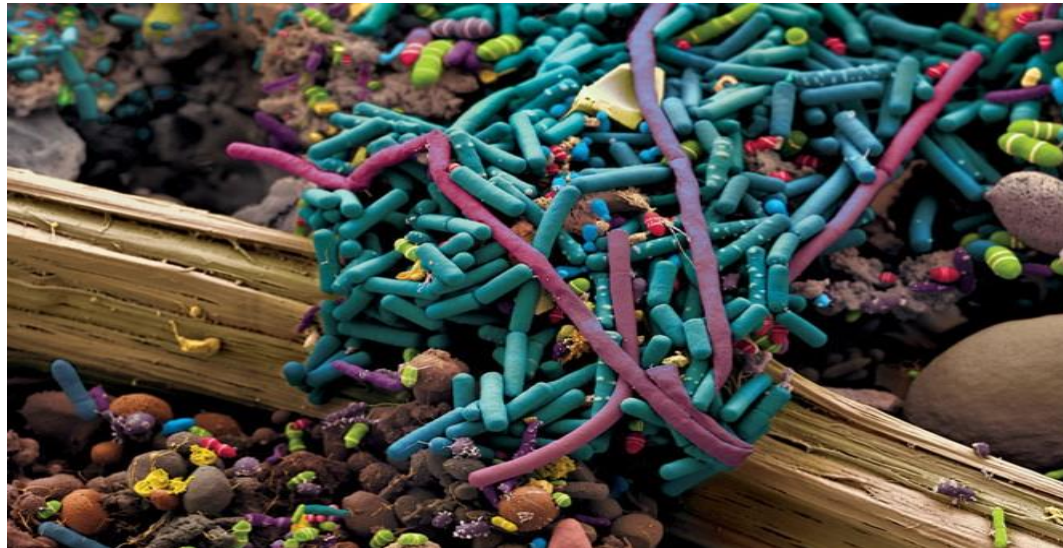


Probiotic colonisation of the aquatic epithelia

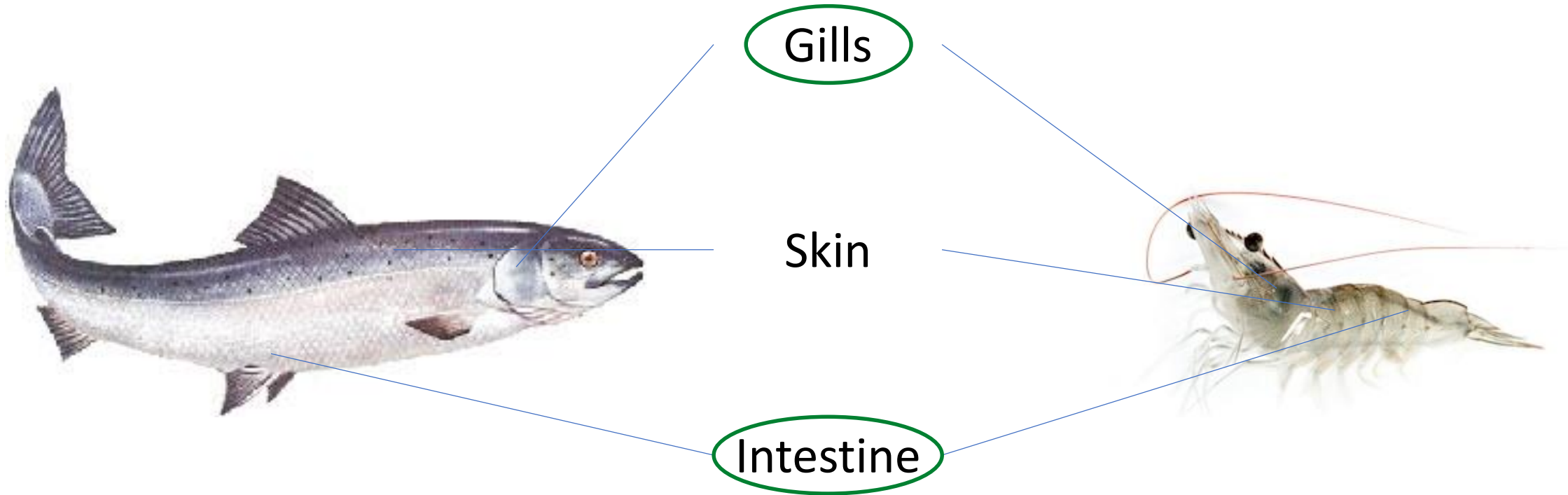
Benedict Standen, PhD

BIOMIN GmbH



<http://oeggerli.com/voyage/>

Mucosal surfaces in aquatic species



“All disease
begins in the **gut**”

Hippocrates
460 – 370 BC



Autochthonous vs allochthonous microbiota

Autochthonous

Native to the intestine

Associated with the intestinal
epithelia

Does not degrade mucins

Remain in the gut for longer periods

Can colonize the gut epithelia +
mucus

Allochthonous

Derived from outside sources

Associated with the intestinal lumen

Can degrade mucins

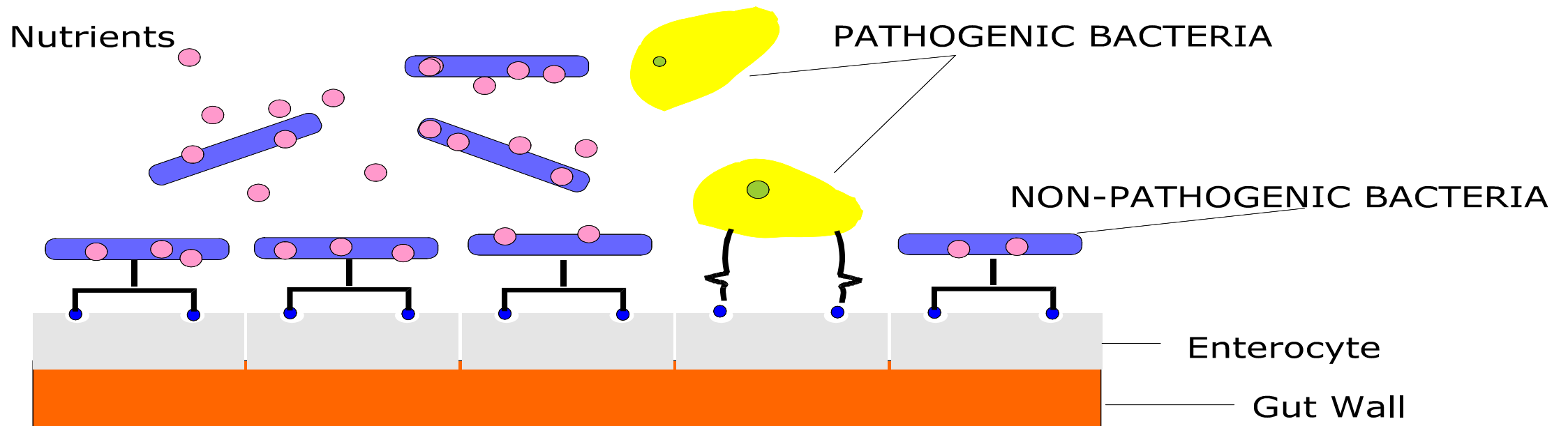
Rapid transit through the intestine

Cannot colonize the gut epithelia +
mucus

Important properties of intestinal probiotics

- Must not be pathogenic to animals (E)
- Must be free of plasmid encoded antibiotic resistance genes (E)
- Must be resistant to wide range of pH and bile acids (E)
- Should be able to colonise the gut (F)

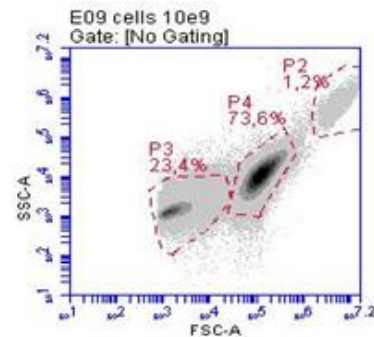
Improving barrier function through competitive exclusion



- If probiotics occupy binding sites, pathogens cannot
- This mechanism is more effective when probiotics are supplied at early development stages

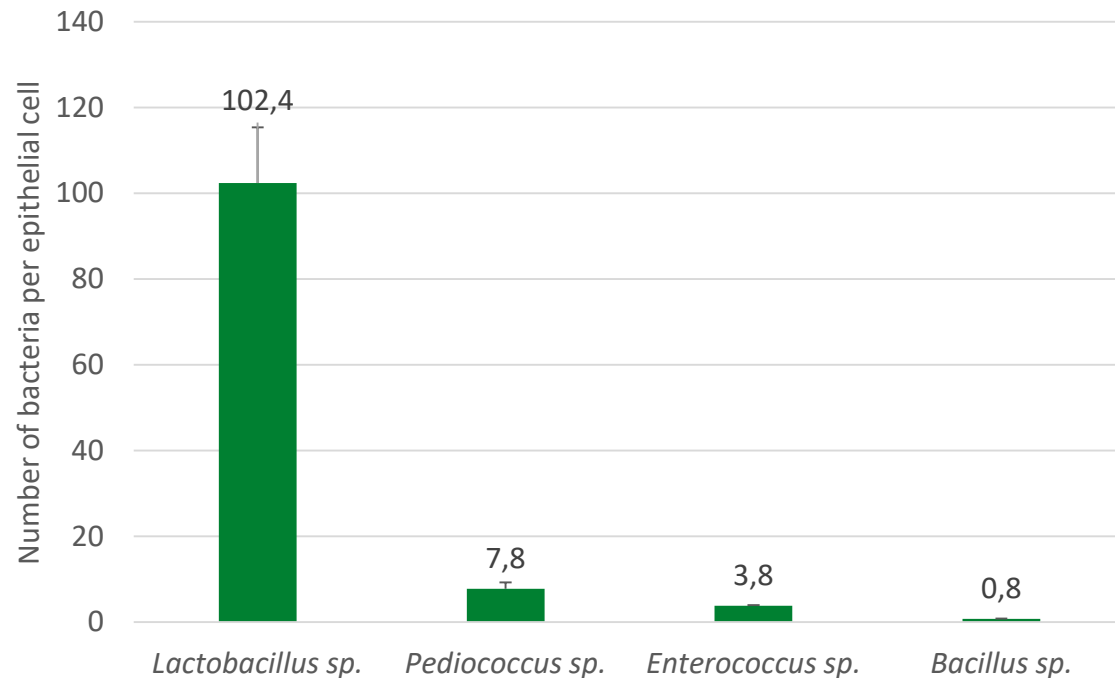
Epithelial attachment - gill

- Probiotics cultured in appropriate medium
- Bacterial cells harvested, washed and stained with CellTracker™ Green
- RTgill-W1 cells grown to subconfluence in wells
- Labelled probiotic bacteria added to epithelial cells at 10^7 cells per well. Heat inactivated bacteria also added at same concentration
- Incubation
- Non-adhered cells were washed away and collected for FACS analysis
- Attached probiotics detached from epithelial cells with trypsin/EDTA
- Bacterial cells resuspended and fixed
- Analyzed with flow cytometer



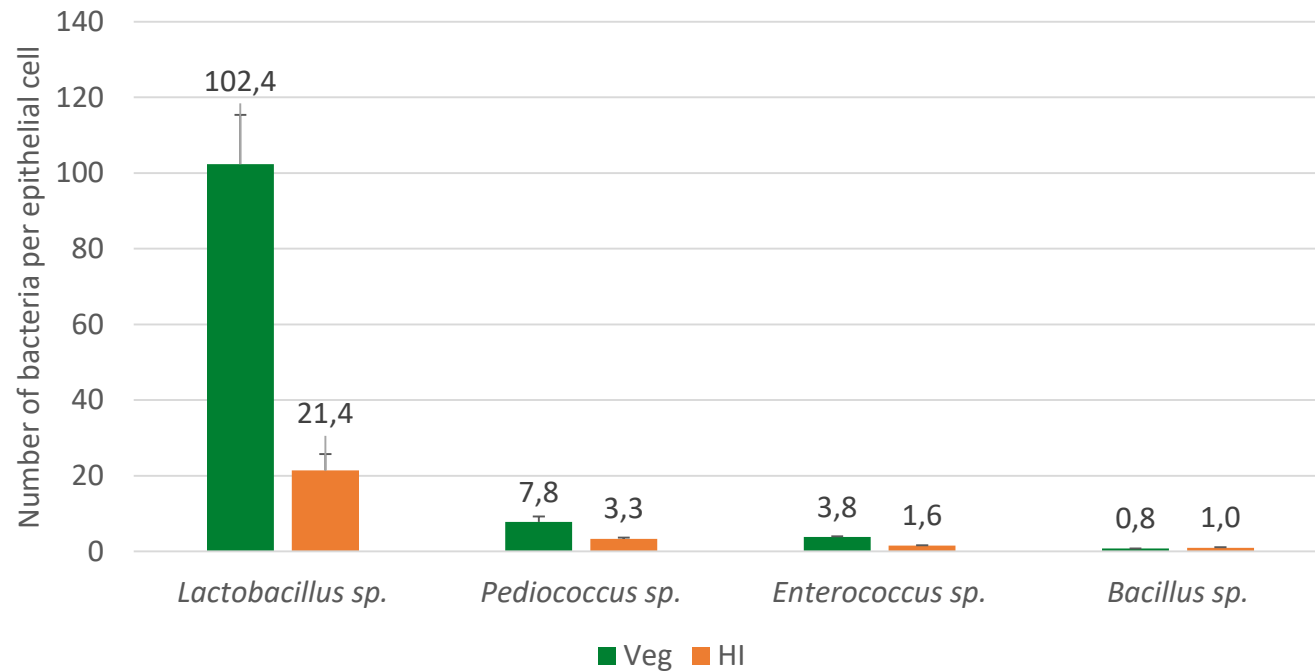
P2 = Epithelial cells
P4 = Bacterial cells
P3 = Instrument 'noise'

Attachment to gill epithelial cells



- Lactic acid bacteria can adhere to gill epithelial cells
 - *Lactobacillus* is best at attaching to epithelial cells
 - *Pediococcus* and *Enterococcus* can also attach, but to a lesser extent
- *Bacillus* can not attach to gill epithelial cells

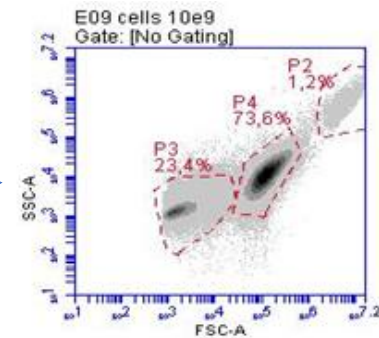
Attachment to gill epithelial cells



- Whilst heat inactivated probiotics can attach (species dependent), the efficacy is considerably lower
- Probiotics **MUST** be alive for epithelial attachment

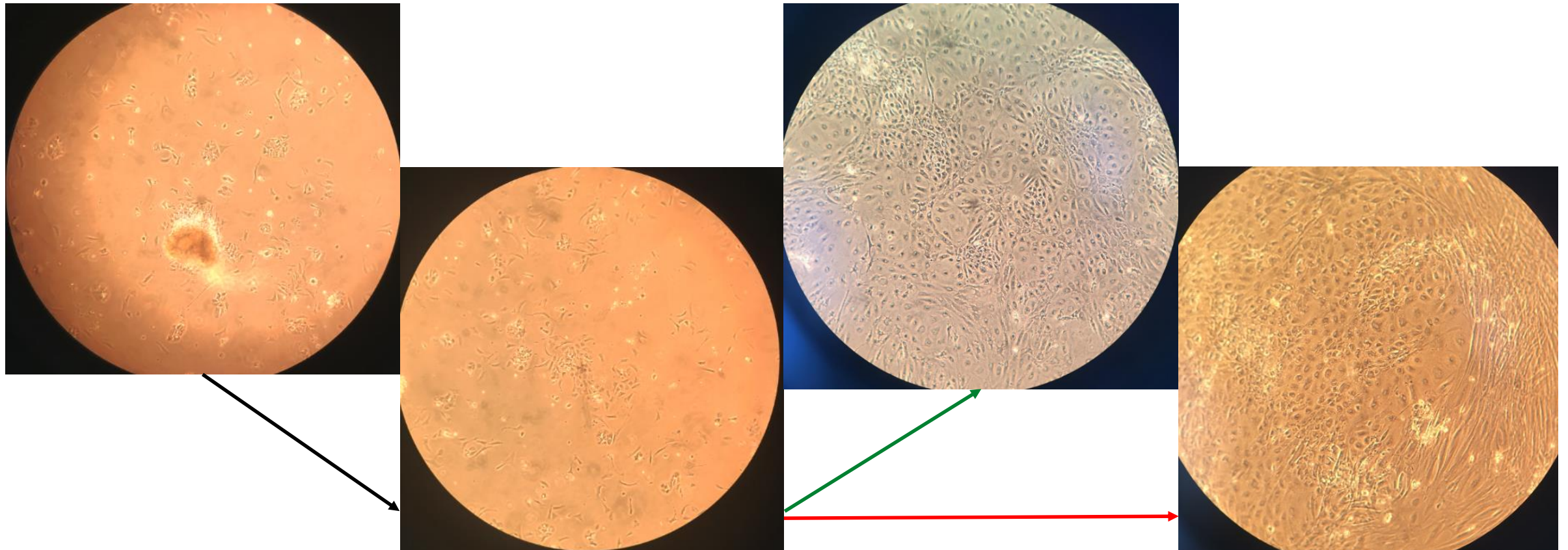
Epithelial attachment - gut

- Probiotics cultured in appropriate medium
- Bacterial cells harvested, washed and stained with CellTracker™ Green
- **Gut cells grown to subconfluence in wells → PROBLEM**
- Labelled probiotic bacteria added to epithelial cells at 10^7 cells per well. Heat inactivated bacteria also added at same concentration
- Incubation
- Non-adhered cells were washed away and collected for FACS analysis
- Attached probiotics detached from epithelial cells with trypsin/EDTA
- Bacterial cells resuspended and fixed
- Analyzed with flow cytometer



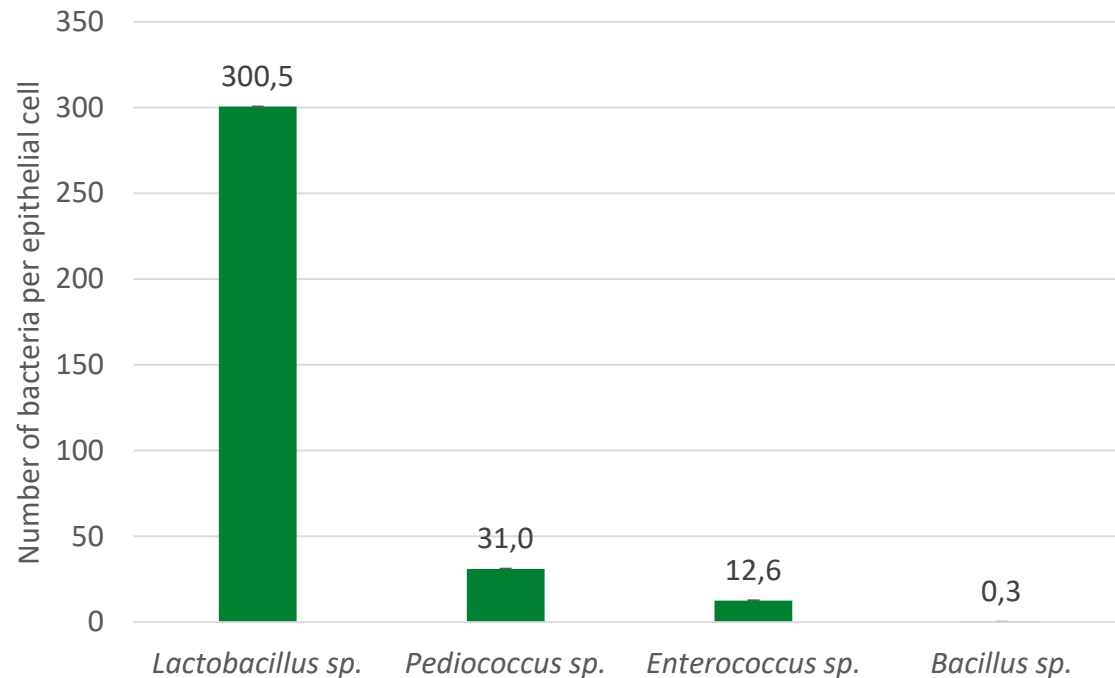
P2 = Epithelial cells
P4 = Bacterial cells
P3 = Instrument 'noise'

Epithelial colonisation – isolating primary gut epithelial cells



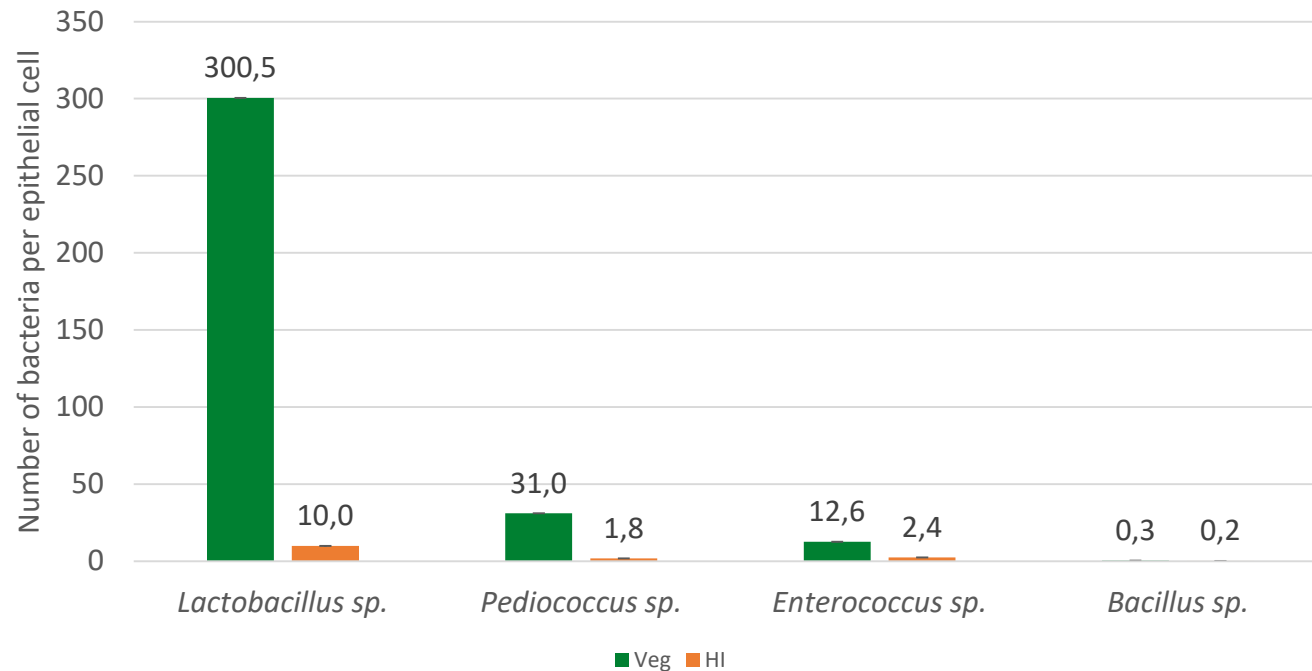
SOURCE: BIOMIN

Attachment to gut epithelial cells



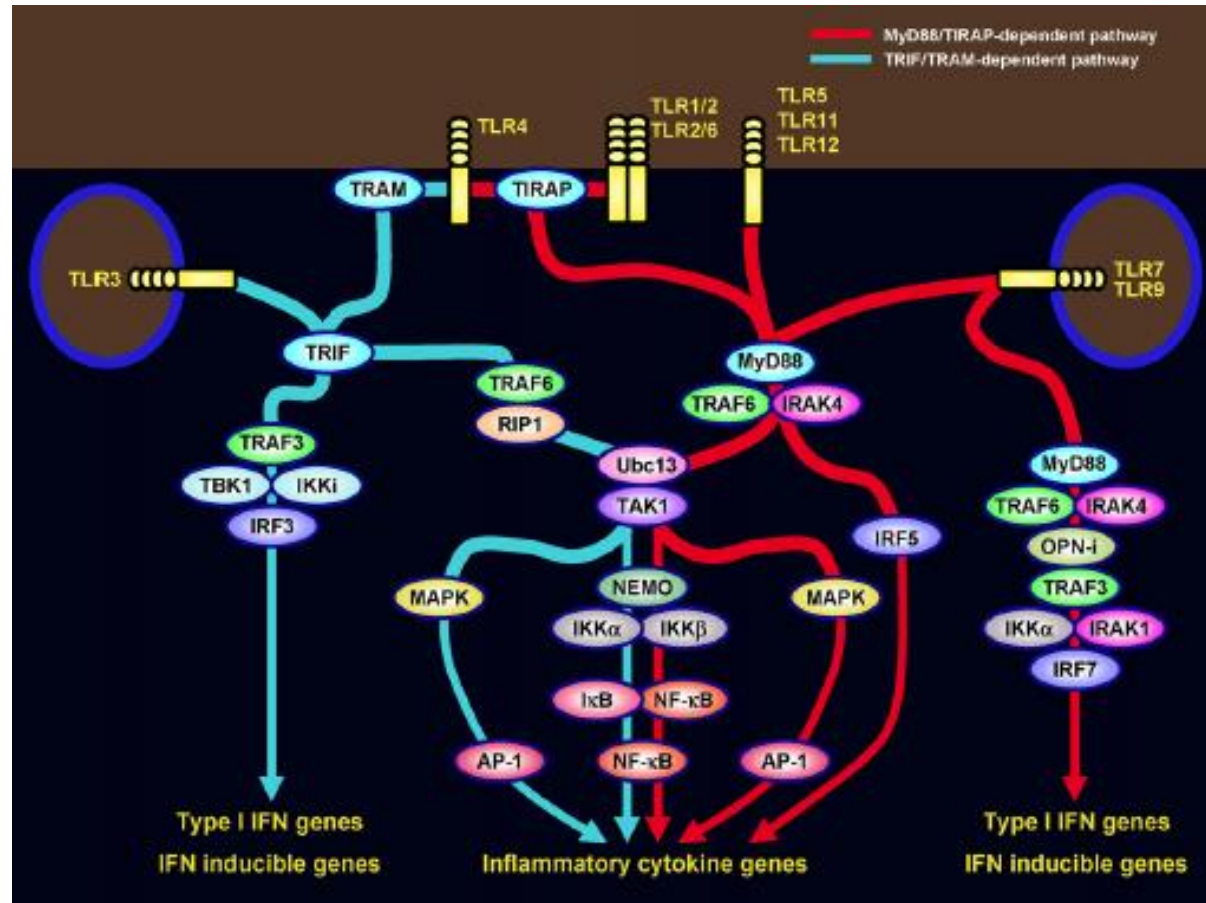
- *Lactobacillus* is very effective at attaching to intestinal epithelial cells
- *Pediococcus* and *Enterococcus* can attach, but to a less extent
- *Bacillus* cannot attach to the intestinal epithelia – good or bad?

Attachment to gut epithelial cells



- Whilst heat inactivated probiotics can attach (species dependent), the efficacy is considerably lower
- HI probiotics may still bring immune benefits – only if molecular ‘barcode’ is conserved
- Probiotics **MUST** be alive for epithelial colonisation

How about the immune system?



SOURCE: Kawai & Akira, 2007

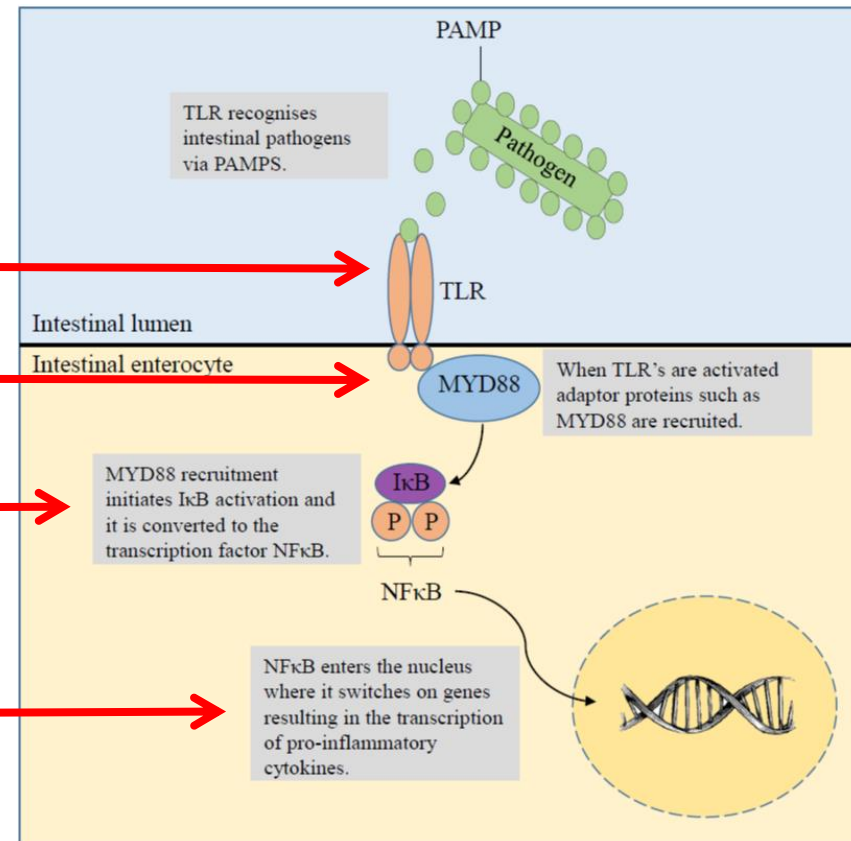
TLR signalling (simplified)

Receptor

Adaptor

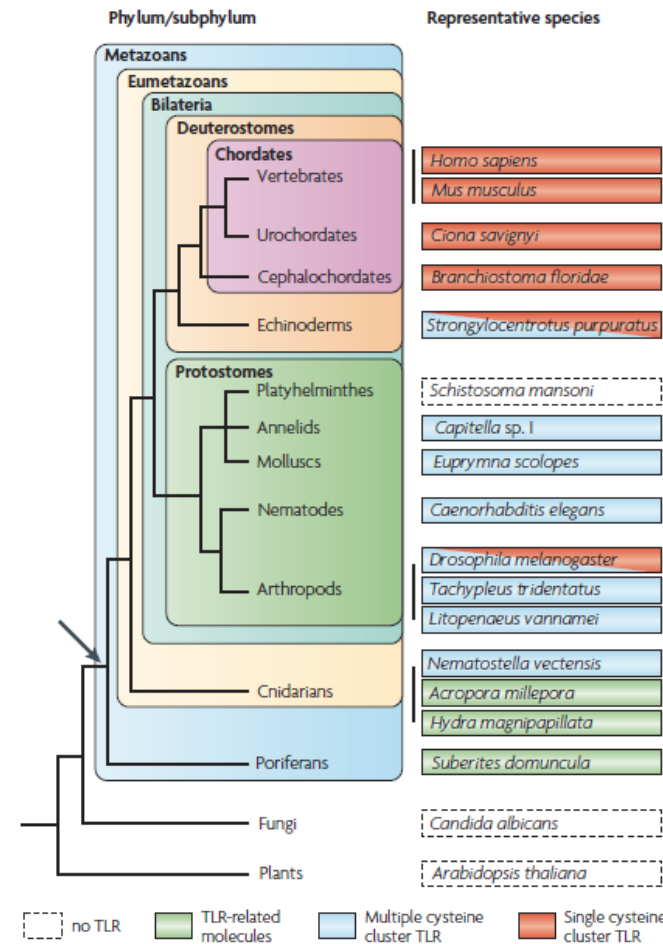
Transcription factor

Cytokines



TLR recognition is conserved

- Toll pathways are conserved in invertebrates
- *Drosophila* is most commonly studied invertebrate
- Toll pathways have important immune functions and involved with development
- TLRs in invertebrates do not recognise microorganisms directly, but use an intermediate molecule

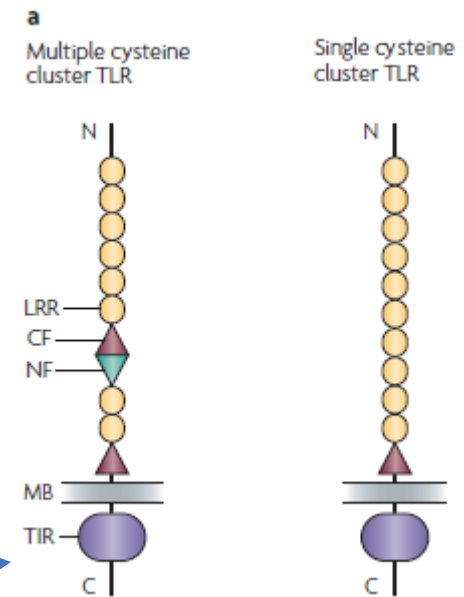


Toll-like receptors — taking an evolutionary approach

François Leulier* and Bruno Lemaitre*

Abstract | The Toll receptor was initially identified in *Drosophila melanogaster* for its role in embryonic development. Subsequently, *D. melanogaster* Toll and mammalian Toll-like receptors (TLRs) have been recognized as key regulators of immune responses. After ten years of intense research on TLRs and the recent accumulation of genomic and functional data in diverse organisms, we review the distribution and functions of TLRs in the animal kingdom. We provide an evolutionary perspective on TLRs, which sheds light on their origin at the dawn of animal evolution and suggests that different TLRs might have been co-opted independently during animal evolution to mediate analogous immune functions.

Leulier & Lemaitre, 2008



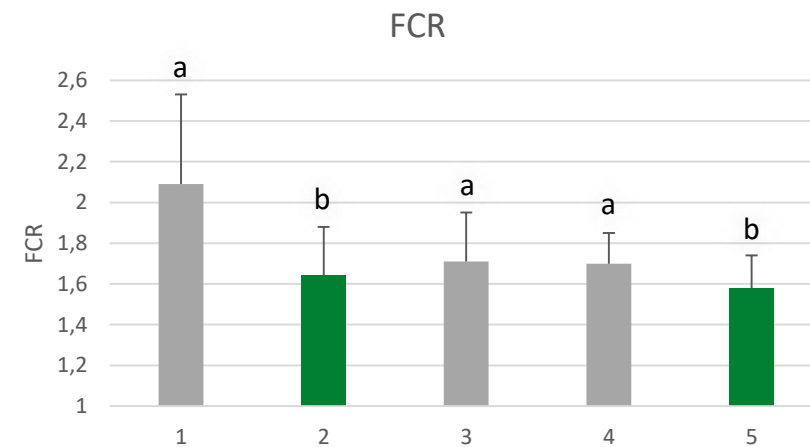
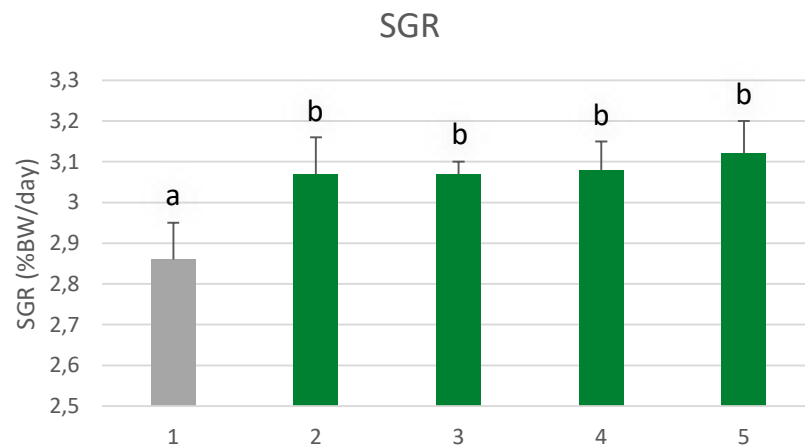
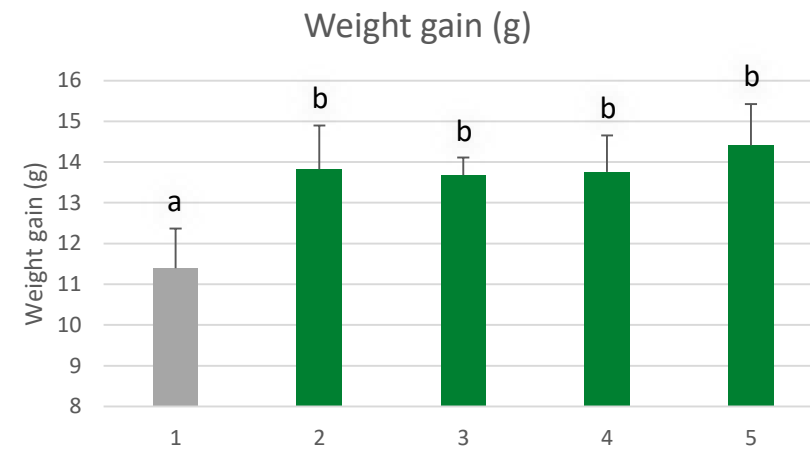
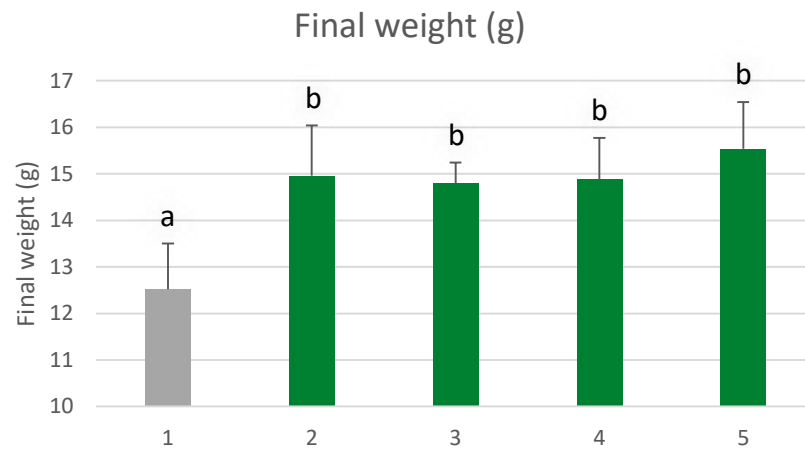
In vivo trial in L. vannamei

- **Objective:** Evaluate the effect of multi-species probiotic in White shrimp with different feeding regimes
- **Treatments ($n = 7$):** Defined below
- **Duration:** 12 weeks
- **Feeding:** 6x per day to apparent satiety
- **Initial weight:** $1.13 \pm 0.01g$
- **Challenge:** *V. parahaemolyticus* (IM injection 4.8×10^4 CFU)

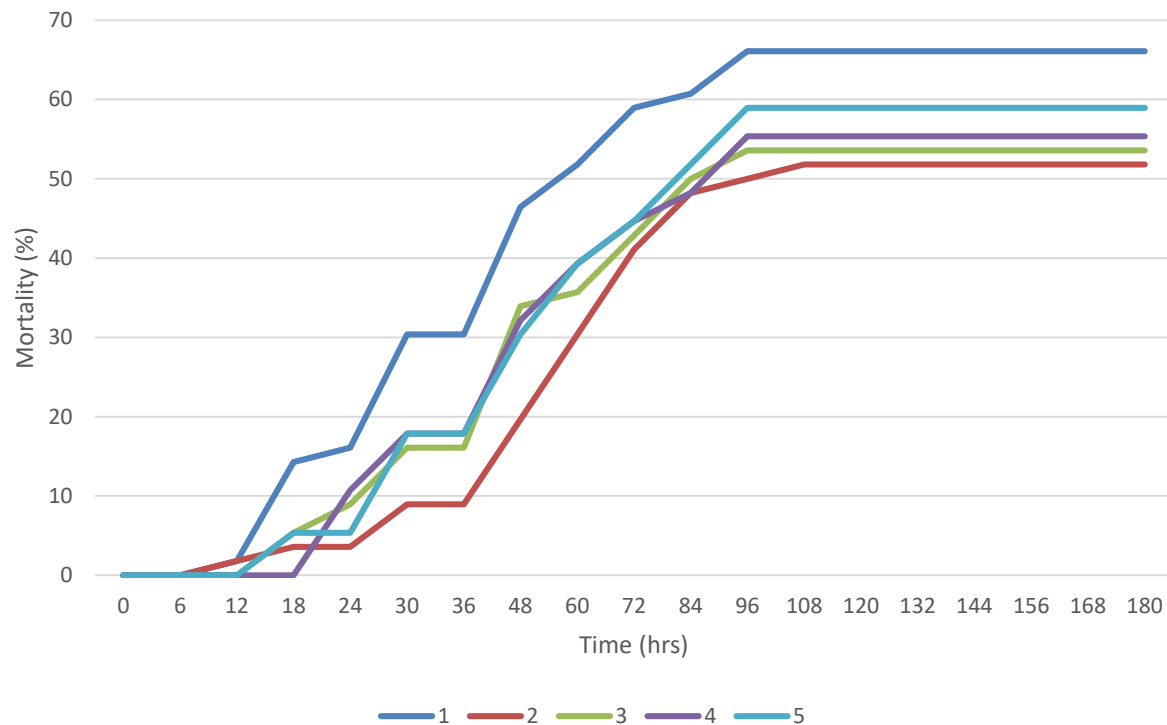
Group	Group code	Probiotic	Feeding regime
Control	1	-	Control feed - continuously fed in a separate RAS
Continuous	2	3g/kg Feed	Supplemented feed - continuously fed
Pulse 1	3	3g/kg Feed	Pulsed: 1 week fed with supplemented feed - 1 week fed with control feed
Pulse 2	4	3g/kg Feed	2week fed with supplemented feed - 2 week fed with control feed
Pulse 3	5	3g/kg Feed	Pulsed: 2 week fed with supplemented feed - 1 week fed with control feed

Day	Control	Continuous	Pulse 1	Pulse 2	Pulse 3
0-6					
7-13					
14-20					
21-27					
28-34					
35-41					
42-48					
49-55					
56-62					
63-69					
70-76					
77-84					

*In vivo trial in *L. vannamei**



In vivo trial in L. vannamei



	1	2	3	4	5
Day	Control	Continuous	Pulse 1	Pulse 2	Pulse 3
0-6	Grey	Green	Green	Green	Green
7-13	Grey	Green	Green	Green	Green
14-20	Grey	Green	Green	Green	Green
21-27	Grey	Green	Green	Green	Green
28-34	Grey	Green	Green	Green	Green
35-41	Grey	Green	Green	Green	Green
42-48	Grey	Green	Green	Green	Green
49-55	Grey	Green	Green	Green	Green
56-62	Grey	Green	Green	Green	Green
63-69	Grey	Green	Green	Green	Green
70-76	Grey	Green	Green	Green	Green
77-84	Grey	Green	Green	Green	Green
Mortality (%)	66.1	51.8	53.6	55.4	58.9

CONCLUSION: Continual supplementation promotes improved growth performance AND increased disease resistance

Is 'true' probiotic colonisation possible?

- To colonise and remain in the gut, multiplication rate must be higher than expulsion rate
- The mucosal layer is not static
- Intestinal cells are being lost naturally all the time – sloughing/ apoptosis
- Probiotic levels must be maintained at artificially high levels for maximum benefits



Continual supplementation of probiotics is necessary

Summary

- Colonisation is a key property for intestinal probiotics
- Different probiotic species (and likely strains) have different capacity for epithelial attachment
- Once attached, probiotics can block pathogens and communicate with the host immune system
- This results in improved immunity and better disease resistance
- Since 'true' intestinal colonisation is unlikely, probiotics must be applied continuously

Thank you!

Gracias por escuchar!

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≡ Biomin® ≡
Naturally ahead

BOOTH #84

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