INSIGHTS INTO EUROPEAN PET FOOD TRENDS AND INNOVATION

PETFOOD FORUM EUROPE

6 May, 2024

Nuremberg, Germany

Can yeast and yeast derivatives help reduce pathogenic pressure in companion animals?

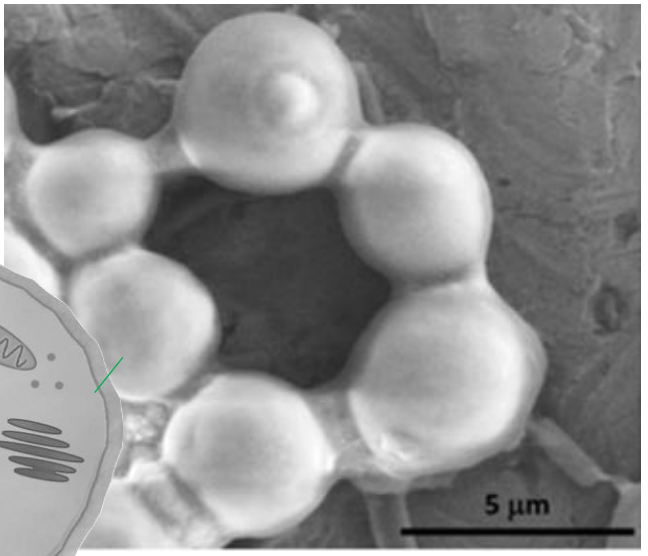
Dr Aleksandra Szopinska, Senior Innovation and NPD Manager, Livalta/AB Agri

#petfoodforum • PetfoodForumEvents.com/Europe

Co-located with Interzoo 2024

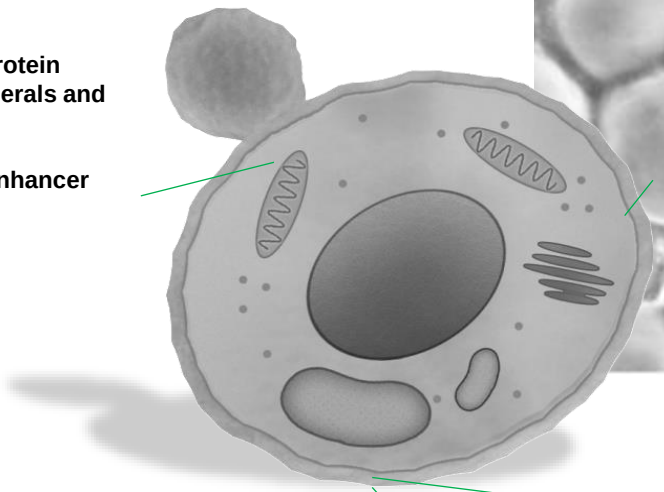
Let me introduce yeast

- *Saccharomyces cerevisiae* is the most widely used yeast species in pet food
- “Generally Recognized as Safe” (GRAS) status for food and feed applications
- Yeast cell can provide both nutritional and functional benefits
- **Cell interior** - nutrition
- **Yeast cell wall -** functional benefits



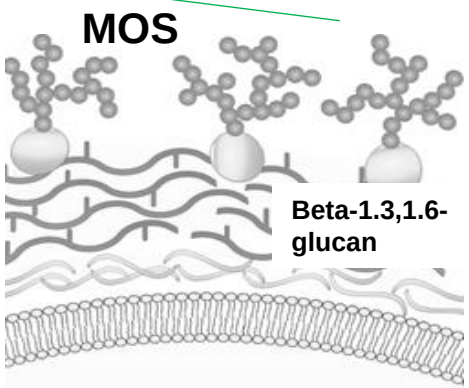
Cell interior

- **Alternative protein**
- **Contains minerals and vitamins**
- **Nucleotides**
- **Palatability enhancer**



Yeast cell wall

- **Binding of pathogenic bacteria**
- **Improvement of intestinal integrity**
- **Enhancer of immune function**
- **Prebiotic**
- **Mycotoxin binding**



PETFOOD FORUM EUROPE

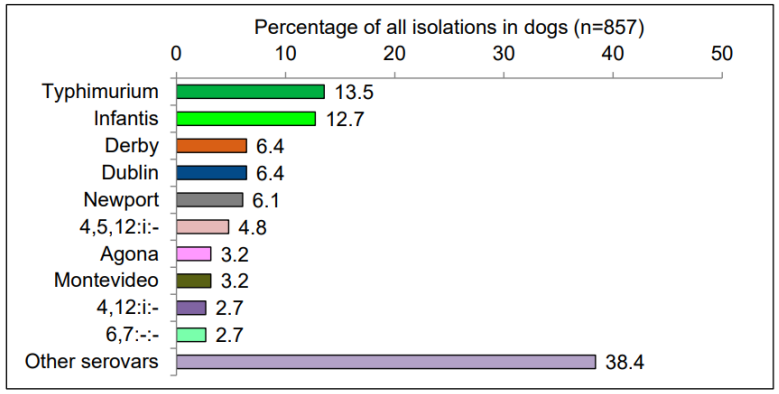
Resources: Jach et al., 2022; Sukhihasri et al., 2013,

Why is yeast important: it can support “gut health” and therefore be part of a strategy to reduce antimicrobial resistance

Antimicrobial resistance: key risk for pets & humans

- Antimicrobial resistance (AMR) - one of the most important human and animal health risks worldwide
- Dogs & cats can become ill when infected with bacteria, but are often asymptomatic carriers – risk of transmission
- Pathogens in pets reported to harbour resistance **to several antimicrobials***

Most common salmonella strains in dogs in the UK (2022)**



Need to reduce the use of antibiotics

- Restrictive measures like the ban by the European Union (EU) of antibiotics as growth promoters for farm animals
- **Prophylactic interventions:**

<u>gut health supporting solutions</u>
(e.g. based on yeast) can help reduce the use of antibiotics

* Marques et al., 2018; Gwenzi et al., 2021
Salmonella in animals and feed in Great Britain: 2022 (publishing.service.gov.uk), **Salmonella in dogs became reportable through amendments to the Zoonoses Order in the UK in 2001

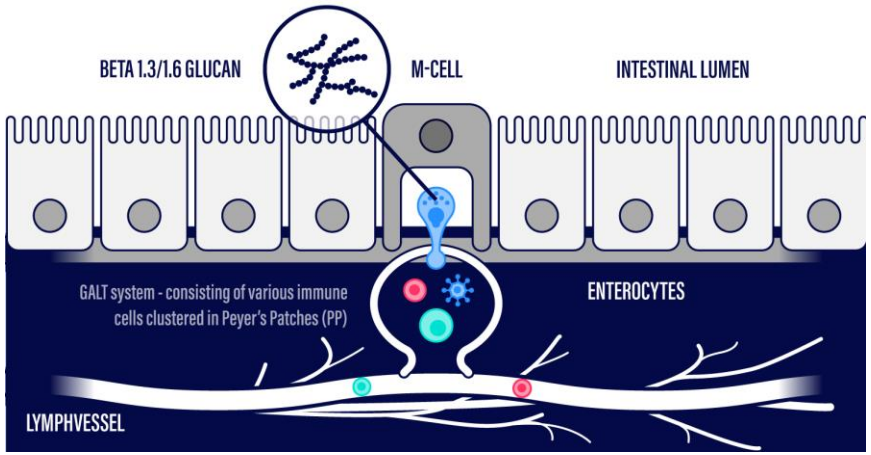
PETFOOD FORUM EUROPE

What is “gut health” and where do R&D activities focus to support it?

What is “gut health”?

- Optimal gut development
- Immune status/response (GALT)
- Gut morphology
- “Healthy” gut microbiome
- Ability to cope with external stress factors (pathogens, toxins, environmental stress factors)

Gut-associated lymphoid tissue (GALT) is an essential component of the body’s immune defense



Simplified schematic view of the intestinal immune system activation.

The gut-associated lymphoid tissue (GALT) system consisting of various immune cells (T-cells, B-cells, dendritic cells and M-cells) in the small intestine clustered in follicles known as Peyer's Patches (PP) (adapted from Volman et al., 2008)

Where R&D activities focus to support “gut health”

High priority R&D Activities globally to support gut health

- Probiotics
- Immune modulators
- Bioactive peptides
- Bacteriophages
- **Postbiotics and antiadhesive agents**

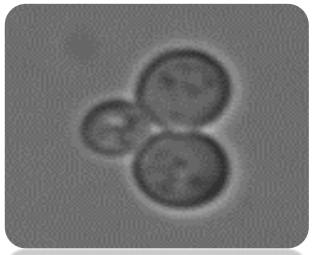
PETFOOD FORUM EUROPE

Yeast sources from dedicated fermentation & co-products

Co-product yeast

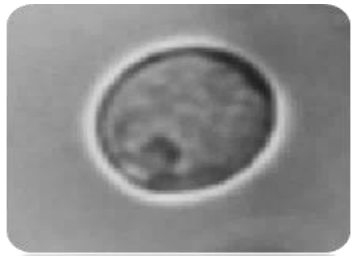
Dedicated fermentation

- Yeast grown as a **biomass**
- High Protein levels (>50)



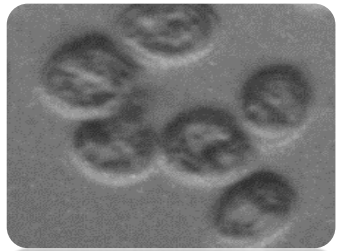
Brewer's Yeast

- **Co- product** from **brewing (circularity)**
- Moderate protein content (40-50%)
- Plant based material present, require pre-treatments



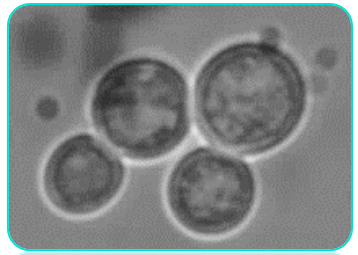
Pot Ale Yeast

- **Co- product** from **whisky production (circularity)**
- Moderate protein content (40-50%)
- Plant material present
- Bacteria metabolites present



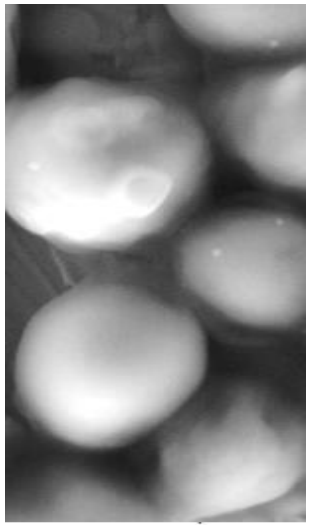
Bioethanol Yeast

- **Co-product** from **bioethanol production (circularity)**
- Moderate protein content (38-42%)
- Pure yeast
- Brazil: 300mills/500kT of spent yeast annually

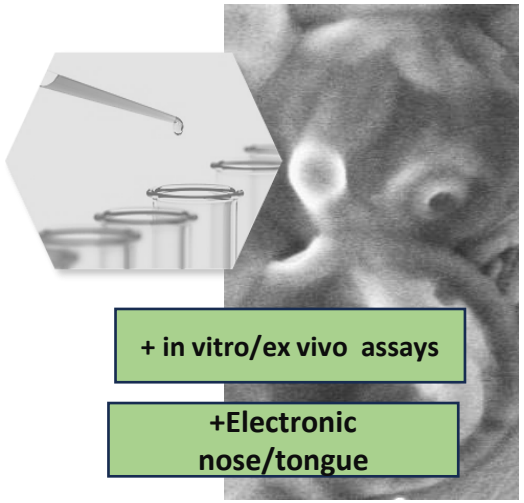


PETFOOD FORUM EUROPE

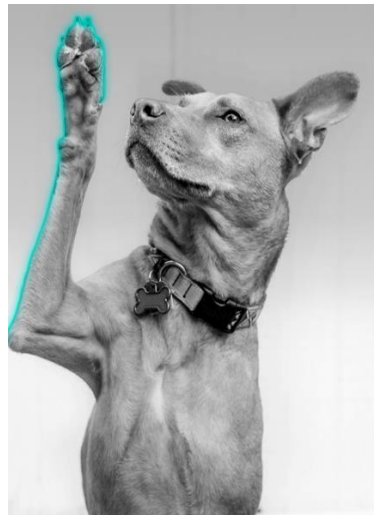
Key steps in the development of new yeast products



+



+



Raw material preselection

The basis to meeting highest quality and technical standards

- ✓ **Responsibly sourced**
- ✓ Pure yeast of consistent composition
- ✓ Strains able to synthetize **high level of bioactive components**

Process development – e.g. enzymatic screen

Process development to consistently achieve:

- ✓ High product/ protein digestibility and access of yeast cell content
- ✓ Improved **functional properties** – e.g. pathogen binding
- ✓ **Improved palatability** (electronic nose and tongue assisted study)
- ✓ Product benefits

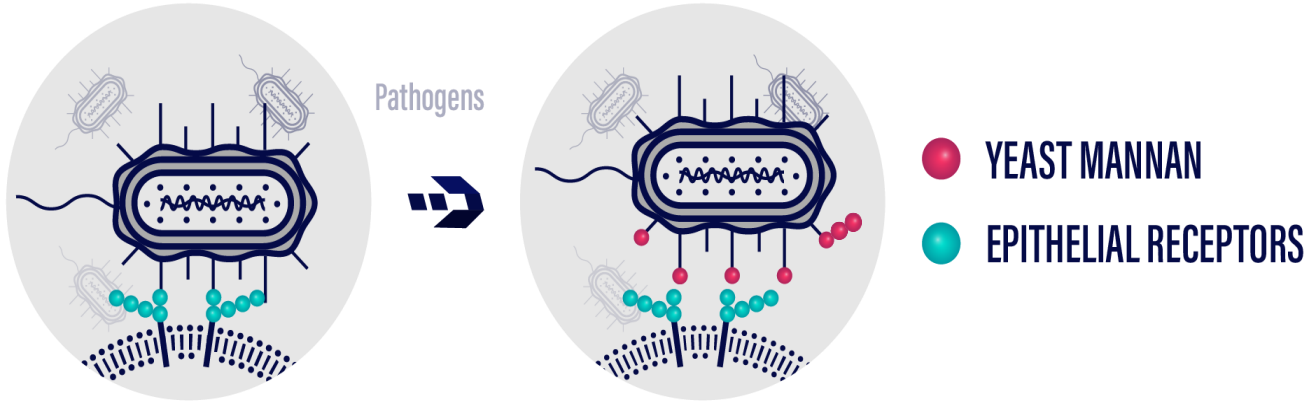
Final decision: do dogs & cats like the products & are the benefits really there?

In use testing

To ensure consistent product characteristics/ functionality/ palatability and health benefits in **animals**

PETFOOD FORUM EUROPE

A model of pathogenic bacteria adhesion inhibition by yeast-based products



More insights to binding mechanisms:

Ganner et al. (2008) discussed he importance of the **three-dimensional structure of mannose chains** on binding behaviour of different yeast-based derivatives

Shoaf-Sweeney and Hutkins (2008) link the adhesion affinity to the presence of **protein-oligosaccharide interactions**, which could lead to increased target avidity.

Ganner et al. (2013) Not only mannan-derived components but also **yeast glucan** content may be an important parameter affecting the capacity of yeast products to bind pathogenic bacteria

Downstream processing (as enzymatic treatment) of yeast can enhance yeast cells components functionality

According to published literature, the binding model assumes that mannose or mannose-derived entities (such as mannan-oligosaccharides: MOS), components of the yeast cell wall, have a high affinity for type I-fimbriae and thus compete with the pathogenic bacteria when present in the gut. References: Althouse et al., 2003; Westerlund-Wikström and Korhonen, 2005, Spring et al., 2000; Lessard et al., 2009; Soltanian et al., 2009)

Measurement of binding interaction: agglutination (microscopy) and *ex vivo adhesion* models

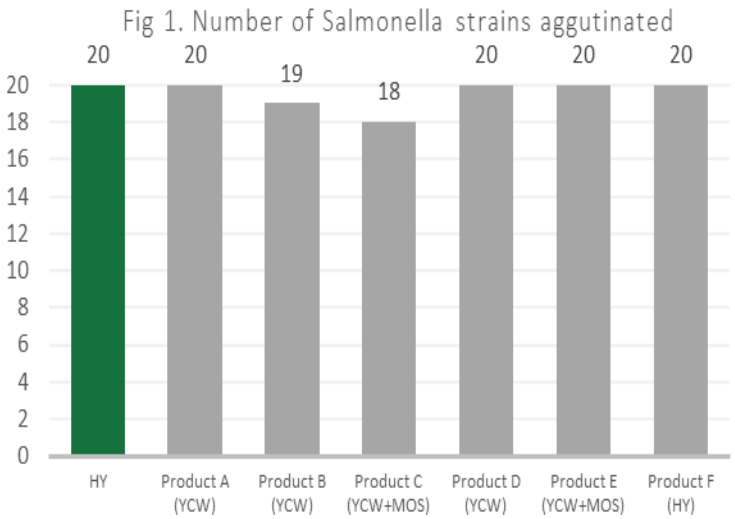
PETFOOD FORUM EUROPE

In vitro agglutination study shows interaction of yeast products with selected pathogenic bacteria strains

Pathogenic strains tested	
Salmonella	E.Coli
S. pullorum	E. coli 2430
S. ohio	E. coli 4923
S. heidelberg	E. coli 1923
S. panama	E. coli 4111
S. ouakam	E. coli ATCC 11229
S. rissen	E. coli ATCC 25922
S. agona	E. coli SB19/02
S. worthington	E. coli 2
S. tennessee	E. coli ATCC 10536
S. sandiego	E. coli SB 469/06
S. minnesota	E. coli SB 30/13
S. infantis	E. coli SB 294/02
S. muenchen	E. coli SB 33/09
S. cerro	E. coli 8
S. albany	E. coli 15
S. mbandaka	E. coli SB 57/13
S. thompson	E. coli SB 11/09
S. adelaide	E. coli ATCC 35218
S. enteritidis	E. coli 3
S. typhimurium	E. coli 4

Products Categories tested in the in the *in vitro* study :

- HY – hydrolysed yeast- Enzymatically treated yeast
- YCW- yeast cell walls
- YCW+MOS - Yeast cell
- walls preparation enriched with additional MOS
- HY- hydrolysed yeast (non- enzymatic approach)



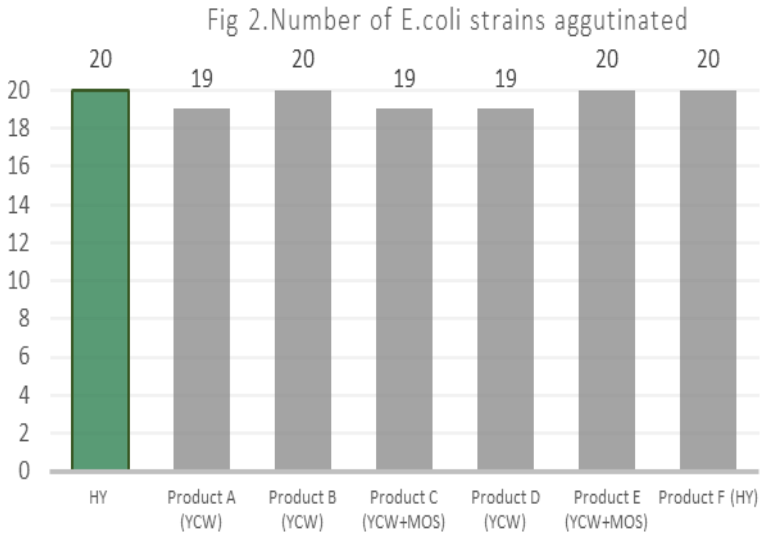
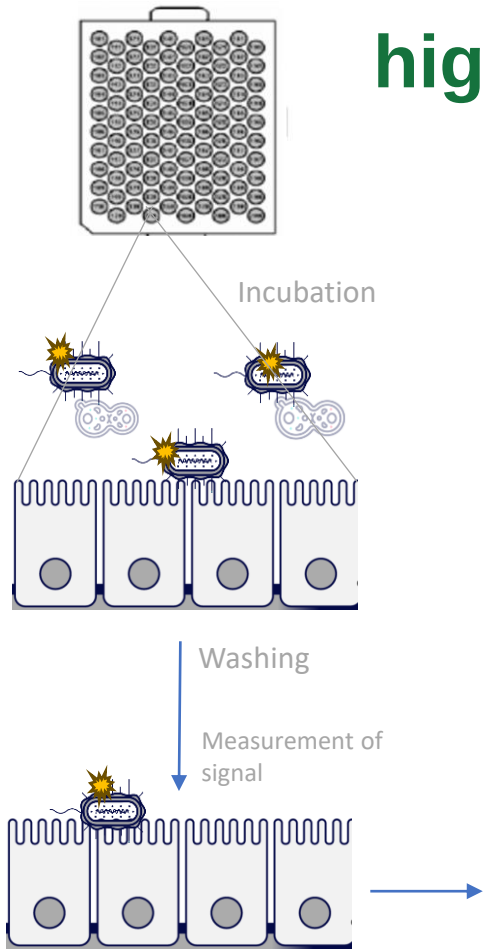
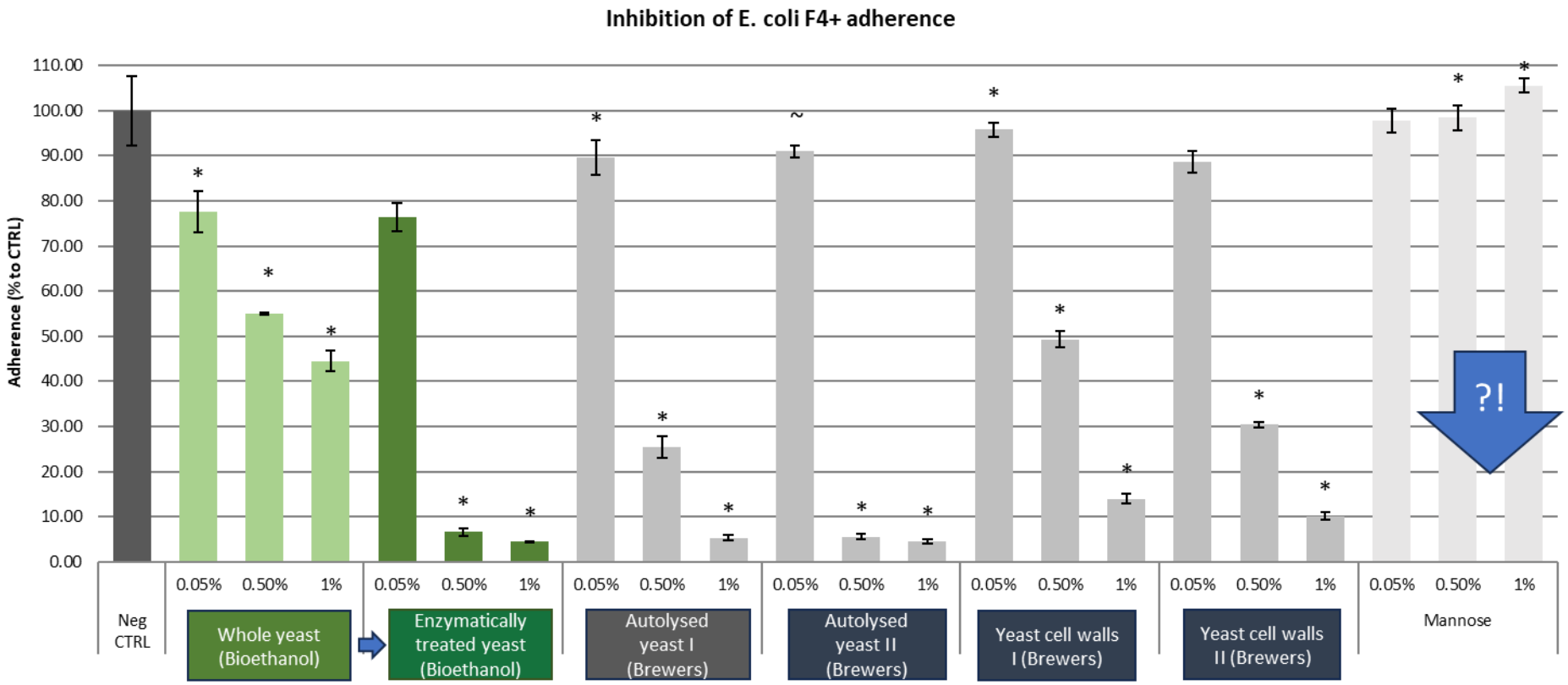


Figure 1.+ 2. Number of pathogenic *Salmonella and E.coli* strains (maximum 20) bound by yeast products other selected yeast-based products.

PETFOOD FORUM EUROPE

Ex vivo bacteria adherence inhibition highlights differences in effectiveness of yeast products

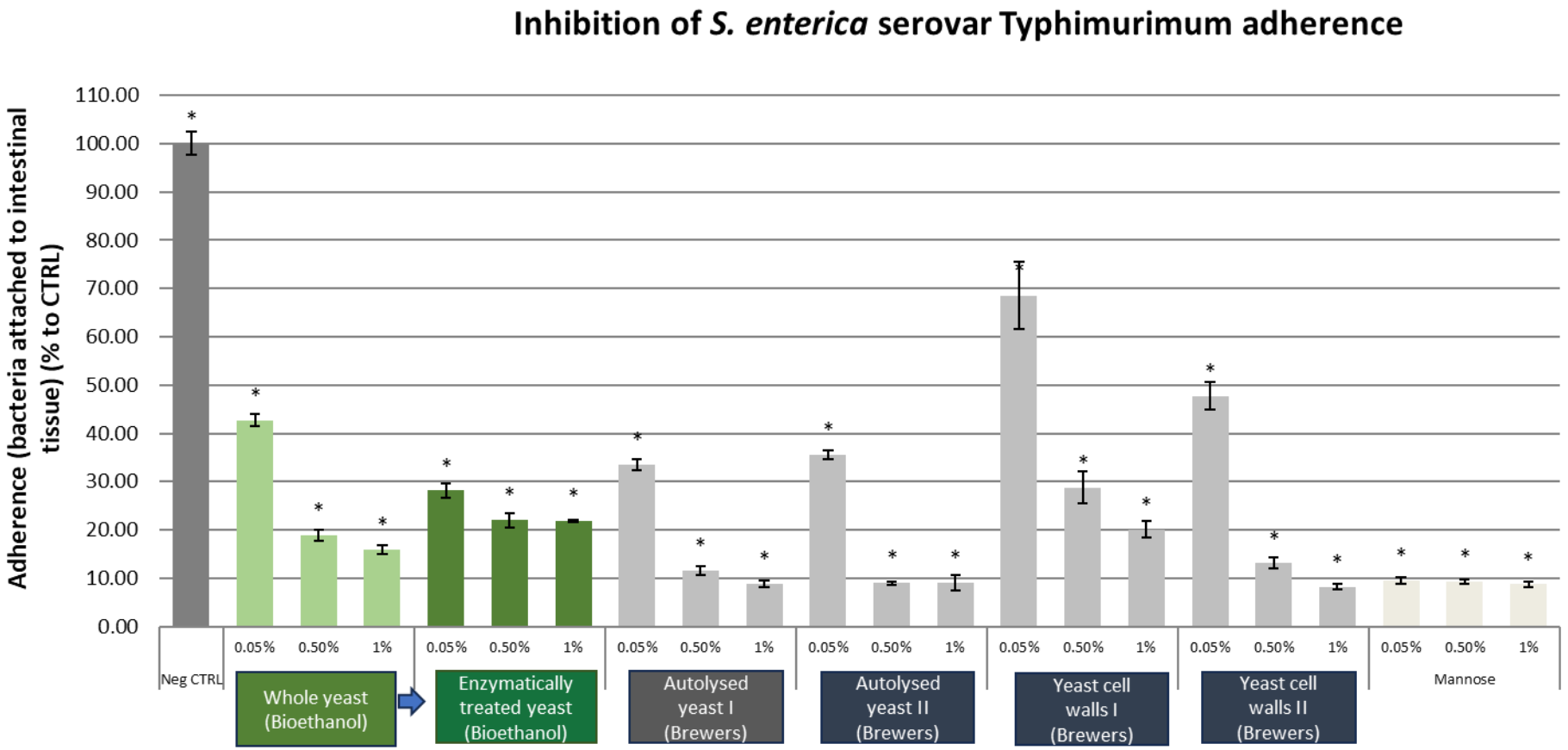




Effect of yeast products on the adherence of E. coli F4+ on piglet intestinal mucosa assessed by ex-vivo study. 100% of bacteria adherence to the intestinal mucosa is observed in the absence of yeast-based test products (Negative Control). * indicate statistical difference to Negative Control at P<0.05. Alimetrics Diagnostics, Livalta R&D

PETFOOD FORUM EUROPE

Ex vivo bacteria adherence inhibition highlights differences in effectiveness of yeast products



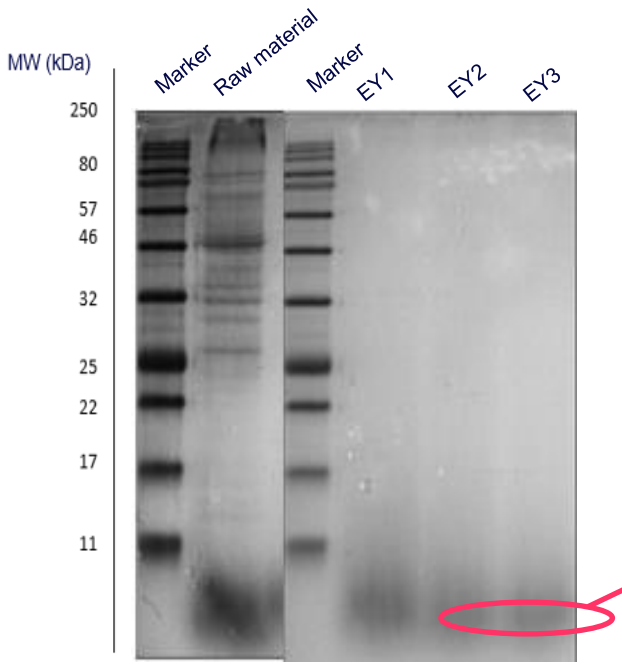
Yeast source	Product type	Total glucan (%)	Total mann an (%)
Sugarcane bioethanol yeast	Inactive Dried yeast	28.0	15.7
Sugarcane bioethanol yeast	Enzymatically treated yeast	28.0	15.7
Brewer's yeast	Autolysed yeast I	24.9	12.0
Brewer's yeast	Autolysed yeast II	19.6	13.1
Brewer's yeast	Yeast cell walls I	37.1	22.6
Brewer's yeast	Yeast cell walls II	25.5	25.0

*Effect of yeast products on the adherence of S. enterica Typhimurium on piglet intestinal mucosa assessed by ex-vivo study. 100% of bacteria adherence to the intestinal mucosa is observed in the absence of yeast-based test products (Negative Control). * indicate statistical difference to Negative Control at P<0.01, Alimetrics Diagnostics, Livalta R&D.*

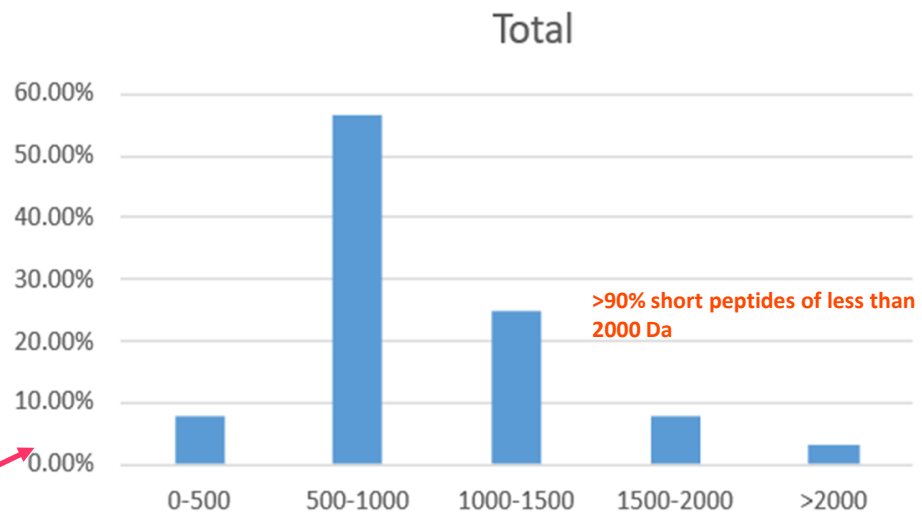
PETFOOD FORUM EUROPE

Enzymatic treatment can enhance nutritional and functional yeast benefits

1. Enzymatic treatment to convert proteins into short peptides



2. Peptides length is below 2kDa in the hypoallergenic MW range



3. Peptides identification study reveal that enzymatically treated yeast may be a source of bioactive peptides

Sequence identified	Activity	Reference
TEKGVFR	**ACE-inhibitory peptide (antihypertensive properties)**	Minkiewicz et al., 2019., BioPEP Database
GHDGKIKIG	ACE-inhibitory peptide (antihypertensive properties)	Minkiewicz et al., 2019., BioPEP Database
LPWFDGM	ACE-inhibitory peptide (antihypertensive properties)	Huang et al., 2021, DFBF dataabse
VSWYDNEYGYSTR	Antimicrobial and fungicidal peptide	Branco et al., 2013, 2017

Fig 1. **Gel electrophoresis (SDS-PAGE)** of protein extracts from intact yeast and enzymatically treated yeast. No high Molecular weight bands > 11kDa can be found.

Fig 2. **Peptides molecular weight distribution**, separated using size exclusion chromatography.

Fig 3. **Peptides i**n Enzymatically treated hydrolysate identified with mass spectrometry. Listed peptides have been previously reported for **bioactive properties.**

PETFOOD FORUM EUROPE

Source: Livalta R&D, Ghent University, Politechnika Slaska

Enzymatically treated yeast have the potential to support Probiotic Bacteria

- **Bacillus subtilis is a spore-forming bacteria used as a probiotic for humans and animals**
- In vitro evaluation the potential synbiotic effect between **enzymatically treated yeast and** probiotic bacteria
- *B. subtilis was inoculated after prior stress (low pH mimicking gastric conditions passage)*
- ***Enzymatically treated yeast has potential to support both growth and survival of probiotic bacteria after gastric pH stress.***

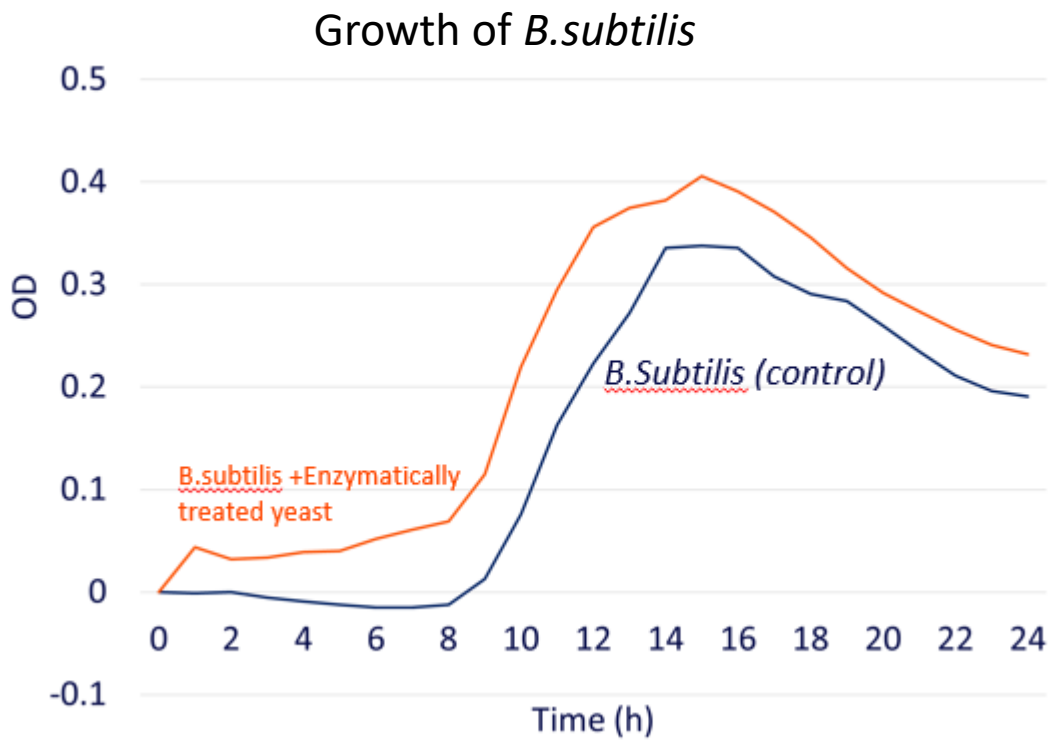


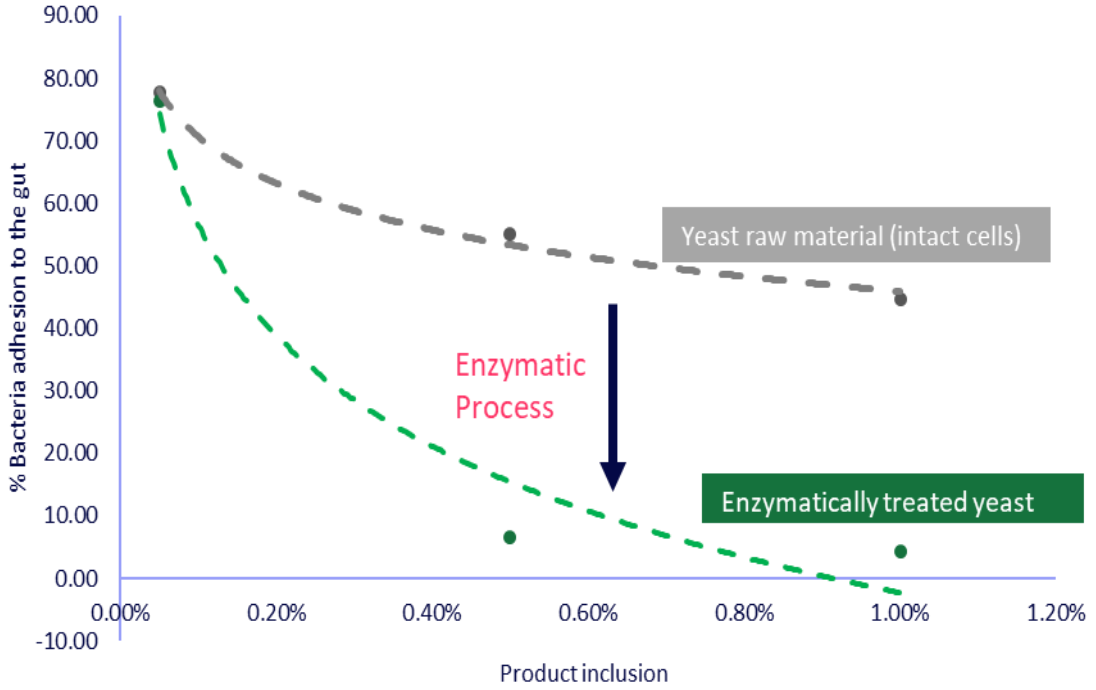
Figure 1. Growth curves of Bacillus subtilis 0.1% glucose or 0.1% Enzymatically treated yeats. Cultures inoculated after prior stress treatment low pH mimicking gastric conditions (methods according to Sumeri et al., 2010) Automated growth analyzer: Bioscreen C (Oy Growth Curves Ab Ltd). Source: Livalta R&D and AMUF, Poland.

- Source: Livalta R&D

What have we learnt so far and what is next?

- There are multiple ways how yeast products can support animal “gut health”
- Yeast based products can help reduce pathogenic pressure by acting as “antiadhesive” agents
- **Cell walls matter** but more factors are involved
- **Raw material selection and processing matter:** Selected enzymatic treatments can develop stronger pathogen inhibition properties (and enhance nutritional benefits)

- **More fundamental research** to establish a clear link between the structural characteristics of yeast cell components and their biological activity
- **More assays for quantitative** measures of product performance mimicking natural conditions
- We need to join forces for a **holistic approach to “gut health”** and the reduction of the use of antibiotics



Ex vivo comparison of efficacy of an intact and processed yeast (Enzymatic Process) to prevent attachment of pathogens (*E. coli F+*) to the intestinal mucosa. Targeted enzymatic treatment of yeast enhance inhibition of bacteria adhesion. Livalta R&D

PETFOOD FORUM EUROPE

The Livalta Team and our companions

Managing Director

Dr Valerie Schuster
and **Shalimar**

Finance

Elaine Workman
and **Luna**

Sales and BD

Katarzyna Swirkowicz
and **Harriet**

Quality & Regulatory

Katharine Dennis
and **Meg**

Operations

Rob Wait
And **Mr Cheeks**

Innovation & Research and Development

Dr Aleks Szopinska
and **Sky**

Dr Hagen Schulze
and **Vasja**

Dr Ingrid Lupatsch
and **Nemo**

Dr Eleanor Wood
and **Dolly**

PETFOOD FORUM EUROPE