Fish Immune Stimulation through the use of Natural and Efficient Yeast Solutions

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What are the Fish natural defenses ?

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Disease is the most important factor for aquaculture development

GOAL 2014 Global Aquaculture industry meetings (Vietnam)

Survey at the end of the sessions.

What is the most important challenge limiting aquaculture?

- **Health and disease management -- 63%**
- Feed -- 4%
- Environmental and social responsibility -- 11%
- Investment capital -- 9%
- Market support -- 1%
- Leadership -- 5%
- Consumer education -- 7%

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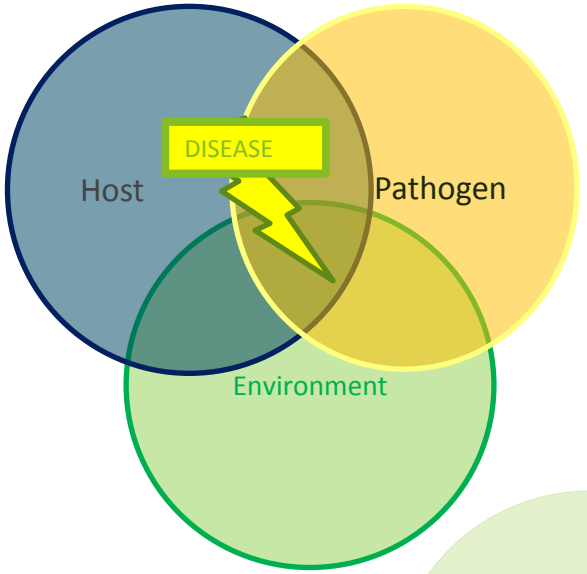
How to fight diseases ?

3 parameters are important in aqua farming



The epidemiological triad (Snieszko, 1974)

Environment

- Better farm management (biosecurity)
- Management of environmental changes (salinity, …)
- Management of water quality (monitoring, bioflocs …)

Pathogen

- Prevention : Vaccination.
- Decrease of pathogen loads
- Treatment : What about antibiotics ?

Animal

- Use good fish fry
- Decrease stress
- **Improve immune status**

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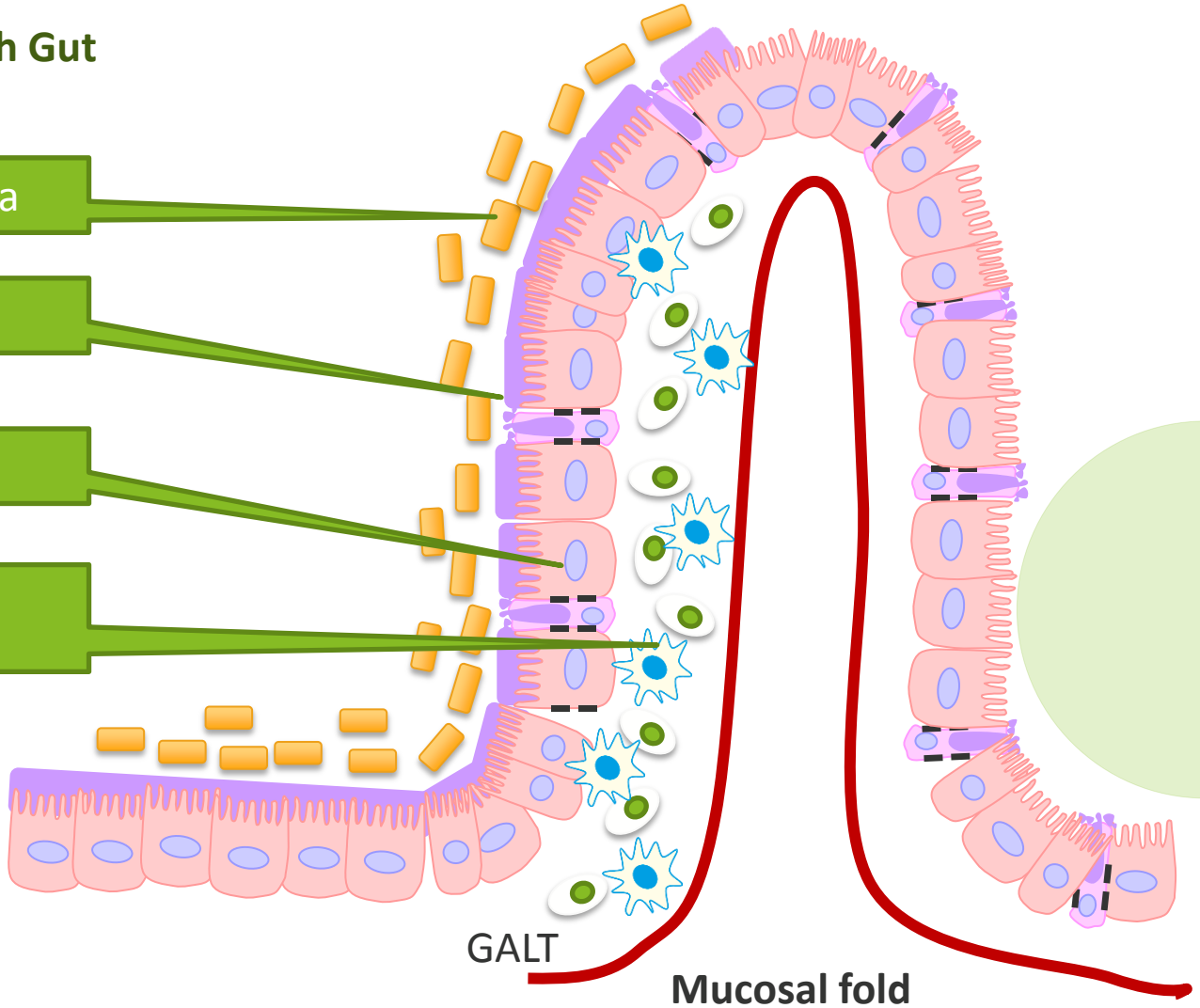
Four barriers in the Fish Natural Defenses

Example : The Fish Gut

1. Commensal bacteria
2. Mucus /goblet cells
3. Epithelium cells
4. Gut Associated Lymphoid Tissue



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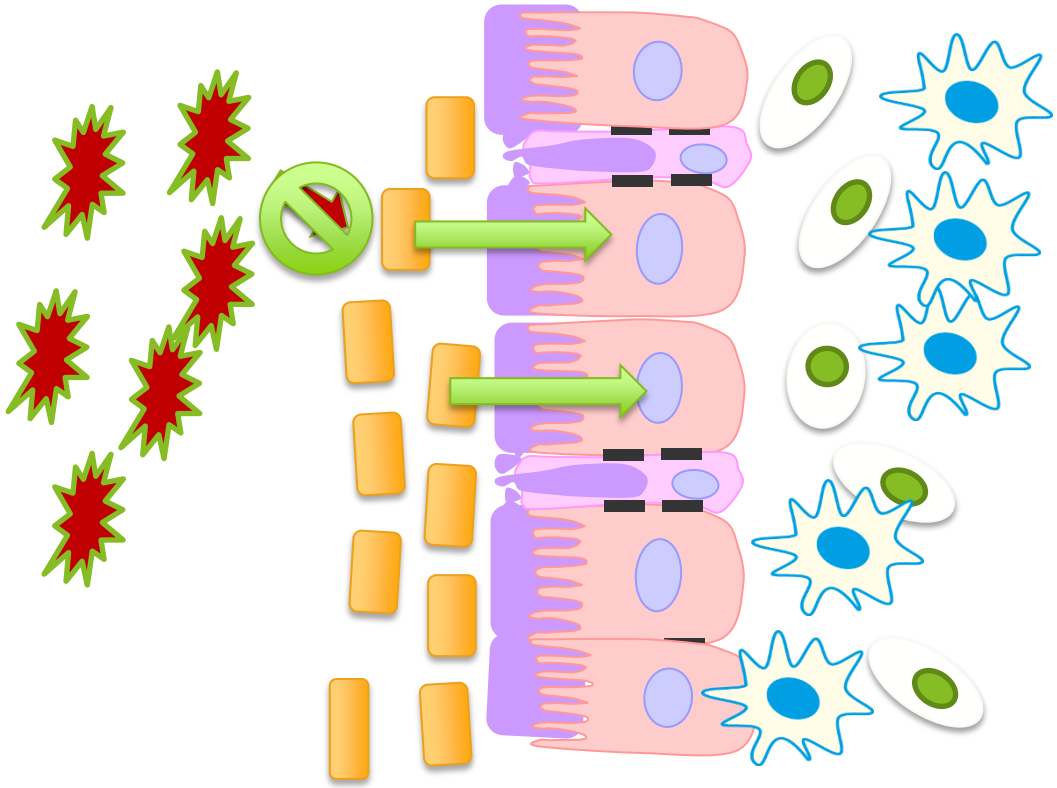
1 – Commensal bacteria – an active barrier

- *Competition with nutrients and binding sites to pathogens*
- *Initiation of secretion of defensin and antimicrobial peptides from epithelial cells*



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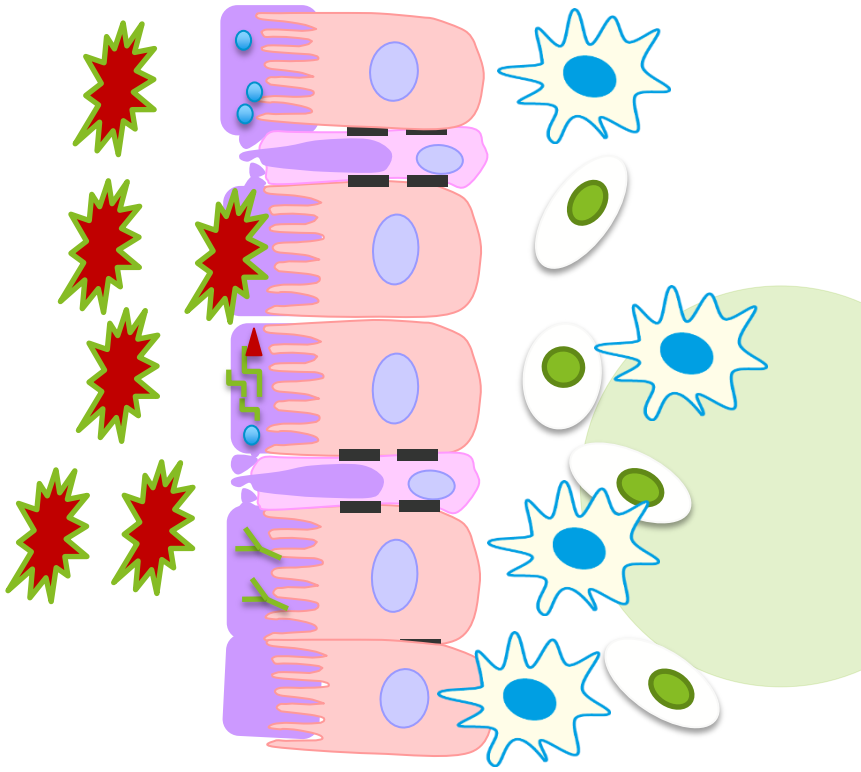
2 – Mucus – A physical barrier

- *Contains mucopolysccharides – glycoproteins*
- ***Prevention of pathogen invasion (bacteria, fungi, parasites)***
- *Prevention of antigenic material translocation (Trapping with IgM, IgZ ?)*
- *Presence of **Lysozyme**, lectins, complement proteins*
- *Gut, Skin, Gills*



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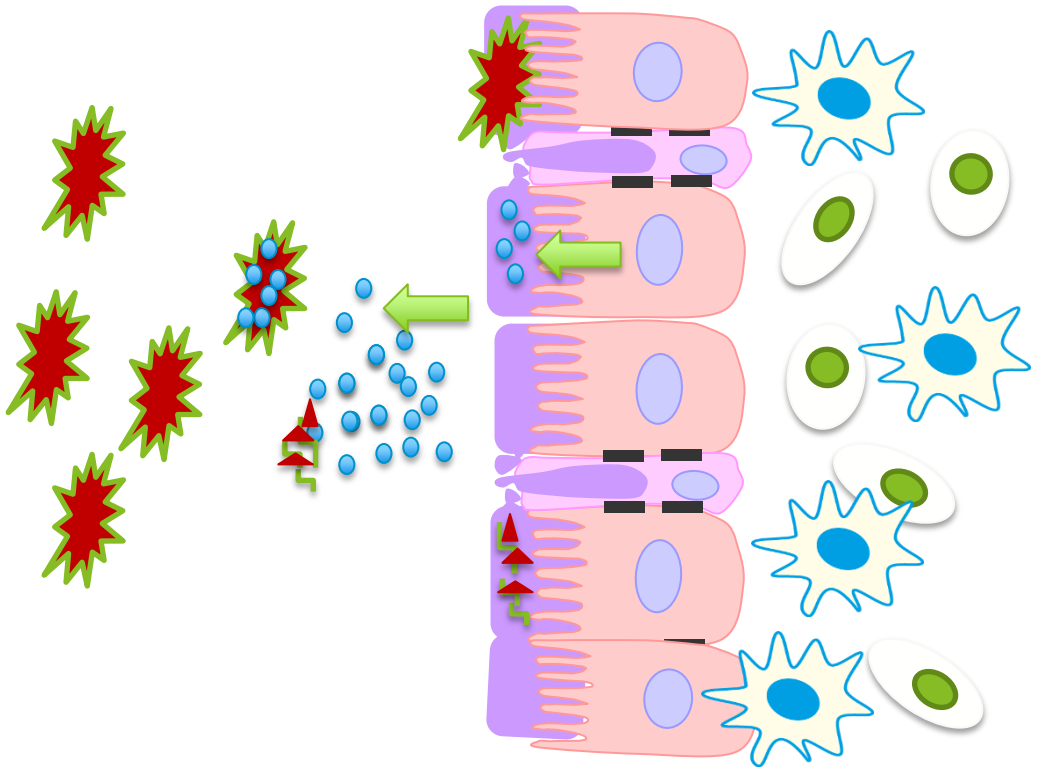
3 – Epithelial cells have also an immunity role

- *Secretion of anti-microbial peptides (Lysozyme, cathepsin, β defensins)*
- *Lysis of pathogen walls*



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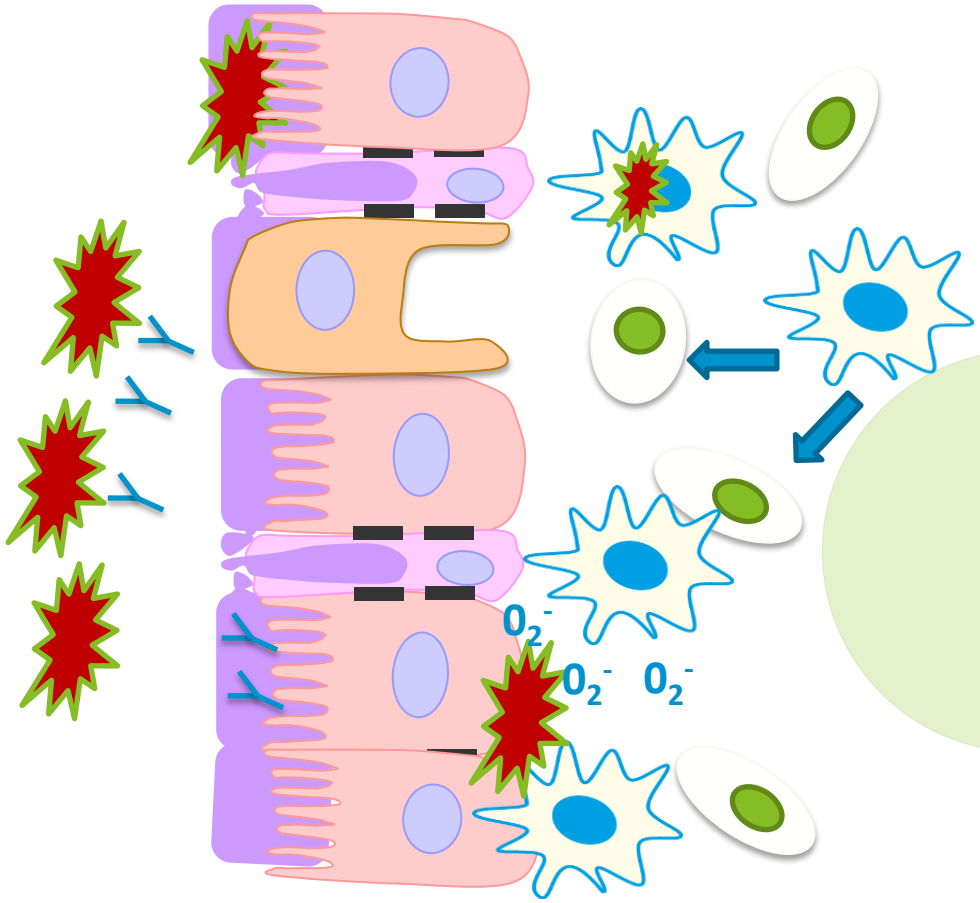
4 – The last barrier : innate immune system

- *Phagocytosis*
- *Respiratory Burst (oxygen species, nitrous oxide)*
- *Activation of adaptive Immune system*



GALT

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What is non specific immunity?

The 4th Barrier

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Fish possesses innate and acquired immunity

- Jawed fish represent the most diverse group of vertebrates.

- First evolutionary group in which adaptive immunity appeared (450 millions years).

- **2 general patterns** for immunity in vertebrates:

  - Conservation of the defensive signal pathways relevant to **non specific immunity**.
  - Restriction of **adaptive immunity** to vertebrates.
    - **Possibility to vaccinate fish**

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Innate Immunity is fast and non specific

Characteristics

- **Non specific** protection
- Rapid onset or activation
- Relatively temperature dependent
- Innate immune systems provide **immediate** defence against infection
- Humoral + cellular response

Role

- Pathogen **recognition**
- Recruitment of innate effector mechanisms (cellular , humoral)
- **Pathogen destruction**
  - Innate immunity wins : resolution of inflammatory process
  - Pathogen wins : prolonged inflammation, acquire immunity is necessary
- Presentation of pathogens antigen to **acquired immunity**
  - Influence T helper polarization

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Immunity in fish

Organs

- Thymus, kidney, spleen, liver, gut associated lymphoid tissues
- No Peyer patches as in terrestrial animals
- Gut Associated Lymphoid Tissue **(GALT)**
- **Head Kidney** assumes hematopoietic functions
- Head kidney contains melano-macrophages centers responsible for phagocytosis, IgM production, antigen processing

Cells

- Similar to higher vertebrates
- Monocytes/**Macrophages**, NK Cells, …
- T Lymphocytes helper (Th) ang cytotoxic (Tc)
- B cells subpopulations (B1, B2).

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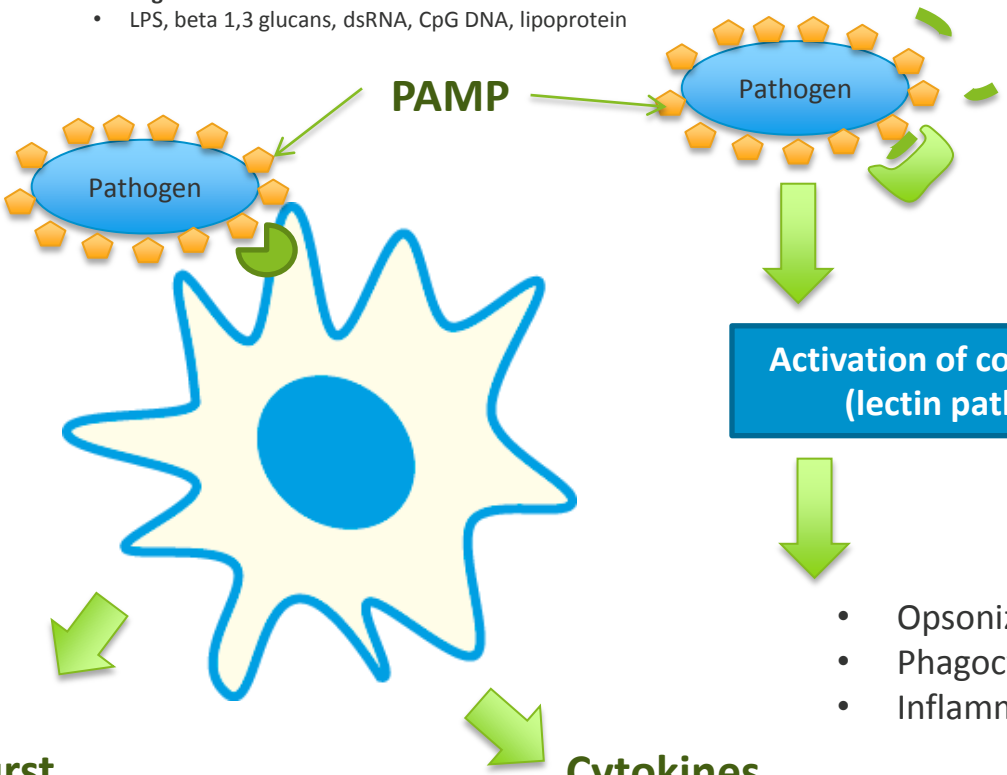
Activation of Innate Immunity

Pathogen Associated Molecular Pattern
- LPS, beta 1,3 glucans, dsRNA, CpG DNA, lipoprotein

PAMP



Secreted PRR

Pattern recognition receptors
- Mannose Binding Lectin, interlectin,…

Membrane PRR
- **Toll Like receptors**
- **TLR**

Activation of complement (lectin pathway)
- Opsonization
- Phagocytosis
- Inflammation

Phagocytosis
Respiratory Burst

Cytokines
TNFα,IL1β
- Inflammation, NFκB
- T helper differentiation (MHCI and MHCII)

Several PRR types on a cell
PAMP-PRR interactions can lead to different immune responses

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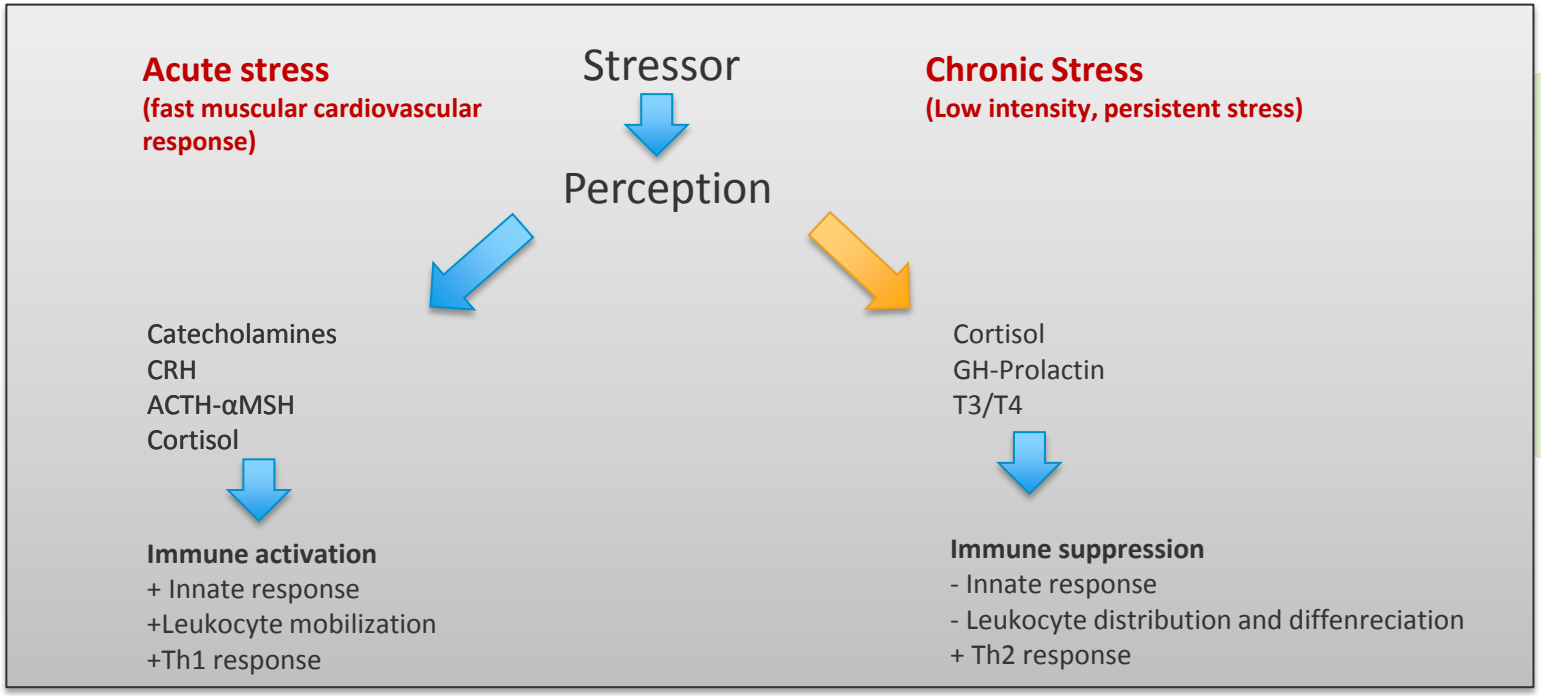
Innate immune system modulation by stress

- Stress response implies the allocation of resources that will affect other functions.
  In particular **immune response can be compromised**.
- Especially in lower vertebrates, **cytokines and neuropeptides**
  can have both roles in neuroendocrine and immune response.
- It depends on the **nature of the stressor**

From Tort 2011 Dev Comp Immun.35.1366-1375



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Immune stimulation

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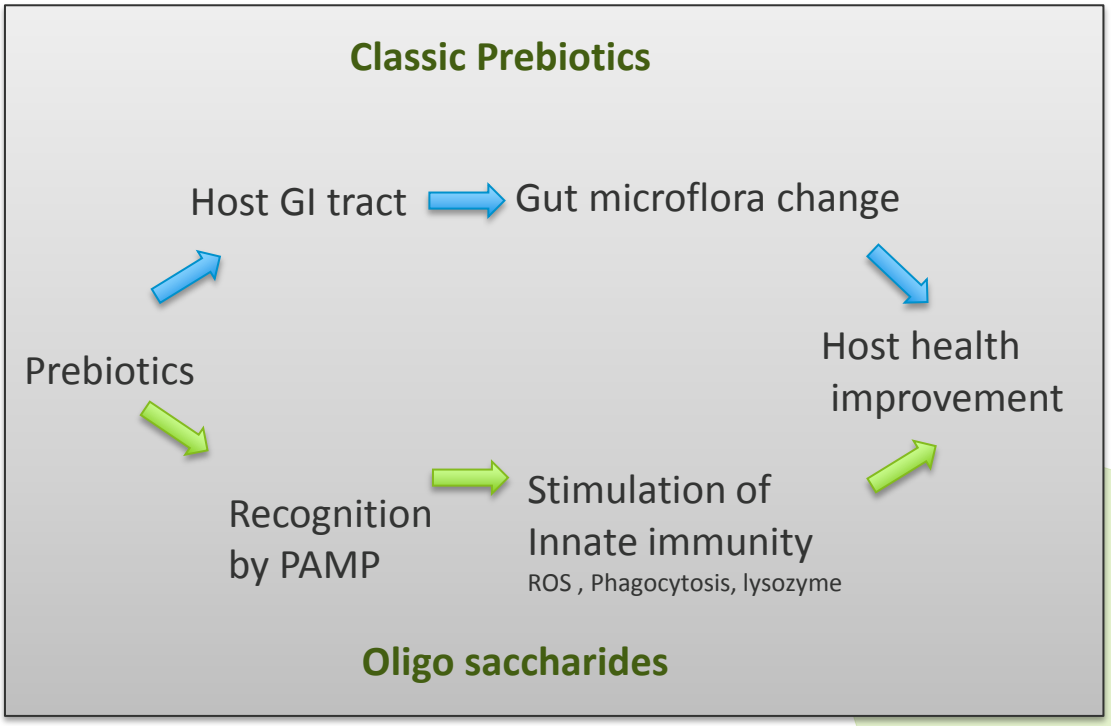
Immune stimulation

- Beta glucans
- Mannanes oligosaccharides
- Chitosan
- Alginates
- Plant extracts



- Nucleotides

  Immune stimulant or dietary supplement ?

Sources : Ringo et al 2012 ; Song et al 2014

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immune parameters that can be stimulated and measured in fish

Parameter	Description	Role
Hematocrit	Number of blood cells	Trigger immune response
Leukocyte numeration	Number and type of immune cells (ex : macrophages)	
Lysozyme activity	enzyme released by macrophages in serum, mucus	Lysis of the peptidoglycan wall of bacteria
Complement	Cascade of precursors activated directly by antigen specific antibodies, lectin, LPS…	Most effective non cellular responses of the immune response (lysis of pathogens, inflammation, phagocytosis)
IgM	Antibodies produced by lymphocytes, recognize pathogens	Neutralize pathogens by binding, triggers processes such as phagocytosis
Respiratory burst	Indication of the oxidative potential of reactive oxygen species such as hydroxide peroxide, superoxide anions and hydroxyl radicals produced by macrophages	Killing and degradation of microbes (measurement by NBT and myeloperoxidase)
Phagocytosis activity	Active host defense occurring in phagocytic leukocytes in spleen, head kidney and lymphoid organs	Killing of microbes after recognition by PRRs (TLRs), ingestion in phagosomes, merging with lysosomes and digestion by proteases (proteolysis)

Most effective test : disease challenge

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(See Song *et al* 2014)

Yeast fractions

Mode of action

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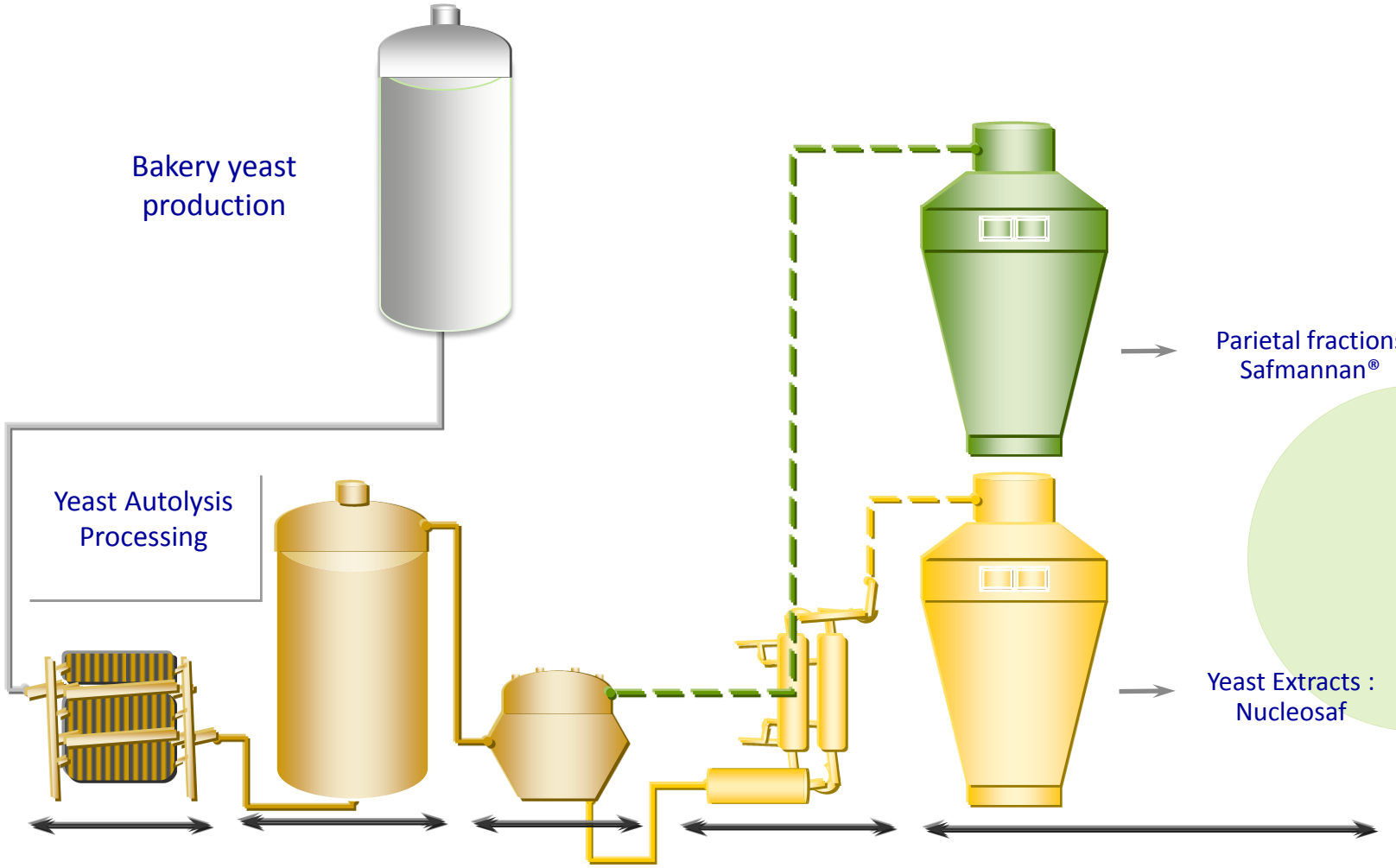
Yeast Extraction is a controlled process



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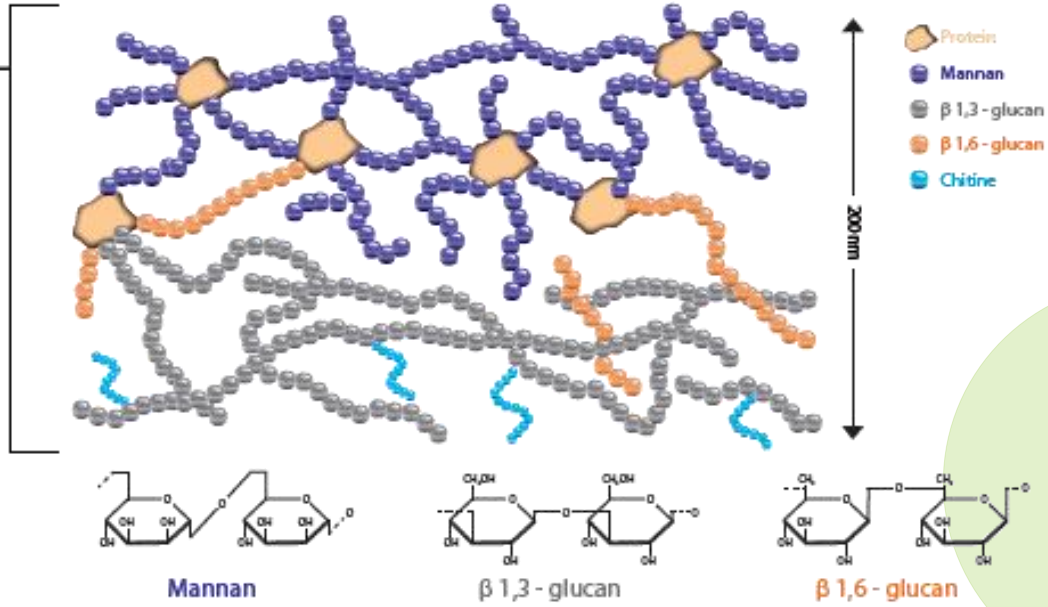
Parietal fractions : a complex composition

Complex of Parietal Fractions
(MEB picture, x1800)



Molecular structure of Parietal Fraction

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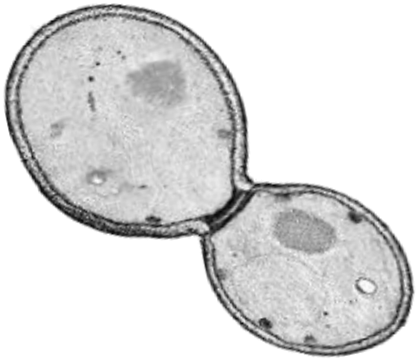
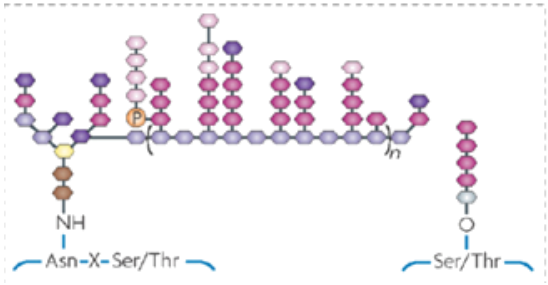
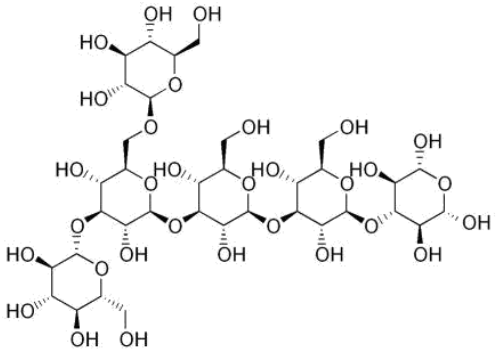
Yeast has specific active components



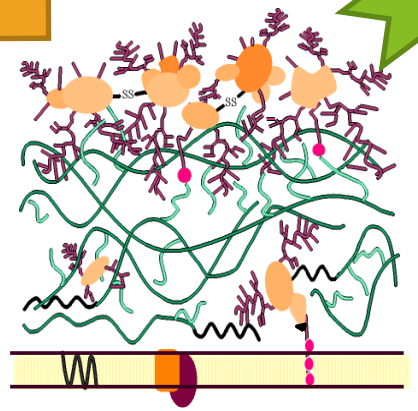


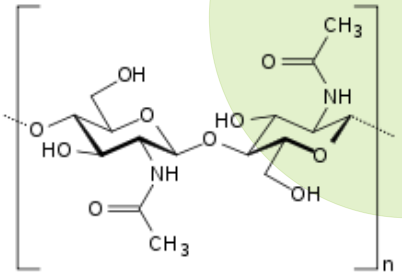
Mannoproteins

(From Netea et al 2008)



β-(1,3 -1,6) Glucans





Chitin

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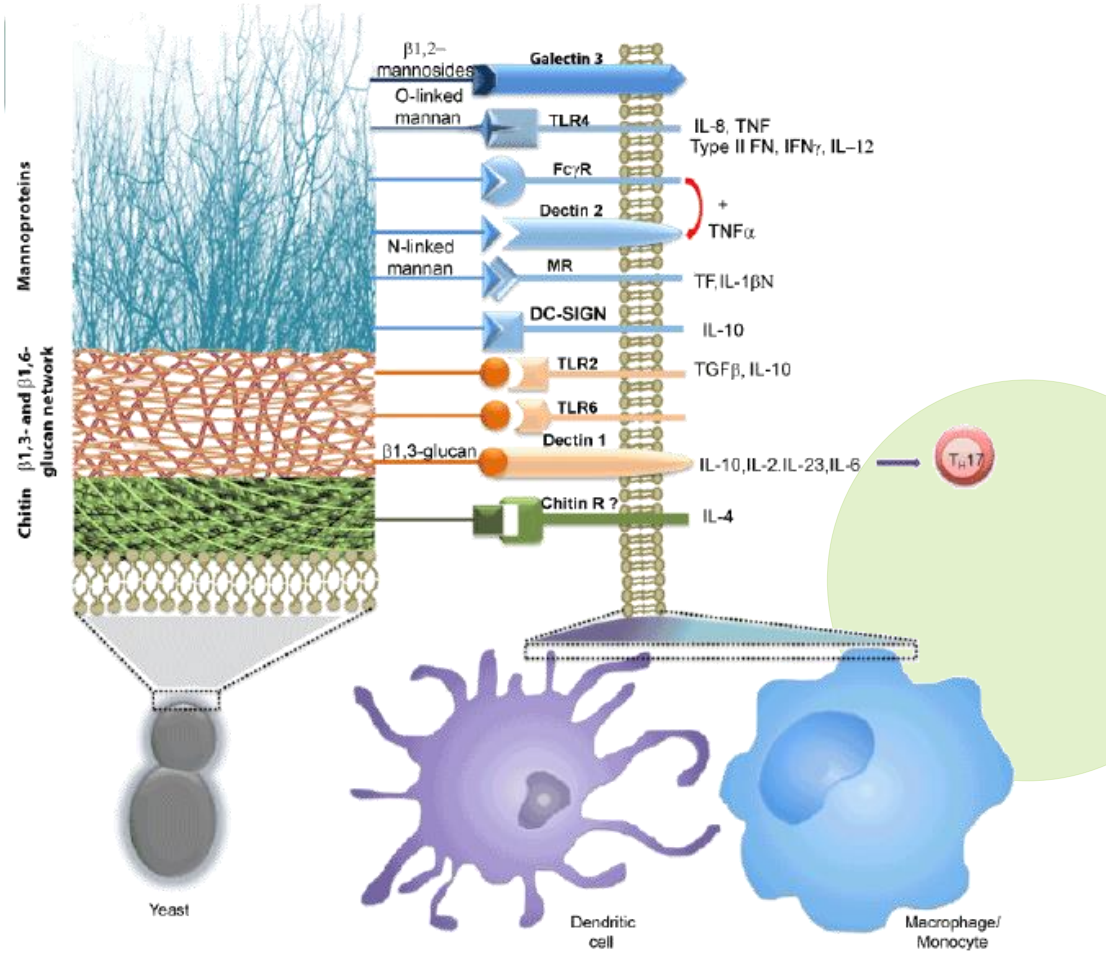
Yeast components activate specific PRRs

- Resulting effect of stimulation with yeast fractions depends of the **composition** of the cell wall.
- Only studies with purest **fractions** can lead to relevant conclusions for a given molecule



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2 important active components

- **Beta Glucans = PAMP**

  - β-1,3/1,6-glucans
  - Insoluble = skeleton

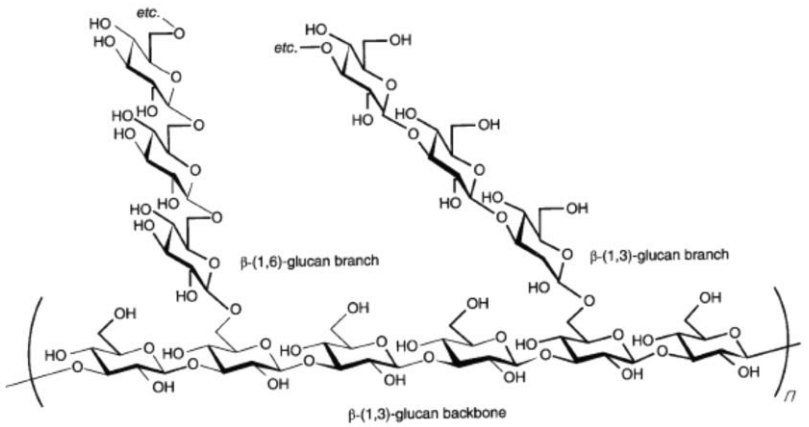

Figure 5.1 Generic glucan structure. Included are the β-(1, 3)-glucan backbone, a β-(1, 6)-glucan branch, and a β-(1,3)-glucan branch. Source: Gannam, A. L. and R. M. Schrock. Immunostimulants in fish diets. Journal of Applied Aquaculture 9: 53–89. Copyright © 1999, Taylor and Francis.

- Fish : stimulation of surface receptors (PRRs) of macrophages (for example CR3, TLR2 or Dectin 1) leading to **phagocytosis, respiratory burst and release of cytokins** (need further research in fish).
- The **branched structure** allows the BG to stimulate several dectin 1 molecules at the same time to potentiate the response.

  A TLR2-Dectin simultaneous activation is more potent.

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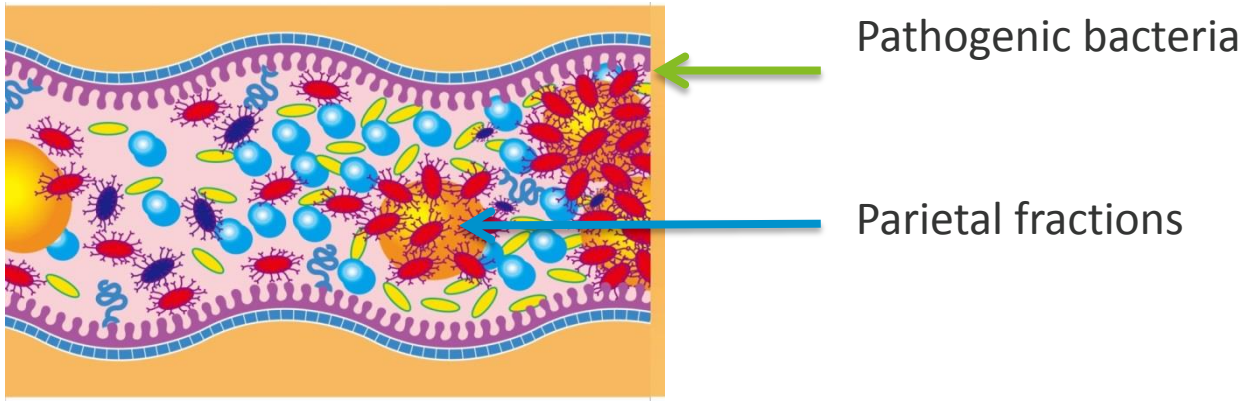
2 important active components

- **Mannan Oligosaccharides**
  - "Prebiotics" (growth of beneficial bacteria)
  - **Binding of pathogens** via mannose site to lectins on the bacterial pili competing with lectin binding site in the gut (mannose)
  - Increase of skin and gut **mucus** production
  - Stimulation of macrophages via mannose binding lectins (MBL) and TLR, triggers **inflammation** processes, via TNF



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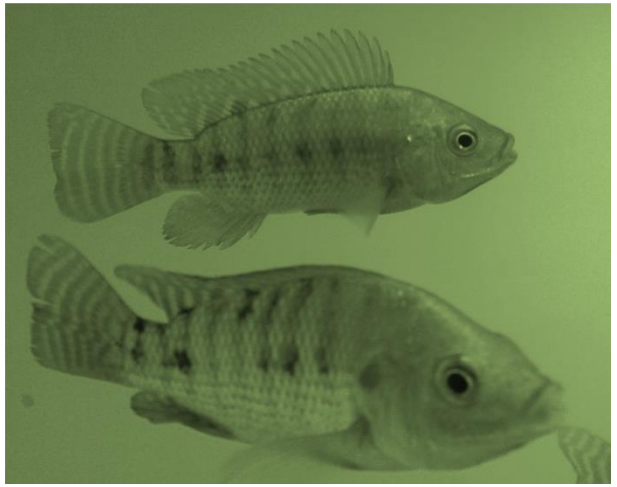
Example of better pathogen resistance

Tilapia



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Mexico

- Mexico – Tilapia
- Safmannan® 3 kg/T
- Commercial feed formula
- 8m^3 hapas, triplicates
- Each cage : 3,600 fish of 6 g average
- 120 days of culture
- **Challenge with *Aeromonas hydrophilia***

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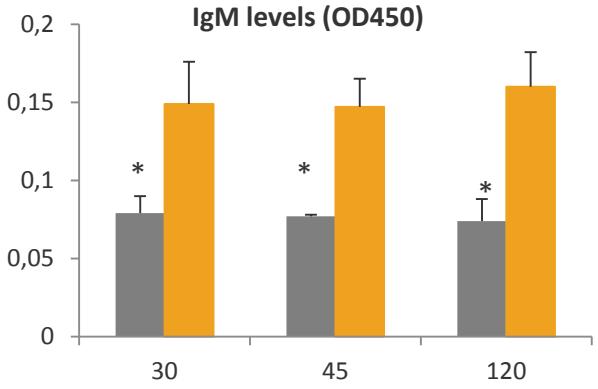
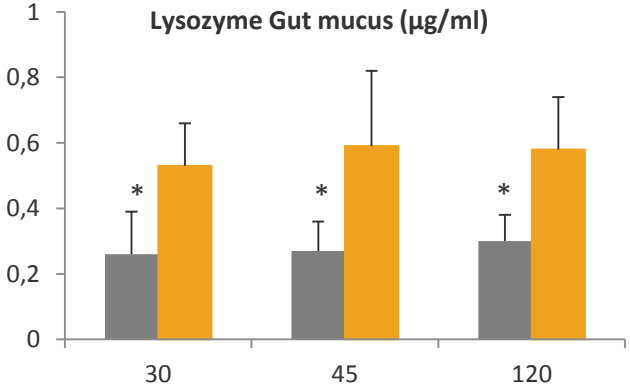
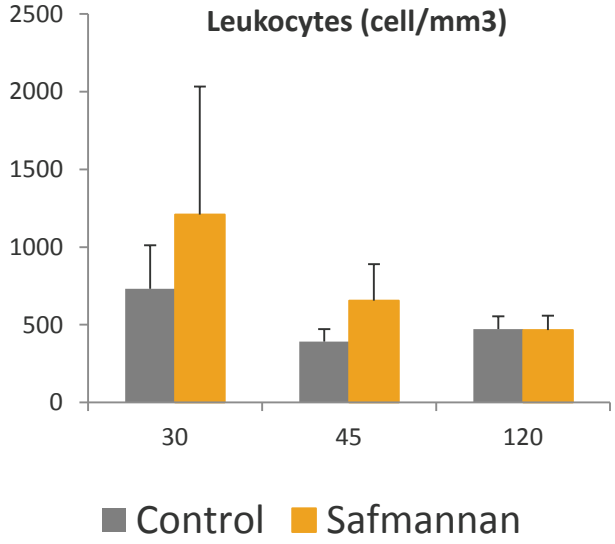
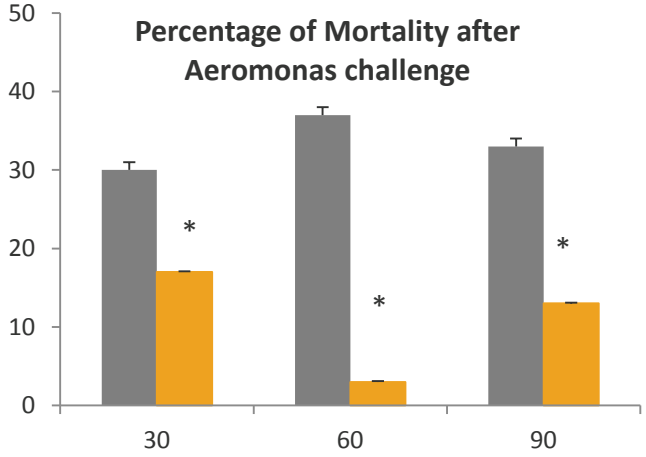
Yeast fractions stimulate innate immunity





Yeast parietal fractions increase innate immunity indicators as IgM and Lysozyme levels





Yeast parietal fractions improve survival after *Aeromonas* challenge

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Thailand

- Pond trial in Cages (2mx2mx1m)
- 30 g tilapia – 100 ind /cage
- Safmannan® at 0.5 and 1 kg/T

- Harvest at 3 months
- Challenge with *S. agalactiae* (20 fish from each cage)
- Histopathology of the intestinal tissue

Composition	first month	second and third month
Fish meal 55%	25.0	**22.0**
Fish meal 64%	10.0	**9.0**
Corn meal	28.0	**23.0**
Casava meal	10.0	**-**
Soy bean meal	12.0	**-**
Peanut bean meal	8.0	**34.0**
Squid meal powder	5.0	**4.0**
Vitamins and minerals	2.0	**2.0**
Soybean sauce by product	-	**6.0**
Safmannan®0.5 kg/T	50	**50**
Safmannan®1 kg/T	**100**	**100**

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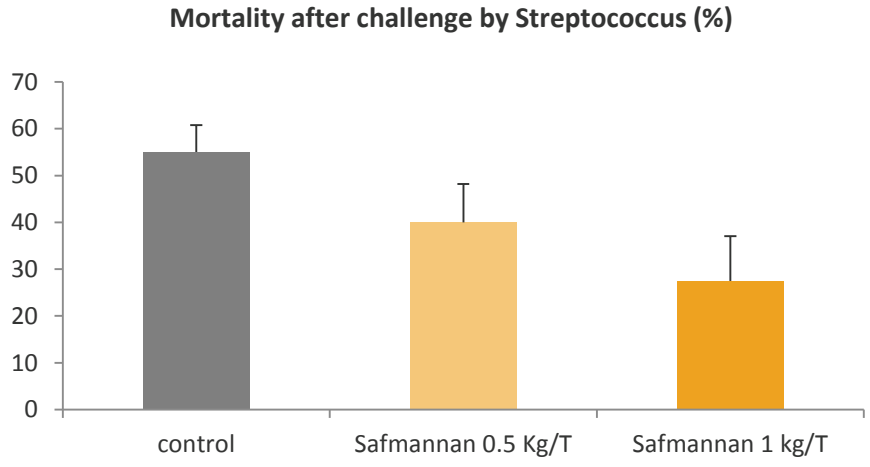
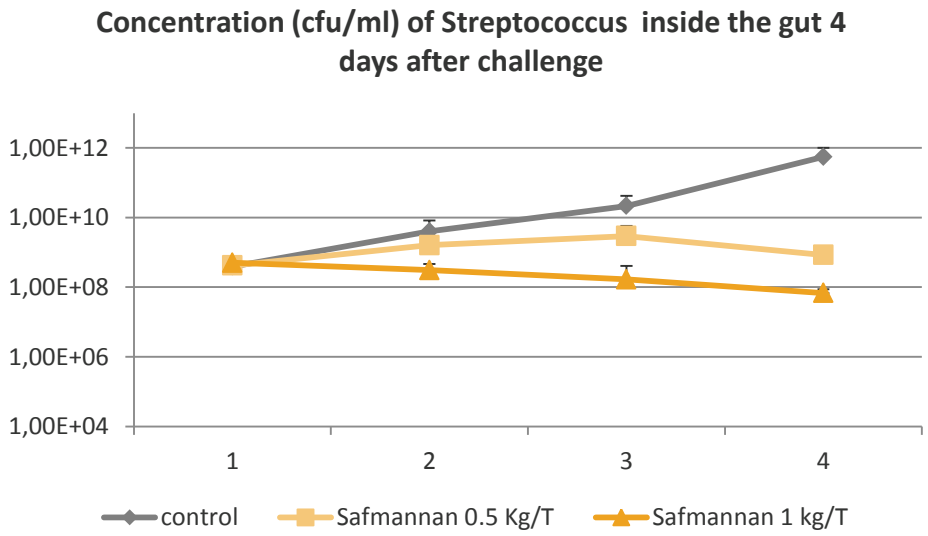
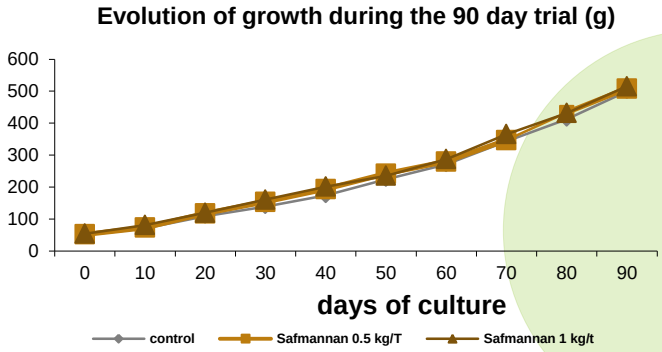
Decrease of Streptococcus infection





- **Decrease of mortality** in the treated groups
- Concomitant **decrease of the pathogenic bacteria** in the gut of treated fish
- The immune stimulation did **not have effect** on growth in near pond conditions (cages in pond)



Parietal fractions at 0.5 and 1 kg/T increase the survival of tilapia challenged by *S. agalactiae* with good growth performances

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Freshwater fish - recommendations

A strong effect in stress situation and on pathogen prevention

Hatchery/Nursery.

- During masculinization as it is a stressful process and tilapia can be subjected to pathogen challenge – **1 to 2 kg/T** (not more than 4 weeks).
- Before transportation to nursery and to grow out farm. Preparation against stress – **1 to 2 kg/T for 2 to 4 weeks.**

Grow out farms

- After transportation, starter feeds. **1kg/T for 1 month.**
- Prevention of pathogens such as Streptococcus **0.5 kg/T** all the time. Increase to **1 kg/T** around the pathogen risk period (hot temperatures for example).

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Examples

Marine Fish

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Japanese seabass (*Lateolabrax japonicus)*

- **Feed Research Institute, Beijing China,**
- Juveniles fish, 18g
- 10 weeks trial, followed by a bacterial challenge (*Aeromonas veronii*)
- Performance and immune parameters
- All feeds with 25% fish meal- 20% Soybean meal, but a positive control with 38,5 FM – 0% SBM
- **4 Safmannan®dosages : 0.25 ; 0.5 ; 1; 2 kg/T on SBM diets**
- Feed with 47-48% CP, 21,4 MJ/kg

Ingredients	FM	SBM	Saf 0.25
Fish meal	38.5	25	25
Soybean protein concentrate	20	20	20
Soybean meal	0	21	21
Wheat flour	21	21	21
Fish oil	6	6.4	6.4
Monocalcium phosphate($Ca(H_2PO_4)_2$)	1	2.1	2.1
Microcrystalline cellulose	10.1	1	0.975
Phospholipid (93%)	2	2	2
Choline chloride (50%)	0.4	0.4	0.4
Vitamin and mineral Premix	1	1	1
Methionine hydroxy analog-Ca (98%)	0	0.1	0.1
Safmannan® (mg/kg)	0	0	250

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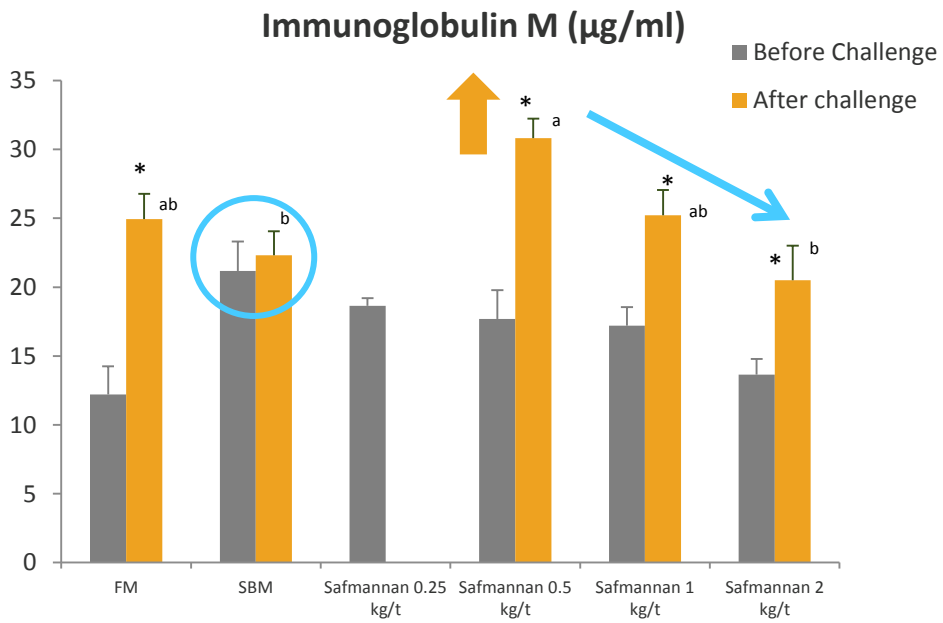
Immune parameters : an optimal concentration



IgM :

Levels already elevated for SBM control BC – **enteritis effect**

A **significant increase** in Parietal fractions 0.5 kg/T, after challenge

A decrease of production of IgM with the increase of the dose

- **SBM control has a pathological issue (confirmed by histology)**
- **Parietal fractions at 0.5 kg/T best to stimulate IgM production.**

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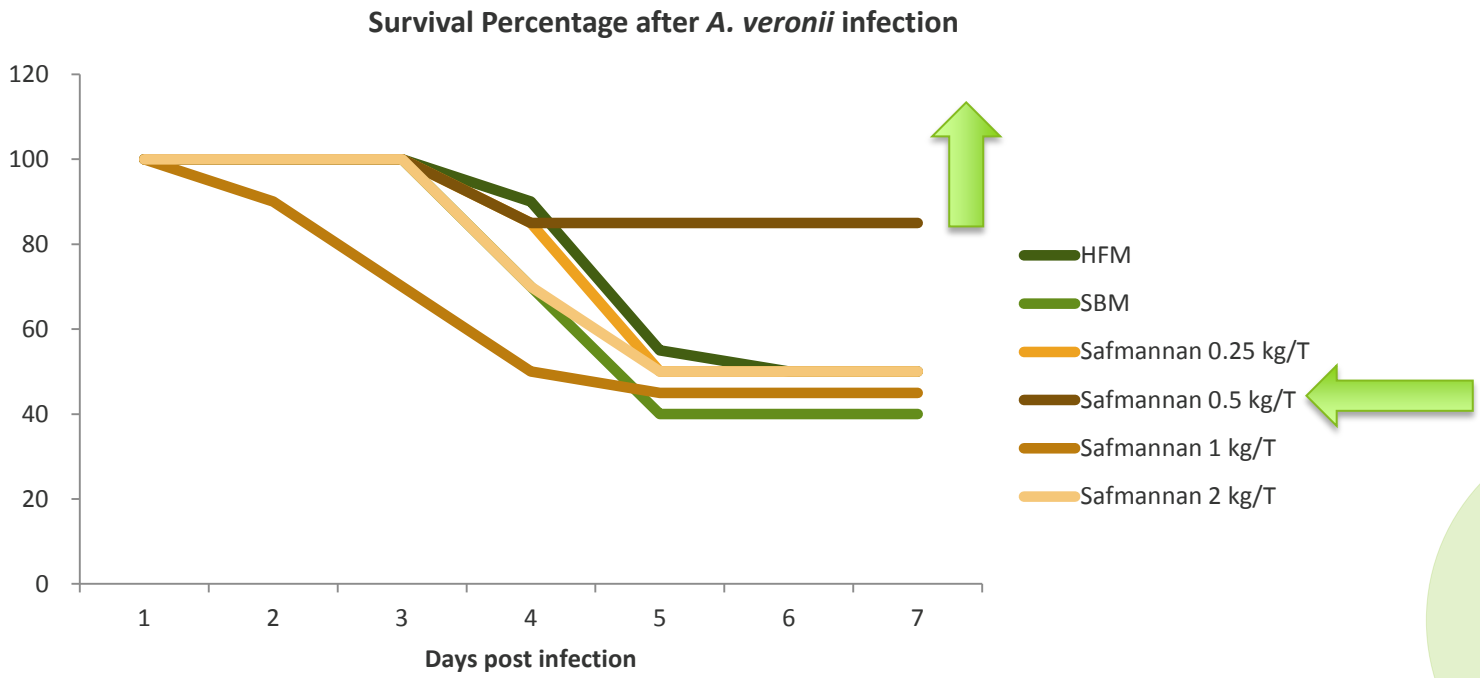
Increase of survival during *Aeromonas* Challenge



Best Immune stimulation obtained with parietal fractions at 0.5 kg/T

(Production of IgM, resistance to bacterial challenge).

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European seabass (*Dicentrarchus labrax*)

Sea bass (HCMR Greece)

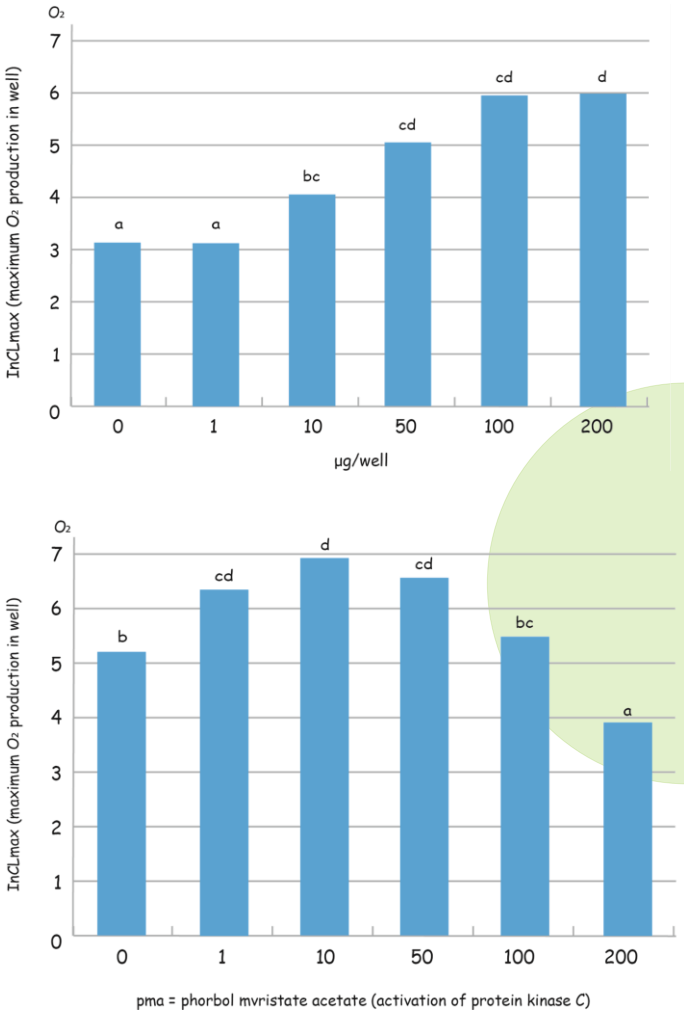
100 g sea-bass

Blood collection: white blood cells

Blood cell in contact with yeast material

Direct stimulation with yeast fractions

Stimulation with yeast fractions after pre stimulation with pma



YCW: Be careful of overstimulation !

Strong or prolonged stimulation with high doses of parietal fractions can lead to an over stimulation of the immune system

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Marine Fish- recommendations

Hatchery/ Nursery

- Boost the immune system in larvae and fry: **0.5 kg/T, all time**
- Adaptation to stress before transportation : **0.5 to 1 kg/T - 2 to 4 weeks.**

Grow out cages

- Prevention of bacterial diseases : **0.5 kg/T** all time.
- Can help fight against parasites as parietal fractions can increase the production of skin mucus.

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Yeast fractions can help to improve disease resistance

- **Fish disease resistance has several components**
  - **The 4 Barriers**
- **Composition of Yeast fractions is important**
  - **Active components : specific PRRs**
  - **Can work on different barriers**
  - **Use a constant product**
- **A good management tool**
  - **Use the correct dosage**
  - **Be careful of over stimulation**

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Thank you for your attention

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