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

Philippe Tacon

18/11/2015

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What are the shrimp natural defenses ?

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Disease is the most important factor for aquaculture development

GOAL 2014 Global Aquaculture industry meetings (Vietnam)

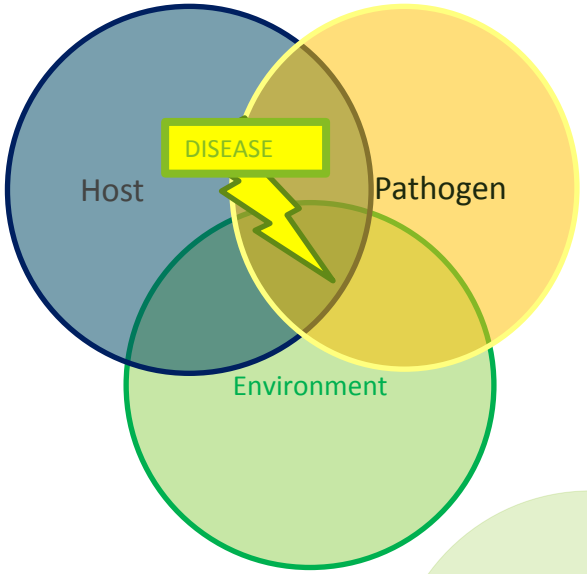
Survey at the end of the sessions.

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%

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 (bioflocs …)

Pathogen

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

Animal

- Post larvae quality
- Decrease stress
- **Improve immune status**

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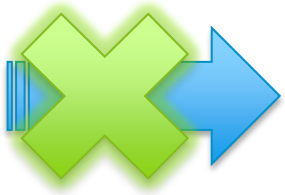
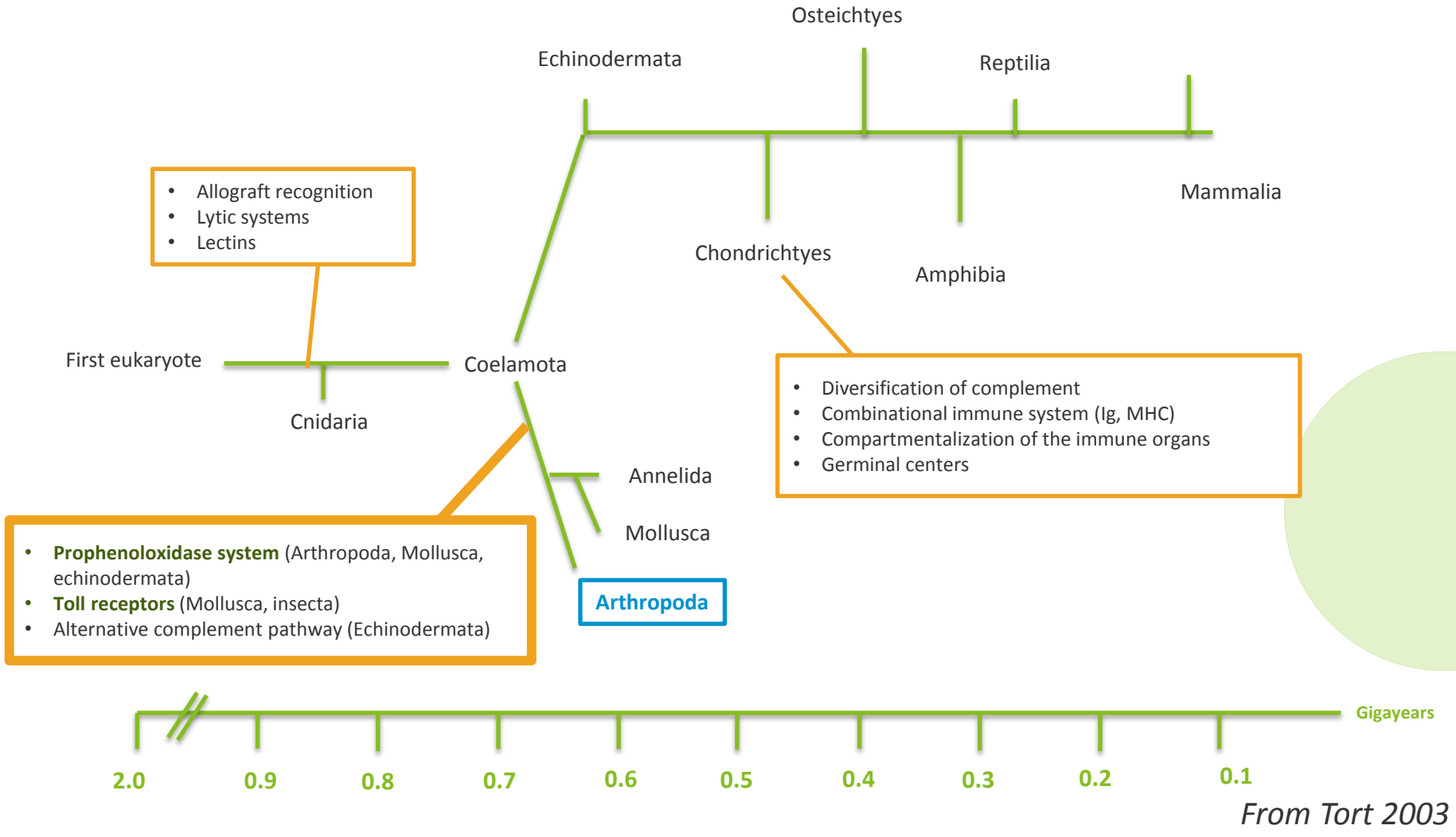
Are shrimp defenses the same as fish ?



Shrimp are arthropods and early in the evolution tree



From Tort 2003

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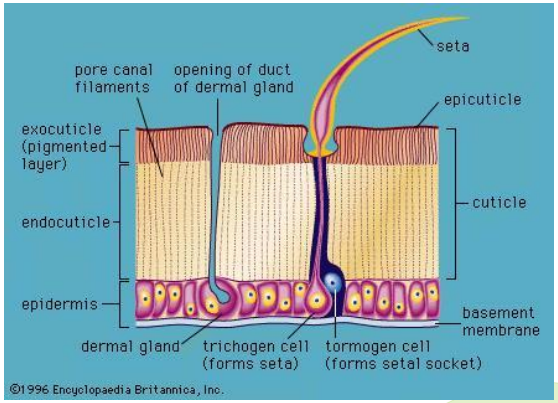
How shrimp defenses work? Barriers ?

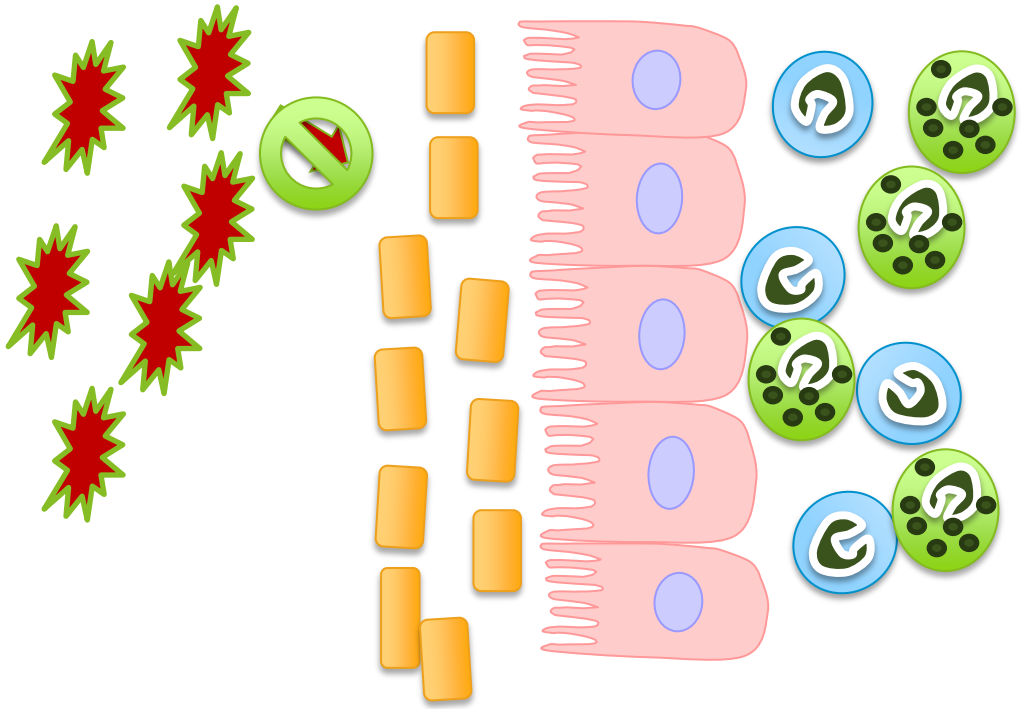
- **First barrier, structural : the cuticle or exoskeleton**
  - Cover : Skin, Gut, Hindgut. (major ways of pathogen entries
  - Rich in chitin (immune defense)
  - Molting a way to get rid of pathogens (?)
  - + regeneration of appendages, rapid wound healing
- **2nd barrier : Commensal bacteria**
  - Microflora of the gut .
- **The 3rd barrier : Innate immunity mechanisms**



Source encyclopdedia Britannica

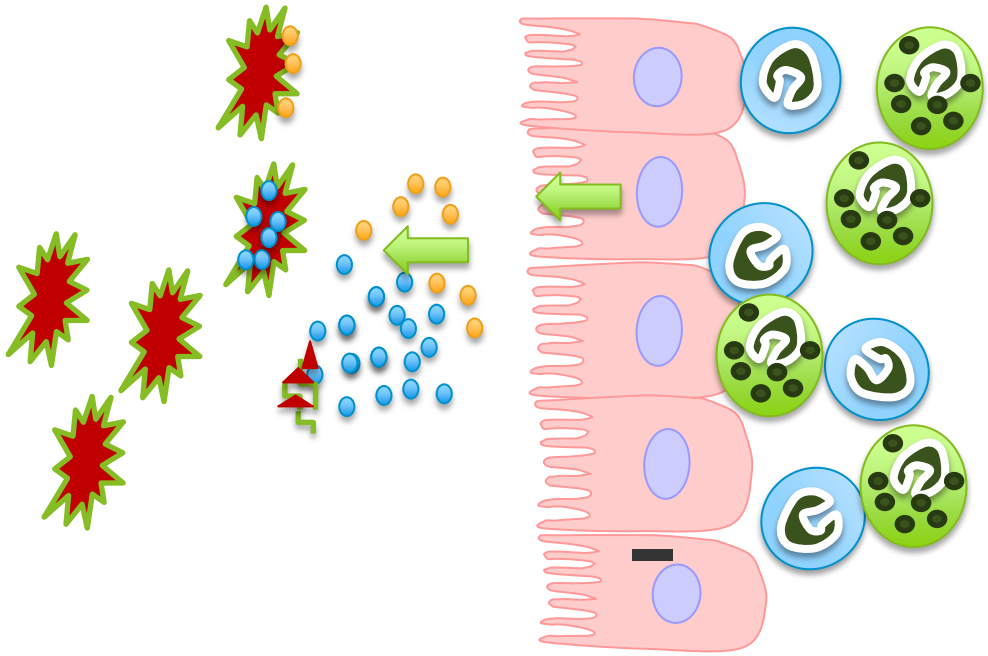
1 – Commensal bacteria – an active barrier

- *Competition with nutrients and binding sites to pathogens*
- *Communication with Innate Immune system to be considered as self.*



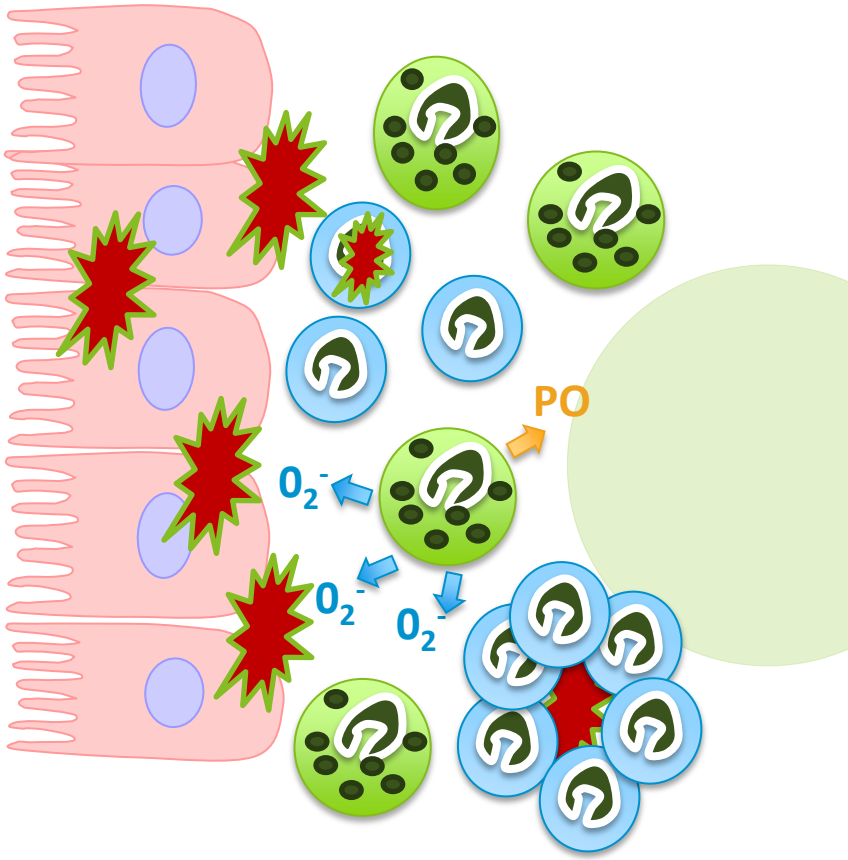
2 – Epithelial cells have also an immunity role

- *Secretion of anti-microbial peptides (Lysozyme, penaeidins, crustins)*
- *Lysis of pathogen walls*
- *Secretion of lectin (recognition of pathogens –bacteria_fungi)*



3 – Innate Immune System

- *Phenoloxidase cascade*
- *Phagocytosis*
- *Respiratory Burst (oxygen species, nitrous oxide)*



Shrimp immune System

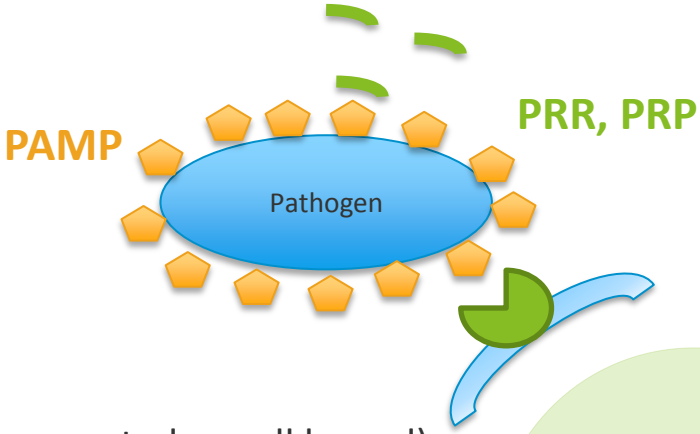
- No adaptive immune system
- Rely mostly on the innate immune system

- A cellular response
- A humoral response

Innate Immunity mechanisms

Innate immune response are triggered by

a specific PAMP – PRR/PRP recognition



PAMP : Pathogen Associated Molecular Pattern

Lipopolysaccharides; beta glucans, Peptidoglycan

PRR, PRP : Pathogen recognition Receptor/Protein (can be secreted or cell bound)

LGBP, GBP, lectins.

Lectins (secreted) contain a carbohydrate recognition domain specific to Carbohydrate sequences in pathogen surface.

Toll Like receptors (TLR) recognizes gram-positive bacteria and fungi responds by leading signaling cascades, and regulating some immune-relative genes

What are the cellular responses ?

- Hemolymph is a **open circulatory system**
- **Hemocytes** free floating in the hemolymph are in direct contact with organs

Haemocyte type	Function in Immunity
Hyaline cell	Phagocytosis
Semigranular cell	• Encapsulation • Phagocytosis (limited) • Storage and release of ProPo • Cytotoxicity
Granular cell	• Storage and release of ProPo • Cytotoxicity

- **Phagocytosis** :
  - internalization and destruction of foreign material
- **Encapsulation**
  - Response that cannot be carried out by phagocytosis
  - Carried out by several hemocytes in cooperation
- **Apoptosis**
  - Programmed cell death (virus infected cells)
  - Caspase play an important role

Johansson *et al* 2000
Syed *et al* 2015

What are the humoral responses ?

- **Released by semi granular/granular cells**

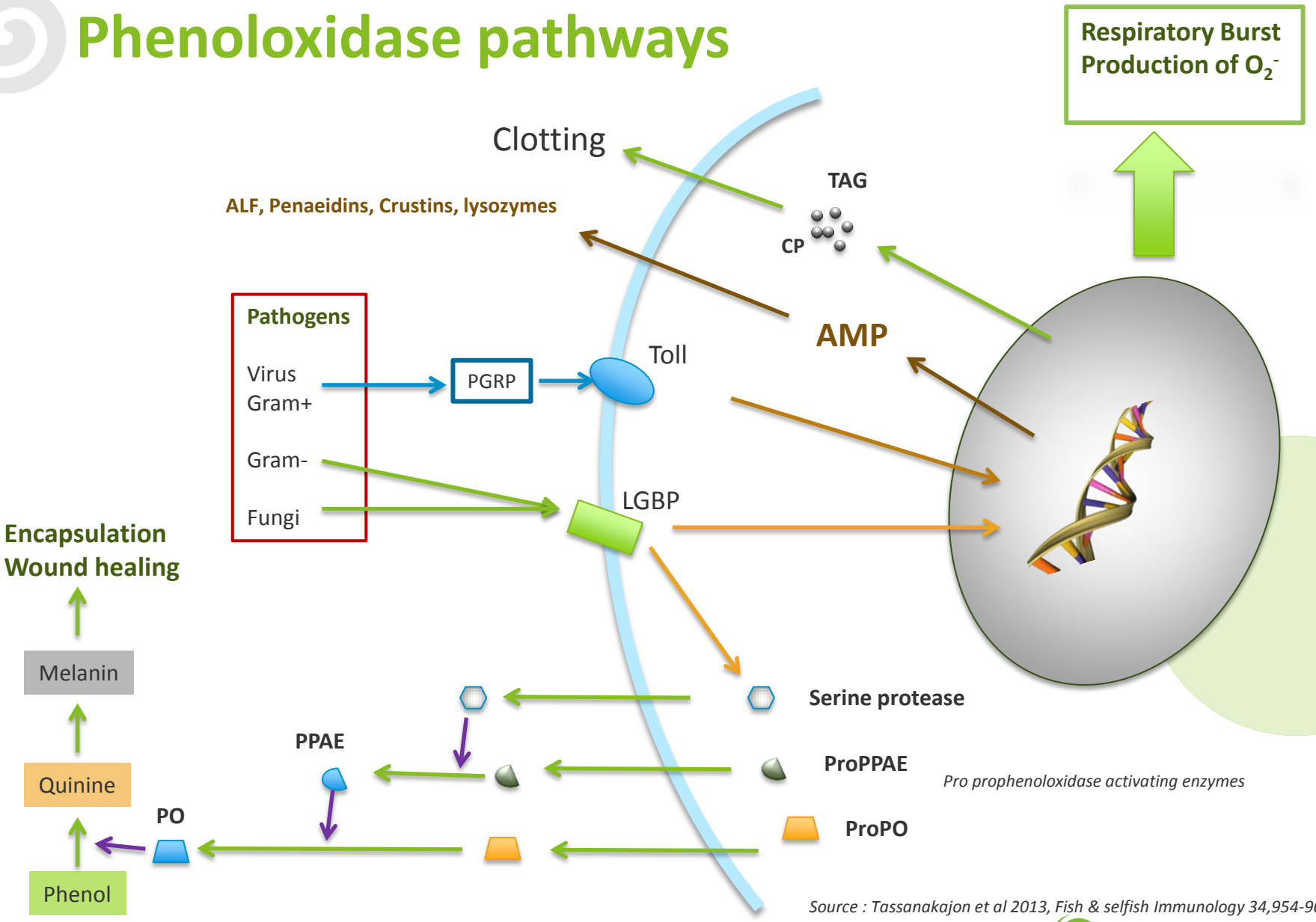
- **Prophenoloxidase** cascade induces melanization
  - Cellular melatonic encapsulation : wound healing, parasite entrapment, microbes killing

- **Clotting**
  - Clotting proteins (Cp) are aggregated upon stimulus of foreign material by the action of transglutaminase and Ca^{2+}

- **Anti microbial peptides.**
  - First line of defense against pathogens
  - Can be effective against a broad range of pathogens (bacteria, fungi…)
  - Penaeidins (anti fungi, anti gram positive bacteria), also a role as cytokin
  - Crustins, mostly but not only against gram positive bacteria. Also a role in aggregation
  - Lysozyme, against gram positive and negative bacteria (especially *Vibrios*)

Amparyup et al 2013, Khader et al 2015

Phenoloxidase pathways



Source : Tassanakajon et al 2013, Fish & selfish Immunology 34,954-967

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Innate immune system is influenced by stress

Stress

- Temperature changes
- Salinity changes
- Metal, heavy metal
- Ammonia

Lower the activity of Phenoloxidase

Decrease Total Hemocyte Count

Lower the activity of Phagocytosis

Increase susceptibility to diseases.

Immune stimulation

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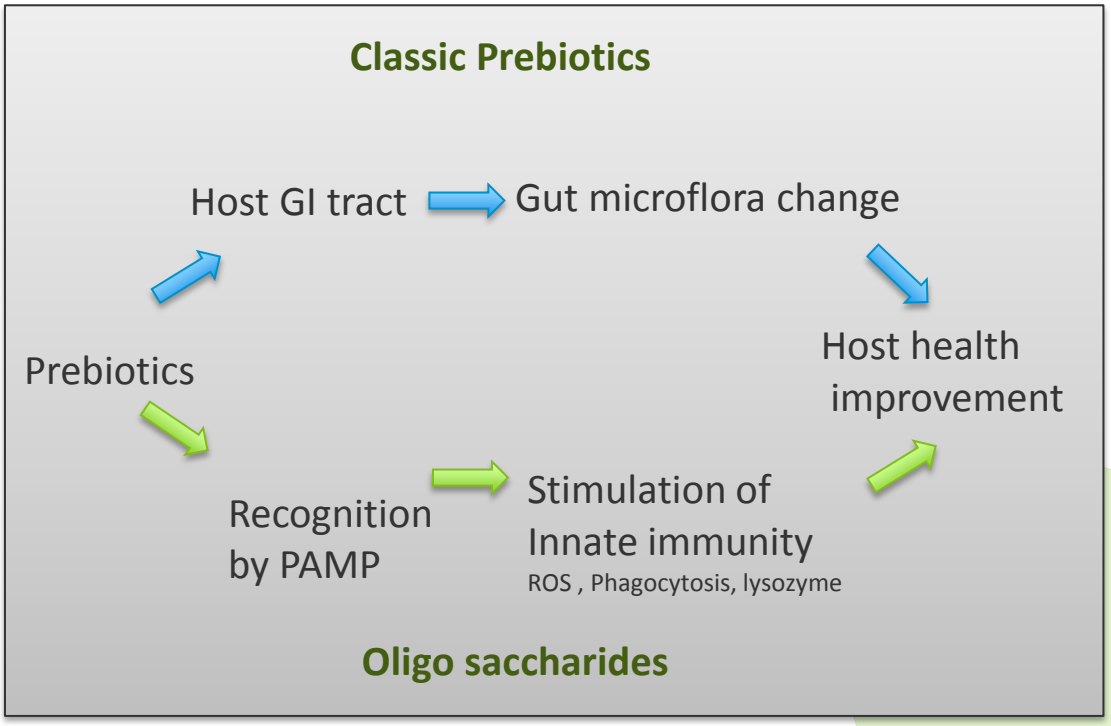
Immune stimulation

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



- **Nucleotides ?**

Are they a stimulant of immunity or a dietary supplement ?

Sources : Ringo et al 2012 ; Song et al 2014

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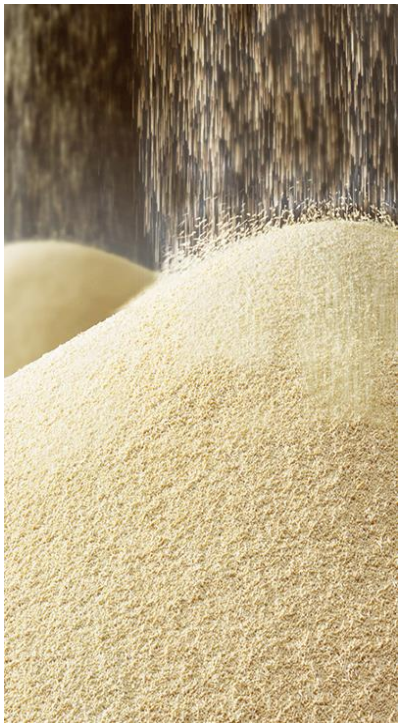
Immune parameters that can be stimulated and measured in shrimp

Parameter	Description	Role
Hemocyte Count	Number and type of immune cells (ex : granulocytes)	Trigger immune response
Lysozyme activity	enzyme released by macrophages in serum, mucus	Lysis of the peptidoglycan wall of bacteria
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)
Prophenoloxidase	Complement system adapted to marine invertebrates. Gives the status of the immune status of shrimp	Enhances microbiocidal activity via respiratory burst and phagocytosis (opsonization). Triggers melanization

(See Song *et al* 2014)

Yeast fractions

Mode of action



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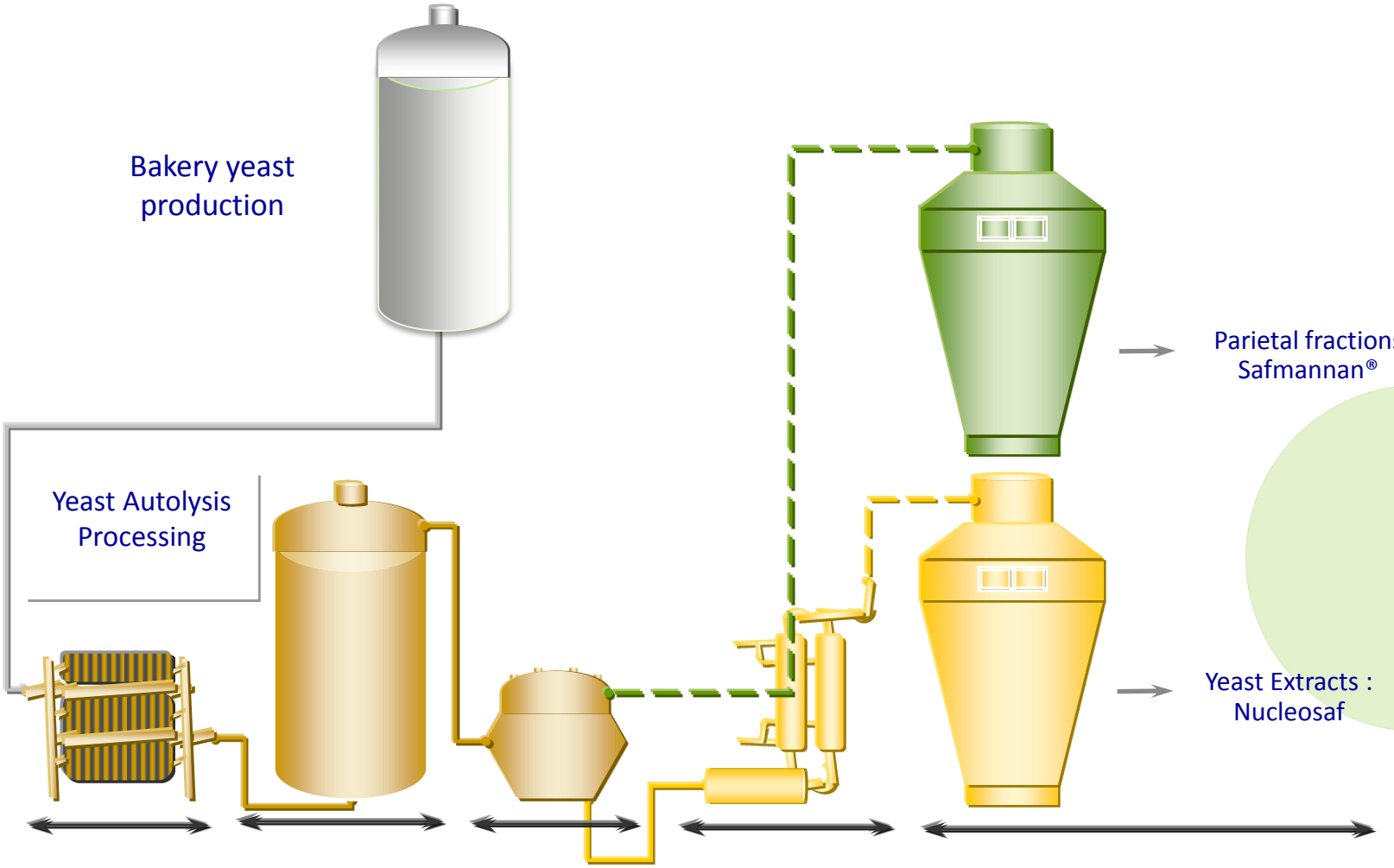
Yeast Extraction is a controlled process



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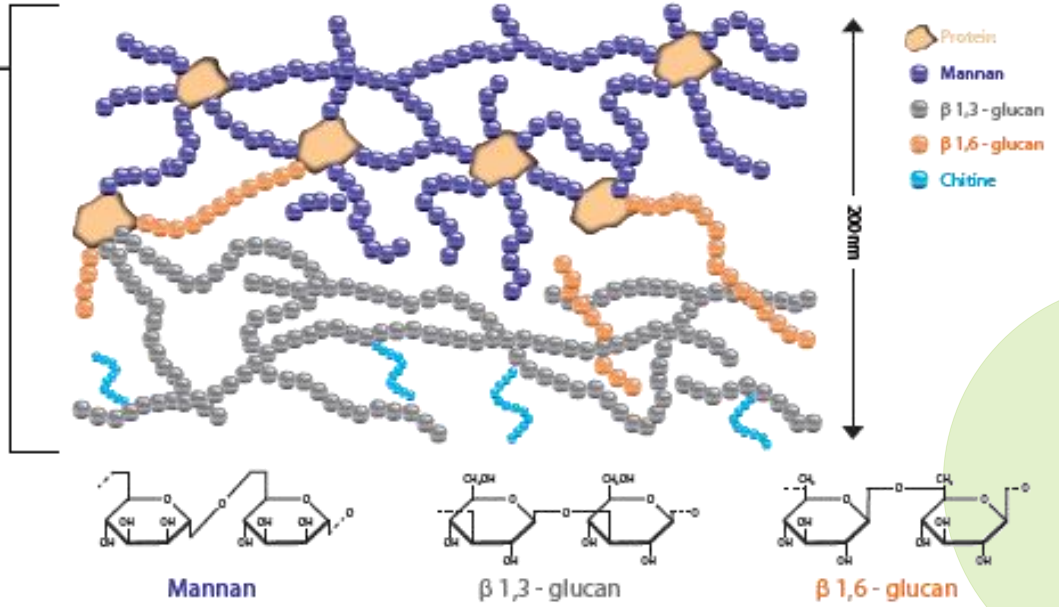
Parietal fractions a complex composition

Complex of Parietal Fractions
(MEB picture, x1800)



Molecular structure of Parietal Fraction

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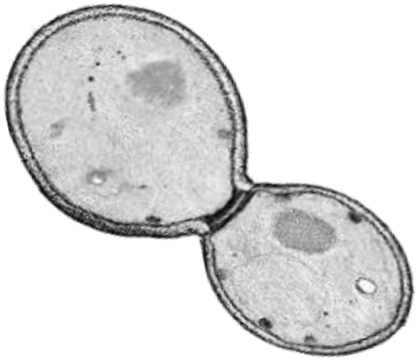
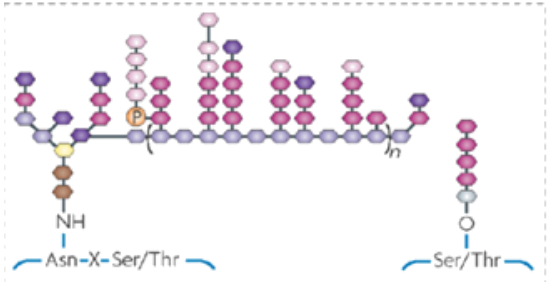
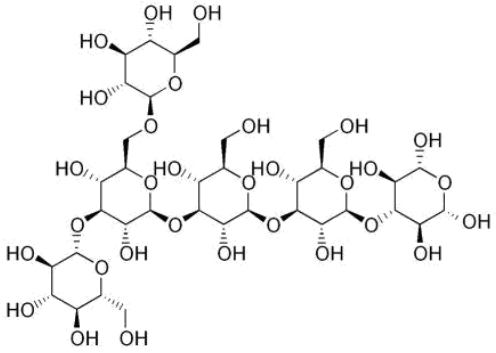
Yeast has specific active components



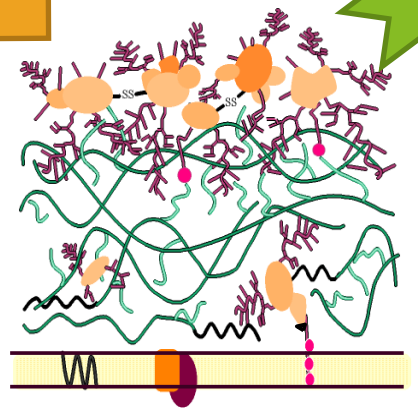


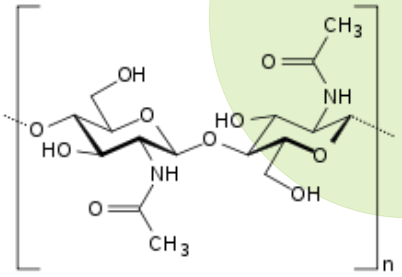
Mannoproteins

(From Netea et al 2008)



β-(1,3 -1,6) Glucans





Chitin

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How are beta glucan working ?

- **Beta Glucans = PAMP**

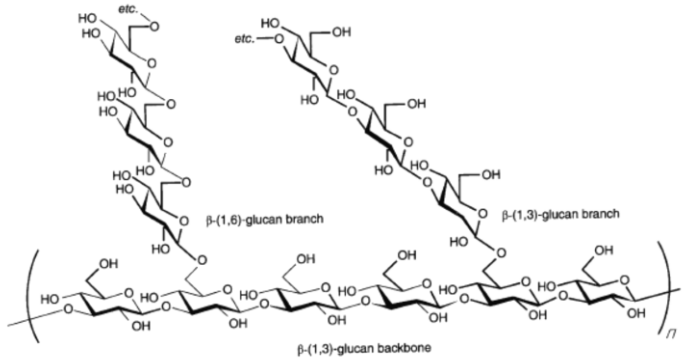
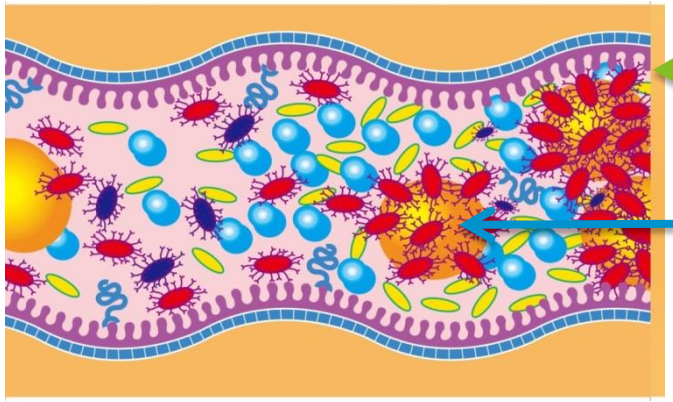


Figure 5.1 Generic glucan structure. Included are the β-(1, 3)-glucan backbone, a β-(1, 6)-glucan branch, and a β-(1,3)-glucan branch. Source: Gannam, A. L. and R. M. Schrock. Immunostimulants in fish diets. Journal of Applied Aquaculture 9: 53–89. Copyright © 1999, Taylor and Francis.

  - **β-1,3/1,6-glucans**
  - Insoluble = skeleton
  - Insoluble form has better activity than soluble form (Ooi and Liu 2000)
  - Beta Glucans are recognized by BGBP, LBGP proteins
  - Induces **Degranulation of hemocytes** (innate immunity barrier)
    - Stimulation of **Phagocytosis**
    - Stimulation of **Phenoloxidase Cascade.**
    - Stimulation of **Respiratoty Burst**

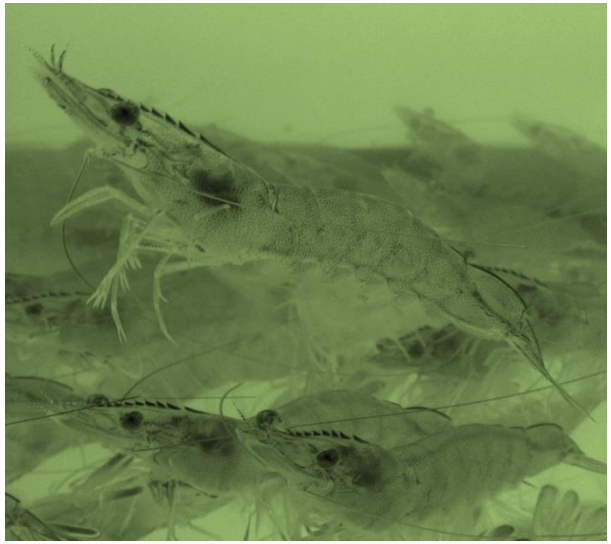
How are Mannans working ?

- **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)
  - Mannan as PAMP ?
  - **Attention : Yeast fractions are not only MOS !**



Example of better pathogen resistance

Shrimp



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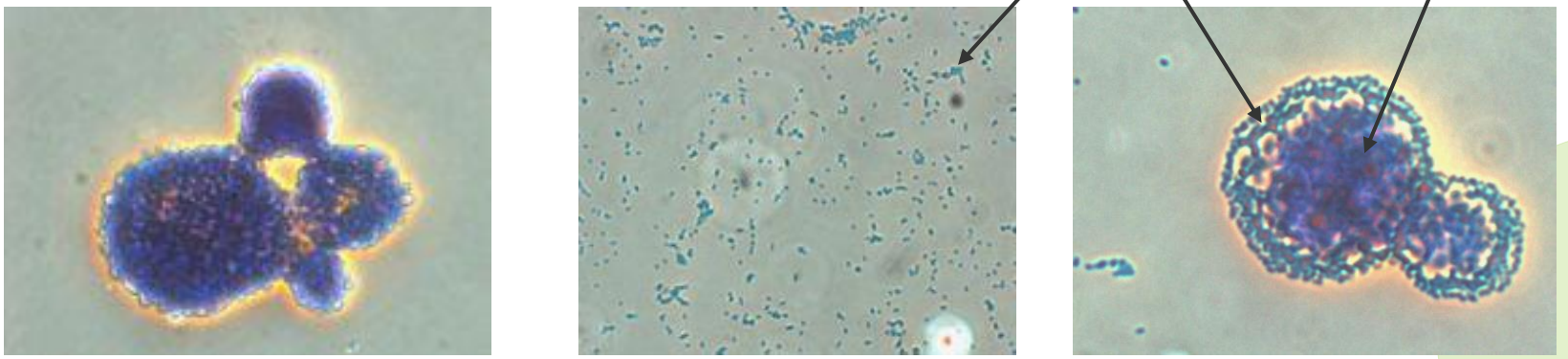
Yeast fractions bind to pathogens

Safmannan®can bind *Vibrio campbelii*, a virulent bacteria in shrimp hatcheries

Safmannan®is put in contact with bacteria for 30 mins



Safmannan®alone | Vibrio alone | Safmannan®+ vibrio

Trial performed at IMAqua laboratory, Univ. of Ghent, Belgium

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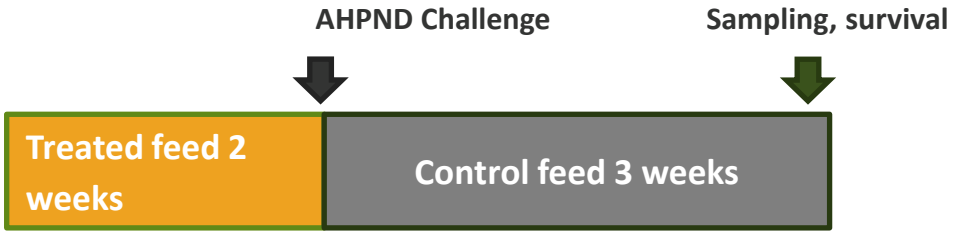
White shrimp – AHPND/EMS Challenge (1)

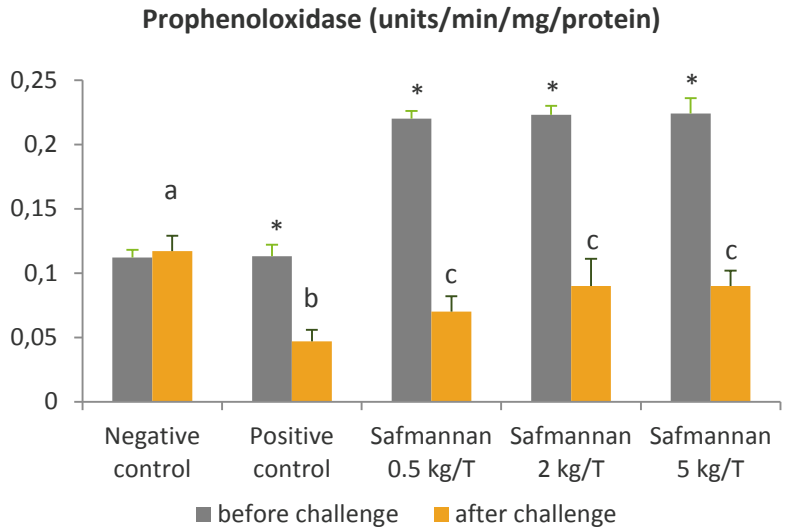
University of Can Tho, Vietnam

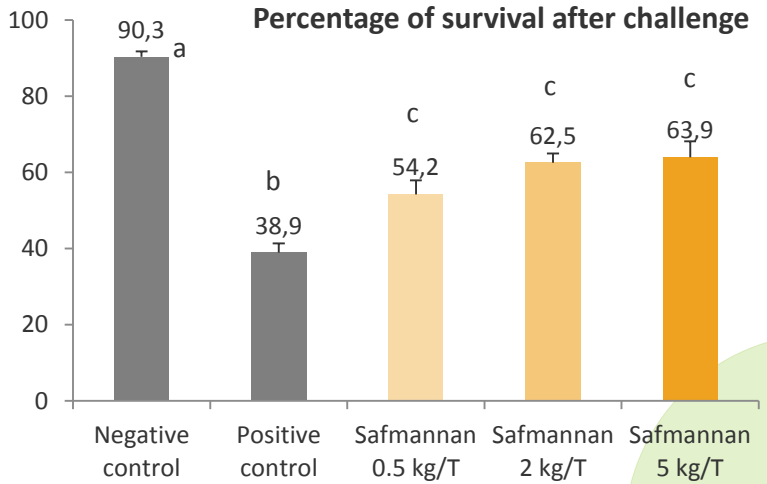
- 2-3 g L. vannamei shrimp
- 30 shrimp/ 30L aquarium
- Triplicates
- Safmannan®, top dressed on commercial feeds at 0.5, 2 and 5 kg/T
- 2 weeks feeding
- Challenge with *V. parahaemolyticus*

(immersion for 15 mins at 10^8 cfu ml^{-1}, *then addition of sea water to reduce density to* 10^6 cell ml^{-1}*)*



White shrimp – AHPND/EMS Challenge (1)





- Safmannan®**increases prophenoloxidase** activity at all concentrations and maintain a high level after challenge.
- Safmannan®**improves survival** after challenge (+60%).

- **Safmannan®at 2 kg/T is the optimum concentration at a short administration time (2 weeks).**

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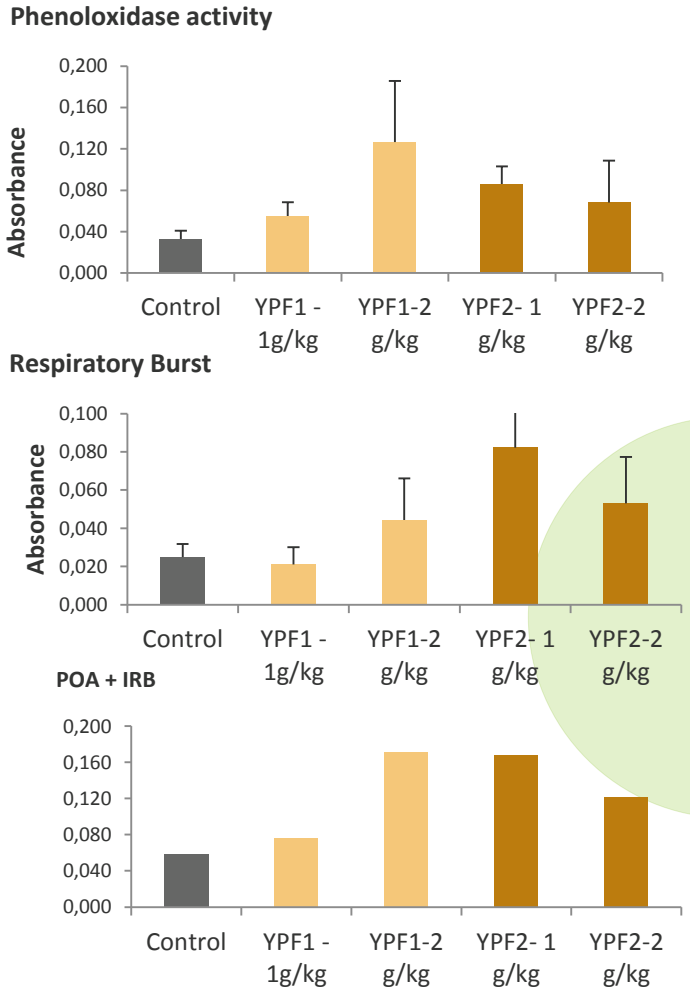
Yeast fractions can vary from one to the other.

Trial performed at IMAQUA, Belgium

- Shrimp 9 g BW
- 15 shrimp/ 50 L aquaria
- Feeding for 14 days
- 2 yeast fractions tested (different processes)
- Measurement of phenoloxidase activity and respiratory burst

- **Different processes give different efficacy levels**
- **For the same efficacy Yeast fraction process 1 needs to be used at double dosage**



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Shrimp- recommendations

Hatchery/ Nursery

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

Grow out ponds

- Prevention of bacterial diseases : **2 kg/T** all time at starter feed (4 weeks) – or during nursery stage-then decrease to **1 kg/T** and **0.5 kg/T**.

Additional applications of nucleotides

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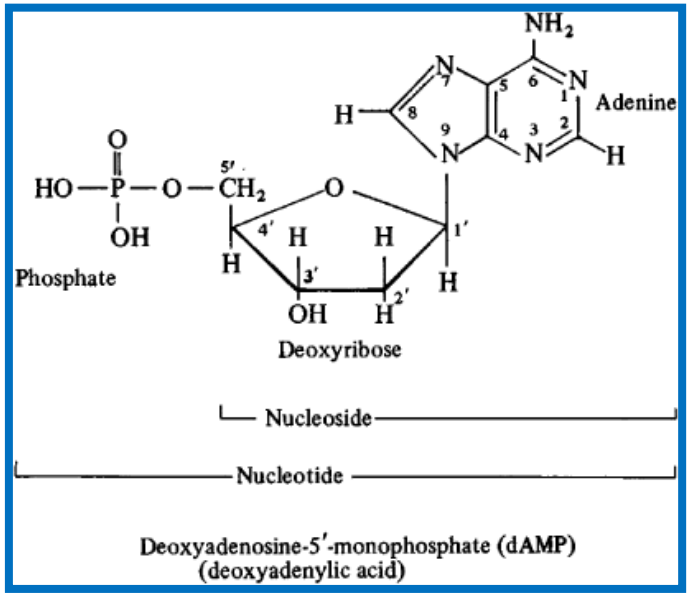
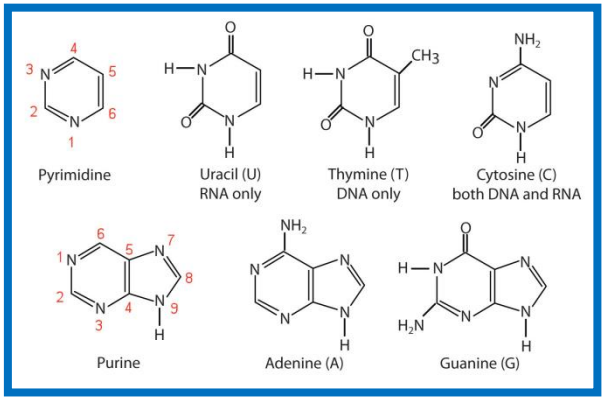
What are nucleotides ?





- Nucleotides are the building blocks of tissue RNA, DNA and ATP
- Essential physiological and biochemical functions (genetic information, coenzymes, cell signaling energy mediators…)
- Consistent beneficial influences on aquatic animals
- Define optimal dosage.

Ringo et al 2012

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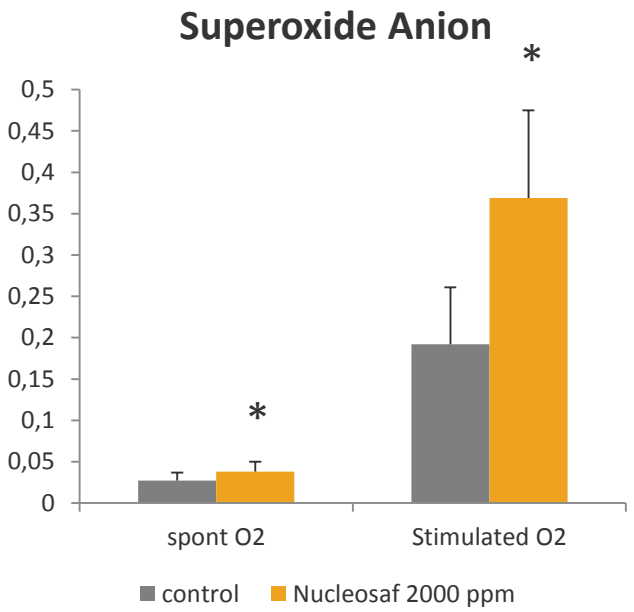
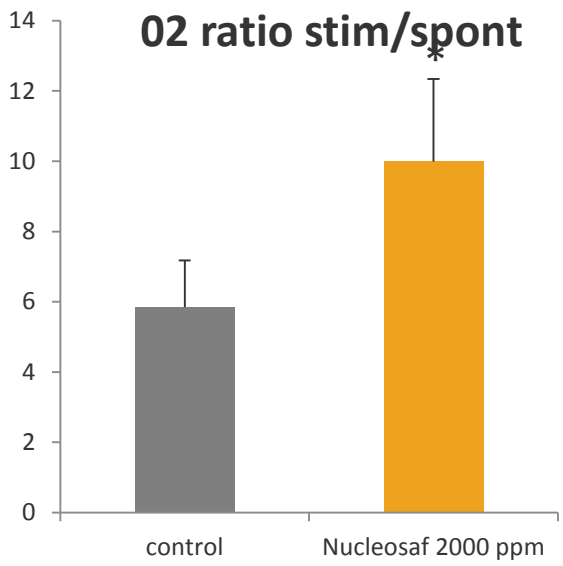
Nucleotides rich products has an effect on immunity

- Thailand –Shrimp (Songkla Univ)

- Dose = 2 g/kg of Nucleosaf.
- Shrimp of 1g.
- 15 shrimps / 45 L aquarium/ 4 replicates per treatment.
- 42 days of experimentation, feeding 4 times a day.
- **Final stress** with a sharp decrease of the salinity from 25 to 8 ppt.

Nucleosaf stimulates immune parameters in shrimp





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Yeast fractions can help to improve disease resistance

- Shrimp disease resistance has several components
  - The Barriers

- Composition of Yeast fractions is important
  - Different Active components
  - PAMP activates PRR-PRP
  - Can work on different barriers (immunity- microflora)
  - Use a constant and defined product

- A good management tool
  - Use the correct dosage
  - Be careful of over stimulation

Thank you for your attention

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