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Probiotics - Concurrent Workshop Repeated

Sunday, 12 June 2011	Start 8:30am	Duration: 50mins	Van Gogh
	Start 9:25am	Duration: 50mins	Van Gogh

Rotorua GP CME 2011 NZMA
General Practice Conference & Medical Exhibition

09-12 June 2011 | Energy Events Centre | Rotorua

Probiotics

Rotorua, New Zealand, 12 June 2011

Anders Henriksson
Danisco Australia

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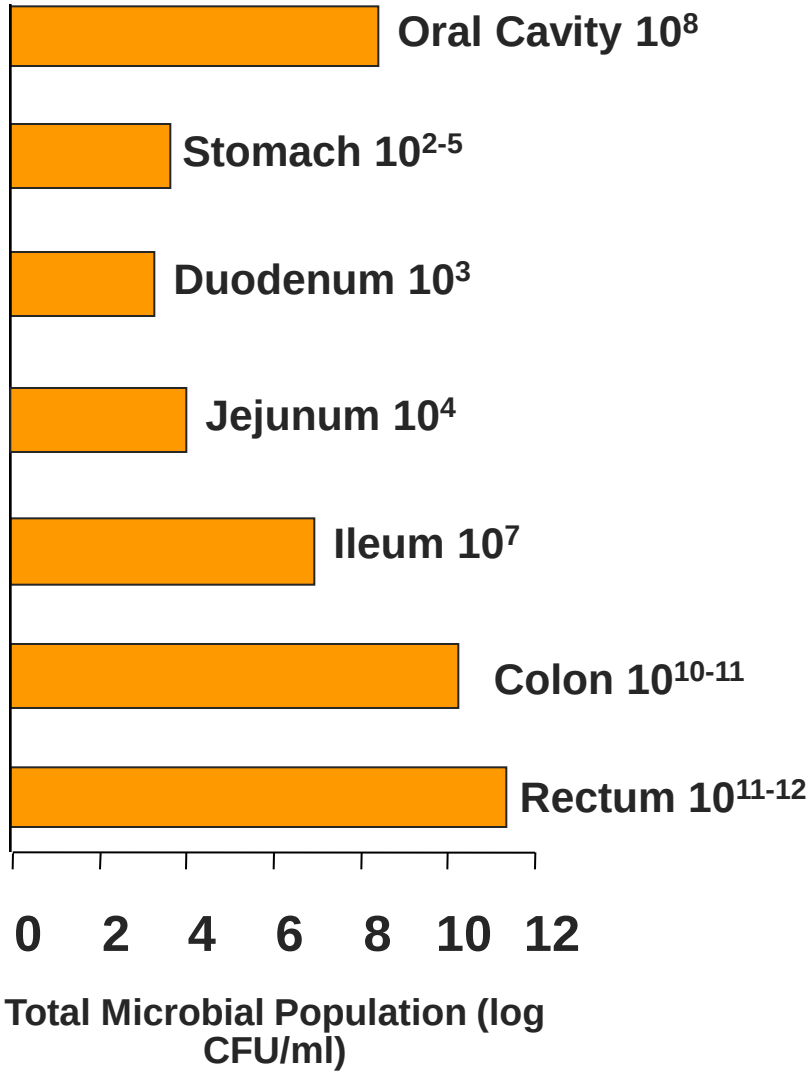
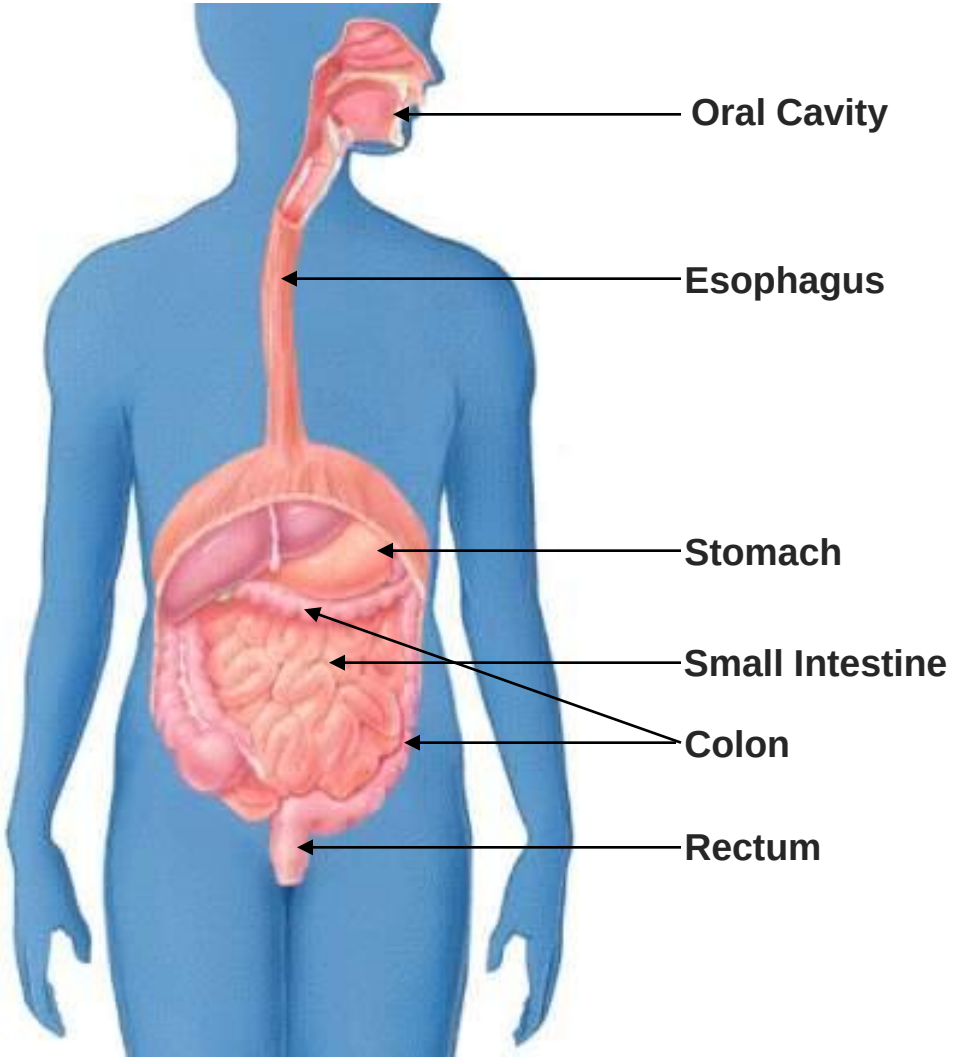
Probiotics – Fact or Fiction?

Overview

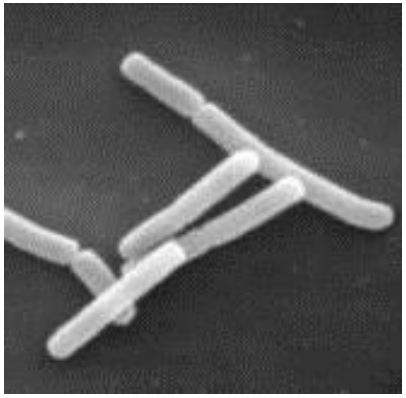
- Probiotics – historical perspective
- Examples of application - management of:
  - Antibiotic associated diarrhoea
  - Eczema
- Stability of probiotics

Introduction to the human gastrointestinal tract

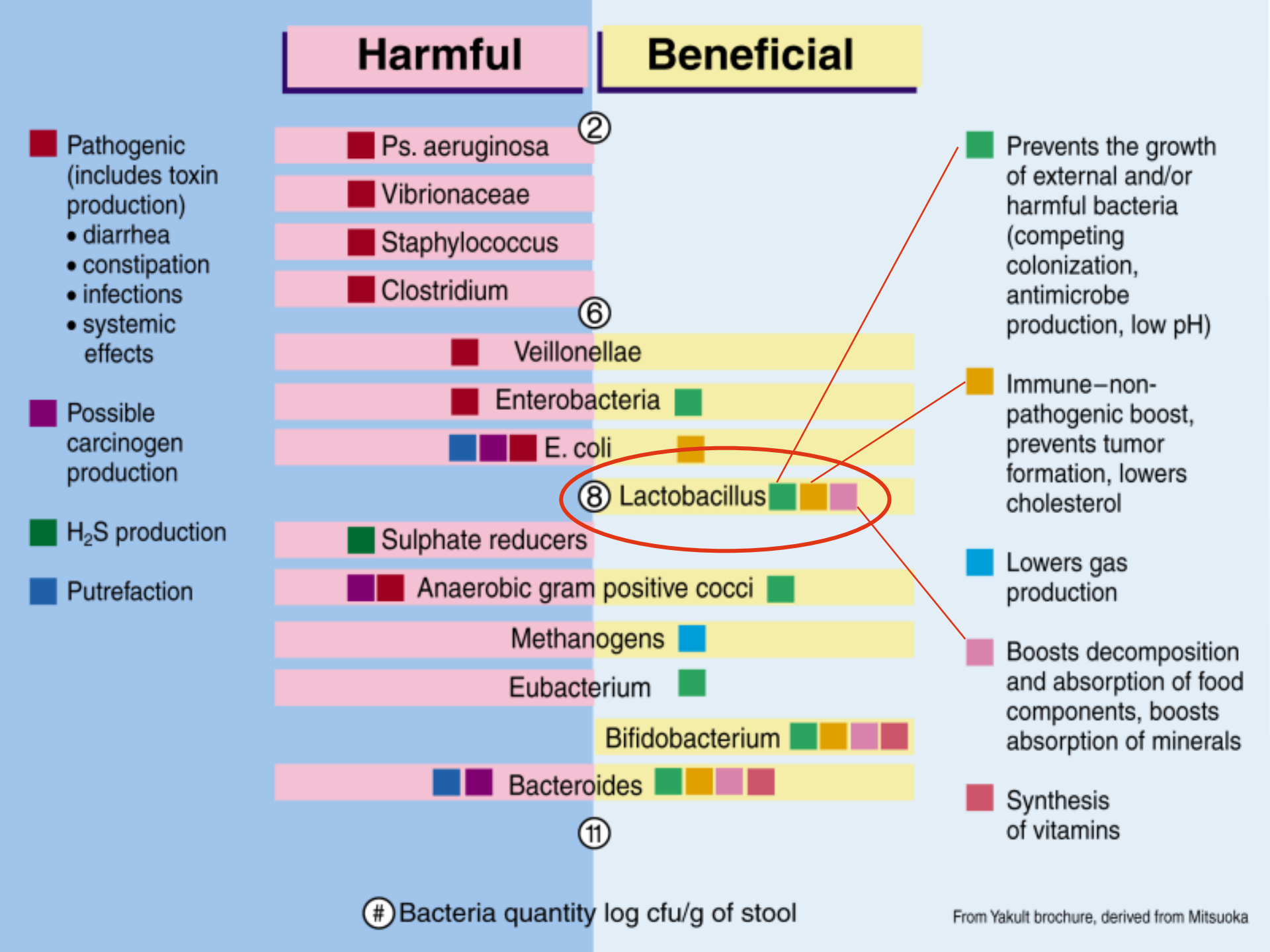




Digestive health
Human microbiota is essential for health

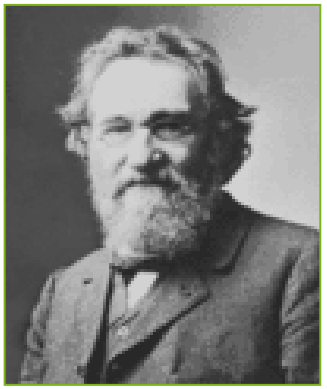


- Each person harbours 1-1½ kg of bacteria, equivalent to 10^{14} microbial cells, epithelial cell linings in the mouth, intestine, vagina, skin
- The intestine alone contains around 1 kg of bacteria with >400 different bacterial species
- These bacteria are metabolically active and have a variety of functions in the intestine
- A good balance of bacteria is essential for optimum digestive health
- In an average lifetime, the gut will handle the equivalent of 65 tons of food and drink, equivalent to the weight of 12 elephants!
- 'Prebiotics' and 'probiotics' can be used to improve the balance of bacteria in the colon


Harmful
Beneficial
Pathogenic (includes toxin production)
• diarrhea
• constipation
• infections
• systemic effects
Possible carcinogen production
H_2S production
Putrefaction
②
Ps. aeruginosa
Vibrionaceae
Staphylococcus
Clostridium
⑥
Veillonellae
Enterobacteria
E. coli
⑧ Lactobacillus
Sulphate reducers
Anaerobic gram positive cocci
Methanogens
Eubacterium
Bifidobacterium
Bacteroides
⑪
Prevents the growth of external and/or harmful bacteria (competing colonization, antimicrobe production, low pH)
Immune–non-pathogenic boost, prevents tumor formation, lowers cholesterol
Lowers gas production
Boosts decomposition and absorption of food components, boosts absorption of minerals
Synthesis of vitamins
Bacteria quantity log cfu/g of stool
From Yakult brochure, derived from Mitsuoka

Lactic acid bacteria and their impact on health - the views of Élie Metchnikoff

- Russian scientist and Nobel laureate **Élie Metchnikoff**, who in the beginning of the 20th century suggested that it would be possible to modify the gut flora and to replace harmful microbes with useful microbes.
- **Metchnikoff**, at that time a professor at the Pasteur Institute in Paris, proposed that the aging process results from the activity of putrefactive (proteolytic) microbes producing toxic substances in the large bowel.
- **Metchnikoff** had observed that certain rural populations in Europe, for example in Bulgaria and the Russian steppes who lived largely on milk fermented by lactic-acid bacteria were exceptionally long lived.



E. Metchnikoff, 1907

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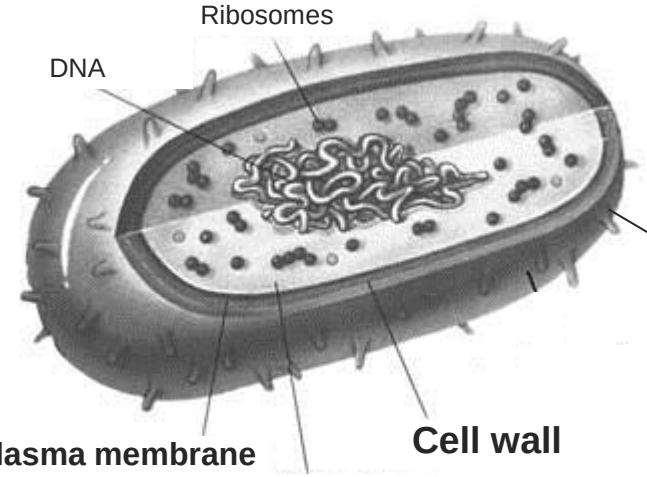
Definition of probiotics

- Substances produced by microorganisms which promote the growth of other microorganisms (Lilly and Stillwell,1965)
- Organisms and substances which contribute to intestinal microbial balance (Parker, 1974)
- A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989)
- A viable mono- or mixed-culture of microorganisms which applied to animal or man, beneficially affects the host by improving the properties of the indigenous microcflora (Havenaar and Huis In't Veld, 1992)
- Living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition (Schaafsma, 1996)
- A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract (Naidu et al., 1999)
- A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host (Schrezenmeir and de Vrese, 2001)
- **Live microorganisms which when administered in adequate amounts confer a health benefit on the host. (FAO/WHO report, 2001)**

The probiotic cell and its composition



Plasma membrane
- Lipids

Cytoplasm
- Enzymes

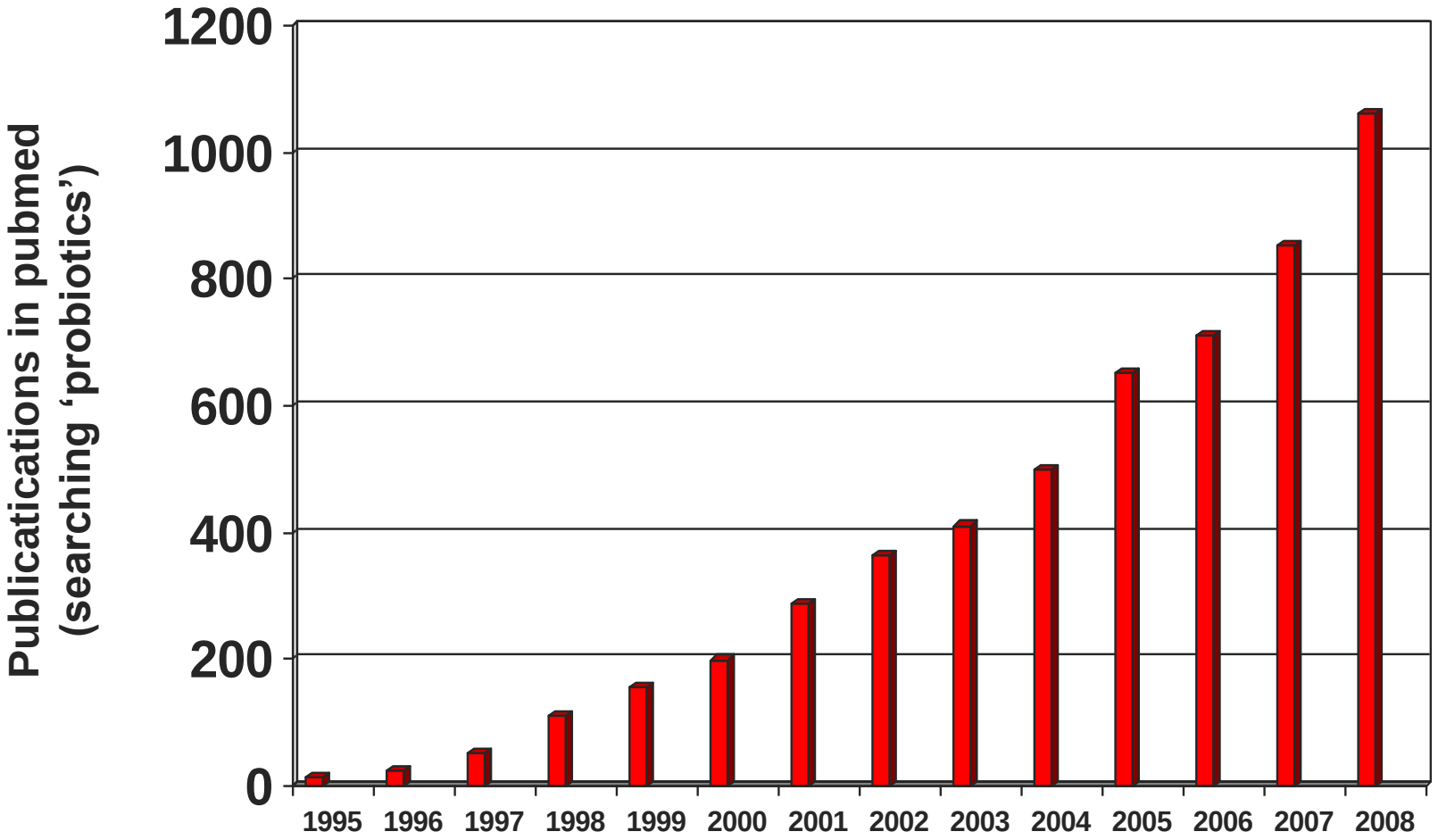
Cell wall
- Peptidoglycan
- Other proteins
- Lipotechoic acid
- Teichoic acid
- Saccharides

Capsules and S-layers
- Protein
- Polycacharides
- Glycoproteins

The cell may release
- Proteins including enzymes
- Glycoproteins
- Polysaccharides
- Oligosacharides
- Hydrogen peroxide
- Organic acids
- Bacteriocins

Components that may effect the immune system and/or microorganisms of the gastrointestinal tract

Interest in probiotics



Overview

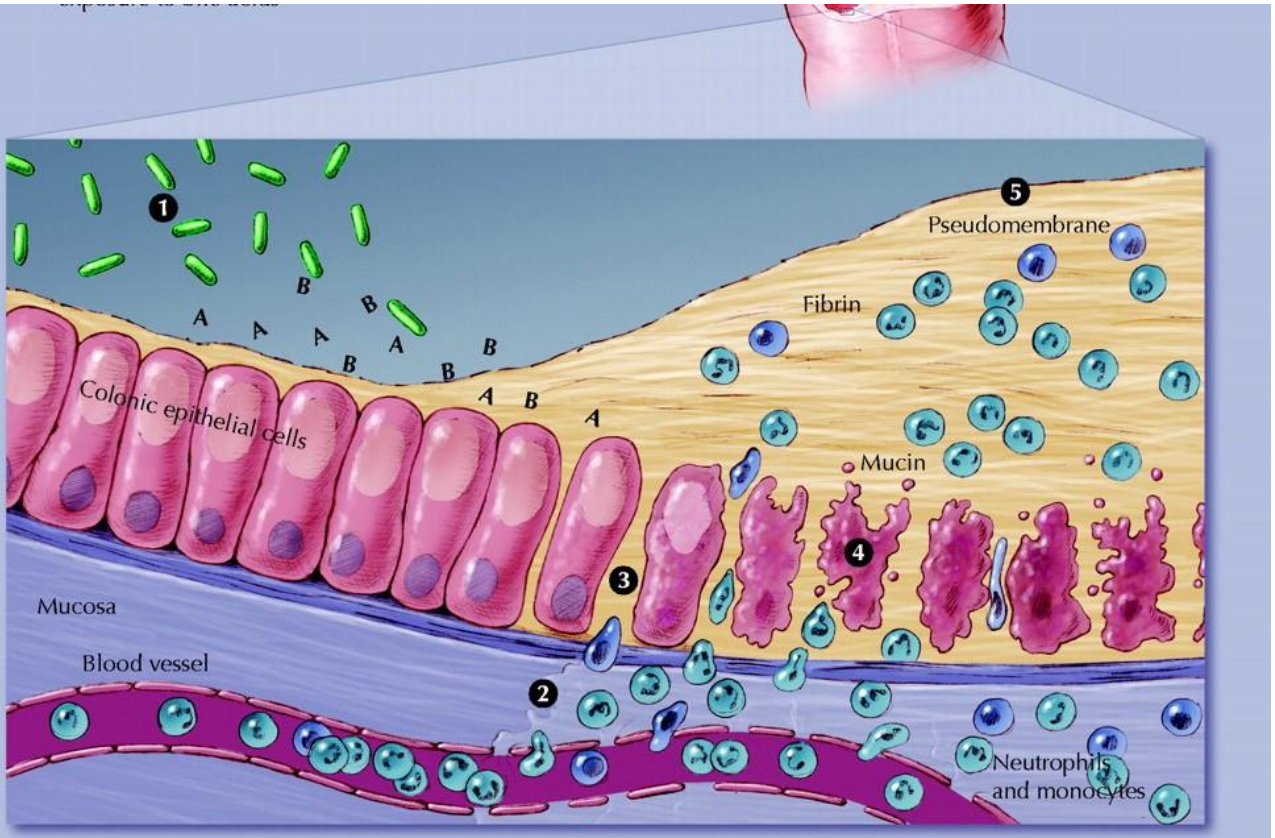
- Probiotics – historical perspective
- Examples of application - management of:
  - Antibiotic associated diarrhoea
  - Eczema
- Stability of probiotics

Colonization resistance

Treatment with antibiotics may weaken colonization resistance, resulting in:

- Invasion by pathogenic bacteria, including *Clostridium difficile*, leading to antibiotic associated diarrhoea and pseudomembranous colitis
- Invasion by yeast, including *Candida albicans*, leading to thrush

Antibiotic assosiated diarrhoea may lead to pseudomembranous colitis



C. difficile vegetative cells produce toxins A and B and hydrolytic enzymes (**1**). Local production of toxins A and B leads to production of tumour necrosis factor-alpha and proinflammatory interleukins, increased vascular permeability, neutrophil and monocyte recruitment (**2**), opening of epithelial cell junctions (**3**) and epithelial cell apoptosis (**4**). Local production of hydrolytic enzymes leads to connective tissue degradation, leading to colitis, pseudomembrane formation (**5**) and watery diarrhea.

Referenc
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Journal of Medical Microbiology

Volume 58, Pages 663-670

"Probiotics to minimize the disruption of faecal microbiota in healthy subjects undergoing antibiotic therapy"

Published May, 2009

Journal of Medical Microbiology (2009), 58, 663–670 DOI 10.1099/jmm.0.47615-0

Probiotics to minimize the disruption of faecal microbiota in healthy subjects undergoing antibiotic therapy

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A novel combination of culturing and DNA-based terminal restriction fragment length polymorphism (TRFLP) analysis was used to investigate the effect of probiotics on antibiotic-induced gut microbiota alterations to determine if a probiotic preparation containing bifidobacteria and lactobacilli, taken during and after antibiotic therapy, can minimize antibiotic disturbance of faecal microbiota. Healthy subjects administered amoxicillin/clavulanate were randomized and concomitantly received a placebo or probiotic mixture. The primary end point was similarity of faecal microbiota as determined by culturing and TRFLP from subjects taking probiotics compared to those taking a placebo measured by comparing data from baseline to post-treatment for each subject. TRFLP analysis revealed a high subject to subject variation in the baseline faecal microbiota. The most common antibiotic-induced disturbance was a relative increase in *Clostridium*, *Eubacterium*, *Bacteroides* and *Enterobacteraceae*. The mean similarity to the baseline increased over time in both treatment groups, although the probiotic group was less disturbed according to both TRFLP and culture data. The culture method revealed that post-antibiotic faecal microbiota in probiotic-consuming subjects were more similar to the baseline microbiota than the control group ($P=0.046$). Changes in *Enterobactereaceae* ($P=0.006$) and *Bifidobacterium* ($P=0.030$) counts were significantly different between the groups. Analysis of TRFLP data reinforced the trend between groups but was not statistically significant ($P=0.066$). This study indicates this mixture of probiotics promotes a more rapid return to pre-antibiotic baseline faecal bacterial microbiota.

Received 6 September 2007
Accepted 25 January 2009

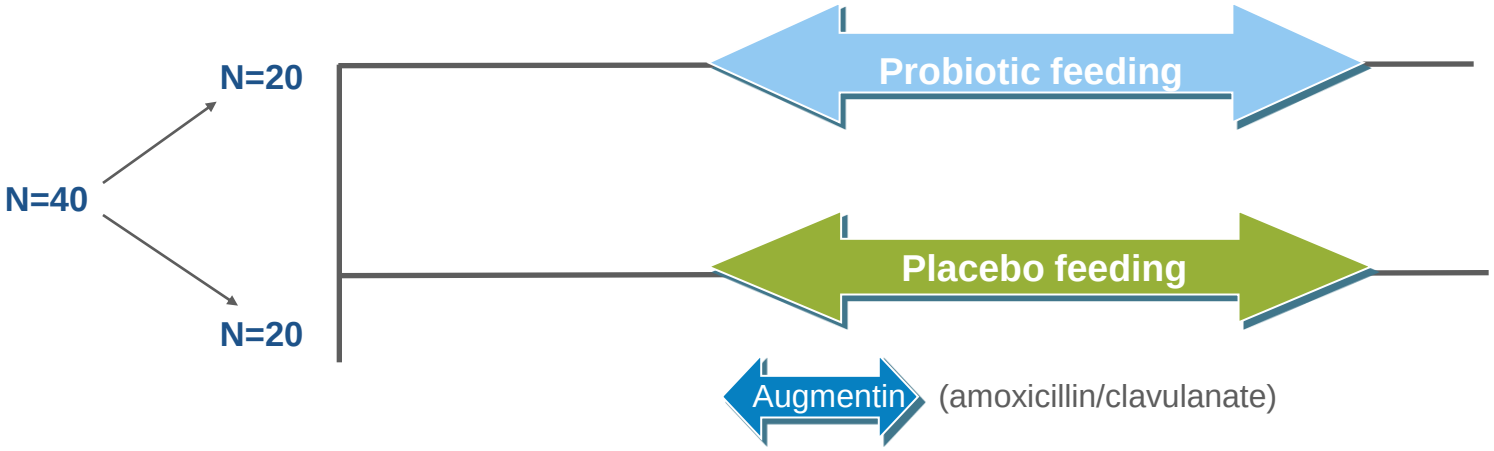
INTRODUCTION

Antibiotic use is often associated with adverse gastrointestinal effects. Attempts to minimize these side effects have included the use of probiotics with the aim of stabilizing intestinal bacterial communities and minimizing possible alterations in microbial community structure.

The microbial environment of the intestine is highly diverse and complex, with an estimated population of over 10^{14} bacteria, of which perhaps 60–80 % are not culturable by conventional microbiology methods (Eckburg *et al.*, 2005). Recent advances in techniques using 16S rRNA sequences, which permit the identification of unique bacterial subspecies, have begun to yield new insights into the diversity, organization and dynamics of gut microbiota in health and disease (Backhed *et al.*, 2005). A recently established method, terminal restriction fragment length polymorphism (TRFLP) analysis, is a practical way to characterize microbiota because species richness and relative abundance are measured rapidly and reproducibly (Blackwood *et al.*, 2003; Kitts, 2001). Because the data from TRFLP are automatically digitized the technique is well

Abbreviations: MANOVA, multivariate ANOVA; TRF, terminal restriction fragment; TRFLP, terminal restriction fragment length polymorphism.

HOWARU® Restore study protocol in brief



Monitored biomarkers through assessment of fecal flora

- Effect of probiotic therapy during and after gut stress by:
  - Levels of lactobacilli and bifidobacteria
  - Levels of *Enterobacteriaceae*, *Bacteriodes* and *Clostridium*
  - First study incorporating TRF analysis with probiotics to determine fecal bacterial communities dynamics and genus specific evaluations

Study conducted at Washington University in St. Louis, MO, under the direction of Dr. Joshua Korzenik

HOWARU® Restore **study result**

Total gut microflora re-established rapidly
Placebo lags even 2 weeks after cessation of gut stress

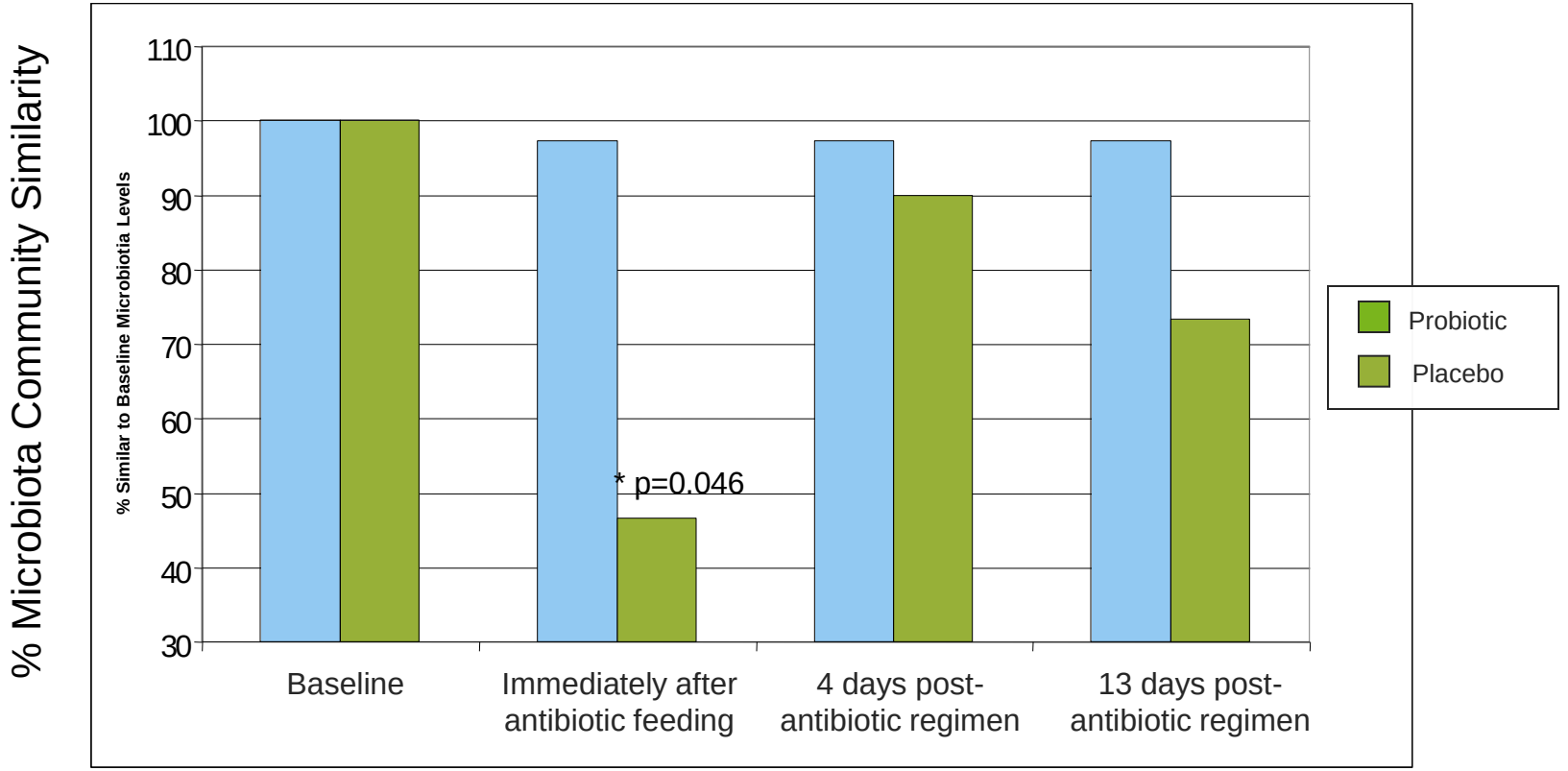


Figure 1. These data support that this probiotic mixture protects the fecal microbiota from disruption by antibiotics, as indicated by greater dissimilarity of microbiota of the placebo group.

HOWARU® Restore study result

- Bifidobacteria levels re-established rapidly
- Placebo shows no improvement 2 weeks after cessation of antibiotic treatment

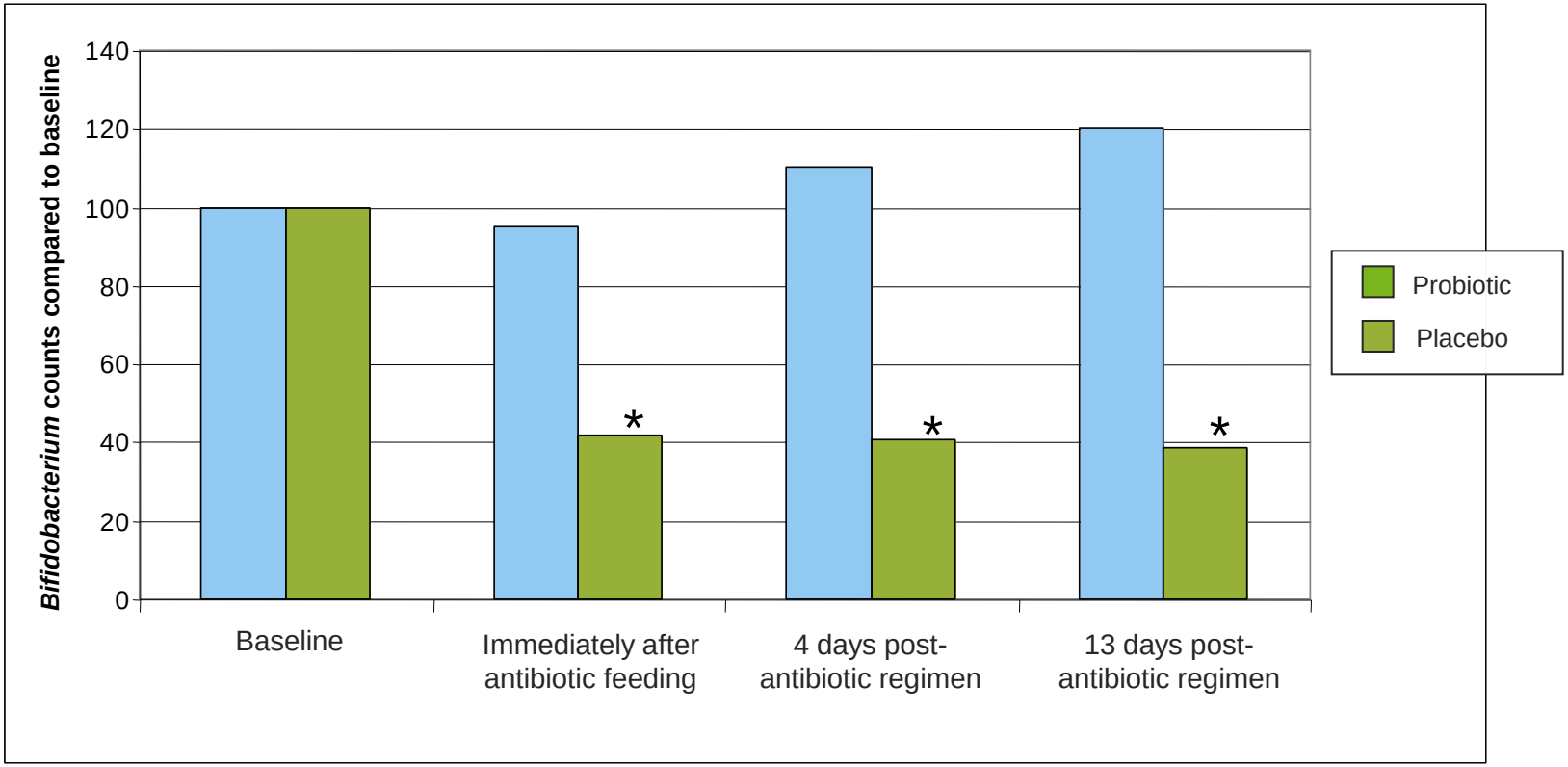


Figure 2. These data support that this probiotic mixture encourages maintenance of bifidobacteria levels in feces in antibiotic-consuming subjects over the post-treatment days (*p=0.030).

The effect of *Lactobacillus acidophilus* on intestinal populations of *Clostridium difficile*

Study design

- **Randomized, placebo controlled cross-over study**
- **Study group**: 31 subjects, 72-103 years of age, median 86 years
- **Treatments**:
  - Run-in (2 weeks), subjects are consuming cheese without probiotics (15g/day)
  - Treatment phase (4 weeks), subjects are consuming cheese containing 10^9 CFU each of *L. acidophilus*
  - Washout phase (4 weeks),
- **Determination of populations:** Faecal levels of *C. difficile*, monitored by PCR, monitored at the end of each treatment period.

Lactobacillus acidophilus reduces intestinal population levels of *Clostridium difficile*

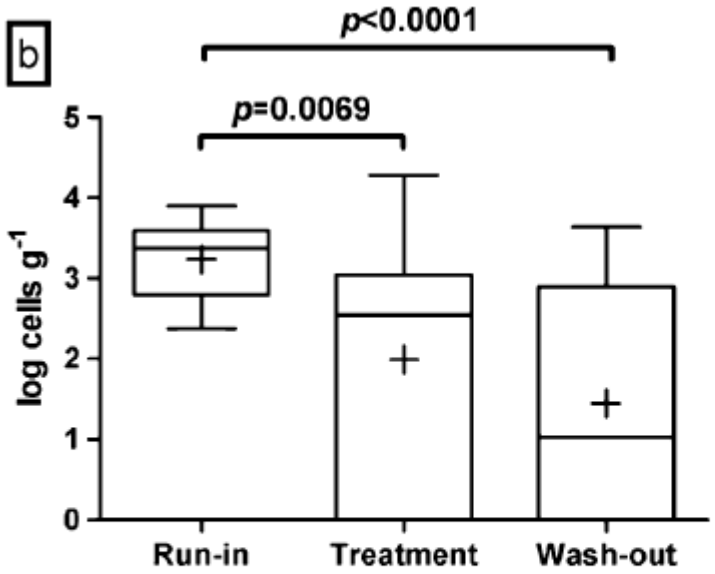
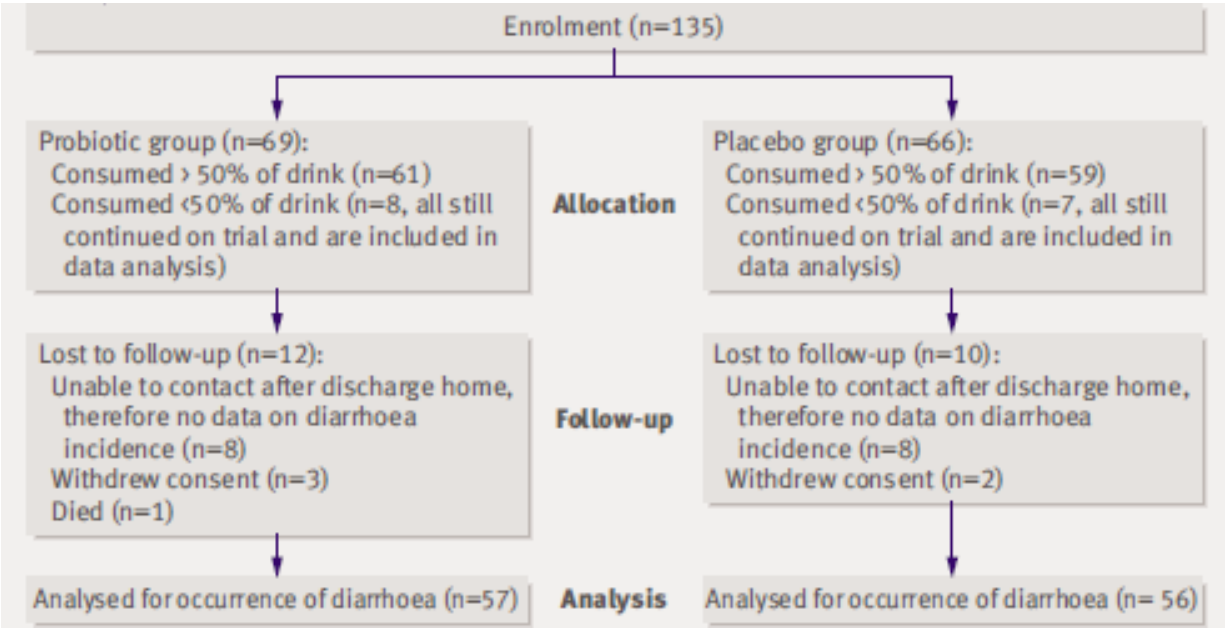


Figure. 2. Faecal levels of *C. difficile* in subjects after a run-in period, after the treatment with *Lactobacillus acidophilus* (10^9 CFU/day) and after a wash-out period.

Lathinen *et al*. (2011) Age,Jan 25.

Use of a probiotic *Lactobacillus* preparation to prevent antibiotic associated diarrhoea (AAD): randomized double blind placebo controlled trial



Hickson *et al* (2007) BMJ

Referenc
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Fewer cases of antibiotic associated diarrhoea in the group receiving probiotics

Table 2 | Total number of cases of antibiotic associated diarrhoea (including cases positive for *C difficile*) and proportion who were positive or negative for *C difficile* toxin (all patients followed up in hospital and after discharge)

	Probiotic	Control	P value*
Diarrhoea			
Yes	7 (12)	19 (34)	0.007
No	50 (88)	37 (66)	
No of patients	57†	56†	
C difficile toxin			
Positive	0	9 (17)	0.001
Negative	56 (100)	44 (83)	
No of patients	56‡	53‡	

*Fisher's exact test.
†22/135 patients lost to follow-up or withdrew.
‡4/113 patients not tested for *C difficile*.

Hickson *et al* (2007) BMJ

Studies assessing the effect of *Saccharomyces boulardii* and AAD

McFarland LV. *Saccharomyces boulardii* in adults

Table 2 Randomized, controlled trials for the prevention of antibiotic associated diarrhea (AAD) using *S. boulardii*

Ref.	Treatment groups	Population	Daily dose: cfu/d (mg/d)	Duration (d)	Follow-up (wk)	AAD in *S. boularii* (%)	AAD in controls (%)
Adam *et al*[37]	*S. boulardii* *vs* placebo	388 hospitalized adults, multisite	4×10^9 (200 mg)	7	0	9/199 (4.5)[a]	33/189 (17.5)
Monteiro *et al*[55]	*S. boulardii* *vs* placebo	240 adults, Portugal	(nr) 4 caps/d	6	0	19/121 (15.7)[a]	33/119 (27.7)
Surawicz *et al*[58]	*S. boulardii* *vs* placebo	180 hospitalized adults at one hospital, Washington	2×10^{10} (1000 mg)	abx + 14 d	2-3	11/116 (9.5)[a]	14/64 (21.8)
McFarland *et al*[54]	*S. boulardii* *vs* placebo	193 hospitalized adults (1 or more beta-lactam antibiotics), 4 hospitals	2×10^{10} (1000 mg)	abx + 3 d	7	7/97 (7.2)[a]	14/96 (14.6)
Lewis *et al*[133]	*S. boulardii* *vs* placebo	72 enrolled, 69 done; elderly patients	4.5×10^9 (226 mg)	14	0	7/33 (21)	5/36 (13.9)
Cremonini *et al*[134]	*S. boulardii* *vs* placebo	43 *H. pylori* + adults on triple therapy	5×10^9 (nr)	14	2	1/22 (5)[a]	6/21 (30)
Duman *et al*[44]	*S. boulardii* *vs* placebo	389 adults in Turkey with *H. pylori* + peptic ulcers, all received triple therapy	2×10^{10} (1000 mg)	14	4	14/204 (6.9)[a]	28/180 (15.6)
Can *et al*[135]	*S. boulardii* *vs* placebo	151 adult inpatients	1×10^{10} (500 mg)	abx	4	1/73 (1.4)[a]	7/78 (9.0)
Cindoruk *et al*[42]	*S. boulardii* *vs* placebo	124 adults with *H. pylori* + dyspepsia	2×10^{10} (1000 mg)	14	6	9/62 (14.5)[a]	19/62 (30.6)
Bravo *et al*[136]	*S. boulardii* *vs* placebo	89 adult outpatients on amoxicillin	1×10^{10} (500 mg)	12	9	3/41 (7.3)	5/45 (11.1)

[a]$P < 0.05$, probiotic *vs* controls. cfu: Colony forming unit; nr: Not reported; abx: Antibiotics.

Overview

- Probiotics – historical perspective
- Examples of application - management of:
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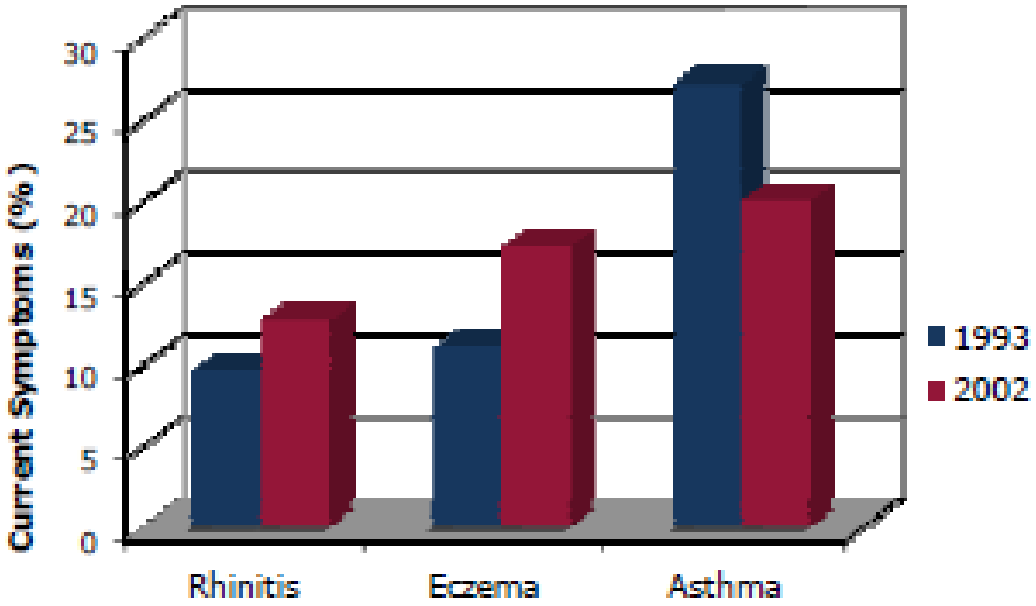
  - Eczema
- Stability of probiotics

The prevalence of eczema, rhinitis and asthma in Australians aged 6-7 years in 1993 and 2002

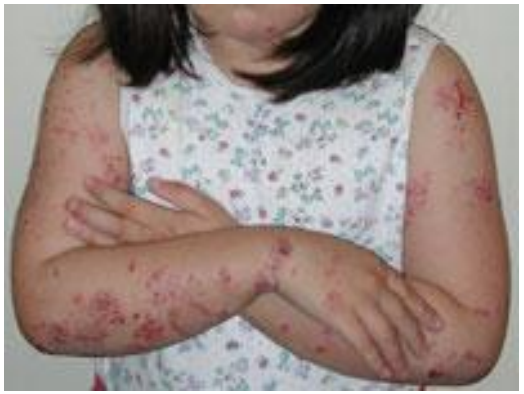


Source: Robertson et al (2004)

Allergies

- Australia and New Zealand have among the highest prevalence of allergic disorders in the developed world
- 4,1 million Australians have at least one allergy
- In 2007, the financial cost of allergies was $7.8 billion in Australia

- One of the earliest signs of allergies is atopic eczema
  - affecting around one in five infants,
  - reducing to around one in six children aged 6-7 years, and
  - one in ten children aged 13-14 years and
  - one in 14 adults (Asher et al, 2006).



Source: Report by Access Economics for the Australasian Society of Clincial Immunology and Allergy " *The economic impact Of allergic disease in AUstralia.; not to be sneezed at*

The hygiene hypothesis

Hay fever, hygiene, and household size

David P Strachan

Department of Epidemiology and Population Sciences, London School of Hygiene and Tropical Medicine, London WC1E 7HT
David P Strachan , MRCP, *lecturer in epidemiology*

Br Med J 1989;**299**:1259-60

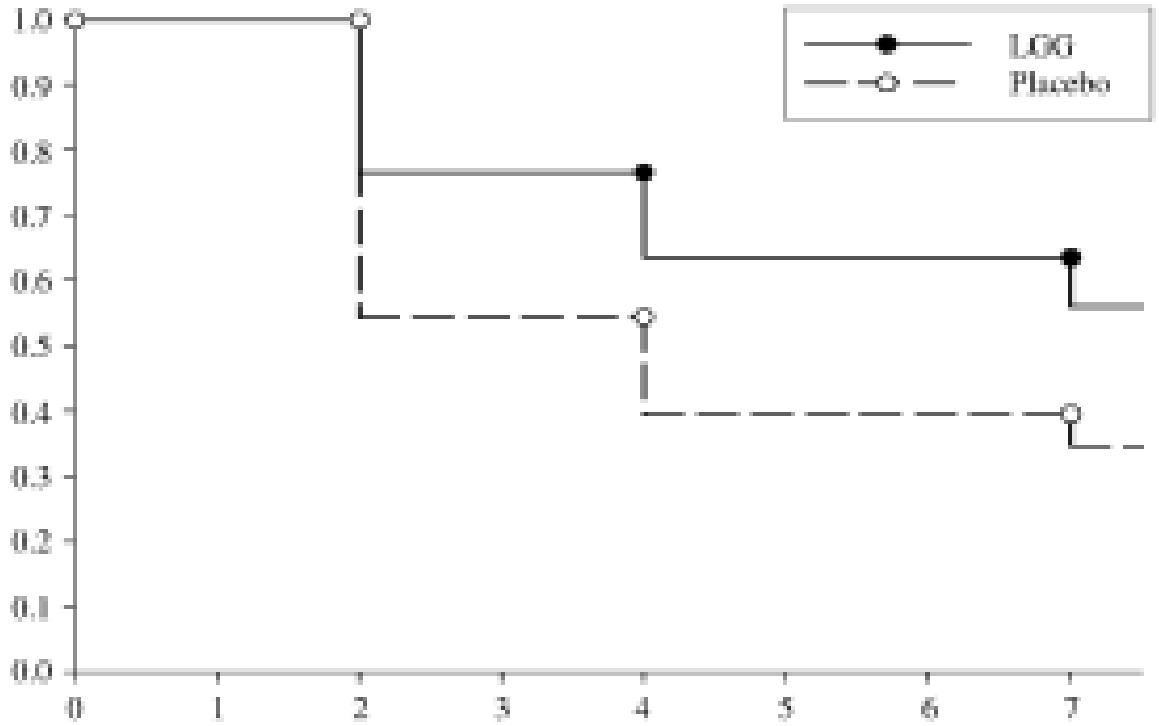
Hay fever has been described as a "post industrial revolution epidemic,"[1] and successive morbidity surveys from British general practice suggest that its prevalence has continued to increase over the past 30 years.[2] Other evidence suggests a recent increase in the prevalence of asthma[2] and childhood eczema.[3] This paper suggests a possible explanation for these trends over time.

Lactobacillus rhamnosus and atopic dermatisis – early studies

Study no	Reference(s)	Strain, CFU/day	Number of subjects (active group + placebo)	Significant reduction of symptoms/incident
1	Kalliomaki et al (2001). Lancet Kallomaki et al (2003). Lancet Kallomaki et al (2007). Lancet	LGG, $2x10^{10}$	159 (two groups)	Yes
2	Kirjavainen et al (2003). JPGN.	LGG, $3x10^{10}$	35 (three groups)	Yes
3	Isolauri et al (2000). CEA	LGG+Bb-12, $3\text{-}8x10^{10}$	27 (two groups)	Yes
4	Majamaa (1997). JCI	LGG, $3x10^{10}$ to infants (??) and $4x10^{10}$ to mothers	27 (two groups)	Yes

Children without atopic eczema

p = 0.008 by log rank test



Kallomaki *et al* (2007)
Lancet

The effect of *Lactobacillus rhamnosus* HN001 on eczema

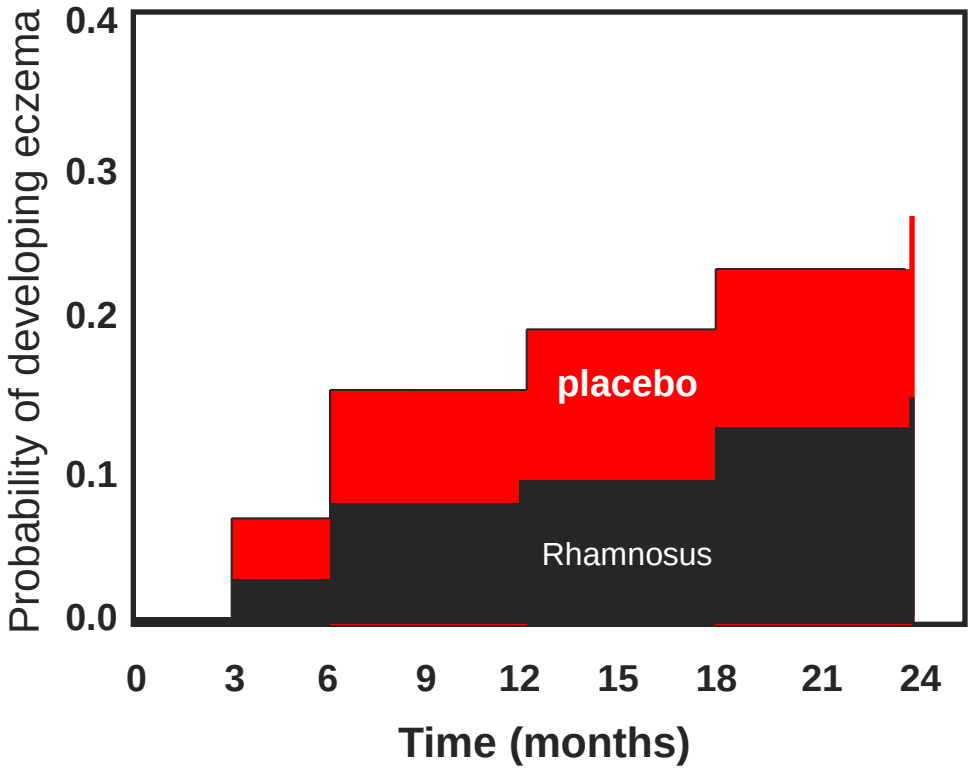
Wellington School of Medecine & Health Science, University of Otago, New Zealand

- Randomized, double-blind, placebo controlled
- Infants with family history of allergy
- 3 treatment arms:
  - ***L. rhamnosus* HN001** at **6 x 10^9** cfu/day,
  - ***B. lactis* HN019** at 9 x 10^9 cfu/day,
  - Placebo
- Around 150 infants per group
- Pregnant mothers treated daily from ~5 weeks pre-term to 6 months post-term for breastfeeding mothers
- Infants treated daily from birth to 24 months old - treatments given as supplement to infant feeds (breast milk, infant formula, weaning food)

K . Wickens , P . Black , T . Stanley , E . Mitchell , P . Fitzharris , G . Tannock , G . Purdie , J . Crane**, 2008,**
A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial . **Journal of Allergy and Clinical Immunology ,** Volume 122 , Issue 4 , Pages 788 – 794

Prescott,S.L.; Wickens,K.; Westcott,L.; Jung,W.; Currie,H.; Black,P.N.; Stanley,T.V.; Mitchell,E.A.; Fitzharris,P.; Siebers,R.; Wu,L.; Crane,J. 2008 Supplementation with *Lactobacillus rhamnosus* or *Bifidobacterium lactis* probiotics in pregnancy increases cord blood interferon-gamma and breast milk transforming growth factor-beta and immunoglobin A detection. **Clinical and Experimental Allergy, 2008** *in press*

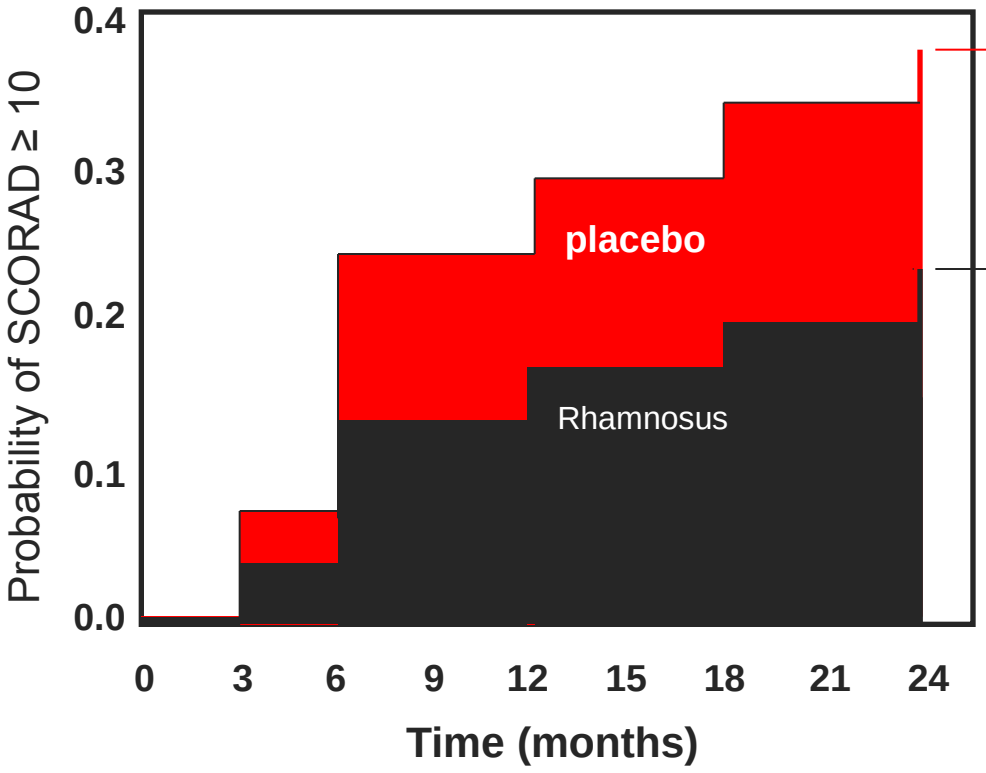
Fewer children developed atopic eczema



Fewer children in the ***L. rhamnosus*** **HN001** group developed atopic eczema in the first two years of life.

Wickens et al. 2008

Less severe atopic eczema



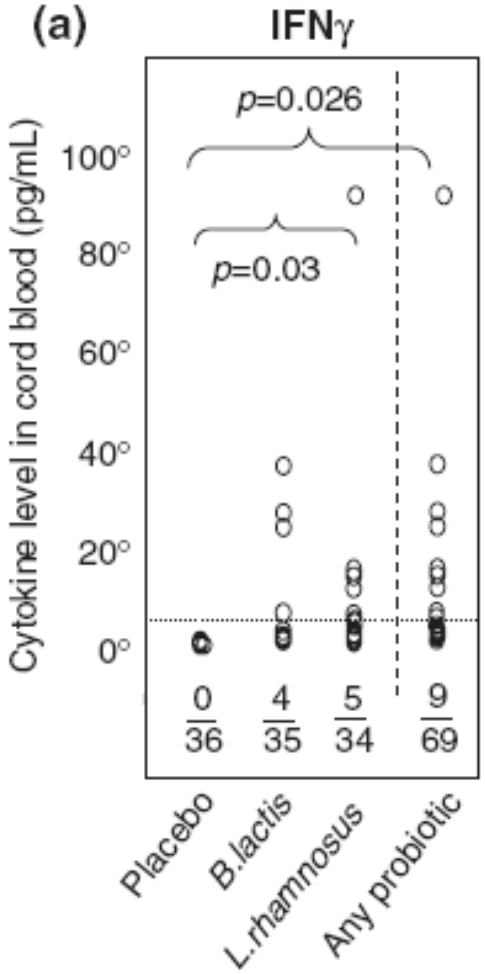
38.7% after 2 years

24.0% after 2 years

Of the children that developed eczema; less severity in the *L. rhamnosus* HN001 group

Wickens et al. 2008

Prescott *et al* (2008)
Treatment with probiotics increases cord blood IFN-γ



In particular treatment with *L. rhamnosus* HN001 gave higher levels of cord blood IFN-γ

Increased IFN-γ has been observed to be associated with reduced allergy risk

Conclusion

At 6 x 10^9 CFU/day:

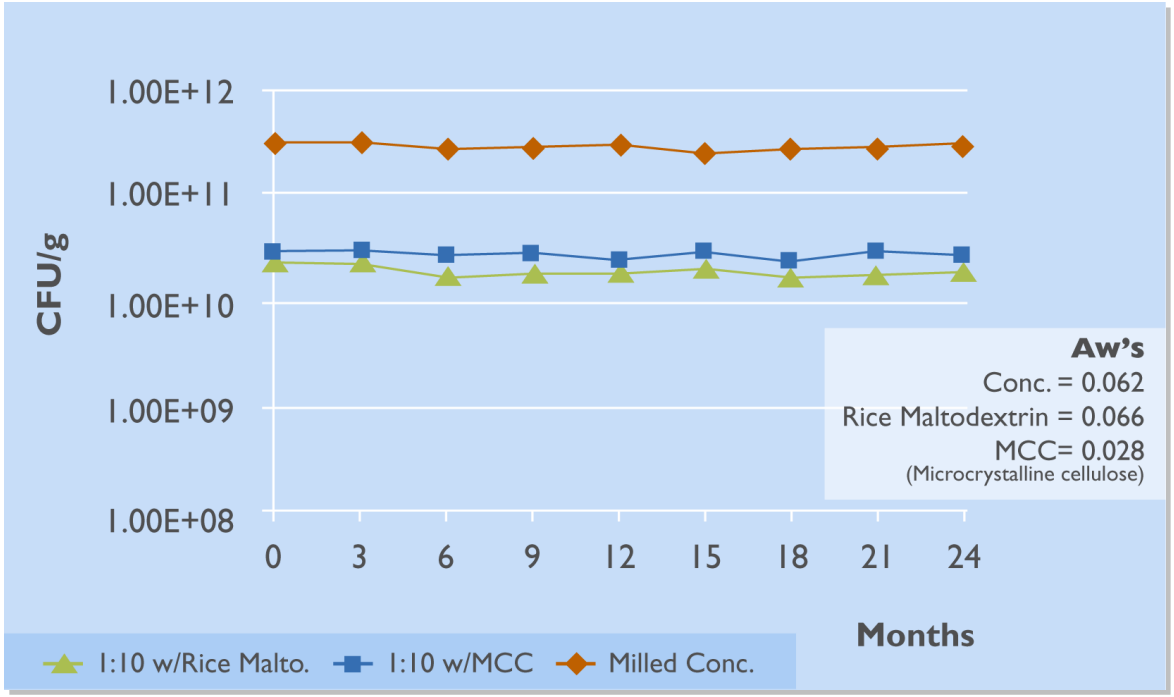
- *Lactobacillus rhamnosus* HN001 treatment gave fewer children with atopic eczema
- HN001 treatment gave less severe atopic eczema
- HN001 may be effective in preventing the development of atopic eczema in high-risk infants
- HN001 induced immune parameters that are considered to contribute to a reduced risk for allergy:
  - Cord blood IFN-γ , Breast milk IgA

Stability of probiotics

- Factors that affect stability of probiotics in capsules
  - How probiotics have been prepared (encapsulation)
  - Storage temperature
  - Water activity

- Survival of probiotics in the gastrointestinal tract
  - Taking probiotics with food – the impact on gastric survival

Probiotic stability
L. acidophilus @ 4°C



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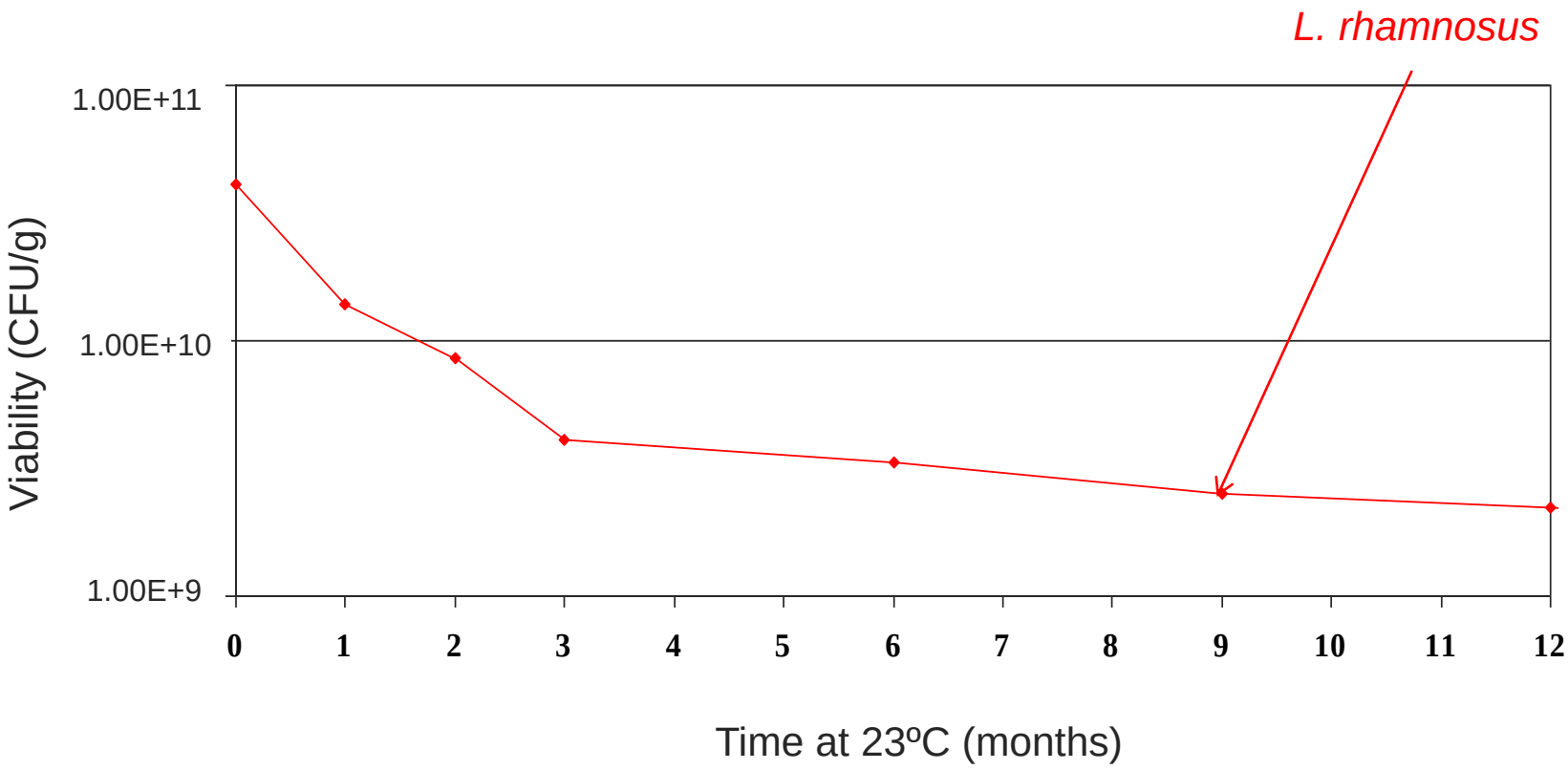
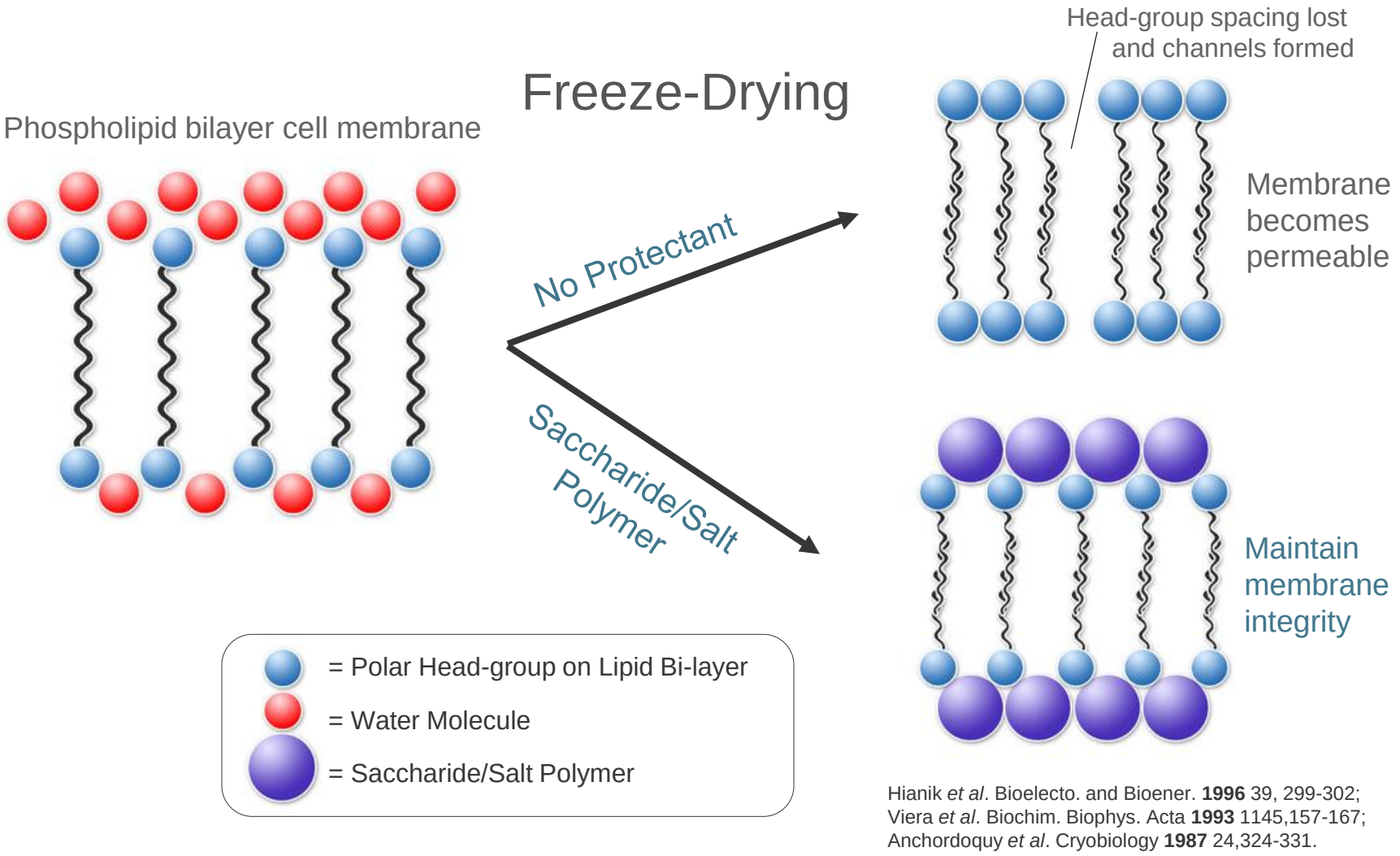
Stability of sensitive probiotic strains



Methods of encapsulation

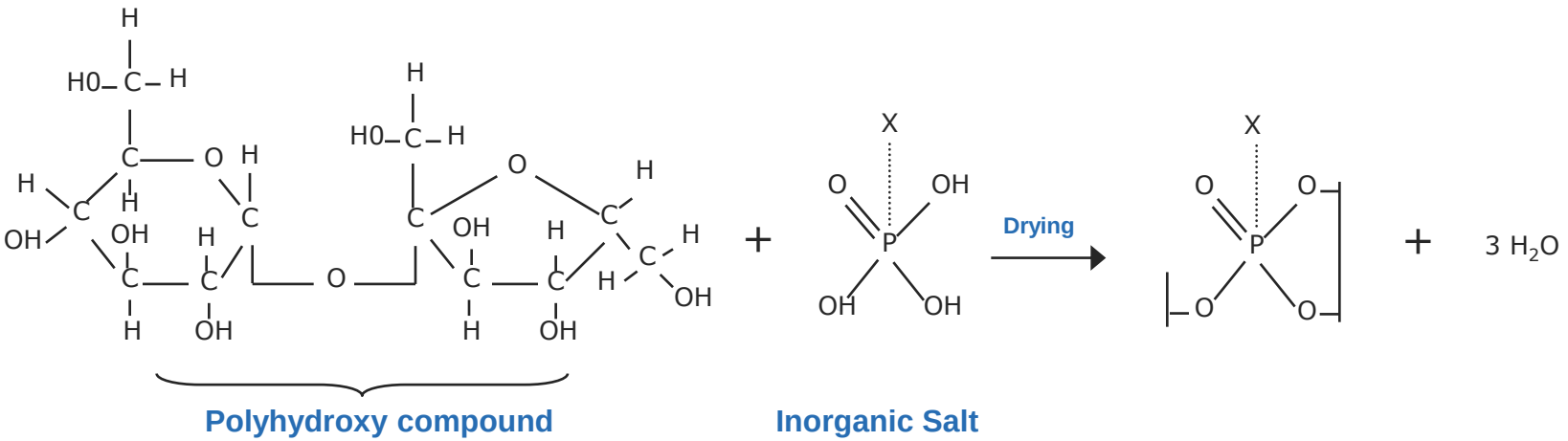
- Protein/carbohydrate coatings
- Alginate coating
- Carbohydrate/protein/fat coating
- Encapsulation with fats
- Polyhydroxy/salt complex

Probiotic stability: Key is to minimize cell membrane integrity during drying and storage



Hianik *et al*. Bioelecto. and Bioener. **1996** 39, 299-302;
Viera *et al*. Biochim. Biophys. Acta **1993** 1145,157-167;
Anchordoquy *et al*. Cryobiology **1987** 24,324-331.

Example of an encapsulation technique

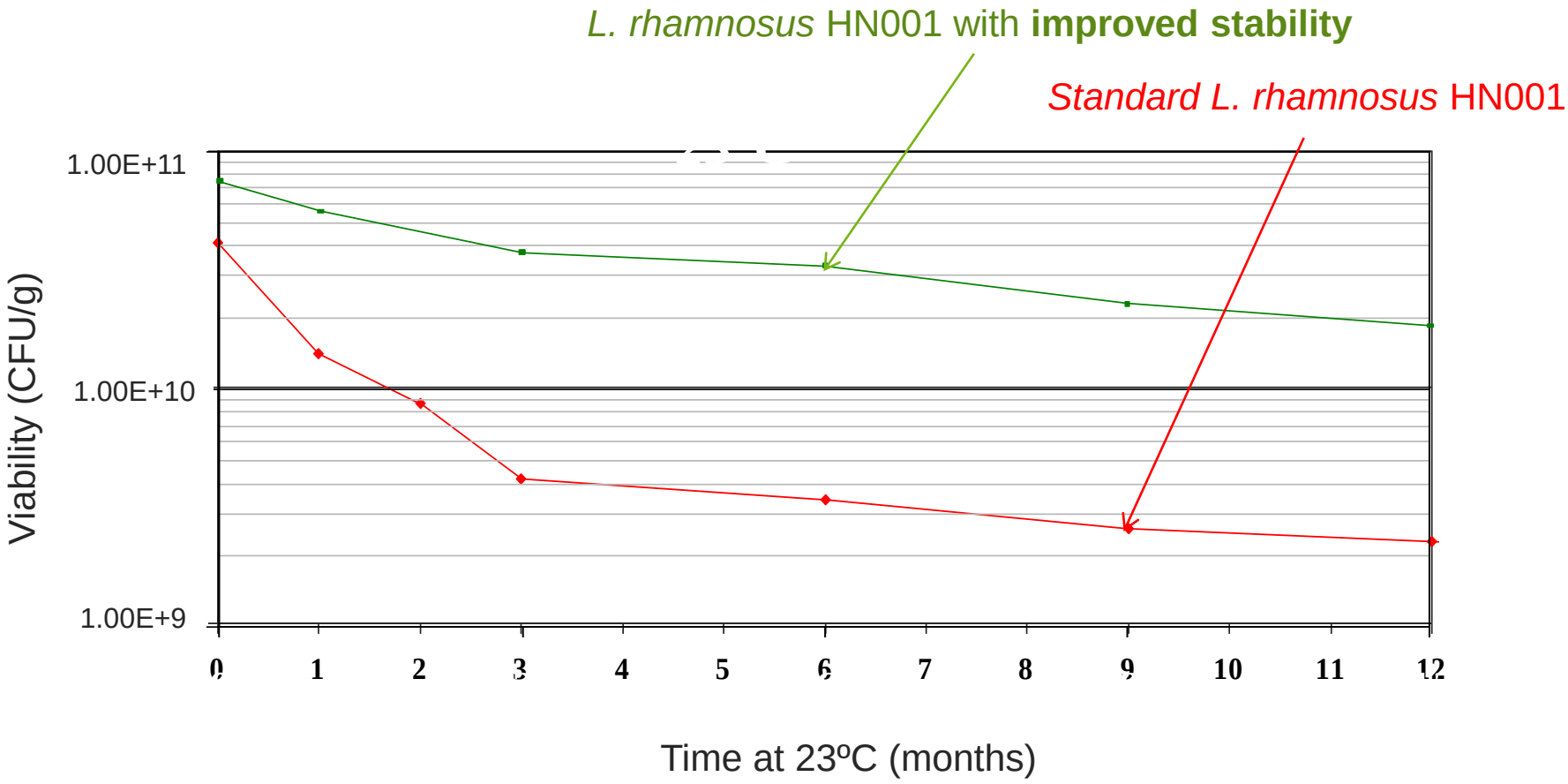


- Protectant is combination of a food-grade and GRAS polyhydroxy compounds plus an inorganic salt

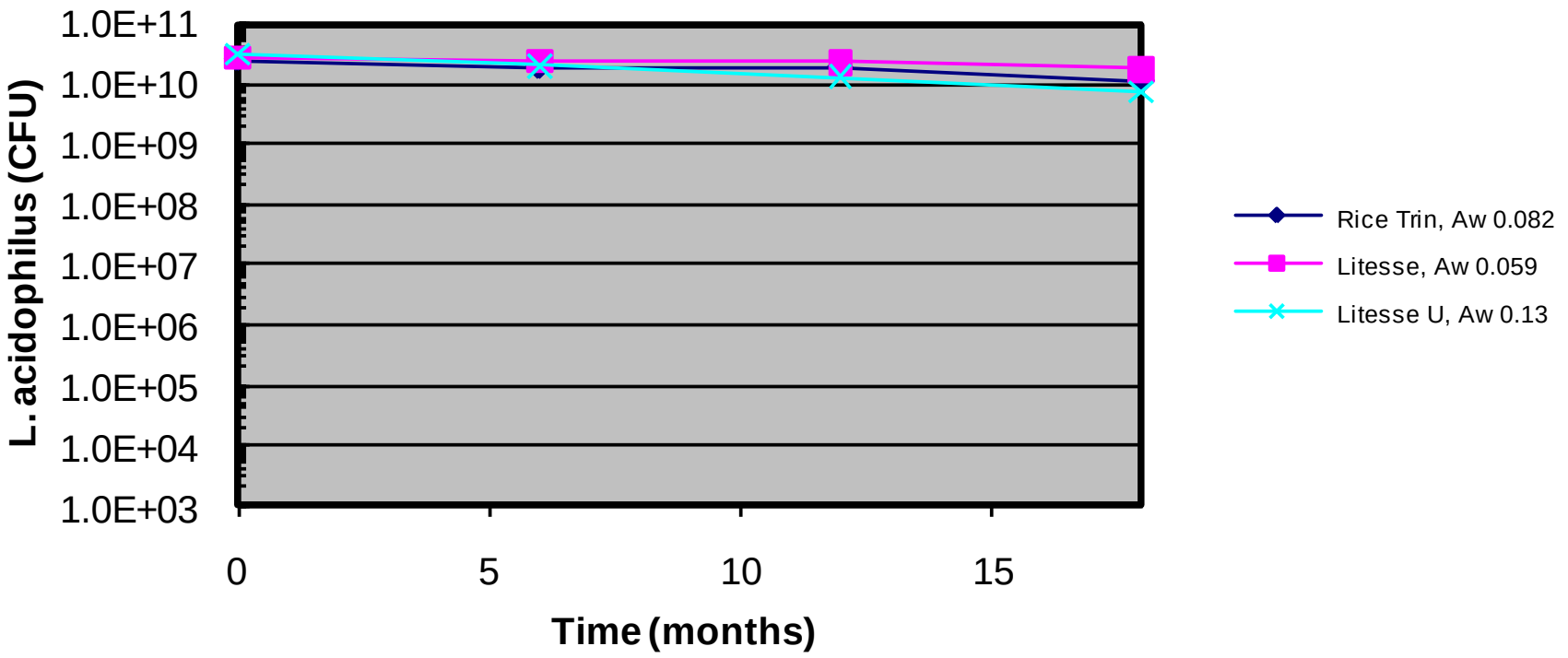
U.S. PATENT 6,653,062

PCT WO 02/09515 A1

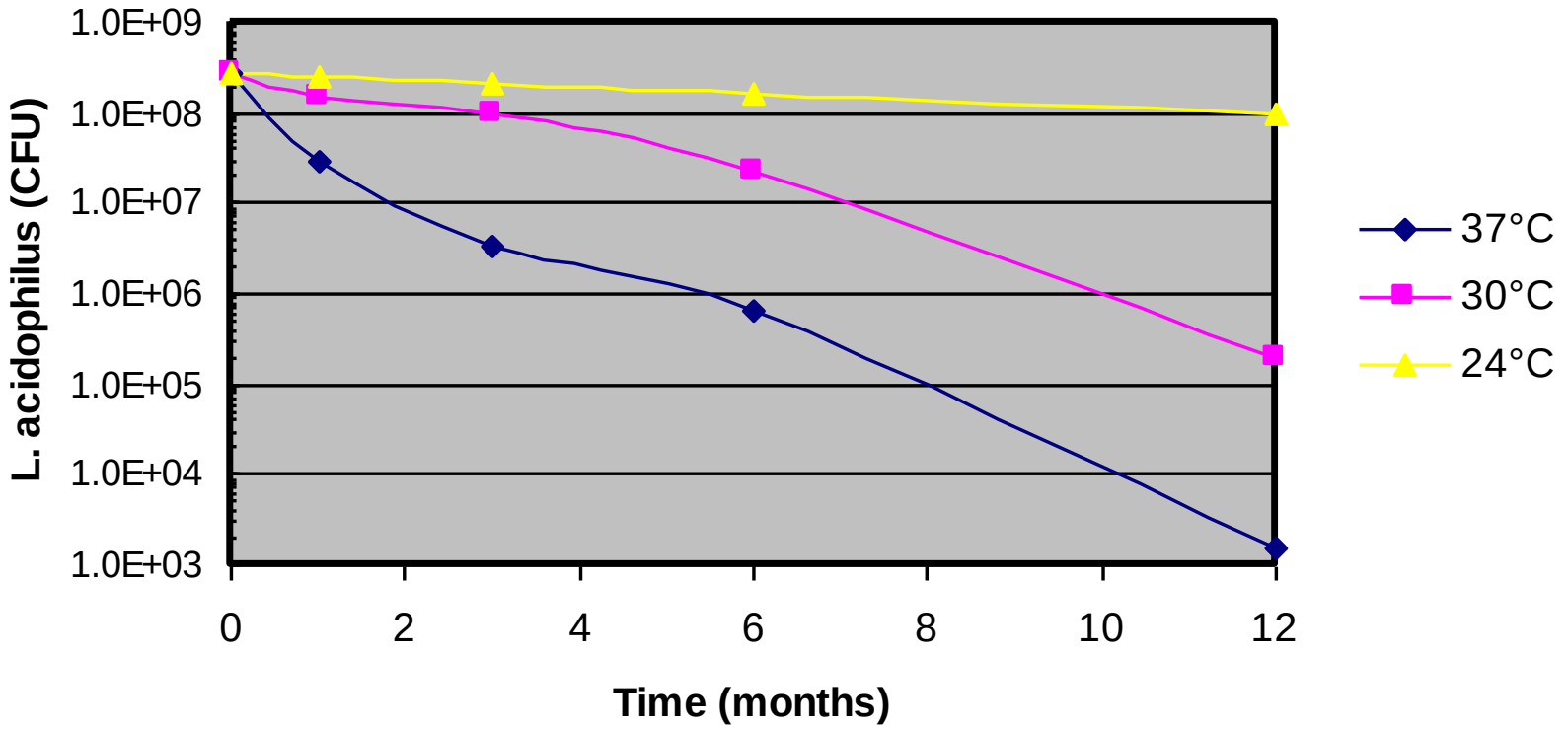
Novel technology to improve stability of sensitive probiotic strains



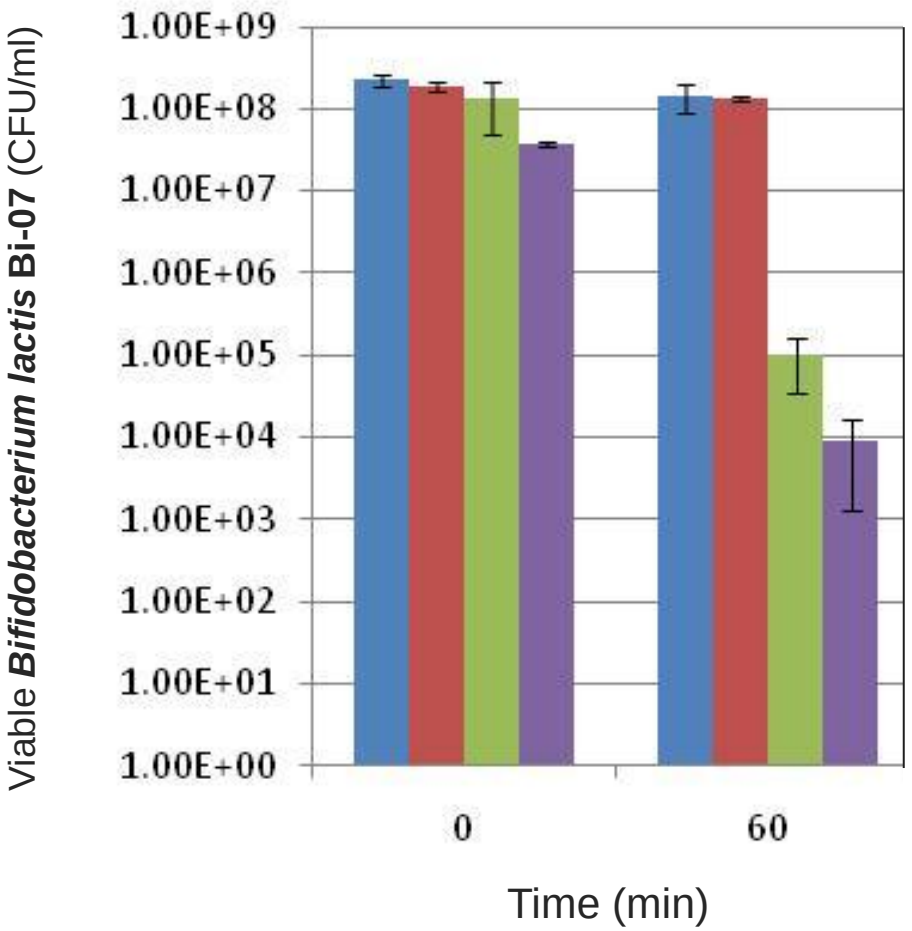
Survival of *L. acidophilus* in carriers with low water activities at 30°C



The effect of temperature on survival of *L. acidophilus* in milk powder (Aw 0.25)



Survival of probiotics in human gastric juice, pH 2.0 (synthetic)



Probiotics delivered with:

- Fresh milk
- Soya milk
- Saline + maltodextrin
- Saline

Summary - probiotics

- Certain strains reduce prevalence and severity of:
  - Eczema
  - Antibiotic associated diarrhoea
  - Rotavirus induced diarrhoea
- Encouraging data indicating that certain strains reduce symptoms associated with IBS and other Functional Bowel Disorders
- Recent developments give probiotic strains with superior stability during storage at ambient temperatures
- Opportunities in complimentary medicine

DANISCO
First you add knowledge...

Time for questions

Summary of HOWARU® Restore research findings

- HOWARU® Restore probiotic blend promotes a **return to normal flora faster** than without probiotic intervention

- HOWARU® Restore probiotic blend **protected against** antibiotic-induced **depletion of *Bifidobacterium*** in feces
  - *Bifidobacterium* sp. reported to have several benefits

- No previous studies for any probiotic has attempted to determine differences in entire bacterial communities

- TRF and culture techniques were complimentary, neither providing complete overall picture of flora changes, each brought different conclusions