Bioactivities of Marine Polysaccharides on the Gut immune systeme - Update

Mustapha M. Berri

Biological Activity of Marine Polysaccharides and Animal Health

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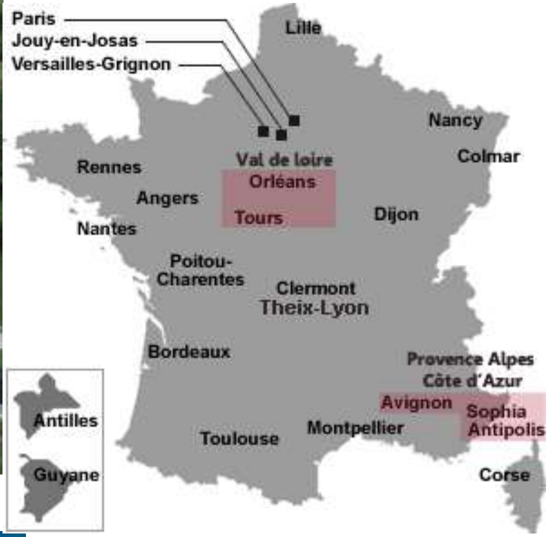


➞ RESEARCH UNIT (UMR ISP 1282)
Host/Pathogen Interactions
Bacteria, Virus, Parasite

Main Research Programs

- **To Characterize Pathogens**

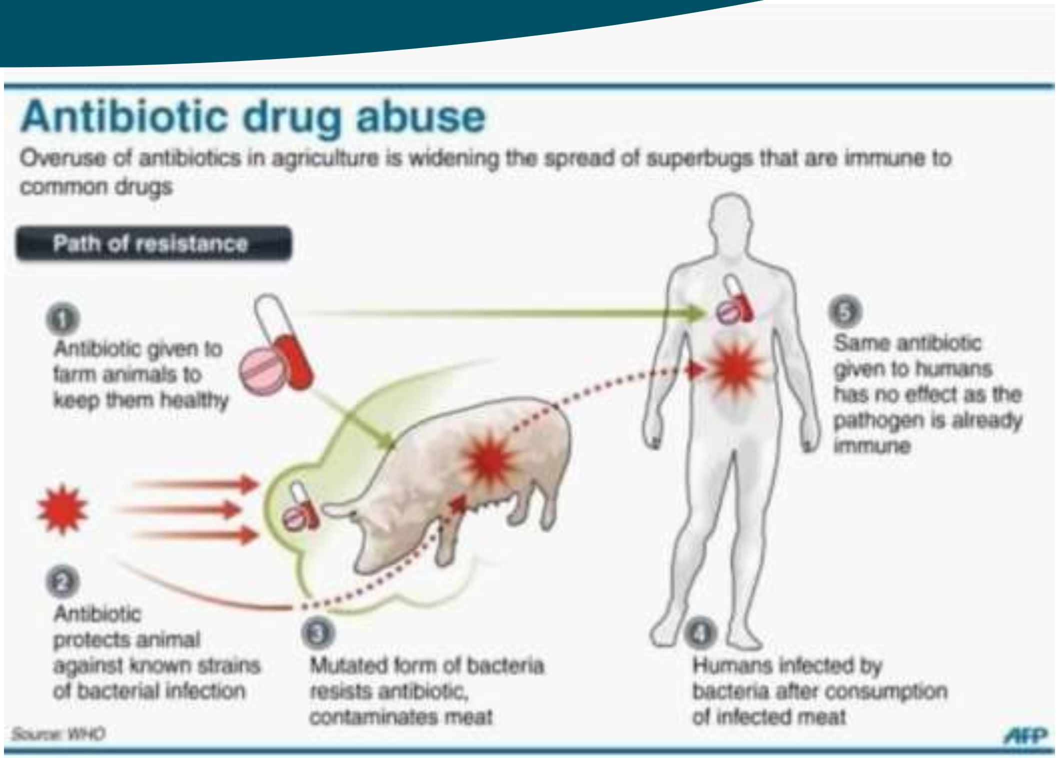
- Diversity, Virulence Factors, Resistance to Drugs

- **To understand Molecular and Cellular Mechanisms of Host/Pathogen Interactions**

- **To analyze the Host Immune Response Towards Microbes**

- **To Evaluate New Prophylactic Strategies and to Test New Molecules**

→ Indiscriminate and Excessive use of Antimicrobial Agents as Growth Promoters in Farm Animal Feed



Ban on antibiotics as growth promoters in animal feed entered into effect on January 1st 2006 (Regulation 1831/2003/EC on Additives for Use in Animal Nutrition)

→Are there really any alternatives to antibiotics use ?

- **Vaccination**
- **Probiotics**
- **Prebiotics**

Algae: A Food Source for Animals and Humans

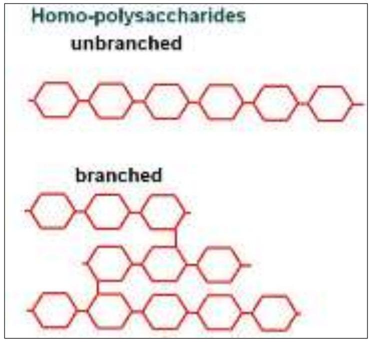
- Algae are the fastest growing plants organisms in nature

- Ability to convert large amounts of carbon dioxide (CO_2) into oxygen
- Today, algae still produce 70% of the earth's oxygen

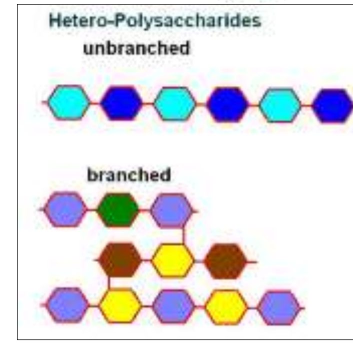
- Ability to triple or quadruple their biomass every day
- One acre of algae can produce the same amount of protein in a year as 21 acres of soybeans or 49 acres of corn

- They produce lipids, protein, vitamins, minerals, fibers and polysaccharides

Classification of the bioactive sulfated polysaccharides



- ❑ They represent 4 to up 76% of dry weight of algae
- ❑ Repetitive structural feature
- ❑ Polymers of monosaccharide units joined to each other by glycosidic linkages
- ❑ Homo and hetero-polysaccharides



◉ Ulvan

◉ Sulfated rhamnose, sulfated aldobiuronic acid

◉ Agar and Carrageenan

◉ Sulfated galactose and 3,6 anhydrogalactose

◉ Fucoidan

◉ Fucose, Xylose,Uronic acid, Galactose, Sulfate

→ Biological properties and potential industrial uses of algal sulfated polysaccharides

◉ Health Benefits

- ✓ Antioxidant
- ✓ Anticoagulation
- ✓ Stimulation of dermal and fibroblast proliferation
- ✓ Anticancer
- ✓ Antimicrobial activity
- ✓ Modulation of the immune response

▸ Potential Industrial Applications

- Nutrition
- Pharmaceuticals
- Functional foods
- Cosmetics

➞Summary of antiviral activities of marine sulfated polysaccharides

Table 1. Antiviral activities of selected marine polysaccharides.

Marine organisms	Specific polysaccharides	Antiviral effects	References
Crustacean	Chitosan	Anti-enteric virus, plant viruses, HIV	[75–81,85,86]
Red algae	λ-carrageenan	Anti-DENV, HSV, HPV, HIV, HAV	[33,35,38–40,46,47]
	κ-carrageenan	Anti-enterovirus, HSV, HIV, IAV	[32,35,39,40,42–44]
	ι-carrageenan	Anti-DENV, HRV, IAV, HPV, HAV	[33,34,36–38,47,48]
Brown algae	Alginate	Anti-HIV, HBV, IAV	[61–66,88–90]
	Fucan	Anti-HIV, DENV, IAV	[67–69]
	Laminarin	Anti-HIV	[70]
Green algae	Ulvan	Anti-IAV	[59]
Shellfish	Shellfish polysaccharide	Anti-HSV, IAV, HBV, HIV	[71–74]
Microalgae	Sulfated polysaccharide	Anti-HSV, IAV	[91,92]

- Direct antiviral Action
- Inhibition of Virus Transcription and Replication
- Inhibition of Viral Adsorption and Internalization
- Inhibition of Virus Fusion with CD4 Protein on the Surface of T lymphocytes
- Improvement of Host Antiviral Immune Responses and Reduce the Mortality Rate of Animals

Wang et al. 2012, Mar. Drugs.

Herpes Simplex Virus (HSV), Duck Hepatitis B Virus (DHBV), hepatitis B virus (HBV), human herpes virus (HSV), influenza A (H1N1) virus (IAV), Human rhinovirus (HRV), hepatitis A virus (HAV), HPV Human Papilloma Virus (HPV)

Marine polysaccharides as antibacterial natural products

- Green algae *Ulva lactuca* (Turkish, British) (Abd El-Baky et al., 2008; Spavieri et al. 2010)

➢Gram-positive and negative bacteria
S. aureus, B. cereus, B. subtilis, K. pneumoniae, Micococcus luteus, Serratia marcescens

- *Ulva*, *Caulerpa* and *Spongomorpha* sps. (India) (Rangaiah et al. 2012)

➢ 18 strains of gram+/- bacteria and fungi
S. aureus, Streptococcus mutans, B. subtilis, Lactobacillus acidophilus, P aeruginosa, E. coli, K. pneumonia, A. niger and C. albicans

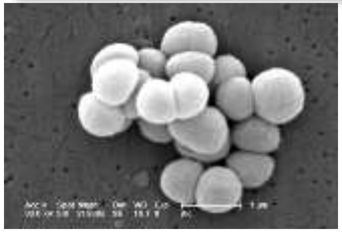
- Marine polysaccharides as antibacterial natural products

- Green algae *Schotia latifolia* Jacq (South of Africa) (Oydemi and Afolayan 2011)

➢*B. cereus, B. pumilus, S. aureus, Mycobacteria aurum, proteus vulgaris, E. coli and K. pneumonia*

- 8 species of green algae Phaeophyceae, Rhodophaceae and Chlorophyceae (Egypt) (Salem al. 2012)

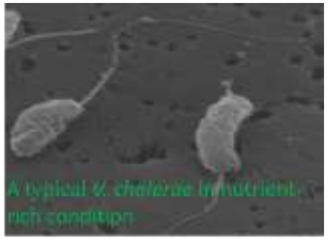
➢ *E. coli, S. typhimurium, Enterococcus feacalis, P. aeruginosa, S. aureus*



S. aureus

S. typhimurium

E. Coli K88



Vibrio cholerea

P. aeruginosa

→ Simplified schematic overview of the immune system

INRA SCIENCE & IMPACT

Breizh Algae Tour — Algae for a better life

Immune System

- Innate/Natural Response
- Adaptive/Specific Response

Cells/System involved

Innate/Natural Response:
Neutrophils
Monocytes/Macrophages
Natural Killer (NK)-cells
Dendritic cells

Cell Mediated Immunity

Tc-cells
Th-cells
(Th0)

Th1 Th2

Humoral Immunity

B-cells

Processes

Innate/Natural Response:

- Phagocytosis
- Antigen Presentation
- Oxidative Burst
- Cytokine Production

Cell Mediated Immunity:

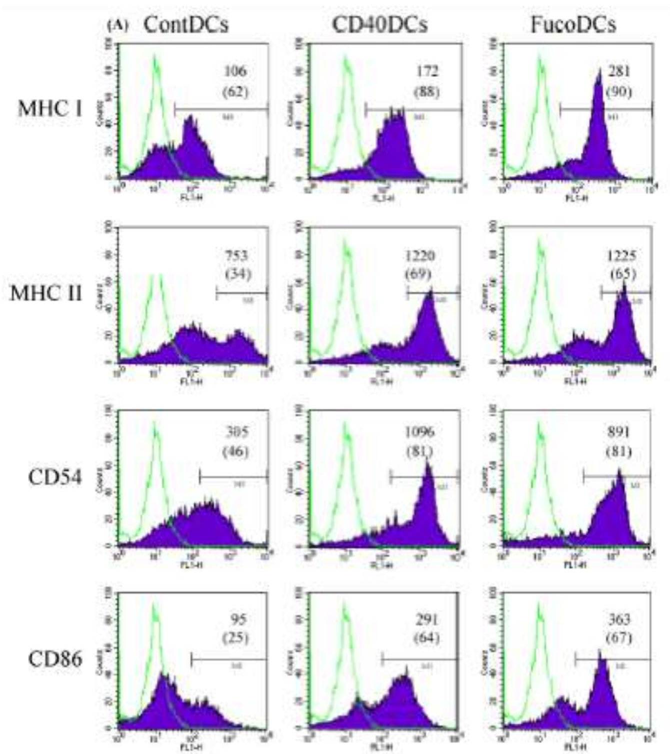
- Cytokine Production (Th1+Th2)
- Macrophage Activation (Th1)
- Lysis of Infected cells (Tc)
- B-cells Activation (Th2)

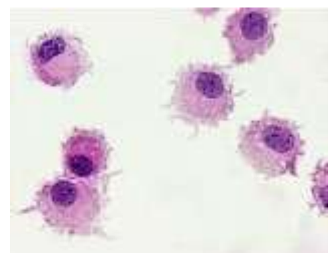
Humoral Immunity:

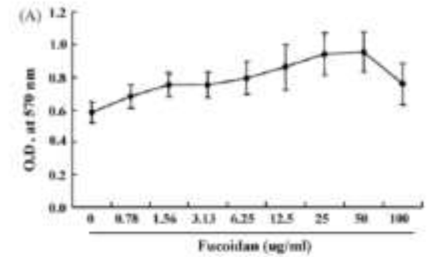
- Antibody Production

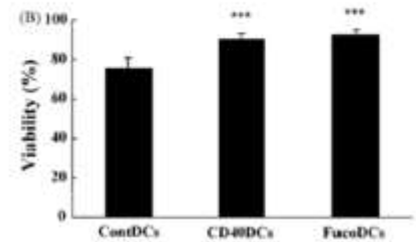
Immunostimulatory effects of marine sulfated polysaccharides on dendritic cells

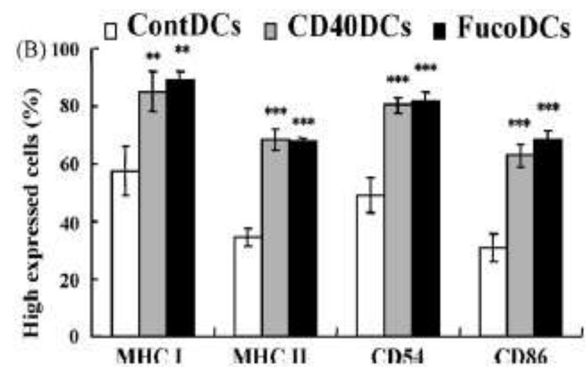
Bone marrow cells, activated with GM-CSF, Fucoidan 50 µg/ml, 1µg/ml of anti-CD40, FACS analysis









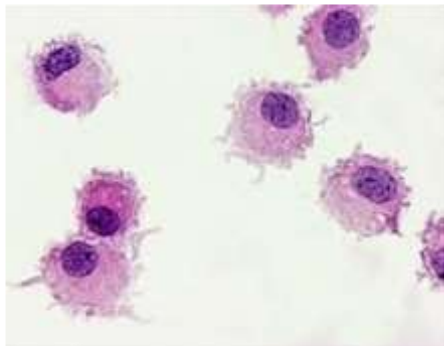


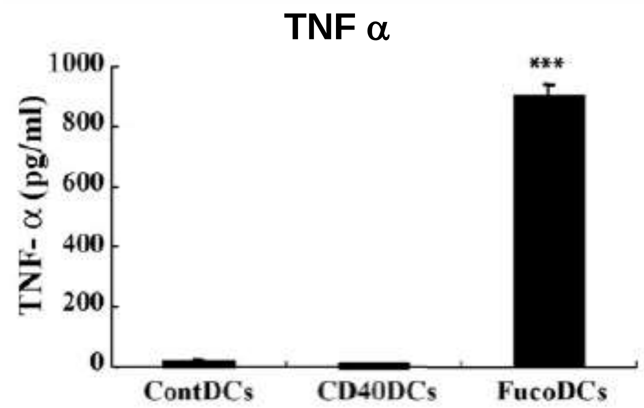
- **Enhanced expression of maturation markers on the surface of fucoidan-treated DCs**

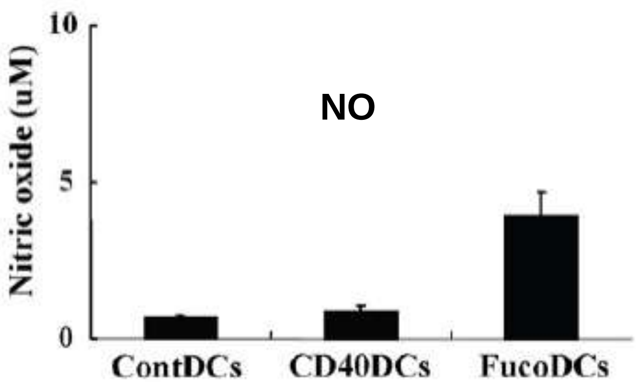
(Kim and Joo, 2012)

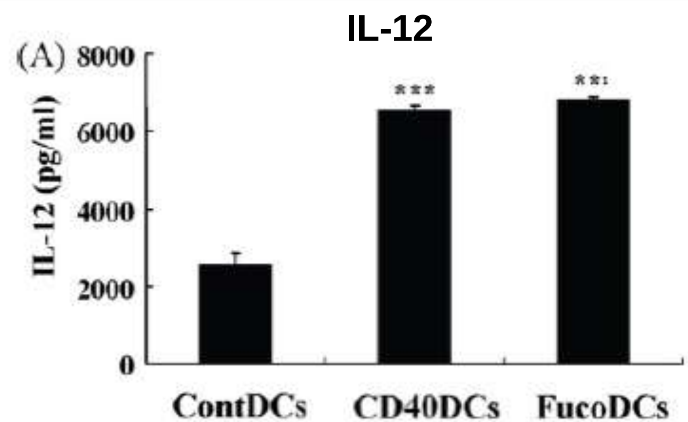
Immunostimulatory effects of marine sulfated polysaccharides on dendritic cells

Bone marrow cells, activated with GM-CSF, Fucoidan 50 µg/ml, 1 µg/ml of anti-CD40, ELISA TNFα, NO, IL-12 on supernatant



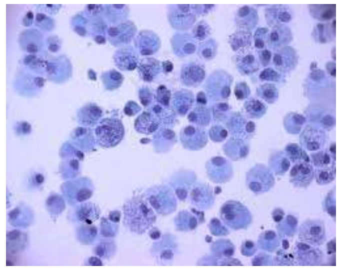




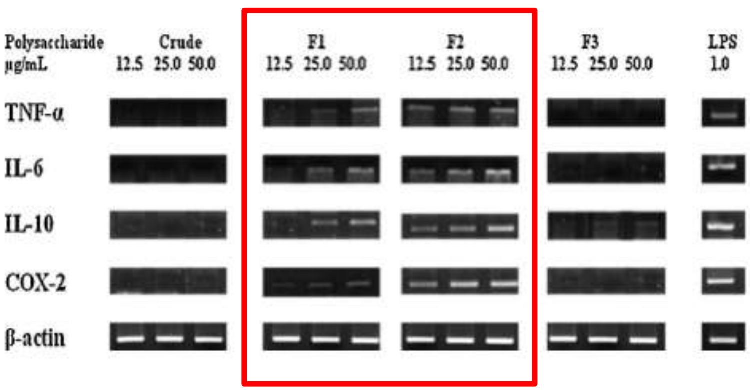


- **Enhanced expression of maturation cytokines of fucoidan-treated DCs (Kim and Joo, 2012)**

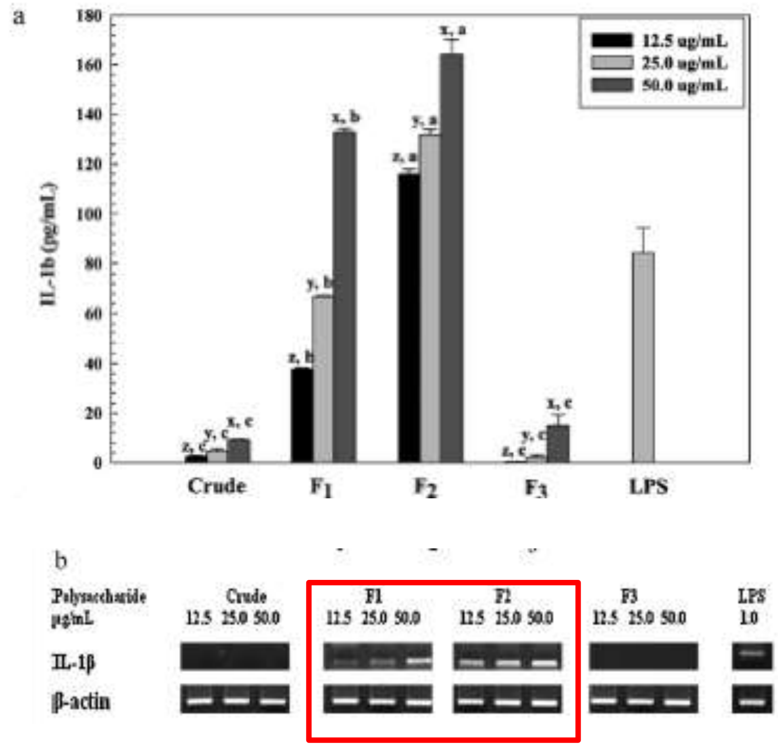
Marine sulfated polysaccharides activate cytokine expression of Raw 264.7 macrophages



(Kim et al. 2012)



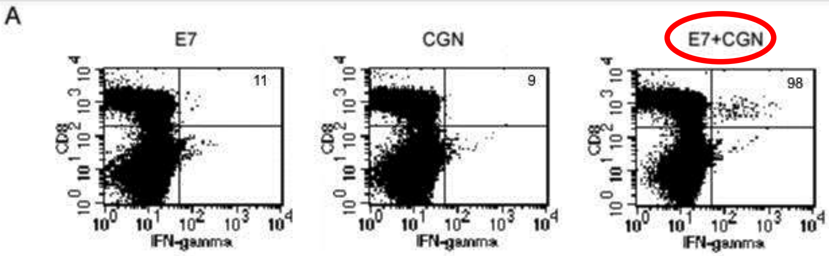
Raw 264.7 cells treated with various concentration of ulvan. RT-qPCR analysis of immune response markers

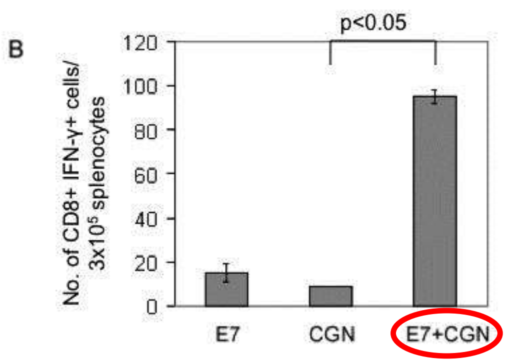


Low molecular weight polysaccharides (<15 kDa) of ulvan stimulate the expression of various cytokine and might be beneficial for immunostimulation

Carrageenan as an adjuvant to enhance peptide-based vaccine potency

C57BL/6 mice vaccinated (s.c.) with 10 µg of HPV E7 peptide vaccine (E7) in combination with 10 µg of carrageenan (CGN) with a boost seven days later

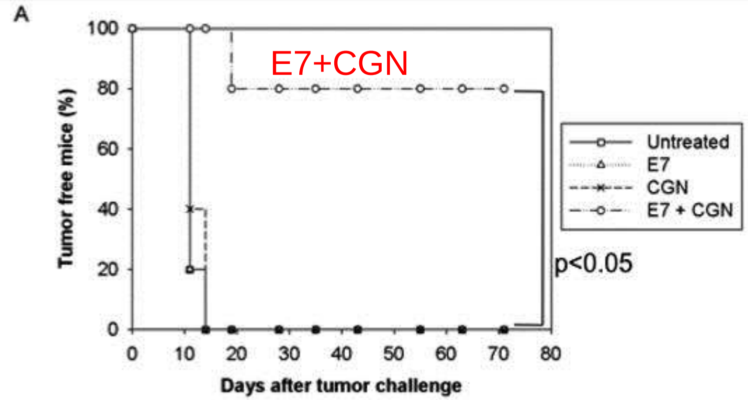


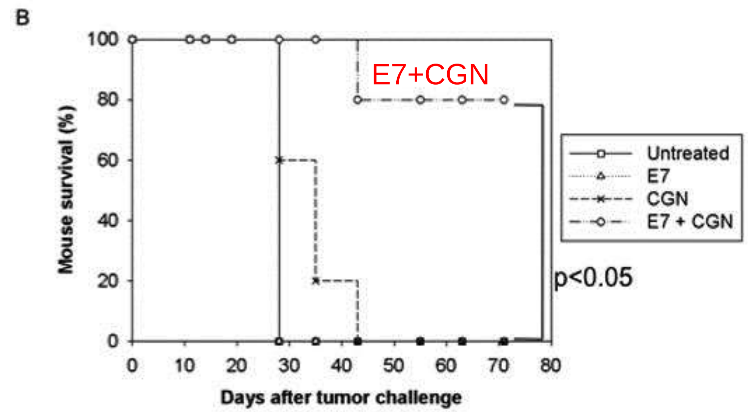


Co-administration of CGN enhanced the E7-specific CD8+ T cells response generated by E7 peptide vaccination

(Zhang et al. 2012)

C57BL/6 mice vaccinated and injected with tumor murine cells (TC-1) one week after the last vaccination

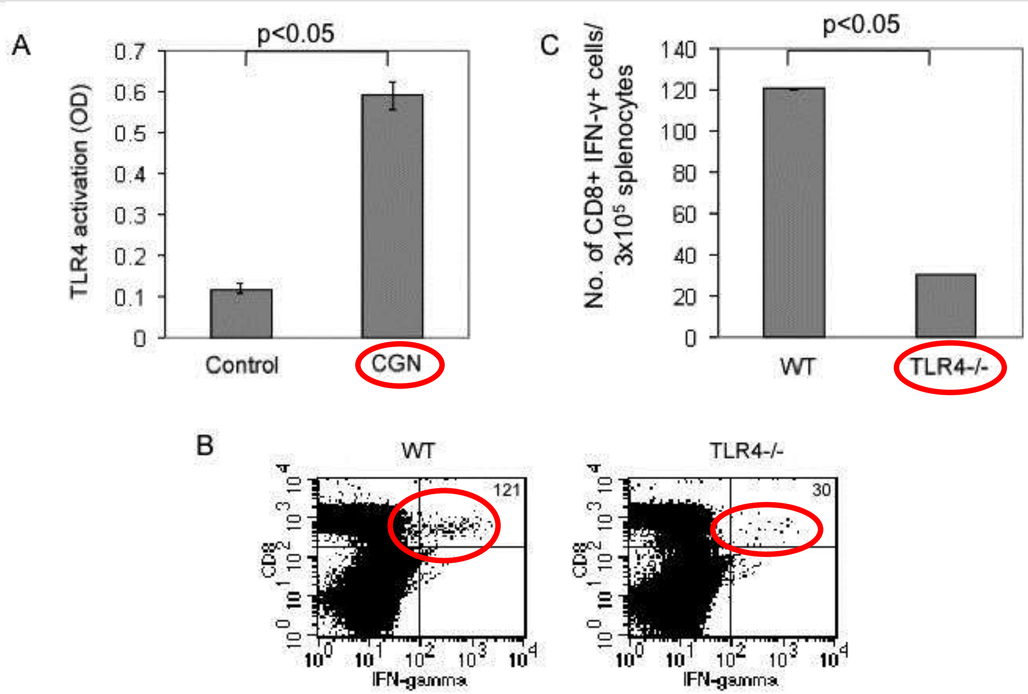




Co-administration of CGN enhanced the protective effect generated by E7 peptide vaccination

Carrageenan as an adjuvant to enhance peptide-based vaccine potency

A) HEK-blue4 cells treated with 1 µg of CGN and analyzed with Quanti-Blue assay and **B-C**) Wt and TLR-/- mice were vaccinated (s.c.) with 10 µg of HPV E7 peptide vaccine (E7) and carrageenan (CGN)



CGN leads to the enhancement of immune response generated by E7 peptide vaccination via TLR4 activation pathway

→ Effect of seaweed extract (SWE) supplementation in pig diet

- Effect of SWE (*Laminaria*) on piglet performance, selected bacterial populations, and cytokine expression in the gastrointestinal tract

- **Notable effect on animal performance and nutrient digestibility**
- **Reduce enterobacteriaceae population and low affect on lactobacillus and bifidobacteria**
- **Immunomodulatory effects with down regulation of IL-α, TNFα and IL-17 in the colon**

- Effect of maternal dietary supplementation with SWE (*Laminaria*) from late gestation until weaning

- **High IgA and IgG in sow colostrum**
- **No effect on IgA, IgG and IgM in sow milk**
- **High IgG in serum of piglet suckling SWE supplemented sow**
- **No apparent effect on intestinal morphology of piglet suckling SWE-supplemented sow**

Antibacterial and immunomodulating activity of Marine Sulfated Polysaccharide (MSP) extracted from Ulva green algae

Olmix Green algae

Water soluble MSP extract

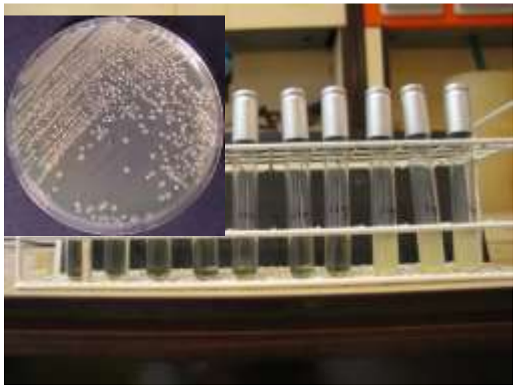
- 11,6% Neutral sugar
- 7,3% Protein
- 12.2% Uronic acids
- 3.6% Sulfated group

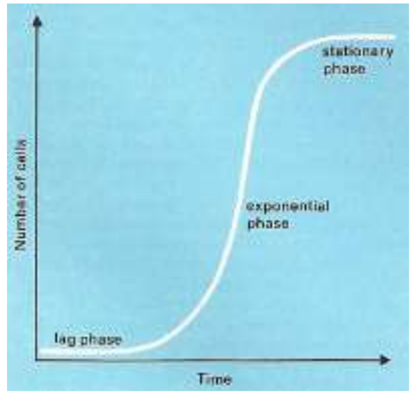
- **Dissolved in ultrapure water or DMEM**
- **Sterilization using filtration/autoclave**
- **Preparation of different concentrations**

- 1- Antimicrobial activity and MIC determination

- 2- Stimulation of immune response mediators

1- Antimicrobial activity and Minimum Inhibitory Concentration (MIC) determination of MSP

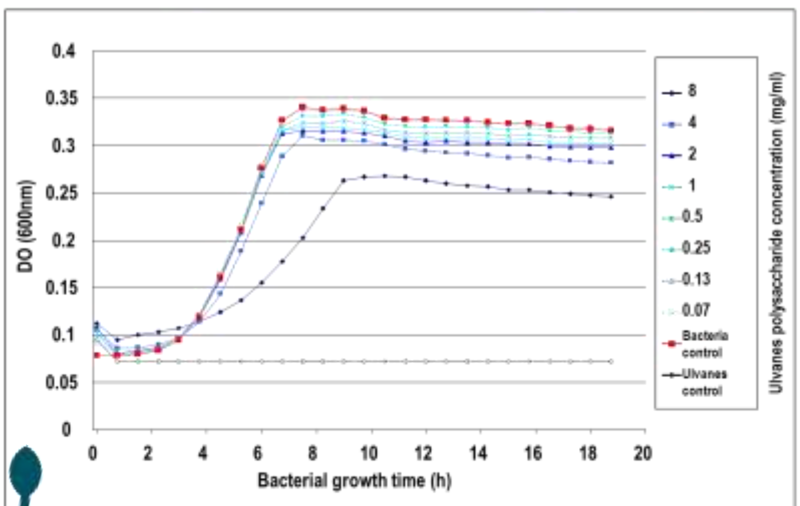




- Tested bacteria strains
- *S. Typhimurium* (bovine septicemia)
- *E. coli K88* (pig enteric colibacillosis)
- *S. aureus* (bovine mastitis),
- *L. Monocytogenes* (rabbit tissue)
- *E. coli 096* (chicken feces)

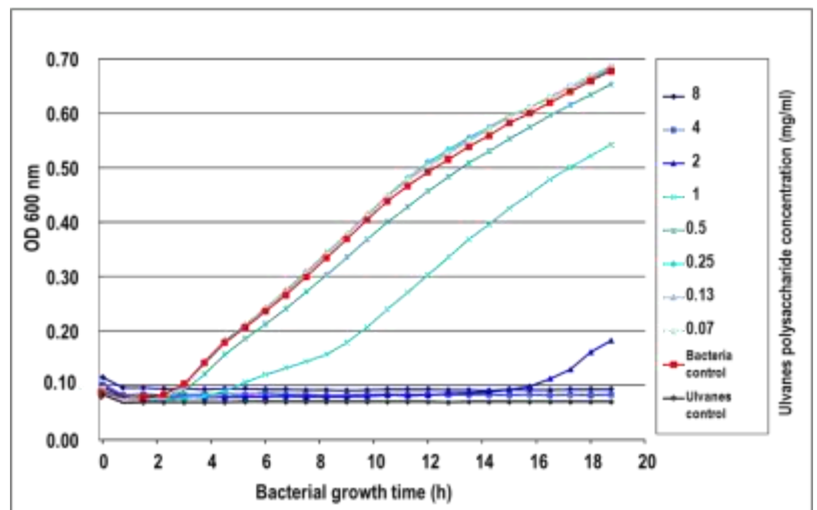
Listeria monocytogenes

Bioscreen assay showed that sulfated polysaccharides inhibited the growth of *L. monocytogenes* at the concentration ranging from 1-8 mg/ml



Staphylococcus aureus

Sulfated polysaccharides exhibited a high inhibition of *S. aureus* growth. A total inhibition was obtained at the concentration of 4 to 8 mg/ml



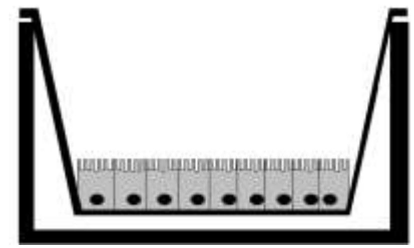
1- Antimicrobial activity and MIC determination of MSP towards all tested bacteria

Bacteria strains	Estimated MIC value (mg/ml)
E. coli 096 (chicken feces) CIRMB P-096	62.4
E. coli K88 (pig enteric colibacillosis) *CIRMB P-945*	63
S. typhimurium (bovine septicemia) CIRMB-940	63
L. monocytogenes (rabbit tissue) CIRMB-711	31.3<MIC<62.6
S. aureus (bovine mastitis) CIRMBP-476	1.9<MIC<3.9

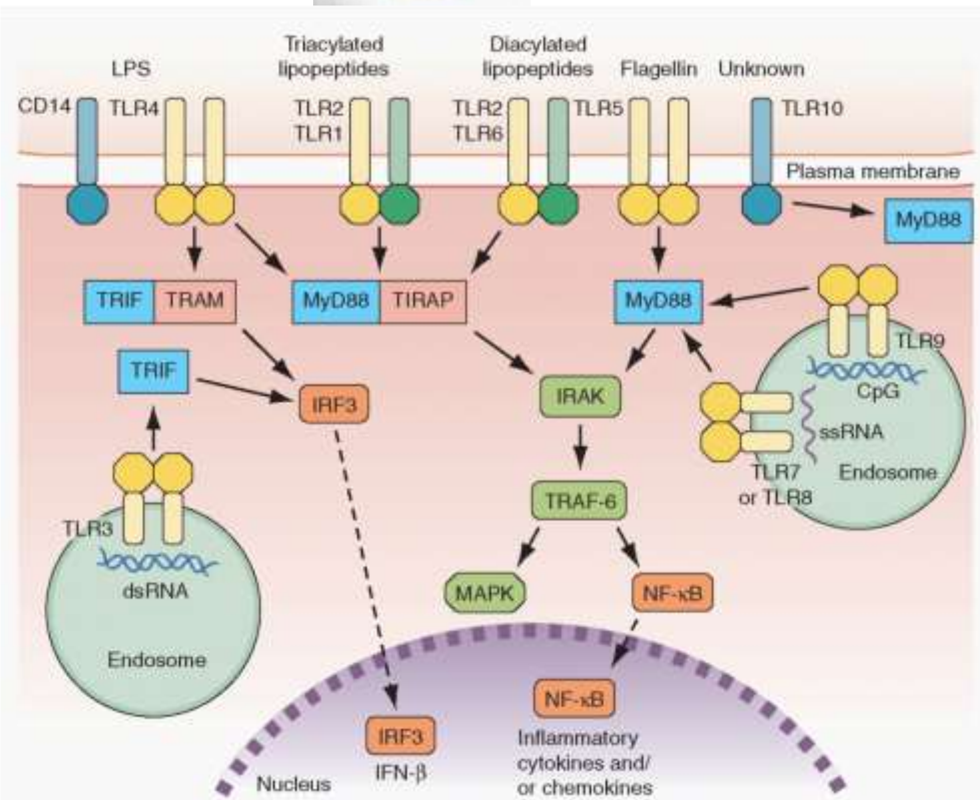
2- Stimulation of immune response mediators with MSP

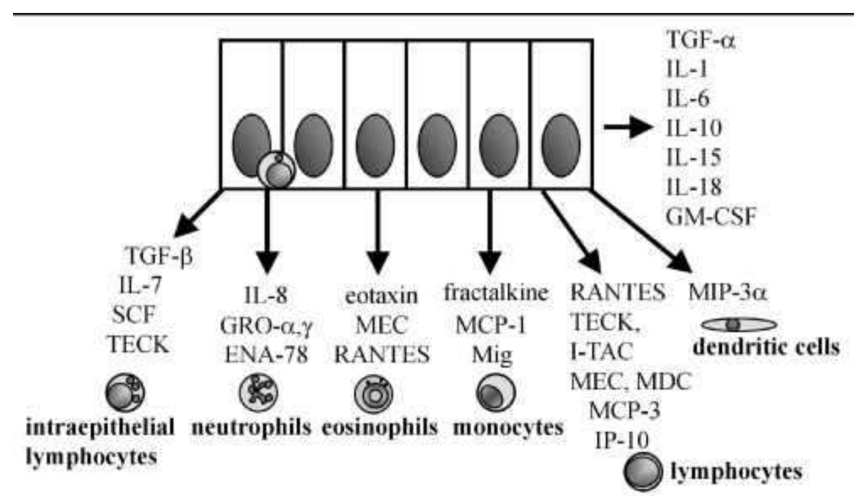
Water soluble MSP extract

- **Dissolved in DMEM**
- **Sterilization using filtration**
- **Preparation of MSP concentrations**

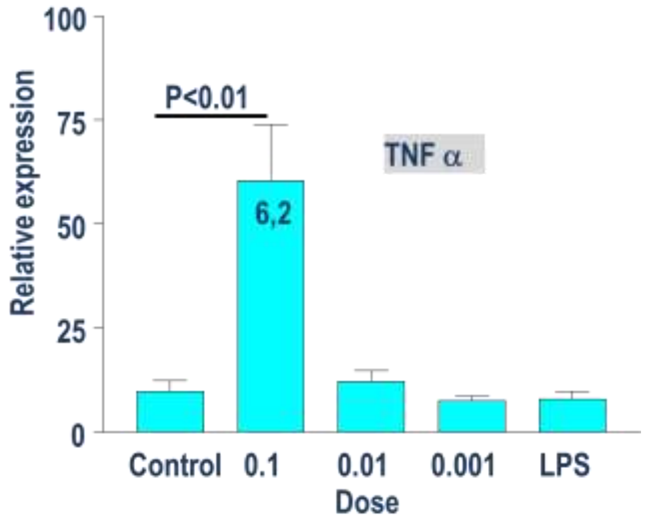


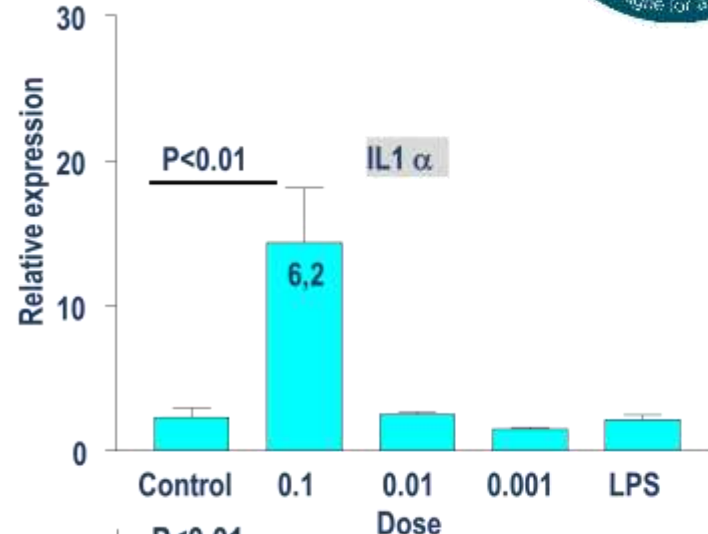
- ✓ IPEC-1 cells differentiation
- ✓ MSP extract treatment
- ✓ RNA purification
- ✓ Cytokines expression analysis

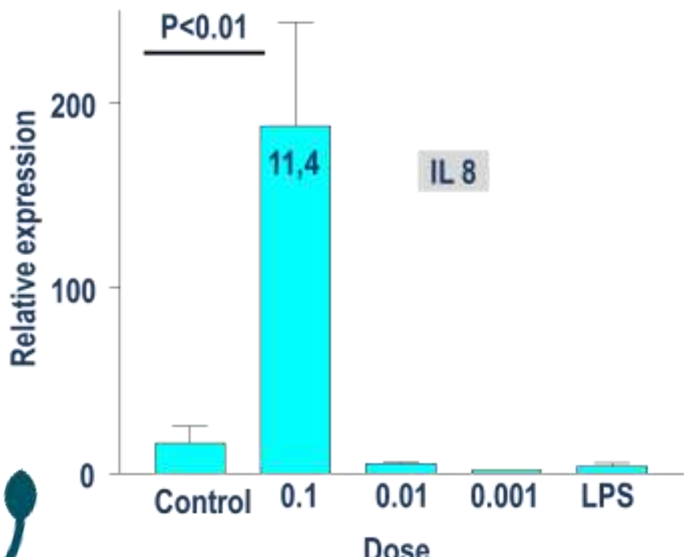


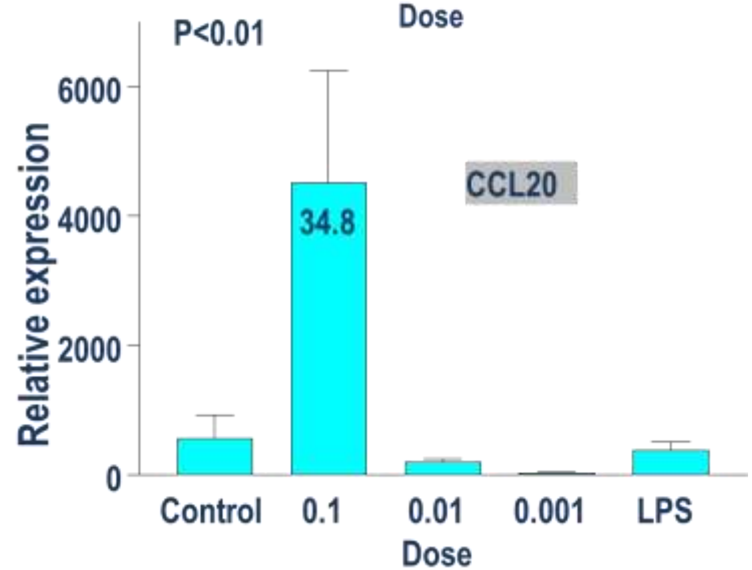


2- Expression increase of immune response mediators by differentiated IPEC-1 cells stimulated with 0.1 % of MSP





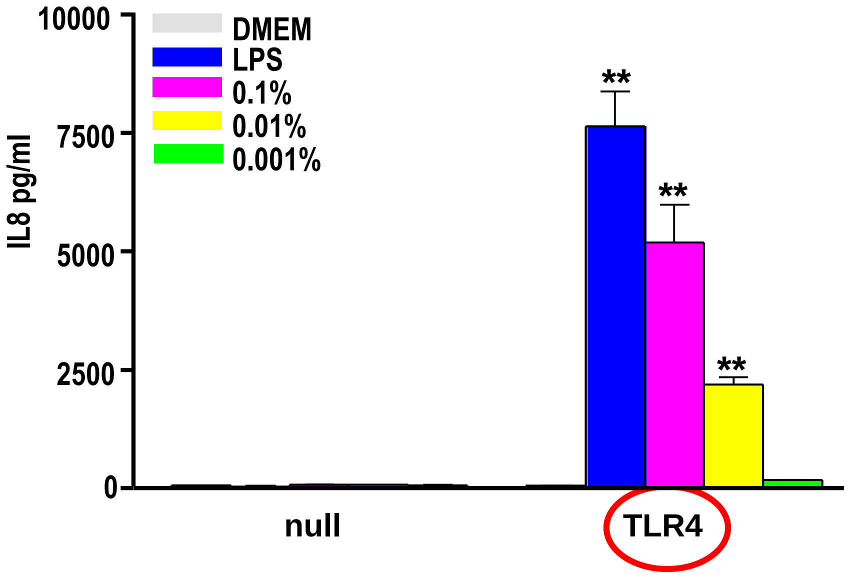


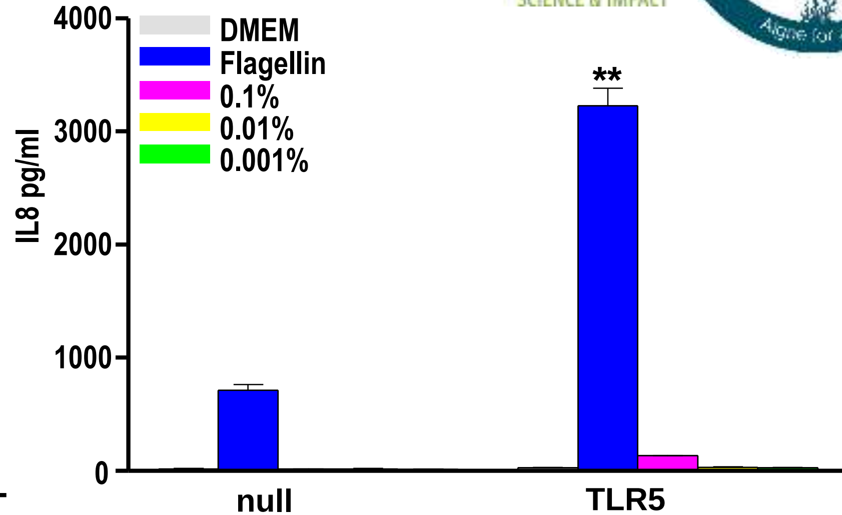


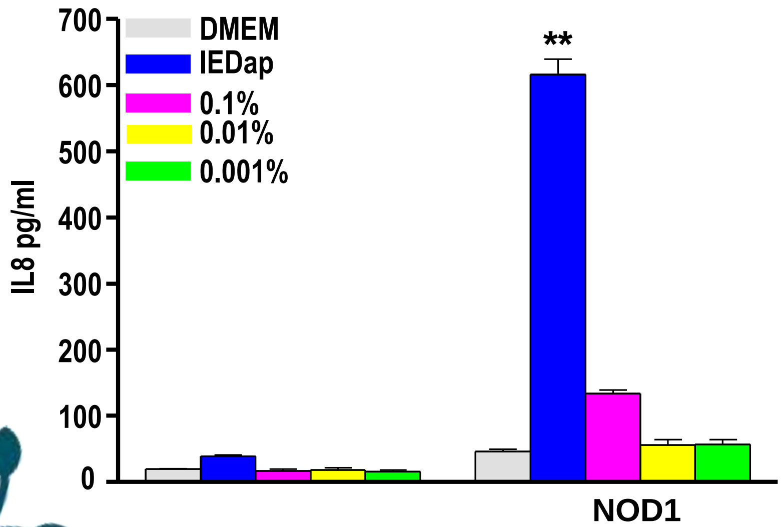
2- Expression fold change of target genes after induction with 0.1% of MSP

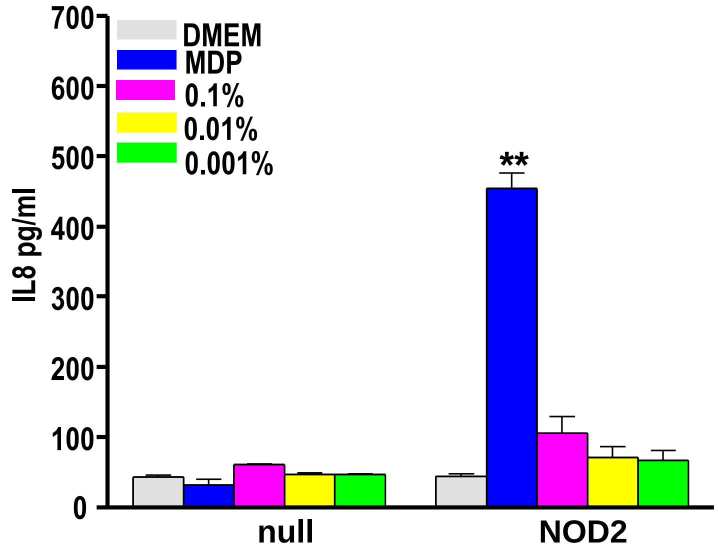
Genes	Main function	Expression fold changes
IL 8	Neutrophil chemotactic factor, phagocytosis	11.4
CCL20	Lymphocytes and dendritic cells recruitment and antimicrobial activity	38.4
TNF α	Phagocytosis and neutrophil chemoattraction	8.3
IL 1β	Proliferation of CD4+ cells, fibroblast, B-cell maturation and proliferation	7.1
IL 6	Anti-inflammatory cytokine, differentiation of T-cells into cytotoxic T-cells, B-cell proliferation and IgA secretion	4
IL 1α	Cell proliferation, differentiation and apoptosis, expression of adhesion molecules and chemotactic factors	2.1
PPAR γ	Transcription factor with anti-inflammatory function, inhibiting TNFα, d'IL1α production	2.4
IL 12 p35 IL 12p40	IFN-γ production and differentiation of Th1 cells, NK cells activation and cytolytic T cells development	1.8 2.4
TGF β	B cells differentiation, switch IgM/IgA, and regulatory T cells induction	1.7
IL 10	B cells differentiation into IgA secreting cells, control of inflammatory responses by stimulating regulatory T cell	1.1
CCL25	Migration and homing of T cells and IgA plasma cells	2.2
CCL28	Recruitment of T cells and IgA plasma cells	1

2- MSP extract stimulated IL-8 production only in human embryonic kidney cells HEK293 that stably express TLR4





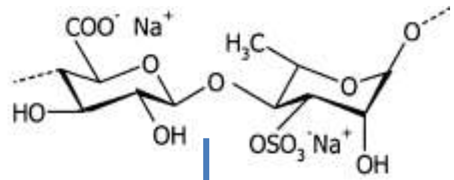


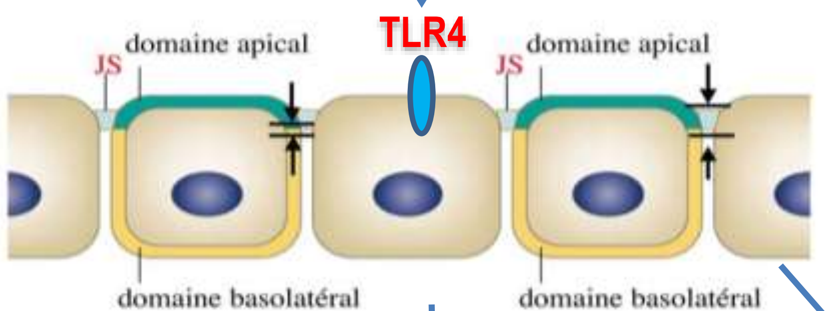


Conclusions

Olmix Green algae

Water soluble
MSP extract





IL-1α
IL-1β
IL6
TNF-α

- Induction of addressins and chemokines
- Lymphocytes, macrophages activation
- Direct antimicrobial activity

IL-8/CXCL8

- Recrutement of PN
- Antimicrobial activity

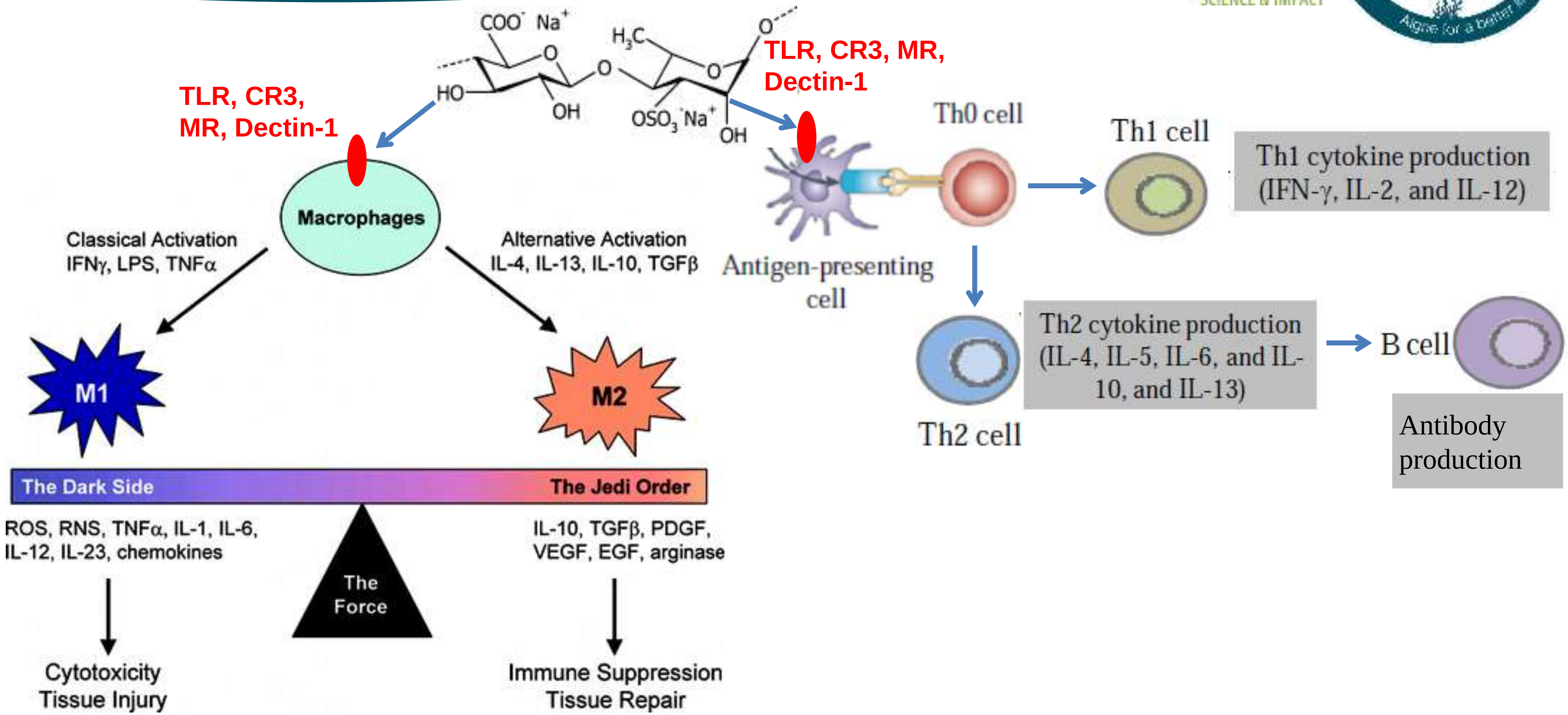
CCL20

- T lymphocytes and DC Recruitment
- Antimicrobial and antiviral activity

PPARg
IL10

- Differentiation, proliferation and apoptosis
- Anti-inflammatory, down regulation of TNFα, and IL1ß production

Algae and microbial polysaccharides both bind to common surface receptors and induce similar immunomodulatory responses



- **Improve mucosal immune response at homeostasis**
  - **Protect against pathogen infection**
    - **Boost immune response as an adjuvant or vaccin**
      - **Immune cells response to algae SP mimics the immune response to microbes = MIMOTOPE**

Acnowledgements

Hervé Demais
Matthieu LeGoff
Pi Nyvall Collen
Marion Gaire
Olivier Biannic

Olmix Group

Henri Salmon

BPI France

Michel Olivier
Cindy Slugocki
Emmanuelle Helloin
Isabelle Jacques
Sebastien Holbert

ISI ULVANS project

Thanks for your attention!