INSIGHTS INTO EUROPEAN PET FOOD TRENDS AND INNOVATION

PETFOOD FORUM EUROPE

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Nuremberg, Germany

#petfoodforum • PetfoodForumEvents.com/Europe

Co-located with

Interzoo 2024

Postbiotics **yeast** *vs* **bacteria**: what is the difference?

Francesca Susca, DVM, PhD | Global Pet Product Manager at Lallemand Animal Nutrition

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LALLEMAND ANIMAL NUTRITION

SPECIFIC FOR YOUR SUCCESS

Lallemand develops, produces and markets high-value yeast and bacteria products.

48 plants including
27 yeast and
9 bacteria plants

ANIMAL NUTRITION	COFFEE	OENOLOGY
BAKING	COSMETICS	PHARMA
BIOFUELS	DISTILLED SPIRITS	PLANT CARE
BREWING	FERMENTATION MEDIA	SAVOURY INGREDIENTS
COCOA	HEALTH SOLUTIONS	SPECIALTY CULTURES

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LALLEMAND ANIMAL NUTRITION

LALPROBIOME, the Lallemand Animal Nutrition's platform for companion animals

- LALPROBIOME is Lallemand Animal Nutrition's platform of innovative microbial solutions tailored for companion animals.
- Leveraging the natural power of yeast and bacteria with cutting edge science to support companion animals' health and well-being, LALPROBIOME provides a wide range of innovative microbial solutions to meet the needs of companion animals and their owners today and for the future.

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LALLEMAND ANIMAL NUTRITION

Bacteria vs Yeast

Bacteria

- Prokaryote organisms (no nucleus)
- Size: ~1 µm
- Reproduction: by cell division
- Presence in the microflora: 99%
- Sensitive to antibiotics

Yeast

- Unicellular eukaryote organisms with cell wall and DNA in a nucleus
- Size: ~ 5-10 µm
- Reproduction: by budding
- Presence in the microflora: 1%
- Resistant to antibiotics

NOT ALL YEAST & BACTERIA ARE EQUAL!

≃ 60 different genera of yeasts → ≃ 500 different species

≃ 10 K different genera of bacteria → > 5 mill different species

Thousands of different strains, each with a unique genetic make-up

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Microbes: do they need to be alive to be beneficial?

The mechanisms underlying probiotic/postbiotic effects are generally attributed to:

The interaction of probiotics with other microorganisms

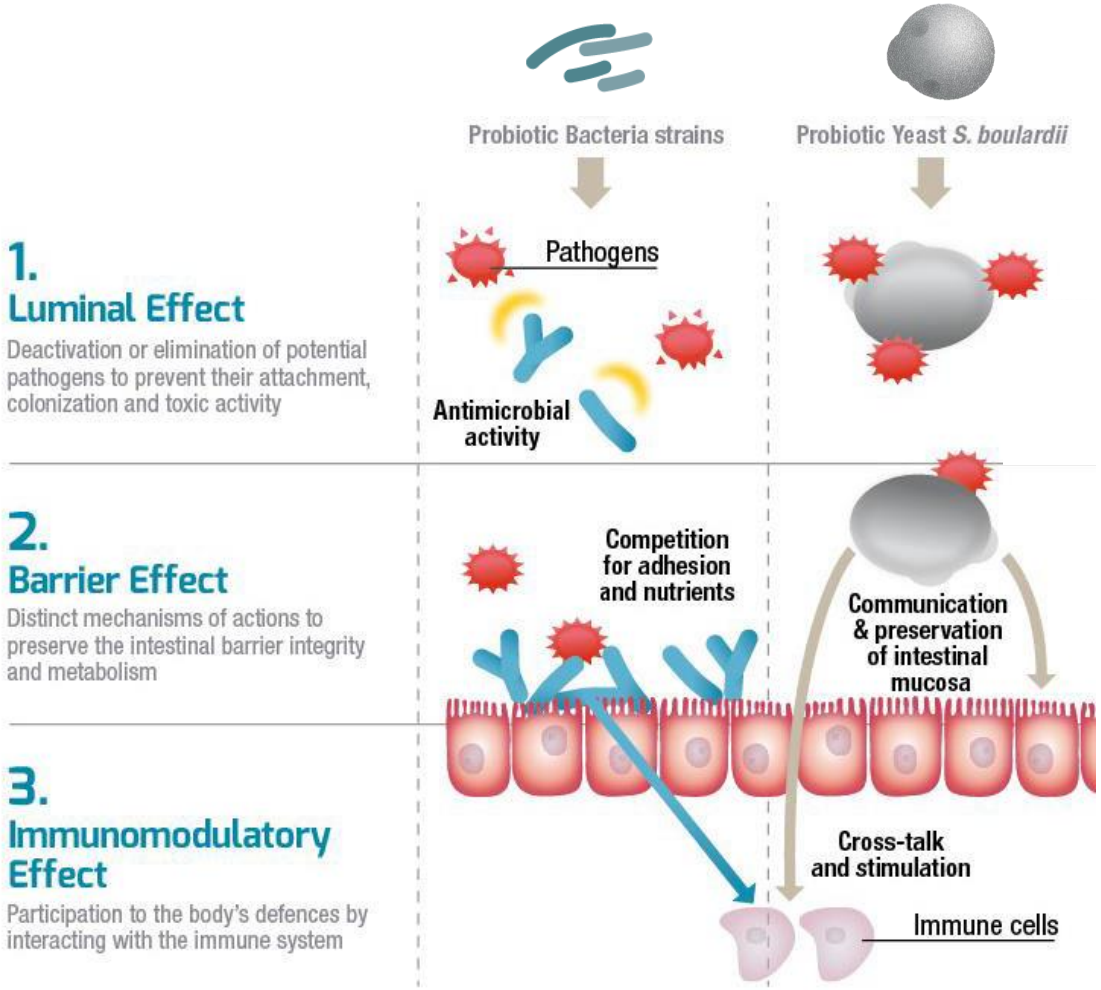
- Competitive exclusion (competition for nutrients)
- Direct inhibition of certain microorganisms
- Increased growth of healthy components of the microbiota (nutritional/environmental proto-cooperation)

Dependent on **viability*** of probiotic cells

The cross-talk of micro-organisms with host cells

- Competition for adhesion sites
- Yeast/bacteria components recognized by gut epithelial cells
- Pathogens binding capacity (yeast)

Independent on **viability*** of probiotic cells



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*Viability: ability to remain alive and capable of growth or reproduction

Postbiotics | Consensus statement

CONSENSUS STATEMENT

Check for updates

The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics

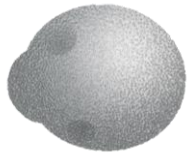
Seppo Salminen[1]✉, Maria Carmen Collado[2], Akihito Endo[3], Colin Hill[4,5], Sarah Lebeer[6], Eamonn M. M. Quigley[7], Mary Ellen Sanders[8], Ragnar Shamir[9,10]

Probiotic microbes: do they need to be alive to be beneficial?

Jasmeet Kataria, Nan Li, James L Wynn, and Josef Neu

An essential symbiotic relationship exists between intestinal cells and commensal bacteria within the human gastrointestinal tract. Alteration or absence of this interaction may play a role in the development of human disease. Use of probiotic organisms has yielded improvement of certain medical conditions, such as inflammatory and infectious gastrointestinal disease, although the mechanisms of benefit remain poorly defined. The administration of live organisms is not without risk, both potential and realized, particularly in certain populations. Therefore, it is of considerable interest to determine if the health benefits of probiotics can be attained without the risks associated with administration of a live organism. Reviewed here is the evidence that heat-killed, ultraviolet-inactivated, and even components of these agents may be just as effective and considerably safer for the host.

« A preparation of **inanimate microorganism** and/or their components that confers a **health benefit** on the host»



POSTBIOTIC Yeast

POSTBIOTIC Bacteria

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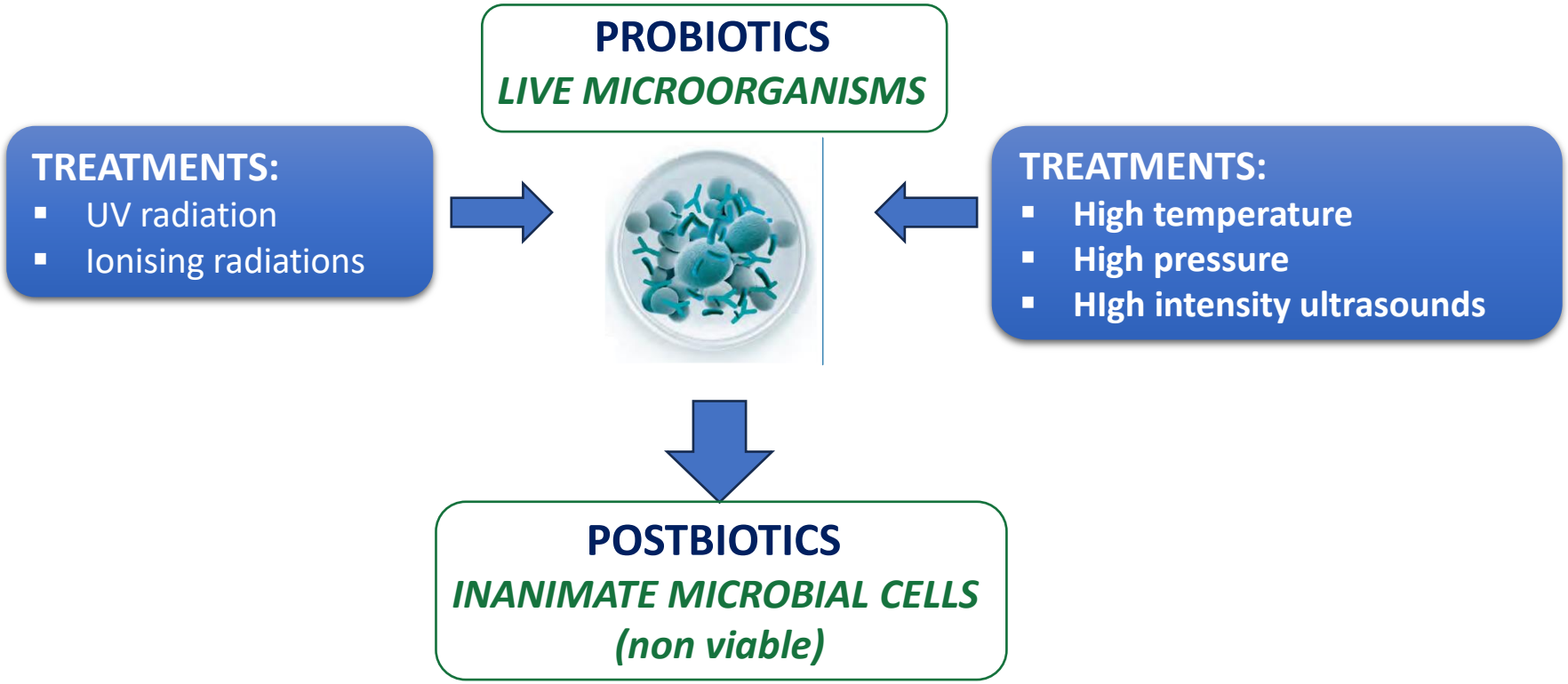
Postbiotics | Inactivation process

- Different methods of inactivation are described and may influence postbiotic biological activities
- Heat treatment is the method of choice.



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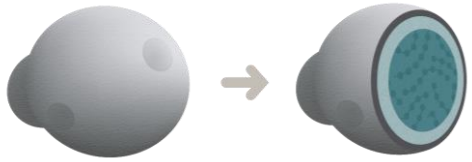
Adapted from Siliciano et al., 2021

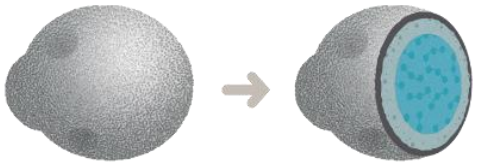
Postbiotic Yeast

WHOLE YEAST CELLS

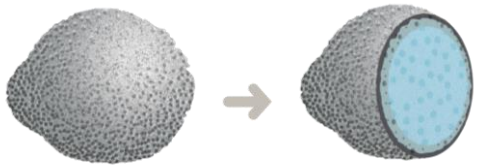
INACTIVATED YEAST



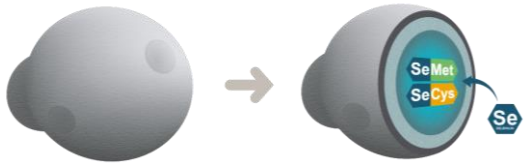
AUTOLYZED YEAST



HYDROLYZED YEAST

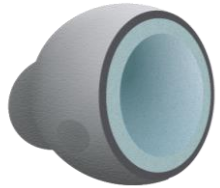


INACTIVATED ENRICHED YEAST



YEAST FRACTIONS

YEAST CELL WALL



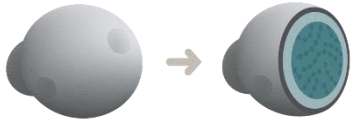
YEAST EXTRACT

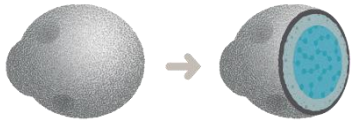
SPECIFIC ASSOCIATION OF YEAST FRACTIONS

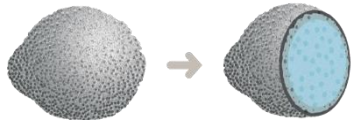
NOT all inactivated or live microorganisms have Post/Probiotic effects

→ Efficacy has to be demonstrated

Postbiotic Yeast | The whole cell yeast







	INACTIVATED YEAST No enzymatic processes	AUTOLYZED YEAST Endogenous enzymes only	HYDROLYZED YEAST Endogenous + exogenous enzymes
Characteristics	Whole cell wall surrounded yeast extract	• Cracked cell wall and intracellular content • Proteins and nucleotides partially fragmented	• Cell wall is broken • Peptides and nucleic acids are fragmented • Hydrolysis oriented and controlled: consistent product
Crude protein content	++	++	++
Digestibility	-	++	+++
Functionality	+	++	+++

Production process has an impact on yeast characteristics and functionality

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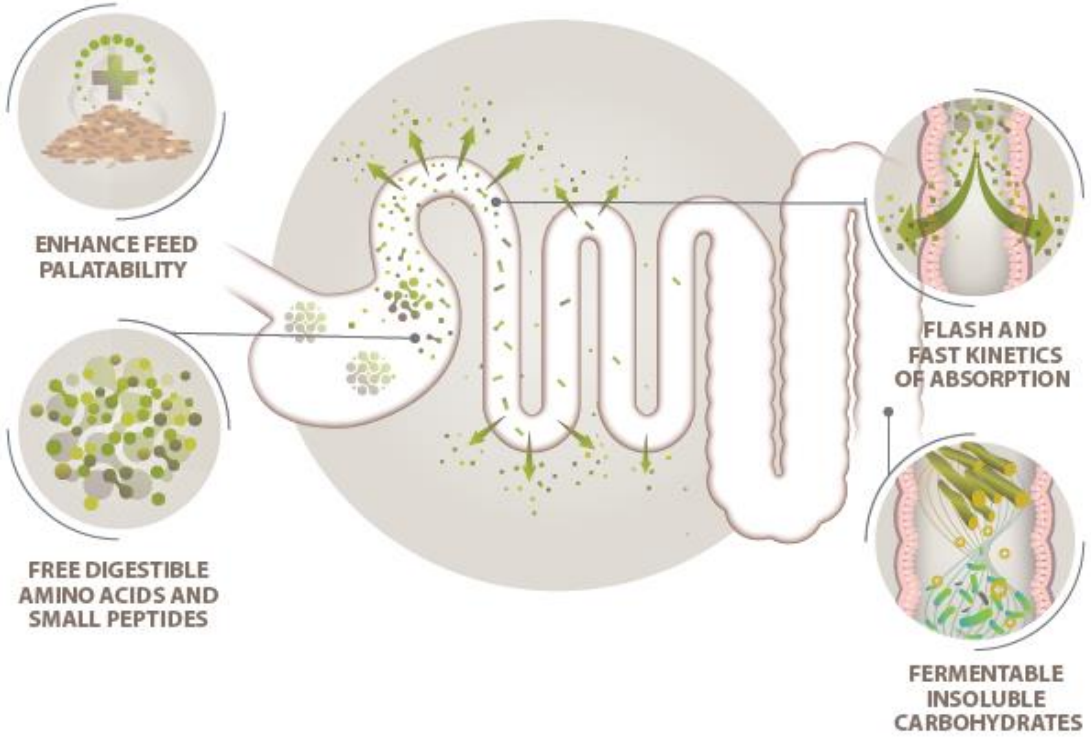
Postbiotic Yeast | The benefits of **hydrolyzed yeast**

DIGESTIVE CARE

Vit B Se Zn Fe Vit D Cr NUTRITIONAL OPTIMIZATION

A **specifically designed hydrolyzed yeast** offers highly digestible and functional nutrients that support digestive care and palatability while contributing to the feed protein balance.

YELA PROSECURE



- **In the upper gut**: highly digestible proteins (free amino acids and small peptides) → **early and fast amino acids absorption**

- **In the lower gut**:
  - ✓ decrease in proteolytic microbial compounds (i.e. ammonia)
  - ✓ fermentable insoluble carbohydrates → improved microbial diversity and activity with **release of SCFAs**

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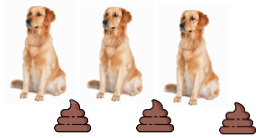
Postbiotic Yeast | The benefits of **hydrolyzed yeast**

DIGESTIVE CARE

Vit B Se Zn Fe Vit D Cr
NUTRITIONAL OPTIMIZATION

***In vitro* trial | Protocol**

3 donors*



3 groups & 3 replicates

Control

Inulin 10g/L

YPSi 10g/L**

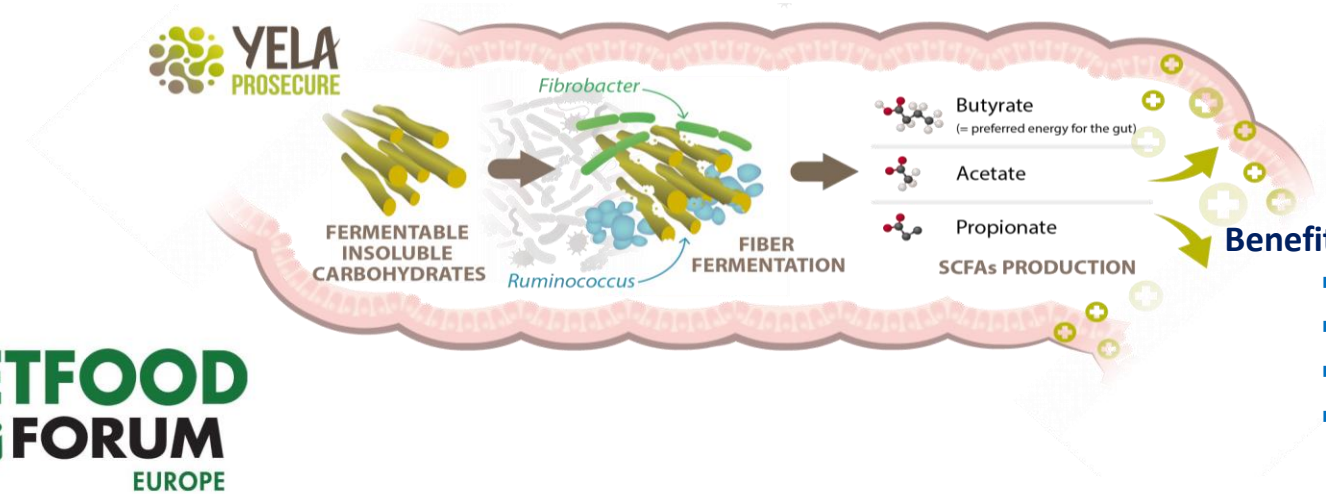
On each replicate SCFAs were quantified by gas chromatography after a 24-hours incubation at 39°C

*Same breed and breeding facility, age (13-17 months), diet since birth
**YPSi: YelaProsecure insoluble fraction

SCFAs	T0	T24 - Control	T24-Inulin	T24-YPSi
Acetate	2.3 ± 0.4^{a}	8.7 ± 0.6^{a}	32.8 ± 19.3^{b}	44.2 ± 2.2^{b}
Propionate	1.6 ± 0.4^{a}	2.2 ± 0.4^{b}	1.4 ± 0.3^{a}	22.2 ± 4.6^{c}
Butyrate	0.4 ± 0.2^{a}	1.2 ± 0.2^{ab}	2.1 ± 1^{b}	6.1 ± 1.9^{c}
Iso-butyrate	0.04 ± 0.04^{a}	0.1 ± 0.08^{b}	0.02 ± 0.01^{a}	0.6 ± 0.1^{c}
Valerate	0.07 ± 0.04^{a}	0.08 ± 0.05^{a}	0.08 ± 0.05^{a}	0.2 ± 0.02^{b}
Iso-valerate	0.09 ± 0.05^{a}	0.3 ± 0.1^{b}	0.06 ± 0.04^{a}	0.8 ± 0.1^{c}
Total SCFAs	4.5 ± 0.4^{a}	12.6 ± 0.8^{a}	36.6 ± 19.7^{b}	74.1 ± 6.9^{c}

- concentration in mM
- significant (P<0.05) difference between groups are shown with different superscript letters.

Results show that this specifically designed hydrolyzed yeast can be source of **functional dietary fiber** able to **support non-selective SCFAs production.**



Benefits of short chain fatty acids (SCFAs):

- Optimal gut integrity
- Closed tight junctions
- Mitigation of inflammation
- Modulation of gut microbiota

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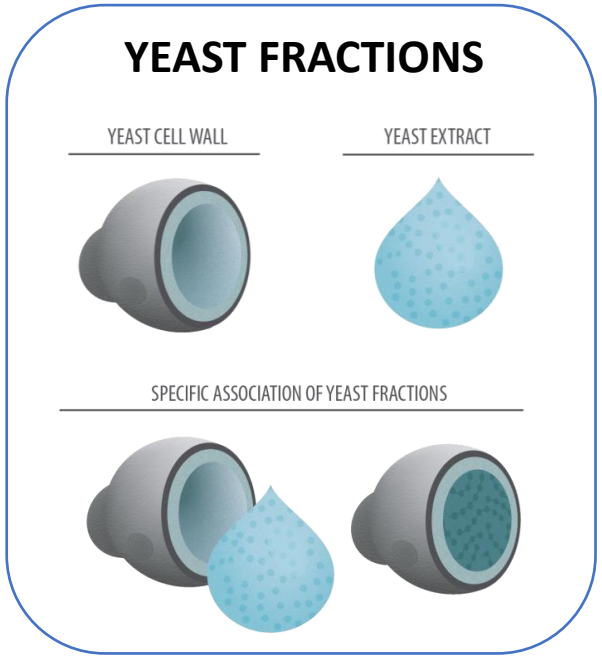
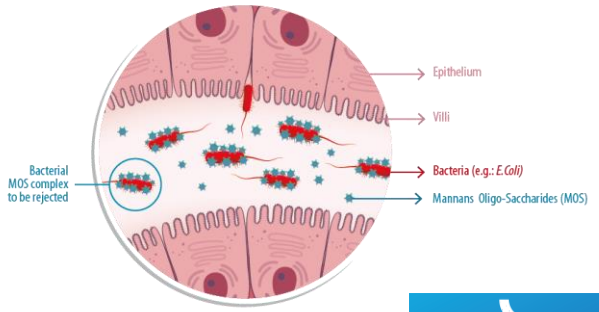
Postbiotic Yeast | The benefits of **yeast fractions**



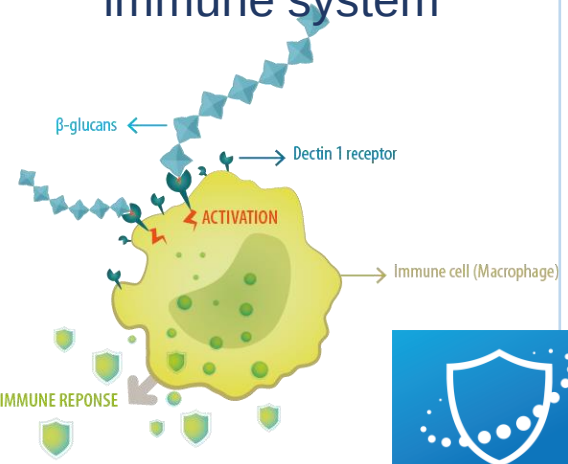
The **insoluble** fraction of the yeast

✓ **MOS** can bind undesirable bacteria



DIGESTIVE CARE

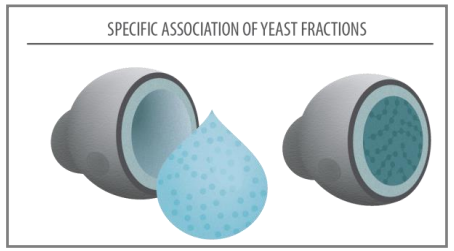
✓ **ß-glucans** helps modulate the innate immune system



IMMUNE SUPPORT

The efficiency of YCWs is not only about MOS and β-glucans levels. Strain, origin and production process may also influence product efficacy and batch-to-batch consistency

Postbiotic Yeast | The benefits of **yeast fractions**

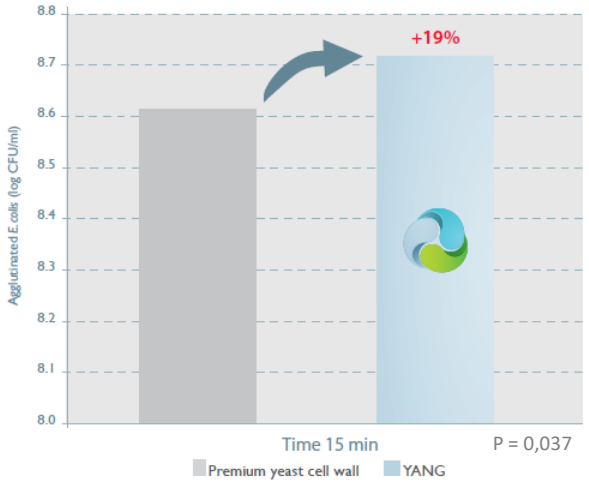


A **synergistic alliance** of yeast fractions

A combination of expertise, science, technology and innovation allow to select inactivated yeast fractions according to their complementarity and expected synergism.

IN VITRO TRIAL

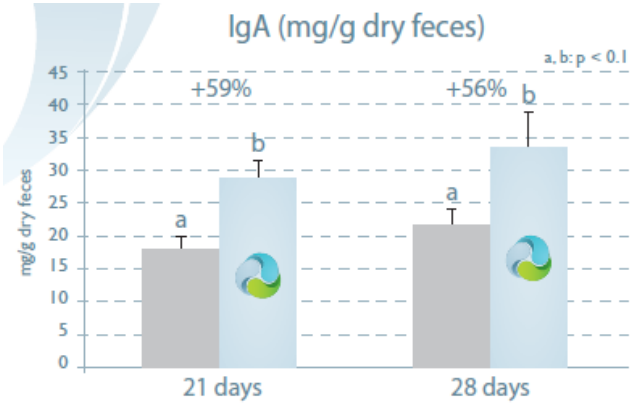
- ✓ Binding capacity: **0,08% YANG** vs **0,2% MOS** Product (Mannans >25%)
- ✓ 5 strains of E. coli isolated from dog/cat faeces at different lifestages
- ✓ Strains collected during episodes of enteritis



DIGESTIVE CARE

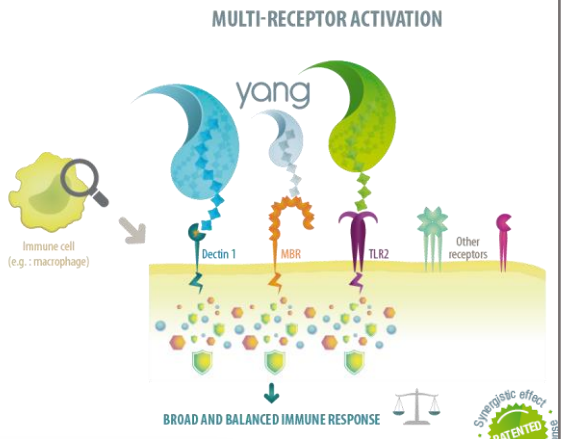
IN VIVO TRIAL

Study conducted by Prof Biagi at DIMEVET of University of Bologna – 2018



DURATION	28 days.
ANIMALS	16 healthy adult dogs - 1 to 8 years old.
TREATMENT	0.8 g YANG /kg petfood.
MEASURED PARAMETERS	Fecal IgA and pH at 21 and 28 days.

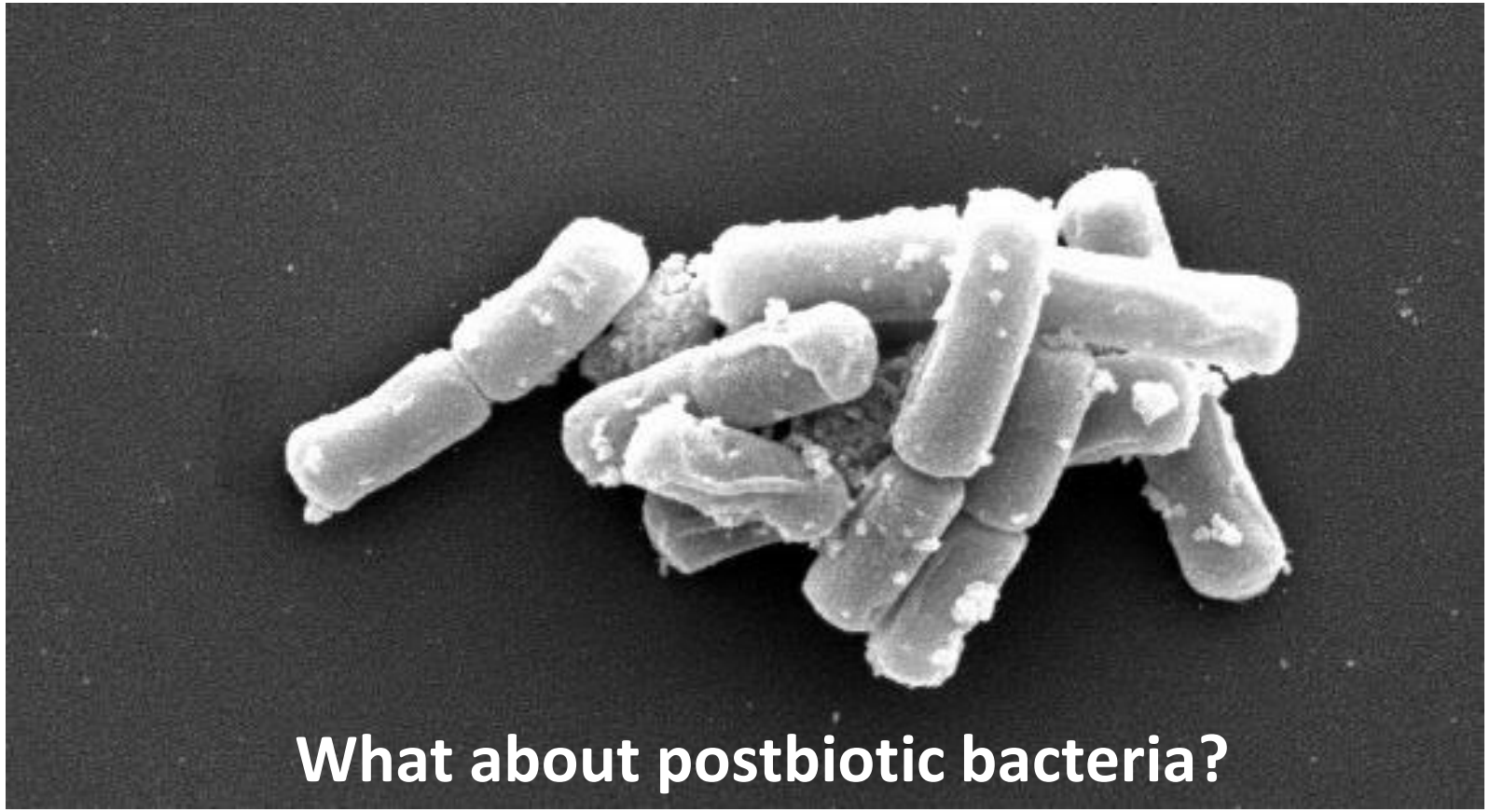
IgA plays an important role in maintenance of gastrointestinal homeostasis by coating the bacteria, favouring a tolerant, non-inflammatory relationship with the host and the homeostatic control of the intestinal redox environment.



IMMUNE SUPPORT

Strains combination + Multi-receptor activation = SYNERGY

Postbiotic Bacteria



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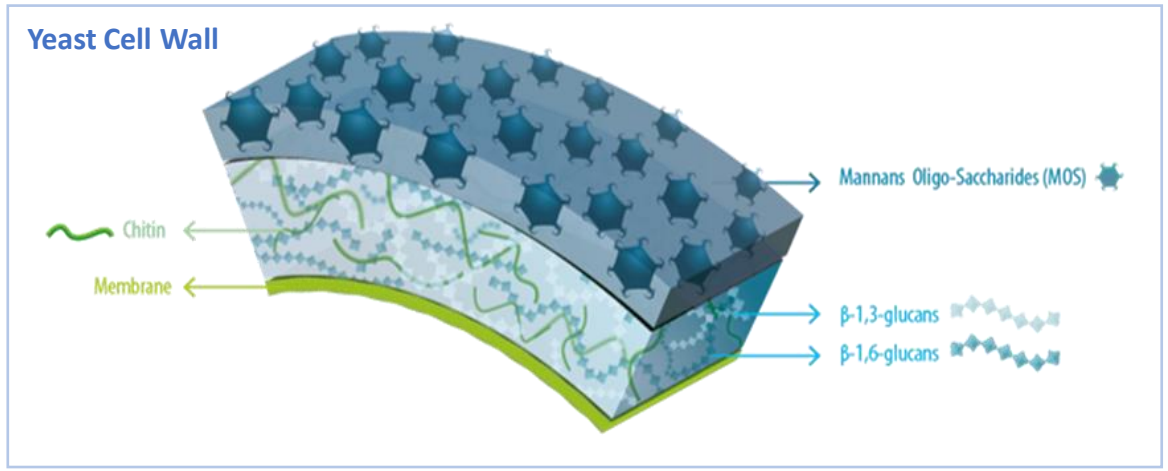
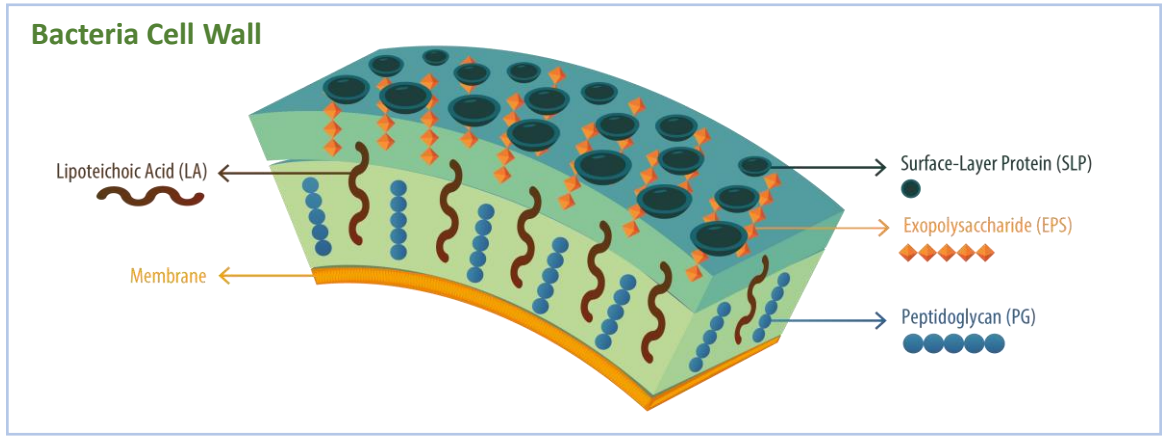
Postbiotic Bacteria | Bacteria cell wall components





Bacteria cell wall and its main components are very different from yeast cell wall:

- **PG** accounting for up to 90% of Gram+ bacteria weight, are **well-known potent activator of immune response.**
- **SLP** are **determinant in the adhesion events** in *Lactobacilli*.
- **EPS** playing a role in intestinal homeostasis via **interaction with intestinal epithelial cells.**
- **LA** is a IL-12 inducer, thus responsible to **activate the innate immune functions.**

The identification of key cell wall components is needed together with the assurance that these molecules maintain their activity after the heat treatment.

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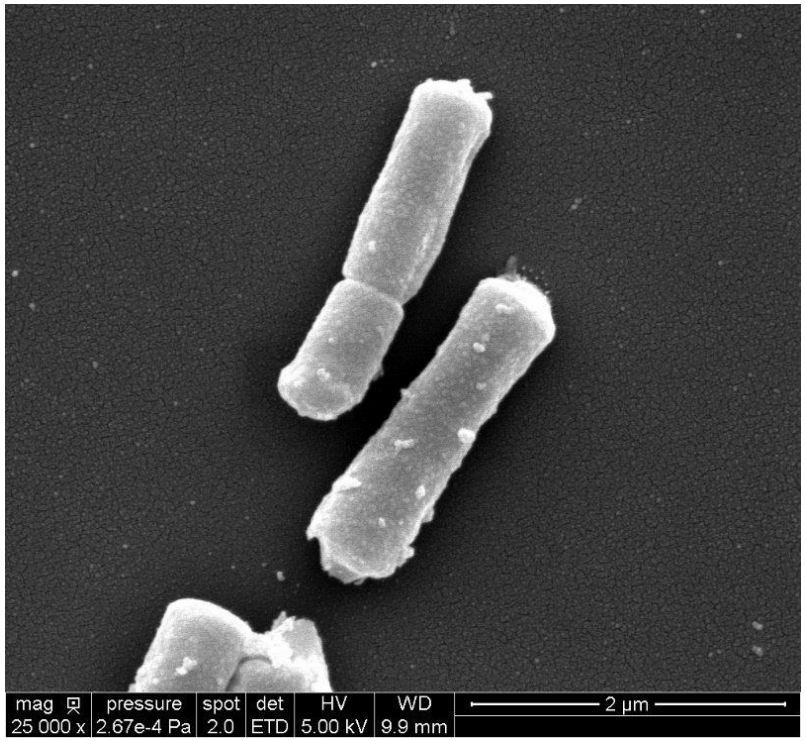
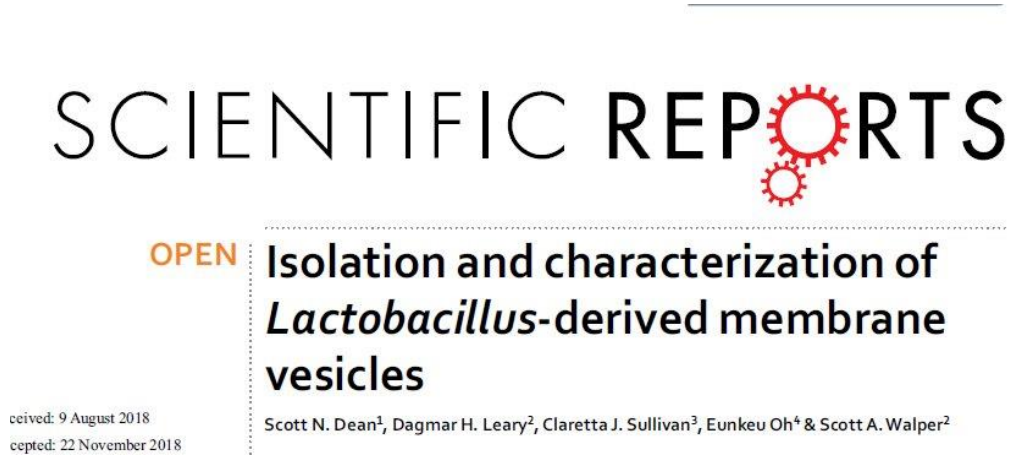
Pique et al., Int. J. Mol. Sci. **2019**, 20, 2534

Postbiotic Bacteria | Other components



- Metabolites, cell fractions, and culture supernatants.
- Cell-free supernatants contain batch culture medium, metabolites, and other secreted products that can cross the mucus layer.
- Microvescicles: cytoplasmic constituents (RNA, protein and DNA)


SCIENTIFIC REPORTS

OPEN

Isolation and characterization of *Lactobacillus*-derived membrane vesicles

ceived: 9 August 2018
cepted: 22 November 2018

Scott N. Dean[1], Dagmar H. Leary[2], Claretta J. Sullivan[3], Eunkeu Oh[4] & Scott A. Walper[2]

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Ref: Pique et al., Int. J. Mol. Sci. **2019**, 20, 2534

Postbiotic Bacteria | Why?

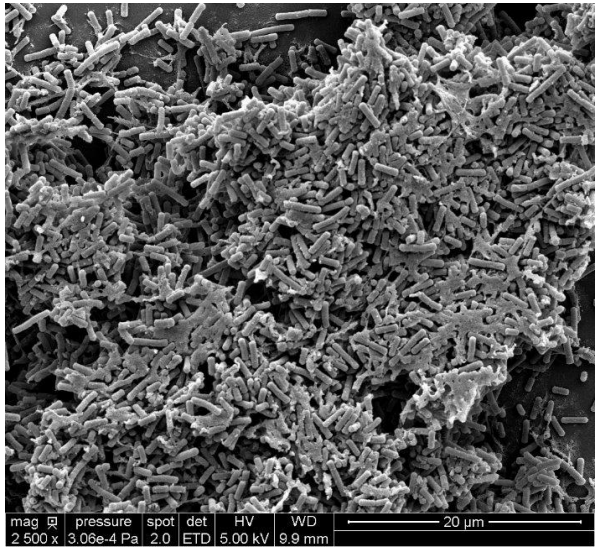

Table 1. Advantages of inactivated bacteria and/or purified compounds in comparison with live probiotics.

Aspect	Advantages
Safety	No risk of translocation from gut lumen to blood, particularly in vulnerable subjects. No risk of acquisition and retransfer of antibiotic resistance genes. No risk of interference with normal colonization of gut microbiota in neonates.
Physiological effects	Release of active molecules from the disrupted inactivated cells, passing through the mucus layers and stimulating epithelial cells more directly. Loss of viability and cell lysis can produce further and more complex beneficial effects.
Pharmaceutical characteristics	Easier to standardize, transport, and store.

Pique et al., Int. J. Mol. Sci. **2019**, 20, 2534

Additional Advantages in the Pet Industry:

- Technological: Stable through all prod process and over shelf life
- Regulatory: Feed materials (EU), market access

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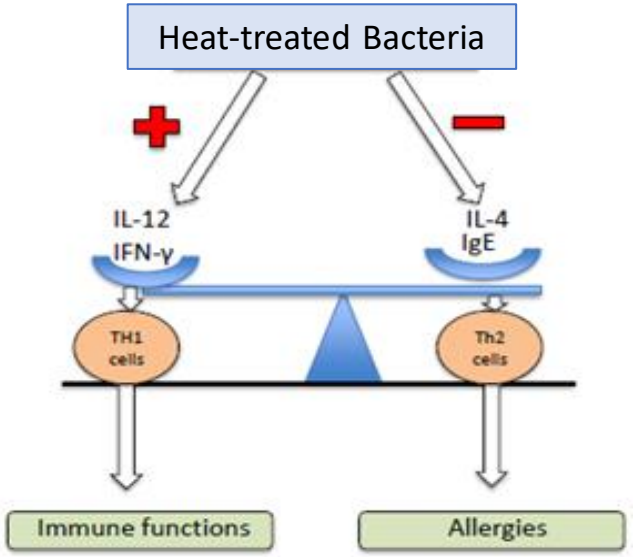
Postbiotic Bacteria | Mode of actions and expected benefits

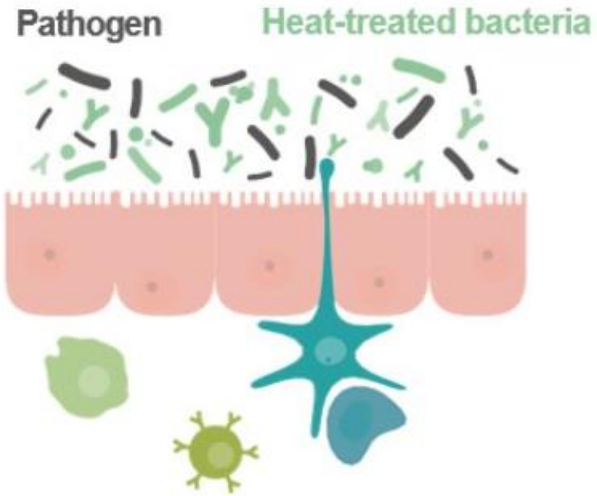
IMMUNE SUPPORT

- PG, SLP, EPS, LA give rise to responses **helping stimulate the immune system.**
- Increase of **antimicrobial peptides**
- Optimization of **CD4:CD8 ratio**
- Able to turn a **Th2 response into a Th1 one**



DIGESTIVE CARE

- **Adhesion to epithelial cell lines**:
  - competition with pathogens for adhesion sites
  - Cross-talk
- **Strenghtening of gut barrier integrity** mainly mediated by EPS and SLP



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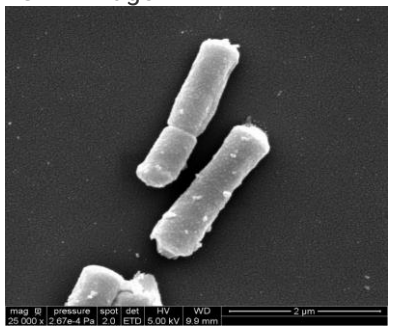
Pique et al., Int. J. Mol. Sci. **2019**, 20, 2534

Postbiotic Bacteria | Dedicated research program

A dedicated research program on a range of selected non-viable bacteria strains aimed to investigate mode of action and benefits of Heat Treated Bacteria

SEM image



LALPROBIOME *L. helveticus* HA-122

The strain-specific gentle heat-treatment process applied:

- enables to preserve the molecular membrane and cell structure whilst inhibiting their capacity to replicate
- ensures the production of microbiologically non-viable, shelf stable, yet functionally active microbial cells.

Adhesion capacity (*in vitro*, internal data)

Strains	HT29* (Colon)	Caco2* (Colon)	FHs74* (Small intestine)
LALPROBIOME *L. helveticus* HA-122	++++	++++	++++
LALPROBIOME *L. paracasei* HA-108	++	++	+++
LALPROBIOME *L. plantarum* HA-119	++	++	+++

Legend: **++++** $X \geq 40 \times 10^6$ cells; **+++** $10^7 \leq X < 40 \times 10^6$ cells; **++** $10^5 \leq X < 10^7$ cells; **+** $10^3 \leq X < 10^5$ cells

*human cells lines

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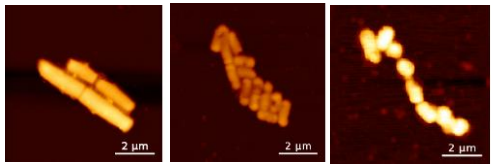
Postbiotic Bacteria | Dedicated research program



Specificity/Quality/ Structure/Function

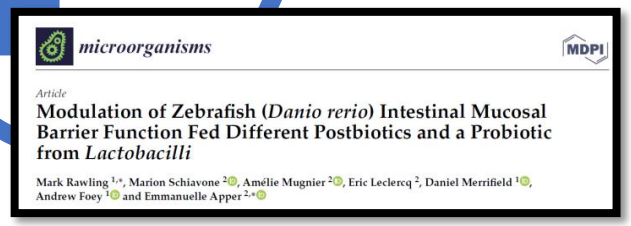
- **Main features** of heat treated bacteria strains
- **Components** explaining their efficacy

In depth characterization

Characterization of the effects

- **Understanding the mode of action on a phenotyping *in vivo* model**
- **Challenged** or not

➔ **Our phenotyping models confirm the potential of the strains tested**



- ***In vitro* and *in vivo* trials in companion animals**

Validation in the target animals

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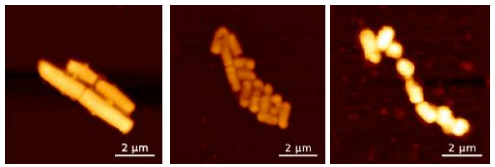
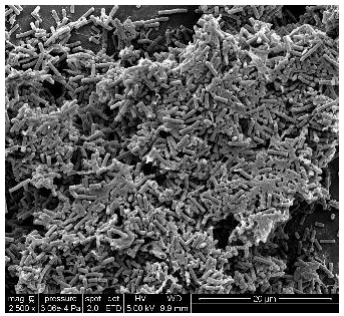
Postbiotic Bacteria | Strain characterization

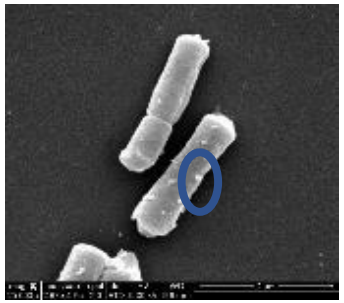


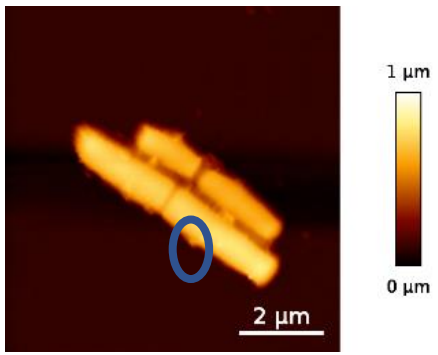
Specificity/Quality/ Structure/Function

- **Main features** of heat treated bacteria strains
- **Components** explaining their efficacy

In depth characterization







- Cells are intact, cell surface integrity is maintained, surface is smooth and rigid (elasticity of 20 MPa)
- Observation of aggregates
- Strain specificity in cell morphology
- Observation of strain specific particles associated with or proximal to the cells at nanometric range

→ Presence of micro vesicles containing proteins described as mediators of Lactobacillus probiotic effect

?

p40 = surface antigen protein
p75 = cell wall-associated hydrolase

→High-resolution AFM structural image

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Postbiotic Bacteria | Proof of concept with phenotyping model

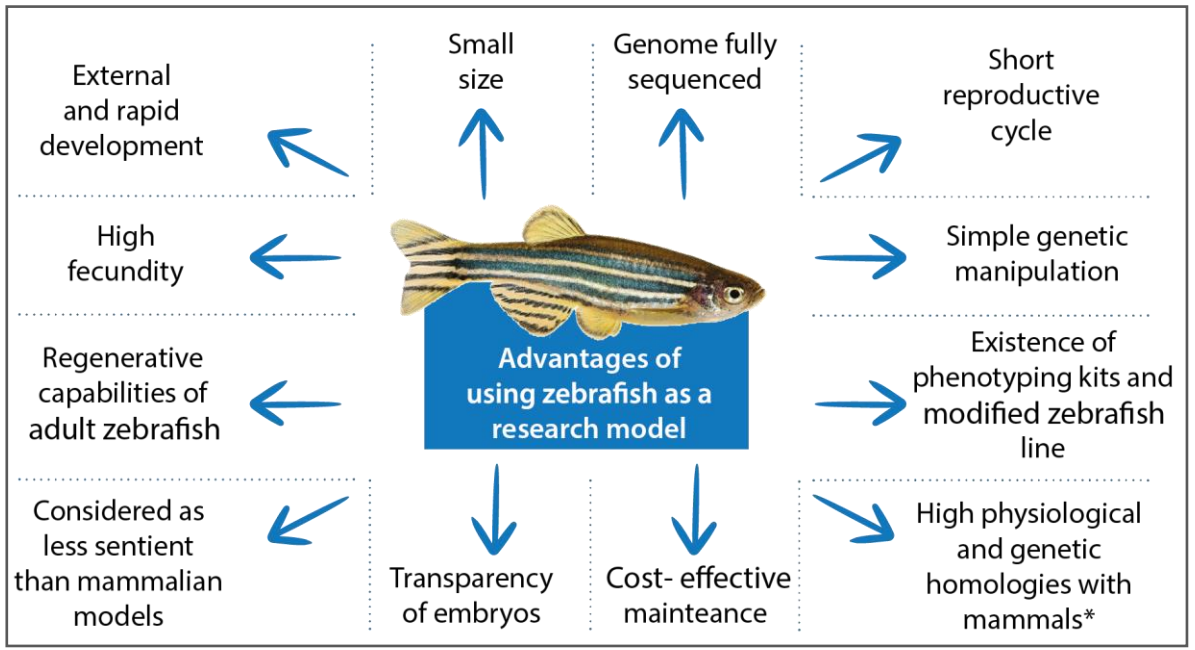
LALPROBIOME

WHY USE THE ZEBRAFISH MODEL IN MAMMALIAN RESEARCH?

- **Understanding the mode of action on a phenotyping *in vivo* model**
- **Challenged** or not

Characterization of the effects

Zebrafish, a model in mammalian research



*genome, immune and nervous systems, nutrient metabolism, intestine structure and functionality, cell differentiation or proliferation

- The replacement of mammalian models by animals considered as less sentient is frequently considered.
- The use of the zebrafish (*Danio rerio*) has exponentially increased among the scientific community over recent decades.

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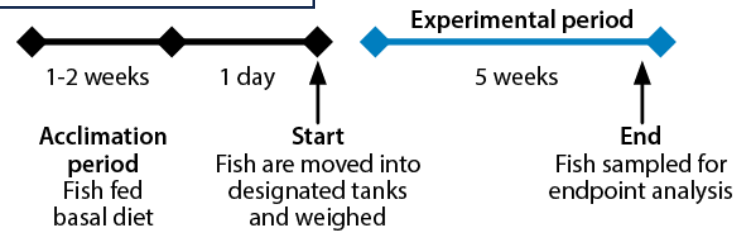
Postbiotic Bacteria | Proof of concept in healthy animal model

PROTOCOL

- **Animals**: Wild-type adult zebrafish (Danio rerio); 25 fish / tank
- **Duration:** 7 weeks
- **Feeding program:**



CONTROL	LHPro	LHPost	LPPost
Basal diet	Basal diet + **LALPROBIOME *L. helveticus* - HA122** at 6x10^6 CFU/g feed	Basal diet + **LALPROBIOME *L. helveticus* - HA122 (Ht)** at 6x10^6 cells/g feed	Basal diet + **LALPROBIOME *L. plantarum* - HA119 (Ht)** at 6x10^6 cells/g feed

- **Measured parameters:**

✓ Goblet cell density (GCD) and coverage (GCC)

✓ Levels and gene expression of innate immune markers (Lysozyme and Cathepsin) and gene expression of tight junction protein (ZO-2a)

✓ Intraepithelial leukocytes (IELs; CD4+/CD8α+) quantification

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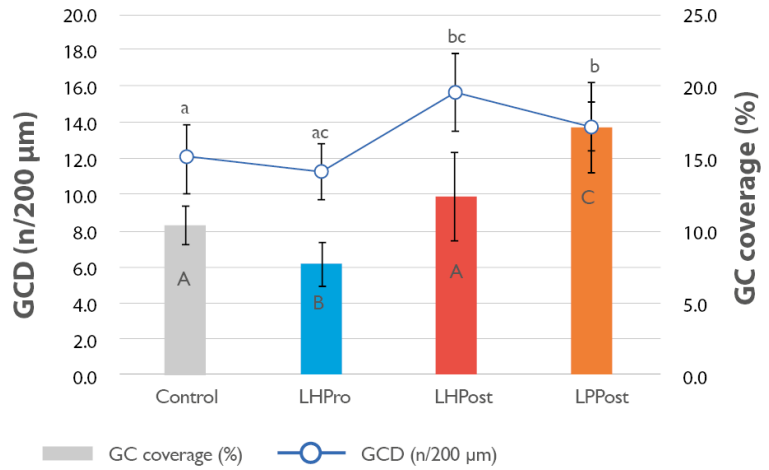
Postbiotic Bacteria | Proof of concept in healthy animal model

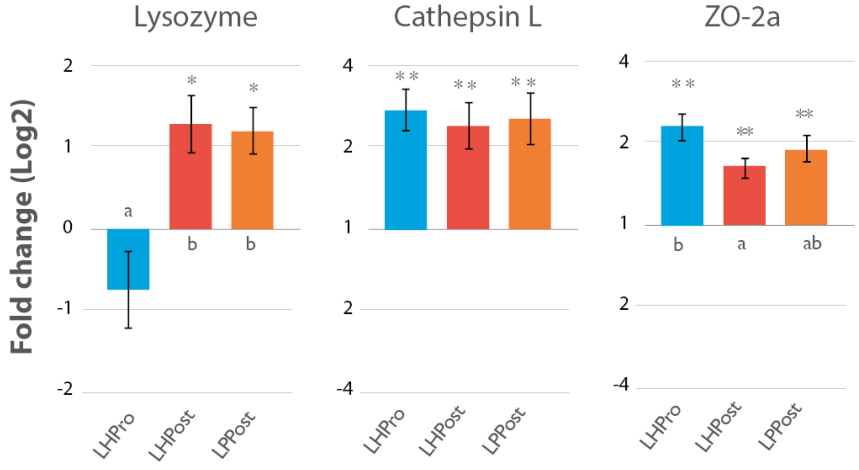
RESULTS

Gut goblet cell coverage (GC) and density (GD)



Both groups supplemented with heat-treated bacteria (LHPost and LPPost) showed an **increase in goblet cell coverage and density** indicative of the ability of **the specific heat-treated strains to enhance the functional mucus barrier**.

Gene expression of innate immune markers and tight junction ZO-2a



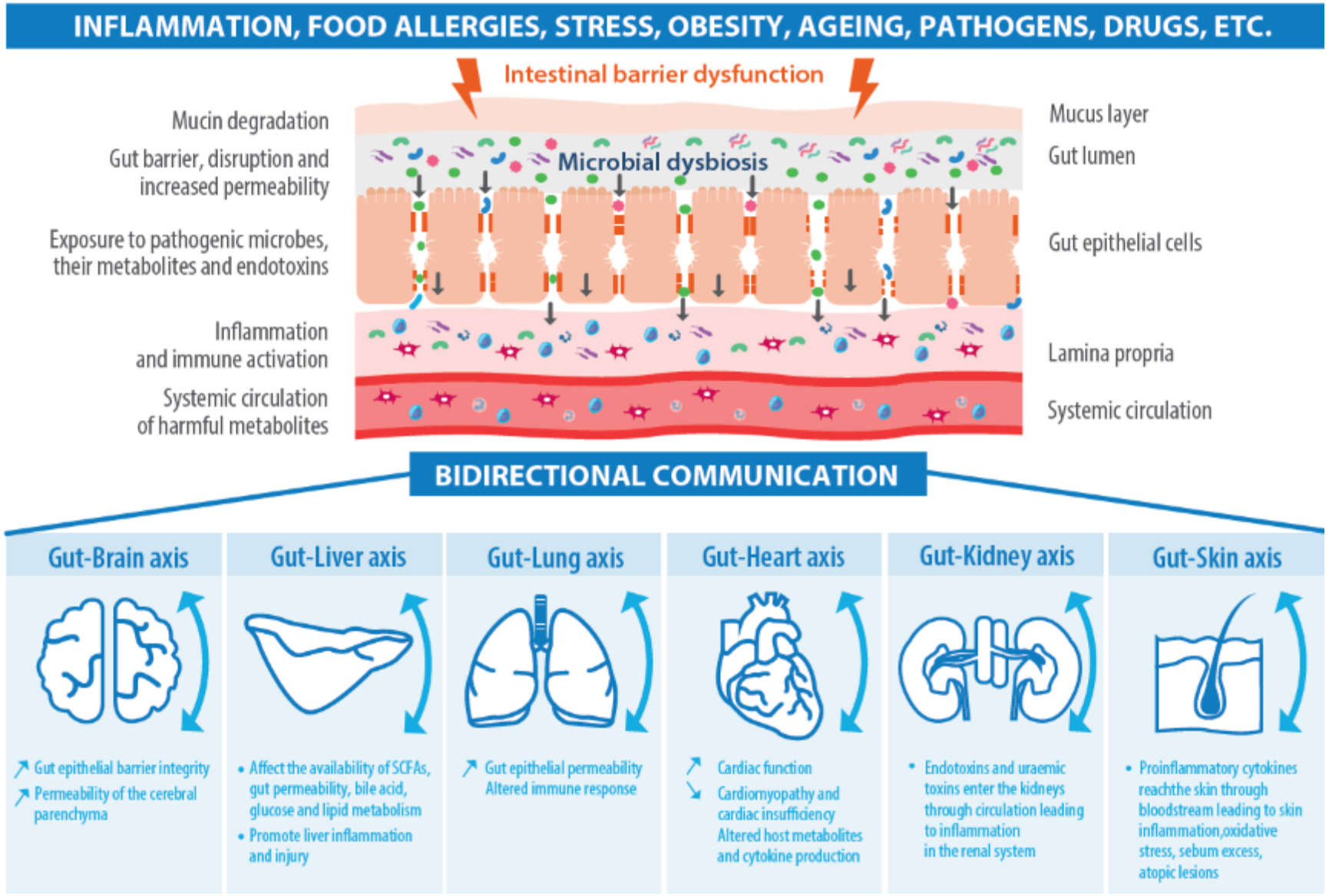
Elevations in protein gene expression levels of lysozyme and cathepsin L were shown in both postbiotic groups while an increased gene expression level of Zonula-Occludin (ZO-2a) was demonstrated in all treatments confirming the **protection and strengthening of the gut barrier in groups supplemented with selected postbiotic strains.**

These results confirm the **protection and strengthening of the gut barrier in groups supplemented with selected postbiotic bacteria strains.**

Postbiotic Bacteria | The importance of gut barrier integrity

Pivotal role of gut barrier and gut microbiota in health and disease.

"All disease begins in the gut"
(Hippocrates, -460 to –377)



Adapted from Saxami et al. (2023)

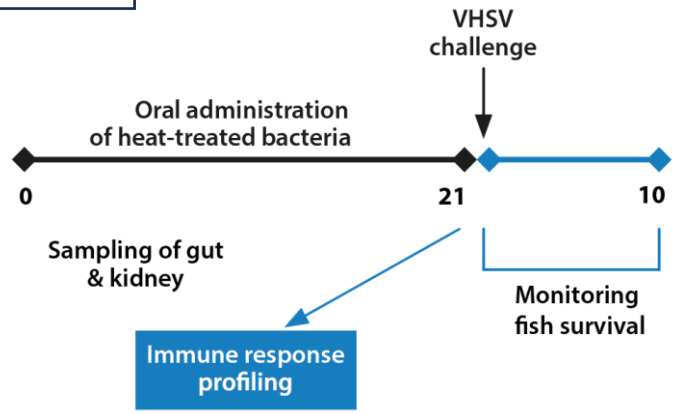
Postbiotic Bacteria | Proof of concept in challenged animal model

Viral challenges

PROTOCOL

- **Animals**: Wild-type adult zebrafish (Danio rerio); 28 fish / tank
- **Duration:** 1 month
- **Feeding program:**

CONTROL 1	CONTROL 2	*L.Paracasei*	*L.plantarum*	*L.helveticus*
Basal diet	Basal diet Challenge with viral hemorrhagic septicemia virus (VHSV)	Basal diet Challenge with VHSV + **LALPROBIOME *L. paracasei* HA-108** at 6x10^6 cells/g feed	Basal diet Challenge with VHSV + **LALPROBIOME *L. plantarum* HA-119** at 6x10^6 cells/g feed	Basal diet Challenge with VHSV + **LALPROBIOME *L. helveticus* HA-122** at 6 x 10^6 cells/g feed



- **Measured parameters:**

✓ mRNA expression of antimicrobial and antiviral factors in gut and kidney

✓ Survival rate after challenge with viral hemorrhagic septicemia virus (VHSV)

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Postbiotic Bacteria | Proof of concept in challenged animal model

IMMUNE SUPPORT

RESULTS

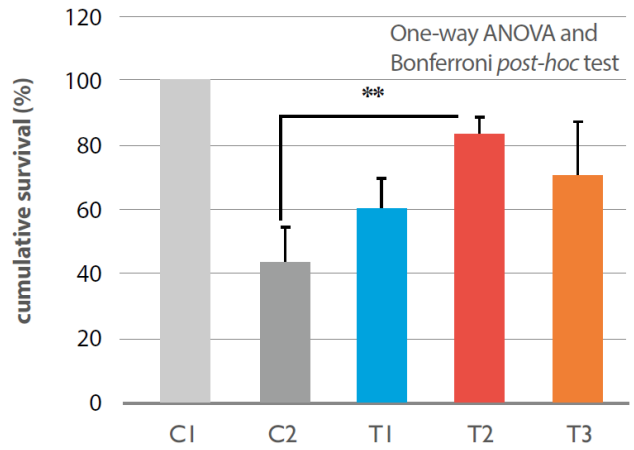
Heatmap of mRNA expression in gut and kidney (21d suppl.)

GENE CATEGORY	GENE NAME	ORGAN	T1	T2	T3
Antiviral	Interferon gamma (ifnγ)	Gut			
		Kidney			
	Interferon gamma1 (ifnγ1)	Gut			
		Kidney			
	Mx	Gut			
		Kidney			
	CD8a	Gut			
		Kidney			
Antimicrobial	β-defensin β1 (def β1)	Gut			
		Kidney			
	Mucin 5.1 (muc5.1)	Gut			
		Kidney			

Heat-treated ***L. plantarum*** **HA-119 enhanced the up-regulation of all the selected immune genes**, dedicated to antiviral and antimicrobial responses, in both gut and kidney.

T1: *L. paracasei HA108*; **T2**: *L. plantarum HA119*; **T3** *L. helveticus* HA122

Fish cumulative survival 10 days post challenge



Significant increase in survival when fish were supplemented with *L. plantarum* HA-119 confirming the **immunomodulatory properties of specific strains of heat-treated bacteria.**

The study shows the **immunomodulatory strain-dependent properties** of selected heat-treated bacteria.

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Legenda

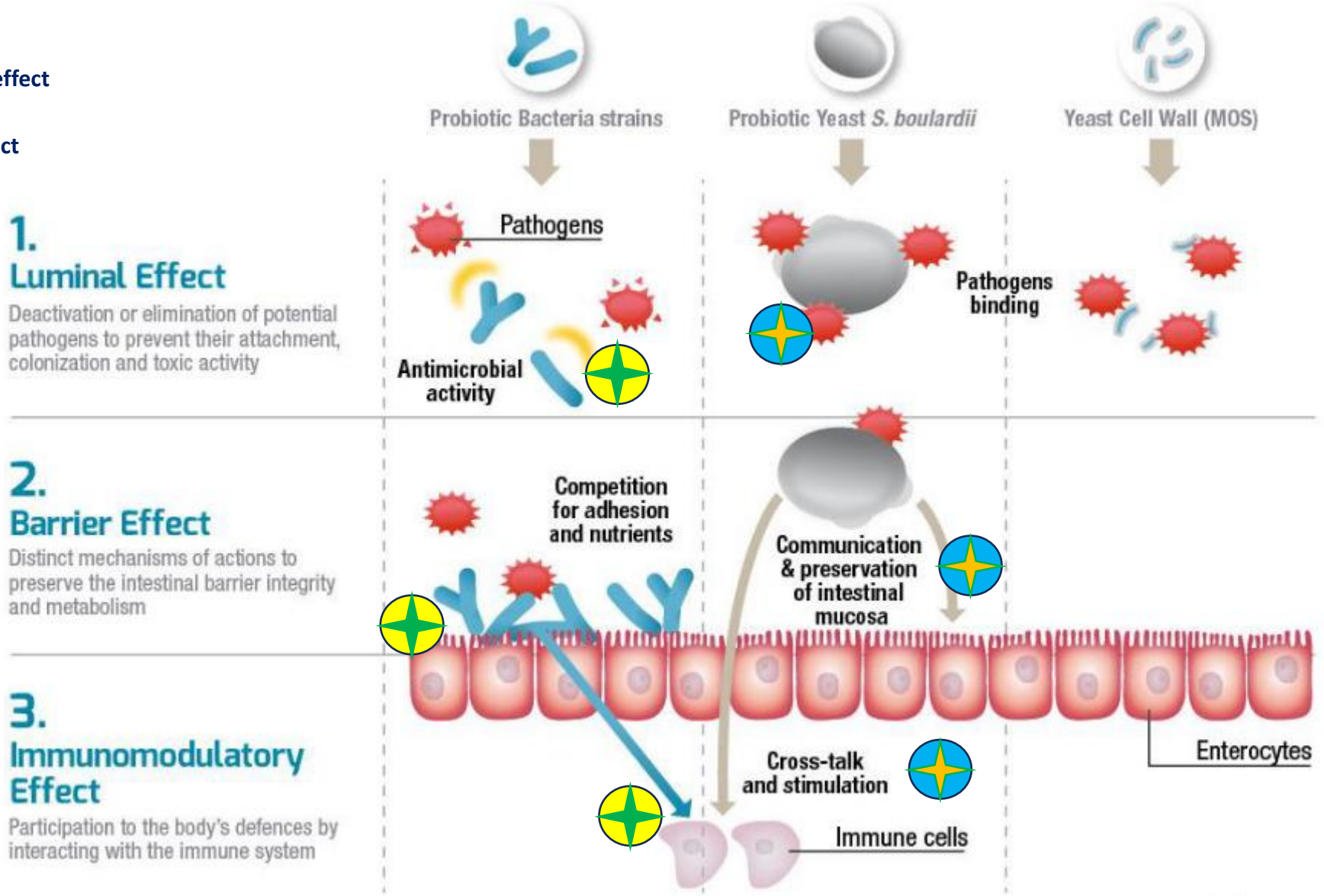
Basal expression	Up-regulation	Up-regulation
1,00 - 1,49	1,50 - 1,99*	> 2,00**

Fold range compared with control groups

Postbiotic Yeast and Bacteria | Conclusions

POSTBIOTIC Bacteria effect

POSTBIOTIC Yeast effect



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Postbiotic Yeast and Bacteria | Conclusions

- Yeast and bacteria have different structure and composition thus different mode of action
- NOT all inactivated or live organisms have Post/Probiotic effects → efficacy has to be demonstrated
- Studies have clearly shown that, no matter if yeast or bacteria:
  - ✓ efficacy **is strain-related**
  - ✓ some **benefits** are **INDEPENDENT from viability**
- As demonstrated for PROBIOTICS, also for POSTBIOTICS we can foresee a **potential complementarity and synergism between yeast and bacteria**

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Thanks for your attention!

For further questions, please contact me at: *fsusca@lallemand.com*

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