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Safety of probiotics

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Lactic acid bacteria – History of safety

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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 - *In vitro*

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For review, see: Vankerckhoven et al (2008) Trends Food Sci Technol 19:102-114

- Taxonomy
  - You can evaluate safety only if you know which strain you are evaluating!
  - Intentional misleading: *Bacillus coagulans* vs. ”*Lactobacillus sporogenes*”
  - Molecular methods as basis for identification
- Adhesion to human tissues
  - Adhesion to mucus / epithelial cells a beneficial feature – Selection criterion
  - Can adhesion be harmful? ***Not a good safety criterion***
- Haemolysis, platelet aggregation – relevant or not?
- Resistance to inactivation by immune system
  - Serum-mediated killing
  - Phagocytosis
- Virulence genes and toxic metabolites
  - *Enterococcus faecium* vs *Enterococcus faecalis* → latter contains virulence genes
  - Enterotoxin production by *Bacillus cereus*, some *Bacillus subtilis*

Taxonomy:
Qualified presumption of safety

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efsa
European Food Safety Authority

The EFSA Journal (2007) 587, 1-16

Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA[1]

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

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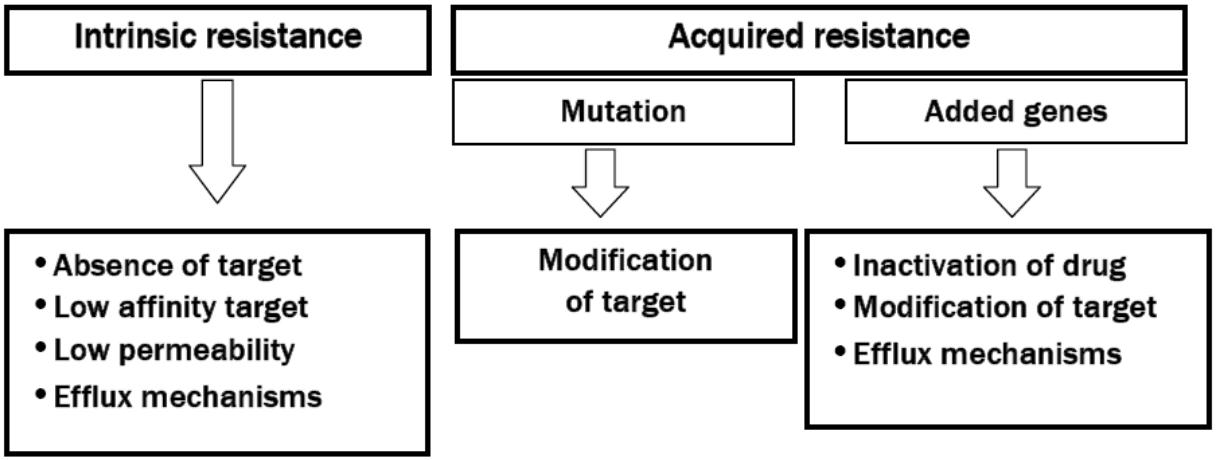

- Antibiotic resistance *per se* not a problem, if it's intrinsic
- Key issue: are there mobilized/transfered resistance elements?
  - Resistance genes within plasmids or transposons; horizontal gene transfer
- *Enterococcus* sp. → many reports of horizintal transfer of resistance (no QPS)
- Removal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

Production of D-lactic acid

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- 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

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- Acute toxicity and tolerance of high doses

  e.g. Zhou (2000) Food Chem Toxicol 38:153-161

- Bacterial translocation (from gut to host tissues):

  e.g. Daniel (2006) Appl Environ Microbiol 72: 5799-5805

  - Healthy animals (adults, neonates)
  - Colitis models
  - Immunocompromized animals

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

    Vankerckhoven et al. (2007) J Med Microbiol 56:1017-1024

- Other models:
  - Liver injury

    Osman et al. (2005) Microb Ecol Health D 17:40-46
  - Intestinal resection

    Mogilner et al. (2007) J Pediatr Surg 42:1365-1371
  - Models for *in vivo* antibiotic resistance transfer

    Mater et al. (2008) J Mol Microbiol Biotechnol 14: 123-125

Can humans eat too much?

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- 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

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- 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 — Mäkeläinen et al (2003) Microbiol Immunol 47:911-914
  - *L. reuteri* ATCC 55730 — Wolf et al (1995) Microb Ecol Health D 8: 41-50
    - gastrointestinal function
  - *Streptococcus salivarius* K12 — Burton et al. (2006) Appl Environ Microbiol 72: 3050-3053
    - oral health
  - *L. acidophilus* LA-CH5, *B. lactis* Bb-12 — Saarela et al. (2007) Int J Antimicrob Agents 29:271-280
    - antibiotic gene transfer
  - *L. rhamnosus* GG — Laitinen et al (2005) Br J Nutr 94:565-574
    - effect on infant growth

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

  e.g. Dekker et al (2009) Int Dairy J 19: 149-154

- Main body of evidence: wide-spread and long-term safe use

Infections by lactic acid bacteria

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Disease	Organism	Identification	Outcome	Reference
AIDS	*L. casei*	?	☺	Abgrall et al. 1997
AIDS (3x)	*L. rhamnosus*	?	☺/☺/☹	Horwitch et al. 1995
AIDS	*L. casei*	AMS-VITEK	☺	Rogasi et al. 1998
HIV	*L. rhamnosus*	?	☺	Schlegel et al. 1998
Leukemia	*L. rhamnosus*	API 50	☺	Chomarat & Espinouse 1991
Leukemia	*Lactobacillus*	?	☺	Cooper et al 1998
Pancreatitis (2x)	*L. rhamnosus*	?	☺/☹	Brahimi et al. 2008
Partial colonectomy	*Pediococcus*	?	☺	Barton et al. 2001
Colonoscopy	*L. rhamnosus*	API 50	☺	Avlami et al. 2001
Enteric fistula	*L. casei*	16S rDNA	☺	Parola et al. 1998
Urolithiasis	*L. jensenii*	PFGE	☺	Chazan et al 2008
Diabetes	*L. casei* group	API	☺	Chanet et al. 2007
Acupuncture	*B. longum*	Metabolic endproducts	☺	Ha et al. 1999
Healthy infant	*B. breve*	DNA-DNA homology	☺	Hata et al. 1988
Healthy senior	*L. rhamnosus*	?	☹	Wolz&Schaefer 2008

Infections by lactic acid bacteria Identification

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Consumption of probiotics vs. *Lactobacillus* bacteremia

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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 (2002) Clin Infect Dis 35:1155-1160

- Sullivan & Nord (2006) found no increase in Lactobacillus bacteremia in Stockholm, Sweden, between 1998 and 2004

Sullivan & Nord (2006) Scand J Infect Dis 38:327-331

- 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)

Salminen et al (2006) Clin Infect Dis 42: e35-344

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

For review, see: **Boyle et al (2006) Am J Clin Nutr 83: 1256-1264**

Do strain differences exist?

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- Clear differences in the number of isolates:
  - Certain *L. rhamnosus*, *B. subtilis* and *S. boulardii* most frequently reported
    Boyle et al (2006) Am J Clin Nutr 83: 1256-1264
  - Also some *L. casei*, *L. fermentum* **Salminen et al (2006) Clin Infect Dis 42: e35-344**
  - 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

Adverse events: The Dutch acute pancreatitis study

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- Clinical study assessing efficacy of a probiotic mixture (”Ecologic 641”) in the treatment of acute pancreatitis → patients in critical condition

  Besselink et al (2008) Lancet 371 (9613): 651-659

- Higher mortality in probiotic group (n=24/153) compared to placebo group (n=9/145)
  - Overal 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)

  Reid et al (2008) Lancet 372 (9633): 112-113

- It is currenlty unclear what caused the observed effects

Conclusions

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- *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

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- 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
  http://europe.ilsi.org/activities/taskforces/diet/probiotics.htm

THANK YOU FOR YOUR ATTENTION!