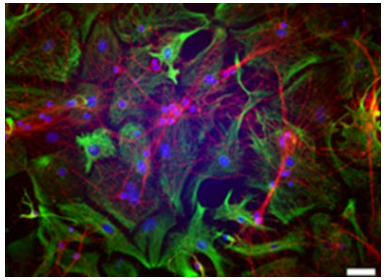



BIC **72%** 46%

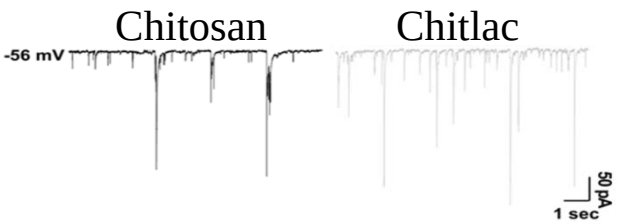
Ag-containing antimicrobial coatings

Neural tissue regeneration

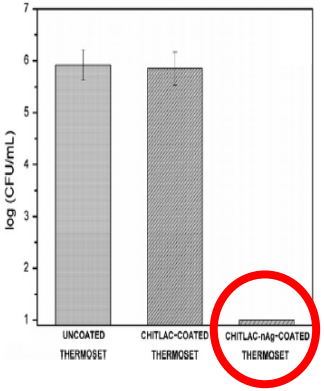
Hippocampal cultures



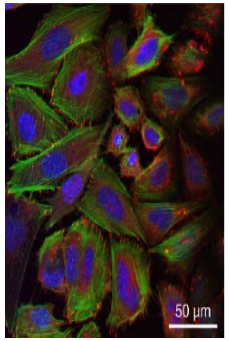
**Spontaneous Post-Synaptic currents**



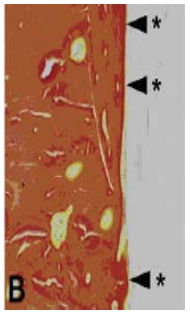

Antimicrobial




*In vitro*




*In vivo*



# Chitlac. Large scale production in medical grade


Endotoxin free

Production up to 1 Kg per batch according to ISO 9001

**biopolLife**

# Mar3bio - Roadmap

Five thematic areas of the strategic research and innovation


**Chemoenzymatic methods for processing**

**Training of Young scientist**
**Communiction / involvemnet of SMEs**

Policy support and stimulation

Biomass production and processing

Enabling techn. Infrastructure

Explortation of the marine environment

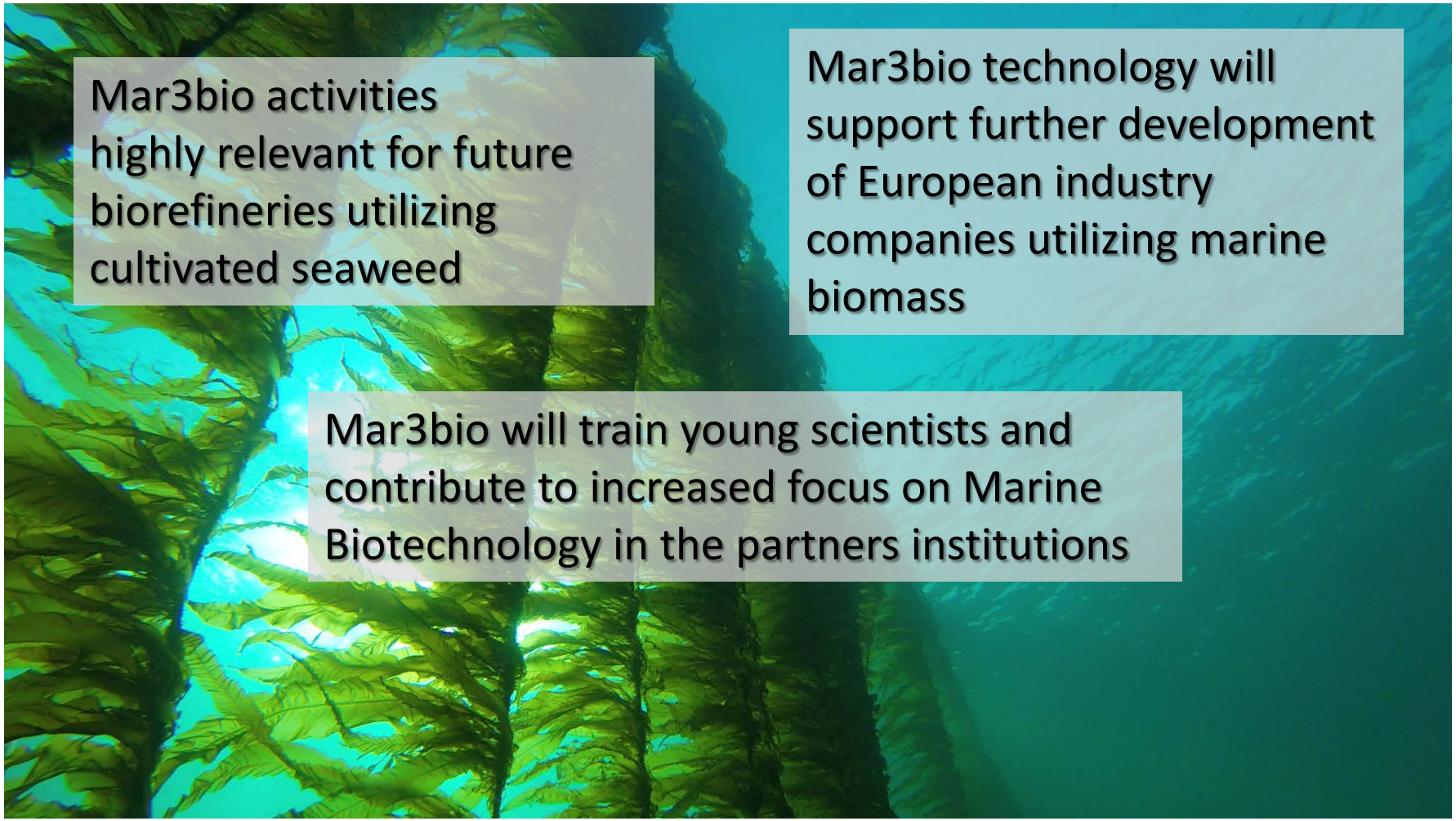
Product innovation

**Chitlac**
**Chito-oligomers**
**Marine - peptides**
**Alginates**
**Fucoidan**
**Laminaran**
**……**
**……**

**Enzymes**

**Enzymes**
**Marine Biomasses**

# Future perspectives



Mar3bio activities highly relevant for future biorefineries utilizing cultivated seaweed

Mar3bio technology will support further development of European industry companies utilizing marine biomass

Mar3bio will train young scientists and contribute to increased focus on Marine Biotechnology in the partners institutions

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Gerd Inger Sætrom
Gundmund Skjåk-Bræk
Olav A. Aarstad

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