The effect of probiotics on animal health: a focus on host’s natural intestinal defenses

Guillaume Tabouret
Animal Health Dept.
Joint Unit 1225 – Host Pathogens Interactions
History of probiotics and definition

Hippocrates of Cos

« The death sits in the bowel; a bad digestion is the root of all evil »

He stated that it was the humble duty of the physician to facilitate that healing power firstly by means of dietary approaches, and if that did was not enough, by means of natural medicines.

The concept of probiotics is back dated over 100 years ago to Elie Metchnikoff (Nobel Prize 1908):

"The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes"

Probiotics consumption alters commensal microflora and resistance to pathogenic bacteria
Roy Fuller (1992): «live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance»

Living microorganisms (either bacteria or yeast) exerting a proven benefit on the target species.

- **Feed regulation**: group of feed additives for stabilizing microbiota of monogastric and ruminants
- **Functional view**: digestive bioregulators
- **WHO**: One, or a few, well defined strains of microorganisms

**Rationale for Probiotics usage**:

- EU limitation of antibiotics usage for growth prospects (but also for therapeutic infect. Dis.)
  - Intensive farming systems
  → need for alternative strategies to strengthen animals' resistance to infections or pathologies associated with farming systems
Probiotics commonly used in farm animals (not exhaustive):

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Strain</th>
<th>Species or category of animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus var. toyoi</td>
<td>NCIMB 40112</td>
<td>Chickens for fattening, laying hens, calves, cattle for</td>
</tr>
<tr>
<td></td>
<td>CNCM I 10121</td>
<td>fattening, breeding does, rabbits for fattening, piglets, saw.</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>NCYC sc 47</td>
<td>Rabbits for fattening, sow, piglets, dairy cows.</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>CBS 493.94</td>
<td>Calves, cattle for fattening, dairy cows.</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>CNCM I- 1079</td>
<td>Sows, piglets.</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>CNCM I- 1077</td>
<td>Dairy cows, cattle for fattening</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>ATCC 53519</td>
<td>Chickens for fattening</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>ATCC 55593</td>
<td>Chickens for fattening, pigs, piglets for fattening</td>
</tr>
<tr>
<td>Pediococcus acidilactici</td>
<td>CNCM MA 18/5M</td>
<td>Chickens for fattening, pigs for fattening, sows,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cattle for fattening, piglets, calves.</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>NCIMB 10415</td>
<td>Piglets, chickens for fattening, calves.</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>DSM 5464</td>
<td>Piglets, chickens for fattening, calves.</td>
</tr>
<tr>
<td>Lactobacillus farcininis</td>
<td>CNCM MA 674R</td>
<td>Piglets, calves, chickens for fattening.</td>
</tr>
<tr>
<td></td>
<td>DSM 10663</td>
<td>Piglets, calves, chickens for fattening.</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>MUCL 39885</td>
<td>Piglets, cattle for fattening</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>NCIMB 11181</td>
<td>Calves, piglets.</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>DSM 7134</td>
<td>Calves, piglets.</td>
</tr>
<tr>
<td>Lactobacillus rhamnosus</td>
<td>DSM 7133</td>
<td>Calves, piglets.</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>NCIMB 30096</td>
<td>Calves.</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>NCIMB 30098</td>
<td>Calves.</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>CECT 4515</td>
<td>Calves, piglets.</td>
</tr>
<tr>
<td>Streptococcus infantarius</td>
<td>CNCM I-841</td>
<td>Calves.</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
<td>CNCM I-840</td>
<td>Calves.</td>
</tr>
<tr>
<td>Bacillus licheniformis</td>
<td>DSM 5749</td>
<td>Sow, piglets, pigs for fattening, chickens for fattening,</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>DSM 5750</td>
<td>turkeys for fattening, calves.</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>DSM 3530</td>
<td>Calves.</td>
</tr>
</tbody>
</table>

Lactobacillus, Streptococcus, Enterococcus, Bacillus, Saccharomyces cerevisiae

Bacterial probiotics have been effective in chickens, pigs and pre-ruminant calves
Yeast and fungal probiotics have given better results in adult ruminants
Probiotics criteria

- Normal component of the target specy microbiota
- Survive and «grow» in their respective ecological niches
- Able to utilize nutrients and substrates in a normal diet
- Capacity to adhere and colonize the epithelial cells of the gut
- Non pathogenic – non toxic
- Able to exert a beneficial effect on the host biology
Probiotics modes of action

1. Enzymatic contribution to digestion

2. Production of inhibitory compounds (antimicrobial): antagonism

3. Competition for chemicals/available energy

4. Competition for adhesion sites (exclusion)

5. Enhancement of the immune response
Direct & Indirect activities

Nutrients competition

Adhesion sites exclusion /receptors competition

Antimicrobials

Aggregation

1. Antimicrobials
2. Adhesion sites exclusion /receptors competition
3. Aggregation
4. Immunostimulation

Immune regulation
Tissue homeostasis
Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118


*Alimentary Pharmabiotic Centre and Department of Microbiology and §School of Pharmacy, University College Cork, Cork, Ireland

Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved March 1, 2007 (received for review January 17, 2007)
Hydrogen peroxide production by *Lactobacillus johnsonii* NCC 533 and its role in anti-*Salmonella* activity

Raymond David Pridmore, Anne-Cécile Pittet, Fabienne Praplan & Christoph Cavadini

Department of Nutrition and Health, Nestlé Research Center, Vers-chez-les-Blancs, Lausanne, Switzerland
ADHESION SITES COMPETITION

Research Article

Competition of *Lactobacillus paracasei* with *Salmonella enterica* for Adhesion to Caco-2 Cells

Alicja Jankowska,¹ Daniel Laubitz,¹ Hanna Antushevich,¹ Romuald Zabielski,² and Elżbieta Grzesiuk³
Probiotic Strains and Their Combination Inhibit In Vitro Adhesion of Pathogens to Pig Intestinal Mucosa

M. C. Collado · Łukasz Grześkowiak · Seppo Salminen

J. Dairy Sci. 90:2710–2716
doi:10.3168/jds.2006-456

Development of New Probiotics by Strain Combinations: Is It Possible to Improve the Adhesion to Intestinal Mucus?

M. C. Collado,*† J. Meriluoto,† and S. Salminen*

*Functional Foods Forum, University of Turku, Itäinen Pitkäkatu 4A, 20520 Turku, Finland
†Department of Biochemistry and Pharmacy, Åbo Akademi University, Tykistökatu 6A, 20520 Turku, Finland

Probiotics binding to mucosa or mucus
Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by *Saccharomyces* probiotics

F. C. P. Tiago,¹ F. S. Martins,¹ E. L. S. Souza,¹ P. F. P. Pimenta,² H. R. C. Araujo,² I. M. Castro,³ R. L. Brandão³ and Jacques R. Nicoli¹

*Journal of Medical Microbiology, 2012*

+, Adhesion observed in 15 min; ++, adhesion observed after 1 h; −, absence of adhesion after 3 h. All bacterial counts in the supernatants of yeast–bacteria associations were significantly different from those of the counterpart control without yeast (Student’s *t*-test, *P*<0.05).

<table>
<thead>
<tr>
<th>Indicator strain</th>
<th>Adhesion/bacterial count [log (c.f.u. ml⁻¹)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Saccharomyces boulardii (live) Saccharomyces cerevisiae UFMG 905 (live) Saccharomyces cerevisiae W303 (live) Saccharomyces boulardii (dead) Saccharomyces cerevisiae UFMG 905 (dead)</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028</td>
<td>6.20 ++/5.62 ++/5.78 − − ++/5.03 ++/5.68</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> (human origin)</td>
<td>6.43 ++/5.77 ++/5.87 − − ++/5.63 ++/5.71</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25723</td>
<td>6.46 ++/5.45 ++/5.83 − − ++/5.61 ++/5.54</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em> ATCC 19430</td>
<td>7.36 ++/6.72 −/6.51 − − ++/5.61 ++/5.43</td>
</tr>
<tr>
<td><em>Shigella sonnei</em> ATCC 11060</td>
<td>− − − − − −</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 19433</td>
<td>− − − − − −</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em> ATCC 15313</td>
<td>− − − − − −</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> ATCC 11778</td>
<td>− − − − − −</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em> (human origin)</td>
<td>− − − − − −</td>
</tr>
<tr>
<td><em>Clostridium difficile</em> ATCC 9689</td>
<td>− − − − − −</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> ATCC 13124</td>
<td>− − − − − −</td>
</tr>
</tbody>
</table>

Elimination with feces
Immunostimulation by probiotics: Yeast – *Saccharomyces* spp.

Live *S. cerevisiae* exerts anti-inflammatory activity
Saccharomyces cerevisiae- and Candida albicans-Derived Mannan Induced Production of Tumor Necrosis Factor Alpha by Human Monocytes in a CD14- and Toll-Like Receptor 4-Dependent Manner

Hiroyuki Tada¹,², Eiji Nemoto³, Hidetoshi Shimauchi³, Toshihiko Watanabe⁵, Takeshi Mikami⁴, Tatsuji Matsumoto⁴, Naohito Ohno⁴, Hiroshi Tamura⁵, Ken-ichiro Shibata⁶, Sachiko Akashi⁷, Kensuke Miyake⁷, Shunji Sugawara¹, and Haruhiko Takada²,¹


- Live Sc. exerts **anti-inflammatory** activity
- One of the main component of Sc. wall is as **pro-inflammatory** as a pathogenic yeast

Secreted immunomodulatory proteins
4 Immunostimulation by probiotics: Lactobacilli

- MAMPs recognition: TLR/NLR/CLR
- Pathogen = non self
- Probiotic lactobacilli = non self

For a similar result?

Strain and species specific variations in the chemical structure of major MAMPS such as LTA or PGN

Immunomodulatory proteins (glycosylation)

→ Different responses in IEC or immune cells

Engineering of various Lactobacilli strains to promote the « desired » effect

From Peter Van Baarlen et al. – Trends in Immunology 2013
Concluding remarks

Microbiota
+ single probiotic well defined strain
+/- pathogen

First level of interactions

Second level of interactions

Third level of interactions

Rather complex...but what if...
Concluding remarks

Microbiota
+ single probiotic well defined strain
+/- pathogen

Microbiota
+ combined / engineered probiotics
+/- pathogen

First level of interactions

Second level of interactions

Third level of interactions

Rather complex...but what if...

A tremendous complexity

+Host inter-species variations
+Host inter-individual variations
Concluding remarks and take-home message

• Existing and convincing proofs of concept of clinical efficacy of probiotics applications for various conditions
  • prevention of antibiotic associated diarrhea
  • prevention severe necrotizing enterocolitis
  • Protection against a variety of pathogens in chicken, pigs…
  • Reduction of shedding of *E. Coli* O157:H7 in cattle and calves
  • improvement of health and production criteria of various livestock animal
  • …. The list in now quite long…

• Several modes of action by which probiotics contribute to human and animal health have been proposed or established

• No single probiotic supplement drives all the mentioned effects.
• There is no common responses to different probiotics even of the same genera (i.e. lactobacili)

Consequently, there is a need for:
• for a rational selection of a specific probiotic for defined targets (individual, specie…) and clinical indication

• for having a better knowledge in the effects (and associated molecular mechanisms) a specific probiotic will have on healthy and unhealthy individuals