Safety of probiotics

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Foods fermented with LAB have been consumed safely for thousands of years
- Including spontaneous fermentation

LAB and *Bifidobacterium* are natural inhabitants of intestinal tract, oral cavity and urogenital tract

LAB are extremely rarely associated with disease or infections, bifidobacteria even more rarely

How to assess safety?
Assessing probiotic safety - \textit{In vitro}


\textbf{Taxonomy}
- You can evaluate safety only if you know which strain you are evaluating!
- Intentional misleading: \textit{Bacillus coagulans} vs. "\textit{Lactobacillus sporogenes}"
- Molecular methods as basis for identification

\textbf{Adhesion to human tissues}
- Adhesion to mucus / epithelial cells a beneficial feature – Selection criterion
- Can adhesion be harmful? \textbf{Not a good safety criterion}

\textbf{Haemolysis, platelet aggregation – relevant or not?}

\textbf{Resistance to inactivation by immune system}
- Serum-mediated killing
- Phagocytosis

\textbf{Virulence genes and toxic metabolites}
- \textit{Enterococcus faecium} vs \textit{Enterococcus faecalis} $\rightarrow$ latter contains virulence genes
- Enterotoxin production by \textit{Bacillus cereus}, some \textit{Bacillus subtilis}
Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA

Opinion of the Scientific Committee

(Question No EFSA-Q-2005-293)

Adopted on 19 November 2007

- Includes a list of microorganisms regarded as safe for consumption
- In the USA: "Generally regarded as safe" (GRAS)
- Major importance: reliable identification and deposition in culture collection
In vitro safety assessments: Antibiotic resistance

- **Antibiotic resistance** *per se* not a problem, if it’s intrinsic
- **Key issue**: are there mobilized/transfered resistance elements?
  - Resistance genes within plasmids or transposons; horizontal gene transfer
- **Enterococcus** sp. → many reports of horizontal transfer of resistance (no QPS)
- **Removal of antibiotic resistance genes**?
  - GMO probiotics, regulatory issues
Production of D-lactic acid

- Some LAB strains produce only L-lactic acid, but many produce both D- and L-lactic acids
  - Metabolism of D-lactic acid by humans is lower than L-lactic acid (different mechanism) → risk of acidosis?

- Probiotics produce only small amounts of D-lactic acid; much less than the normal microbiota of the humans (including infants)

- Normally, lactic acid produced is consumed by other gut microbes

- Numerous studies of D/L-lactic acid producing LAB in humans, adults and infants → no adverse effects

- D-lactic acidosis is rare condition in infants with short bowel syndrome
  - No association with probiotics

- D-lactic acid producing probiotics are safe for adults and infants
  - In the special group of SBS patients, caution is required

For review, see: Connolly & Lönnerdal (2004) NUTRAfoods 3(3): 37-49
In vivo safety assessment: Animal models

- Acute toxicity and tolerance of high doses

- Bacterial translocation (from gut to host tissues):
  - Healthy animals (adults, neonates)
  - Colitis models
  - Immunocompromized animals

- Endocarditis
  - Probiotics 100 to 10,000-fold less likely to cause infections than Staphylococci and Streptococci

- Other models:
  - Liver injury
  - Intestinal resection
  - Models for in vivo antibiotic resistance transfer
Can humans eat too much?

• No observed adverse effect level 50 g/kg body weight (mouse)
• Safety margin for humans (1/100) ½ g/kg body weight
• For 70 kg person: 35 g pure probiotic bacteria
• 35 g ≈ 3500 x 10^9 bacteria
• (100 g yogurt ≈ 10^9)
• ≈ 350 kg yogurt
Human safety studies

- Separate safety / tolerance tests often not done prior to use in foods
  - QPS, GRAS status

- Examples of tolerance tests:
  - **B. longum** 46 and **B. longum** 2C
  - **L. reuteri** ATCC 55730
    - gastrointestinal function
  - **Streptococcus salivarius** K12
    - oral health
  - **L. acidophilus** LA-CH5, **B. lactis** Bb-12
    - antibiotic gene transfer
  - **L. rhamnosus** GG
    - effect on infant growth

- Numerous clinical trials with no adverse effects, also in infants

- Main body of evidence: wide-spread and long-term safe use
# Infections by lactic acid bacteria

<table>
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<tr>
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Salminen et al (2002) found no increase in *Lactobacillus* bacteremia in Finland between 1995-2000 despite strong increase in *Lactobacillus rhamnosus* GG consumption during the same time period.


Salminen et al (2006): Identification of 85 blood isolates of LAB:
- *L. rhamnosus* (n=46), *L. casei* (n=12), *L. fermentum* (n=12), *L. jensenii* (n=3), *L. gasseri* (n=3), *L. salivarius* (n=3)


But, rare cases of bacteremia or fungemia associated with probiotic intake have been reported in (severely) ill patients.

Do strain differences exist?

- Clear differences in the number of isolates:
  - Certain *L. rhamnosus*, *B. subtilis* and *S. boulardii* most frequently reported
  - Also some *L. casei*, *L. fermentum*
  - Apparent lack of *L. acidophilus*, *Bifidobacterium*, others

- Differences in the detection methods?

- Thorough screening only in some regions → regional bias?

- Differences in the clinical situations in which probiotics are used?
  → biased towards strains used frequently with certain diseases?

  - Lack of virulence factors, ”mechanisms of adverse effects”
  - In total, adverse events very rare
Clinical study assessing efficacy of a probiotic mixture ("Ecologic 641") in the treatment of acute pancreatitis → patients in critical condition

Higher mortality in probiotic group (n=24/153) compared to placebo group (n=9/145)
- Overall mortality 11% (normally between 10-30%)
- Higher bowel ischemia in probiotic group (9 vs 0 cases)
- No difference in infections between the groups

Probiotic treatment associated with higher mortality- What was the cause?

However, organ failure rate significantly higher in probiotics group (n=20) than in the placebo group (n=7) before the treatment!
- Organ failure correlates also with bowel ischemia (haemodynamic disturbance)

It is currently unclear what caused the observed effects
Conclusions

**In vitro safety assessments:** Taxonomy, antibiotic resistance

**In vivo safety assessments:**
- Are animal models validated?
- Human safety studies recommended especially probiotics other than *Lactobacillus* and *Bifidobacterium*, which can be considered safe

**Overall safety record of probiotics is excellent**
- Compares well with other foods, drugs etc.:
  - side-effects and adverse events of different foods
  - side-effects of medicines, environmental compounds
  - fermentation originally used to preserve food, reduce adverse effects
- Long history of safe use for LAB: always part of human nutrition and microbiota
- *Lactobacillus* and *Bifidobacterium* safe also for infants
  - Early colonizers of infant gut; also present in human milk; infants exposed to these microbes also during birth
Conclusions

- No safety concerns for healthy consumers

- In certain severe clinical conditions:
  - Consider probiotic administration carefully, depending on health status of patient

- ILSI Probiotic Task Force focuses also on safety of probiotics
  
  [Link to ILSI Probiotic Task Force](http://europe.ilsi.org/activities/taskforces/diet/probiotics.htm)

THANK YOU FOR YOUR ATTENTION!