



Development of probiotic dairy products for an allergy target

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Probiotics

Live microorganisms administered in adequate amounts which confer a beneficial health effect on the host

FAO/WHO (2001) Expert Consultation
<http://www.fao.org/es/ESN/Probio/probio.htm>

Must be alive

Do not need to survive GI transit

Do not need to alter GI microflora

Can be any microbe (food use – focus on lactobacilli/bifidobacteria)

Must have evidence documenting a benefit on human physiology

Can be a food ingredient, dietary supplement or drug

Oral administration not required

Considerations for development of probiotic dairy product

- Define target
- Strain(s) selection
- Dose
- Safety
- Labeling probiotic foods in the US
- Standards for probiotic containing foods

Allergy Target

- What product do you want to market?
- Prevention of onset of allergic disease
 - Pregnant women
 - Babies <6 months
- Control of symptoms
 - Infants
 - Children
 - Adults

A decorative graphic consisting of a thick, dark gray L-shaped bar. It starts as a vertical bar on the left side of the slide and then turns 90 degrees to become a horizontal bar extending to the right, positioned to the left of the main text.

Strain selection

Same species, different function



Sled dog

- cold-hardy
- good endurance
- tough feet



Guard dog

- protective
- muscular
- strong teeth
- ferocious bark
- courageous



Hunting dog

- soft mouth
- good swimmer

All dogs not ideal
for all purposes

Strain selection

Strains with human studies on allergy documenting effects:

- *L. rhamnosus* GG
 - *B. lactis* Bb12
 - *L. rhamnosus* 19070-2 / *L. reuteri* 122460
- Other strains – supported by animal studies

What about dead bacteria?

- Dead bacteria are not probiotics
- Certain physiological effects have been documented for dead bacteria
 - Immune adjuvant effect
 - Delivery of lactase
 - IBS
- How the cell is killed is important
 - Heat-killed have more limited impact
 - Irradiated cells retain some activity

Most controlled clinical studies use high levels of live bacteria – most of these studies do not compare effectiveness of live vs. dead bacteria

Richmeliwitz, et al. 2004. Toll-Like Receptor 9 Signaling Mediates the Anti-inflammatory Effects of Probiotics in Murine Experimental Colitis. *Gastroenterology* 126:520-528

- Documented down regulation of inflammatory response in mouse model of colitis
- Effect mediated by DNA from irradiated blend of probiotic strains (VSL#3)
- Publicity for this paper made inaccurate conclusion that probiotics don't need to be alive for effects
- Some limitations in this model system
- Effects not documented in humans
- Many different mechanisms likely to be responsible for probiotic effects – one of which might be DNA segments

This paper provides important insight into possible mechanisms of the impact of bacteria on health, and provides compelling justification for determining the role of viability in observed effects

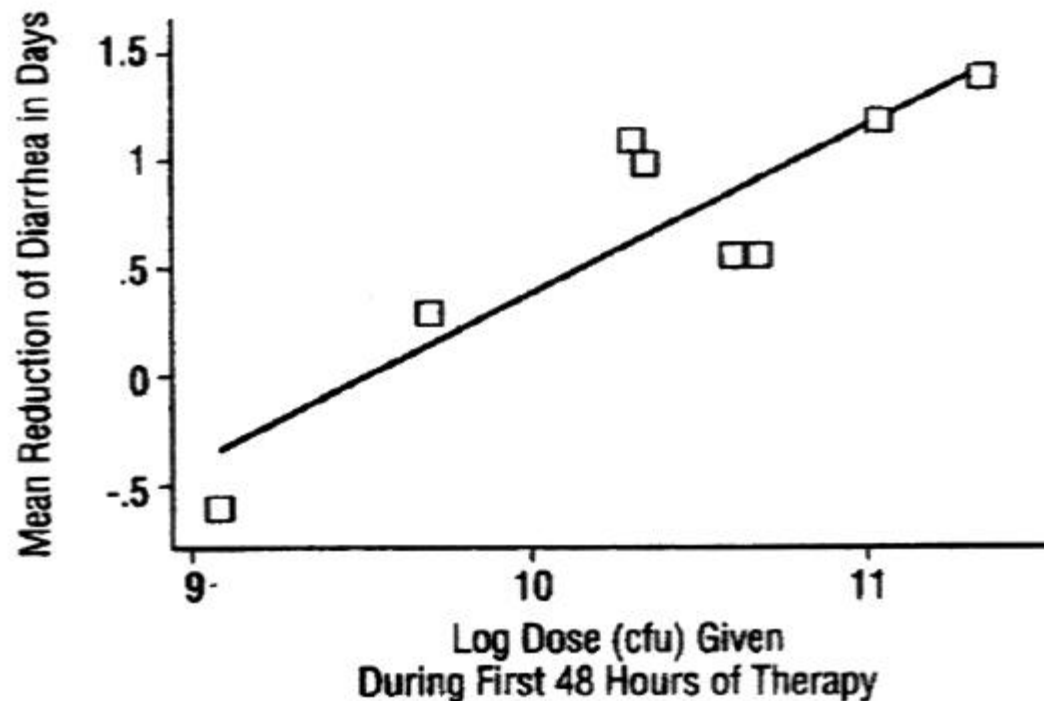
Live vs. dead cells tested with allergy endpoint

- Kirjavainen et al 2003. J Ped Gastroenterol Nutr 36:223-7
 - Viable vs. heat inactivated *L. rhamnsosus* GG
 1. EHF (n=8)
 2. #1 + GG ($\sim 3 \times 10^{10}$ cfu/kg BW/d) (n=14)
 3. #2 heat killed (n=13)
 - 7.5 wk mean treatment time
 - Study aborted due to GI symptoms in heat killed group (5 of 13)
- Trapp et al 1993. Int J Immunother IX:53-64.
 - Yogurt, no-yogurt or heat-killed yogurt for 12 months
 - Heat-treated vs. 'live' yogurt - $\sim 2 \times 10^{10}$ /d ST/LB
 - Reduced nasal allergy incidence in college age subjects in 'live' yogurt only



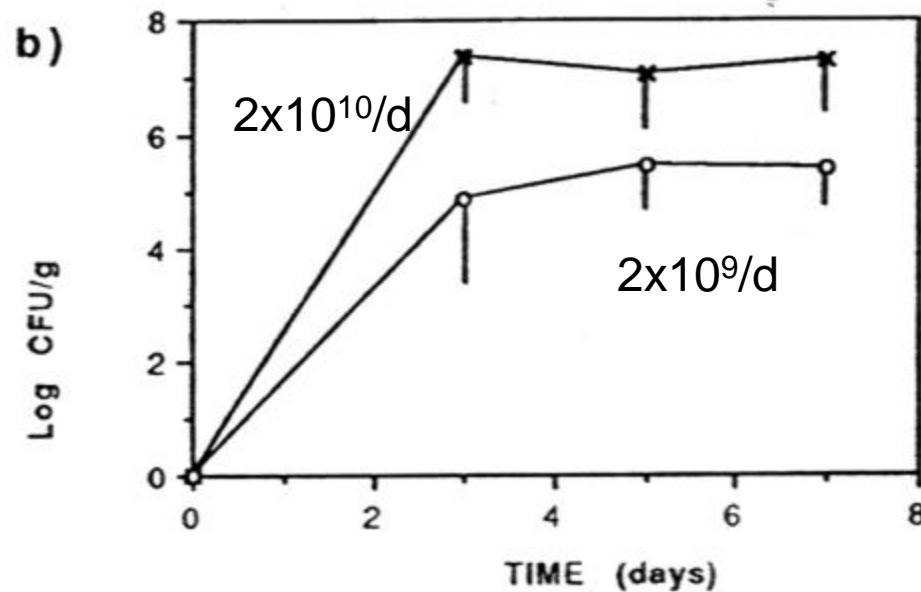
Dose / Levels

Relationship between *Lactobacillus* dose and reduction of diarrhea in children



Van Niel, et al. 2002. *Lactobacillus* therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* **109**:678-684

Fecal LGG concentrations in health human volunteers
after daily consumption of LGG in fermented milks
Dose: 2×10^9 and $2 \times 10^{10}/d$



There was no recovery of LGG in feces at feeding levels of $10^6 - 10^8/d$. At 10^9 , 2 of 7 volunteers showed LGG in feces. At 10^{10} all were colonized. Saxelin et al. 1991. Microbial Ecol Health Dis 4:209-214.

Fecal *L. reuteri* levels in children after 3 wk feeding

■ 14% colonized	}	10^6 cfu/d
■ 10^2 /gm feces		
■ 67% colonized	}	10^8 cfu/d
■ 10^4 /gm feces		
■ 100% colonized	}	10^{10} cfu/d
■ 10^7 /g feces		

Health Benefit

Daily Dose

Day care center antibiotic
use/infections/dental caries

10^8

Antibiotic associated diarrhea

$\geq 2 \times 10^8$

↑ fecal lactobacilli (LGG)

10^9

↑ fecal *L. reuteri*

10^{8-9}

↓ Incidence atopic eczema

10^{10}

Lactose digestion

10^{10}

IBS abdominal pain

2×10^{10}

Infant diarrhea

10^{11}

Remission of pouchitis

10^{12}

Daily Dose in Allergy Studies

- No dose studies conducted in humans with probiotics and allergy incidence or symptoms
- Incidence:
 - Kalliomaki et al. 2001 – 10^{10} cfu/d *L. rhamnosus* GG
- Symptoms:
 - Rosenfeldt et al. 2003 – $2 \cdot 10^{10}$ /d each *L. rhamnosus* 19070-2, *L. reuteri* 122460
 - Kirjavainen et al. 2001 – $8 \cdot 10^{10}$ cfu/kg BW/d *B. lactis* Bb12
 - Kirjavainen et al. 2003 – $3 \cdot 10^{10}$ cfu/kg BW/d *L. rhamnosus* GG
 - Aldinucci et al. 2002 - $2 \cdot 10^9$ yogurt starters (ST/LB)
 - Trapp et al. 1993 - $\sim 2 \cdot 10^{10}$ cfu/d yogurt starters

Dose

- Dose should be determined based on studies which show a physiological effect
- Levels may be different with different strains
- Dose must be delivered through END of shelf life
- Current situation with probiotic dairy foods:
 - Not labeled with dose or strain
 - Levels not communicated



Safety



"Uh-oh."

Generally Recognized as Safe

- GRAS (in US food law): applies to a **use**, not to a substance alone
 - Some are GRAS for 'general food use'
- You can't say "Lactobacilli are GRAS"
- You can say "Lactobacilli are GRAS for use as fermentation agents in foods"
- Published GRAS list is not considered complete
 - Most species used as probiotics are not listed on the current GRAS list
- Self-determinations of GRAS status are not public documents
- GRAS notifications are public, but generally FDA does not update GRAS list with items that it allows through notification

Safety: Lactobacilli and Bifidobacteria

- Generally considered very low pathogenic potential
 - Normal commensals
 - Some species present in high numbers in foods
 - History of safe use for some species in foods and supplements
- Dozens of infections documented due to lactobacilli and bifidobacteria
 - Only in patients with underlying illness
 - Source of microbe in most cases is commensal

Safety Standards in USA

- Food: reasonable certainty of no harm
 - Generally intended for all to use
- Dietary supplement: unsafe if significant or unreasonable risk of illness or injury under the conditions of the recommended use
 - Burden of proof with FDA to prove *unsafe*

Association Between Probiotic Consumption and Infection

■ Rautio, et al. 1999.

- Liver abscess caused by *L. rhamnosus*
- 74-year old hypertensive, diabetic woman
- Consumed 500 ml GG drink/day
- Strain isolated from infection indistinguishable from GG; same DNA finger print from strain from infant with no exposure to GG products

■ Mackay et al. 1999

- Endocarditis caused by *L. rhamnosus*
- 67-year old, history of mitral valve prolapse and tooth removal
- Chewed dried, mixed-strain probiotic preparation, containing *L. rhamnosus*, *E. faecalis*, *L. acidophilus* and others
- Strain isolated from infection indistinguishable from strain in probiotic supplement

Association Between Probiotic Consumption and Infection

- Kunz et al. 2004. J Ped Gastroenterol Nutr 38:457-8
 - 2 cases *Lactobacillus* bacteremia in infants with short bowel syndrome administered dried *L. rhamnosus* GG
 - Both cases resolved with antibiotic treatment
 - Case 1: GG administered at 3 mos; bacteremia 3 weeks later; strain typing not done
 - Case 2: GG administered at 2.5 wks; bacteremia 5.5 mos later; GG confirmed in blood



Standards for probiotic products

Worldwide efforts for developing standards for products containing probiotics

■ Codex 243-2003

- Stipulates microbes for fermented milks/yogurts
 - Minimum of starters at end of shelf life: 10^7 /g total
- Additional labeled microbes
 - Minimum 10^6 /g total

■ National Yogurt Association petition

- Currently, yogurt can be pasteurized
- Petition would define yogurt as having $\geq 10^7$ at time of mfg/ 10^6 at end of shelf life of total starters
- No minimum for other labeled microbes (i.e., probiotics)

■ FAO Guidelines

- Defines probiotic
- Establishes requirements for efficacy and safety

■ International Dairy Federation

- New project to develop methods (*in vitro* and *in vivo*) to evaluate the functionality and safety of probiotics as per FAO recommendations

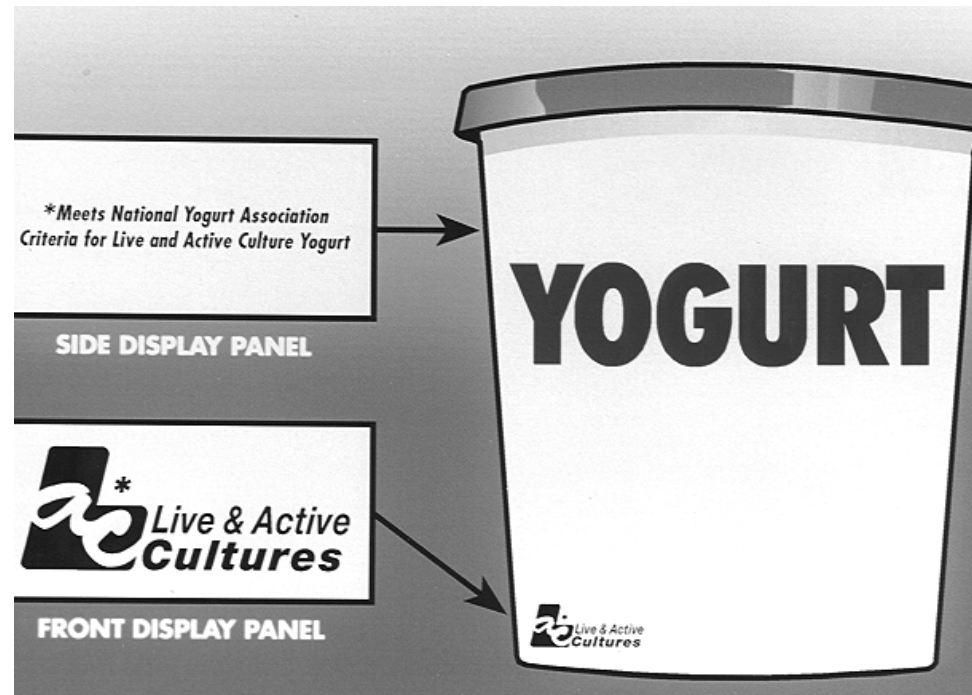
Focus of standards:

- Safety
- Efficacy
- Proper labeling for count and microbe
- 3rd party verification of label accuracy
- Standard methods

Live Active Culture Seal

It isn't enough

- National Yogurt Association
- Minimum level of lactic bacteria – 10^7 /g at end of shelf life
- Doesn't specifically apply to probiotic content
- Starter cultures usually $\sim 10^{8-9}$ /g
- Probiotic bacteria?



Are standards needed?

- Industry needs to do a better job formulating and labeling probiotic products (foods and supplements)
- Proactive commitment on part of industry needed



Product Labeling

Labeling a probiotic food

- Structure/function statement is allowable, if the statement is truthful and not misleading
 - Many such statements on dietary supplement probiotics
 - Few on probiotic foods
 - Examples:
 - “Supports your body’s natural defenses”
 - “Helps digestion”
 - “Supports a healthy intestinal tract”
- Claim on prevention of allergy or management of allergy unlikely acceptable for S/F claim
- Qualified health claims
 - None currently allowed

Establishing Efficacy

- Sources of information:
 - Experience – traditional uses
 - Animal studies
 - Case reports
 - *In vitro* experiments
 - Clinical trials
- Plausible biological mechanism

Slide courtesy of Jim Heimbach

To develop a successful probiotic product

- Properly formulate to provide health benefits
 - Scientific basis for product is essential
- Properly formulate to provide product of interest to consumers (taste, convenience, price)
- Properly label
 - Genus, species, strain of probiotic
 - Levels delivered by product through end of shelf life
 - What benefits product will provide
- Safe
- Good communication on benefits

Some useful sites for additional information

- www.mesanders.com – will post copy of this talk by Friday
- www.usprobiotics.com
- Live 'Forum' with ability to submit questions on probiotics to experts: www.danonevitapole.com
- www.isapp.net – International Scientific Association for Probiotics and Prebiotics
- www.probiotics-amsterdam.org/workshop