Probiotics, Prebiotics, and Synbiotics

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Yogurt: A Wonder Food?

by the American Institute for Cancer Research

The body benefits from a healthy supply of "good" bacteria in the digestive tract, according to many researchers. A lack of beneficial bacteria among the microorganisms in the gut is thought to contribute to many health problems, including cancer.

Probiotic Foods

Yogurt / Kefir (fermented milk drink) / Sauerkraut

Tempeh (cake made of fermented cooked soybeans)

Miso (fermented soybean paste)

YAHOO! SEARCH

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Probiotics
Definition

- Nutritional supplement
- Contains 1 or more cultures of living organisms
  - Typically bacteria or yeast
- Modify the endogenous microflora
- Have a positive effect on the host

Prebiotics
Definition

- Nondigestible food ingredients
  - Fructo-oligosaccharides (chicory, inulin)
  - Lactulose
- Positively affect the endogenous flora
- Stimulate the growth of one or a limited number of bacterial species
  - FOS⇒Bifidobacteria
  - Lactulose⇒Lactobacilli

Synbiotics
Definition

- A probiotic organism in combination with its prebiotic food
- Providing both the organism and substrate at the time of ingestion may offer improved chance of survival in GI tract

Intestinal Flora

- 10^{12} viable bacteria/gram of large bowel content
  - More than total cells in the human body
  - More than total humans who have ever lived
- At least 17 families
- At least 50 genera
- 400-500 species in any single person
- 80-90% unculturable

The Normal Human Microflora - Birth

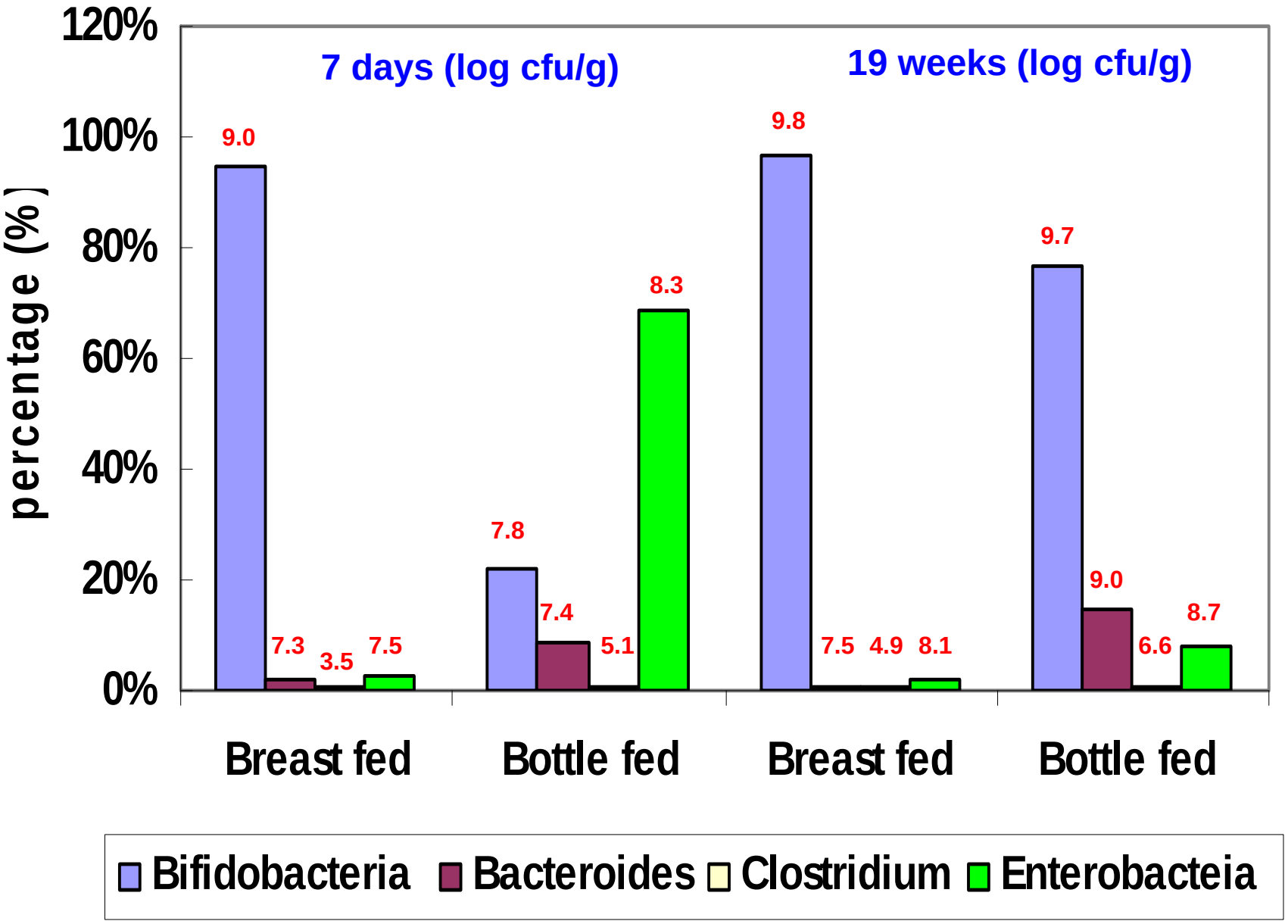
0 to 2 days: The newborn infant is microbiologically sterile. Its first flora is acquired from vaginal, skin and rectal microflora of the mother

Dominant Types:

- **Lactobacilli:** Mainly *acidophilus* types
- **Streptococci:** Non-haemolytic
- **Enterobacteria:** Various including *E. coli*

2 days to Weaning: Highly dependent on breast or bottle feeding

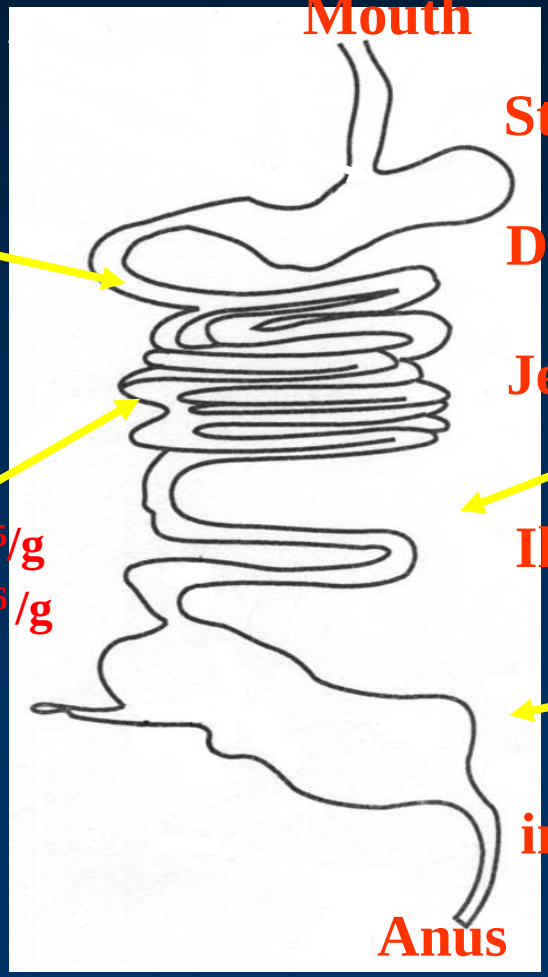
	2 - 7 days		1 – 19 weeks	
	Breast fed (log cfu/g)	Bottle fed (log cfu/g)	Breast fed (log cfu/g)	Bottle fed (log cfu/g)
Bifidobacteria	9.0 (95%)	7.8 (22%)	9.8 (97%)	9.7 (77%)
Bacteroides	7.3 (2%)	7.4 (8.7%)	7.5 (0.5%)	9.0 (15%)
Clostridium	3.5 (<1%)	5.1 (<1%)	4.9 (<1%)	6.6 (<1%)
Enterobacteria	7.5 (3%)	8.3 (69%)	8.1 (2%)	8.7 (8%)


7 days (log cfu/g)
19 weeks (log cfu/g)
120%
100%
80%
60%
40%
20%
0%
percentage (%)
9.0
7.3
3.5
7.5
7.8
7.4
5.1
8.3
9.8
7.5
4.9
8.1
9.7
9.0
6.6
8.7
Breast fed
Bottle fed
Breast fed
Bottle fed
Bifidobacteria
Bacteroides
Clostridium
Enterobacteia

The Normal Human Microflora - Adult

- The intestinal microflora acquires adult characteristics and is fully formed by two years of age
- It is important to note the variation in number and type of microbial species in the different parts of the intestine

Typical Microbial Flora of the Gastro-intestinal Tract of Man



Lactobacilli 1-100/g

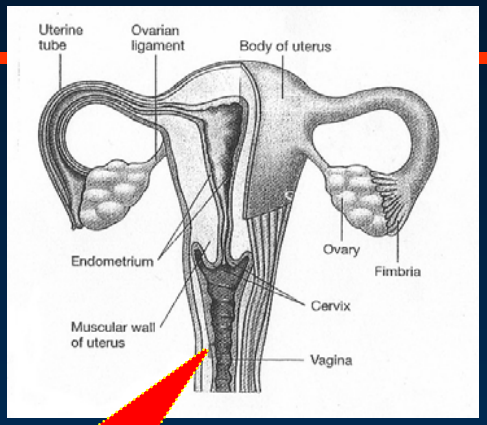
Lactobacilli $1x10^2$/g
Streptococci $1x10^4$/g

Lactobacilli
Streptococci
Enterobacteria
Bifidobacteria
Yeasts
$1x10^5$/g
$1x10^6$/g

Bifidobacteria
Lactobacilli
Streptococci
Bacteroides
Enterobacteria
$1x10^5$/g
$1x10^8$/g

Bacteroides
Eubacteria
Bifidobacteria
Anaerobic cocci
Clostridia
Lactobacilli
Enterobacteria
Streptococci
$1x10^{10}$/g
$1x10^{11}$/g

The Protective Effect of the Human Microflora – Prevention of Candidiasis



Normal condition

Candida overgrowth

Occasional *Candida* cell

Vaginal epithelial surface

Protective lactobacilli depleted

***Candida albicans* overgrow and change to 'hyphal' form – some invade cells**

Numerous lactobacilli attached to epithelial surface prevent attachment and overgrowth of *Candida albicans*

Inflammatory symptoms

Metabolic Activities of the Normal Flora

- **Synthesis of vitamins:**
  - **B-vitamins: B_{12}**
  - **Folic acid**
  - **Biotin**
  - **Riboflavin**
  - **Vitamin K**
- **Conservation of nitrogen:**
  - **30% of urea produced in liver is released into colon (70% urea released in urine)**
  - **Bacteria recycle urea into amino acids - available to host**

Metabolic Activities of the Normal Flora (cont)

- Synthesis of short chain fatty acids (SCFA's):
  - The colonic epithelial cells deliver approximately 50% of their energy requirements from butyrate produced by the microflora.
  - Estimated that 5-10% of the total body energy is from SCFA's
  - Colonic cells deprived of butyrate begin to atrophy within approximately 5 days. This decreases integrity of mucosal barrier and causes mucous permeability to increase dramatically
  - Relatively low numbers of colonic flora (notably eubacteria and peptococci) produce butyric acid: estimated production is 100mmol/day

Luminal Nutrition and Intestinal Conditioning

- G.I tract mucosa is only body tissue with a systemic and luminal nutritional source
- Over 50% of nutrition of small and large intestinal mucosa is luminal

Energy Source						
	Glucose	Glutamine	Butyrate	Aspartate	Acetate	Propionate
Duodenum	30%	60%	-	10%	-	-
Jejunum	20%	70%	-	10%	-	-
Colon	5%	5%	60%	-	20%	10%

Metabolic Activities of the Normal Flora (cont)

- **Detoxification and toxin production:**

The microbial flora can detoxify and positively transform many substances:

- **Heavy metals (by binding and by enhancing excretion from systemic circuit)**
- **Biotransformation of plant polysaccharides releasing lignases and phyto-estreogens (iso-flavones) by colonic flora (colon and breast cancer)**
- **Release of quercetin by bacterial β-galactosidase from fruit carbohydrates. Quercetin & rutin have been shown to be powerful anti-mutagens and can also show cancer-promoting properties**
- **Plant polyphenols such as those contained in grape skin/seed extracts are only absorbed in the colon following interaction with microflora**

(Lemaire, 2004 – personal comm)

- **Evidence that depending on the type of flora, release of dietary glucosinolates(isothiocyanates) has effect of stimulating or repressing cytochrome P450 in liver (Nugon Bauden *et al*, 1998)**

Microbial Flora and Production of Carcinogens.

The microbial flora are vigorous producers of carcinogens:

- **Fecapentaenes: most potent carcinogens found in the intestine. Formed from derivatives of mainly saturated fats**
- **Heterocyclic amines: can be produced either from high temperature cooked fats or cigarette smoke interacting with protein. Potent tumour producer in prostate, colon and breast. One meal of high cooked fat/protein as in BBQ can produce 200 x normal level of heterocyclic amines in bowel**
- **Bile acids, nitrosamines, diacylglycerol**
- **Most active microbes are *Bacteroides* species**
- **Vegetarians have lower levels of *Bacteroides*, significantly lower levels of carcinogens and significantly lower levels of CRC.**

Probiotics

Preparations of one or more components of the normal microbial flora designed to contribute to the population dynamics, or metabolic/immunological balance, of the intestinal flora, and so confer health benefits to the host

Possible Probiotic Benefits

Nutr Clin Care. 2004 ; 7(2):56-68

Intestinal Disorders

Diarrhea
- Antibiotic-associated
- Traveler's Pathogen-induced
- or infectious

Gastroenteritis

Irritable bowel syndrome

Inflammatory bowel disease
- Crohn's disease
- Ulcerative colitis
- Pouchitis
- Lactase digestionOther

Other Medical Disorders

Cancer / Hyperlipidemia

Helicobacter pylori infections

Hepatic diseases

Genitourinary tract infections

Improved immune function

Food substitute in allergies

Nutritional Supplement for Weight Gain

Faliure-to-thrive

Cancer cachexia

AIDS Pancreatitis/cystic fibrosis

Inflammatory bowel disease

Probiotics

Potential Mechanisms of Action

- Inhibition of adhesion
- Immunomodulation
- Production of antimicrobial substances
- Modification of toxins or toxin receptors
- Competition for nutrients
- Reduction in bacterial translocation
- Anti-inflammatory signaling within the epithelium

History

Probiotics: 19th Century

- Pasteur (1877)
  - Observed antagonistic interaction between bacterial strains
  - Suggested that non-pathogenic bacteria should be used to control pathogenic bacteria

Probiotics: Early 20th Century

- Metchnikoff (1907)
  - Observed that lactic fermentation of milk arrested putrefaction
  - Suggested that consumption of fermented products would offer the same benefit to humans
  - Felt that longevity in Bulgarian peasants was due to ingestion of “soured milks”

Probiotics: Mid to late 20th Century

- 1950's, Ferdinand Vergin publishes article discussing effects of antibiotics on beneficial intestinal bacteria

- 1980's Fuller establishes first definition of probiotics

Commonly Used Bacterial Strains for Probiotic Purposes

Nutr Clin Care. 2004 ; 7(2):56-68

Lactobacillus Species	*Bifidobacterium* Species
L. acidophilus *L. bulgaricus* *L. casei* *L. Rhamnosus GG* *L. plantarum*	*B. Adolescentis* *B. animalis* *B. bifidum* *B. breve* *B. infantis* *B. longum* *B. thermophilus*
Other Lactic Acid Bacteria	**Non-Lactic Acid Bacteria**
Enterococcus faecium *Streptococcus thermophilus*	*Bacillus subtilis* *Escherichia coli strain nisslle* *Saccharomyces boulardii* *Saccharomyces cerevisiae*

Selection Criteria for Probiotic Isolates

- Must be considered totally non-pathogenic
- Should be species indigenous to the target host
- Should be capable of ‘colonisation’ of digestive tract
  - resists stomach acid, bile salts,
  - capable of attachment to epithelial cells
  - capable of immunomodulation in-vitro
- Scientific evidence especially clinical data is desirable to support use
- Commercial release of the product should be covered by thorough Quality Assurance programme

Advances in Probiotic Research

Other trial work recently published has shown probiotics at high potency to:

*** General stimulation of immunity and maintenance of intestinal balance** **4-8 billion**

*** Reduce incidence of neonatal allergy** **2-6 billion**

*** Reduce severity of established allergy** **10-40 billion**

*** Reduce severity of inflammatory bowel disease** **100-400 billion**

*** Prevent dysbiosis from antibiotic use –** **30-100 billion**

Summary of Trials

THE TWO ESSENTIALS:

- **Important to have functional effective strains**
- **Adequate numbers are essential**

Higher numbers produce a:

- ✓ **More profound therapeutic effect**
- ✓ **More consistently**
- ✓ **Faster!**

New Consortium

Recent and current trials have been using the:

CONSORTIUM

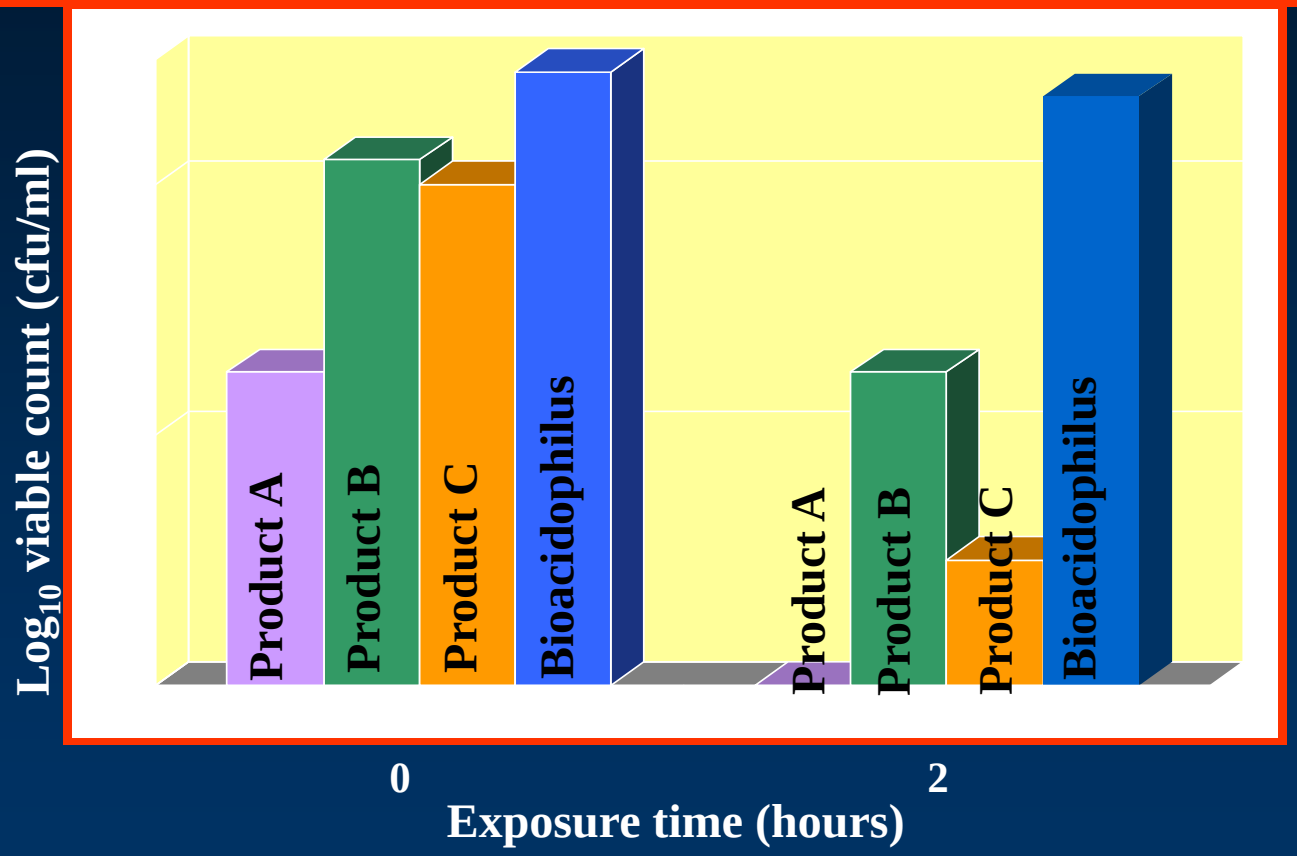
Lactobacillus acidophilus ⟹ **Two strains** → **CLT 21**, **CLT 60**

Bifidobacterium bifidum ⟹ → **CLT 20**

Bifidobacterium lactis → **CLT 34**

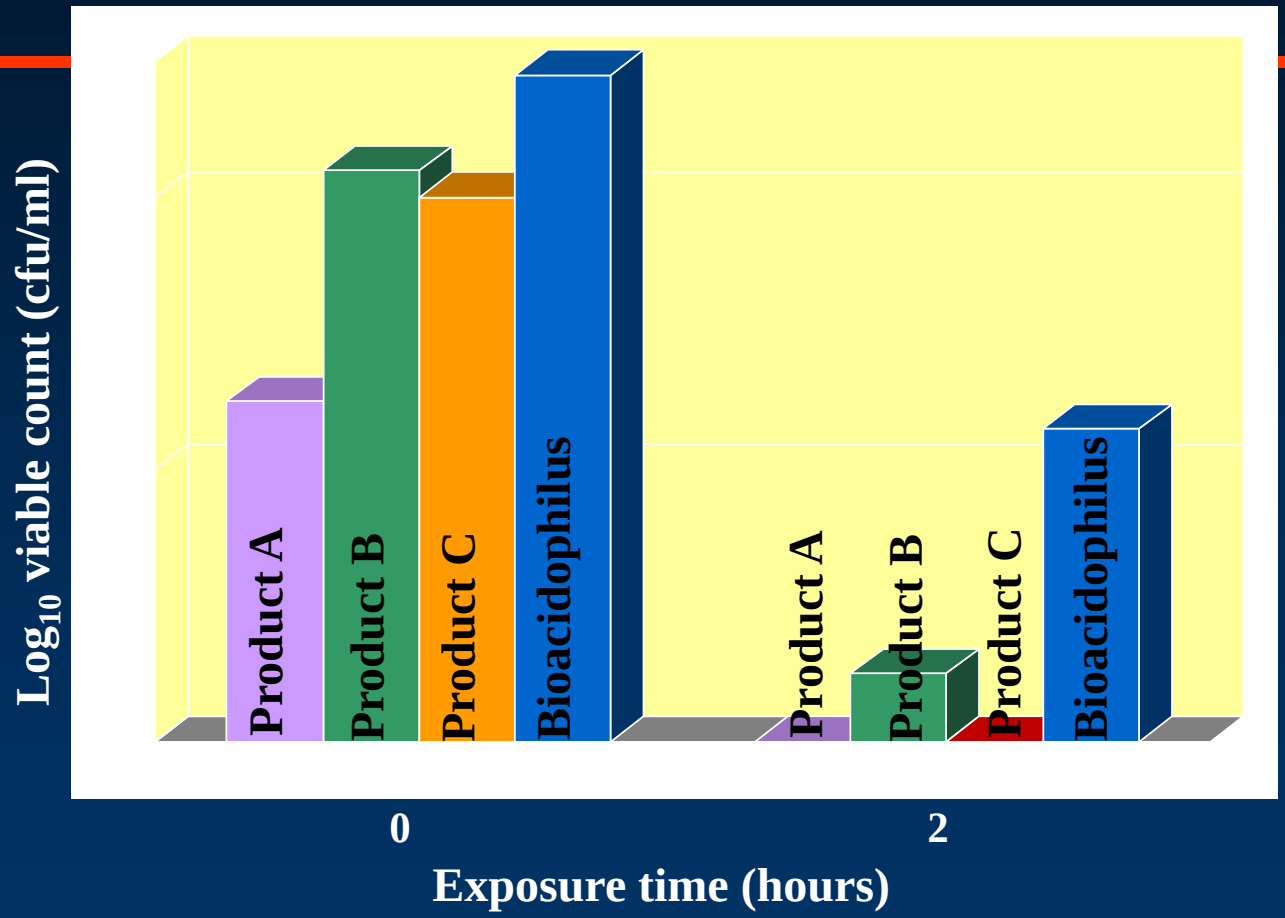
Following screening of numerous human strains, two new strains of *L. acidophilus* and *B. bifidum* were inseparable in terms of efficacy from the original two strains

Passages of Probiotics through the G.I. Tract



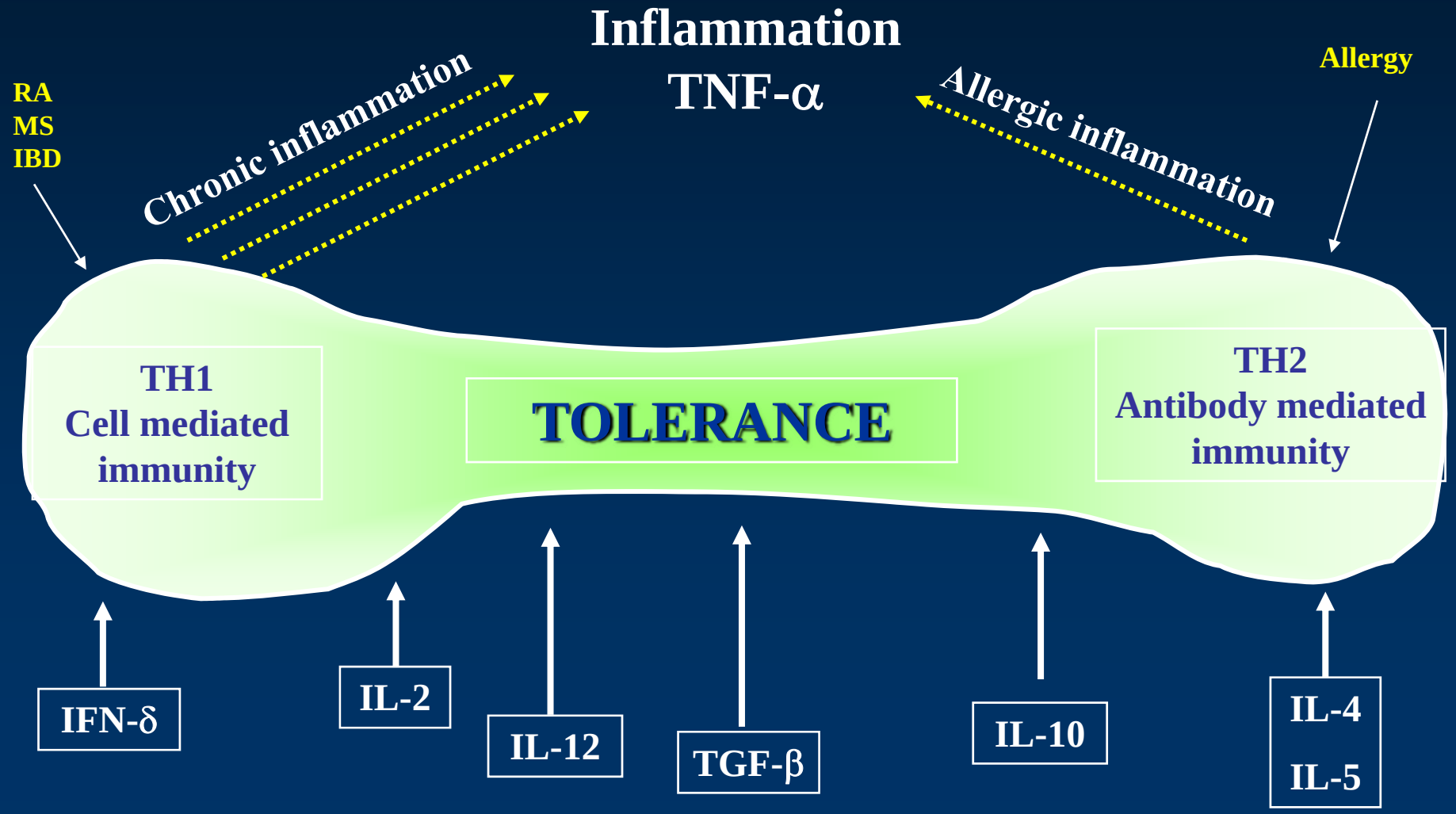
The Acid Test: At a high pH of 4.2, Bioacidophilus remains the most viable probiotic

Passages of Probiotics through the G.I. Tract

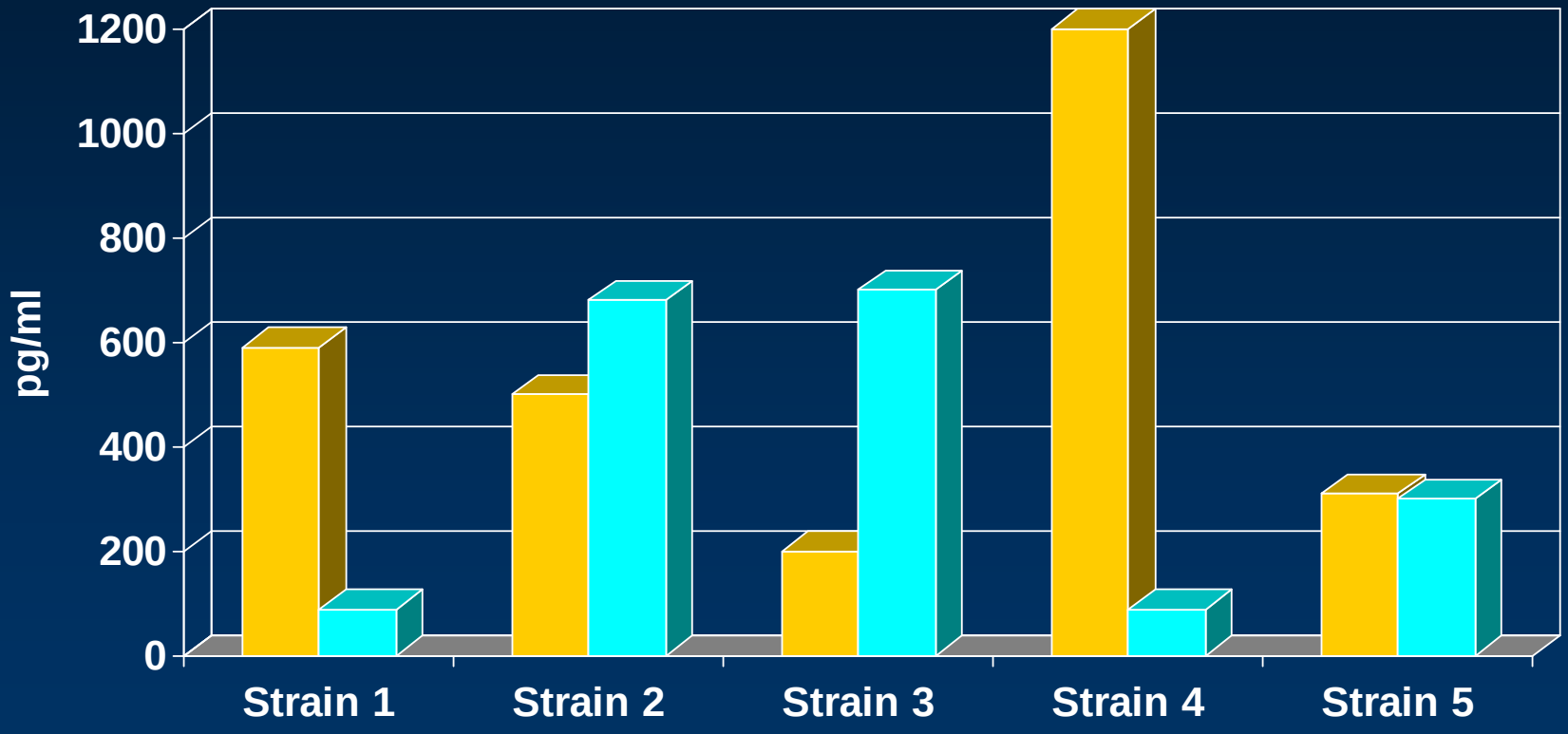


The Acid Test: At a low pH of 2.6, Bioacidophilus remains the most viable probiotic

Major Cytokine Influence on Immune Response/Tolerance



Effect of Different Probiotic Strains on *In-vitro* Stimulation of Cytokines in Peripheral Mononuclear Blood Cells (Cont..)

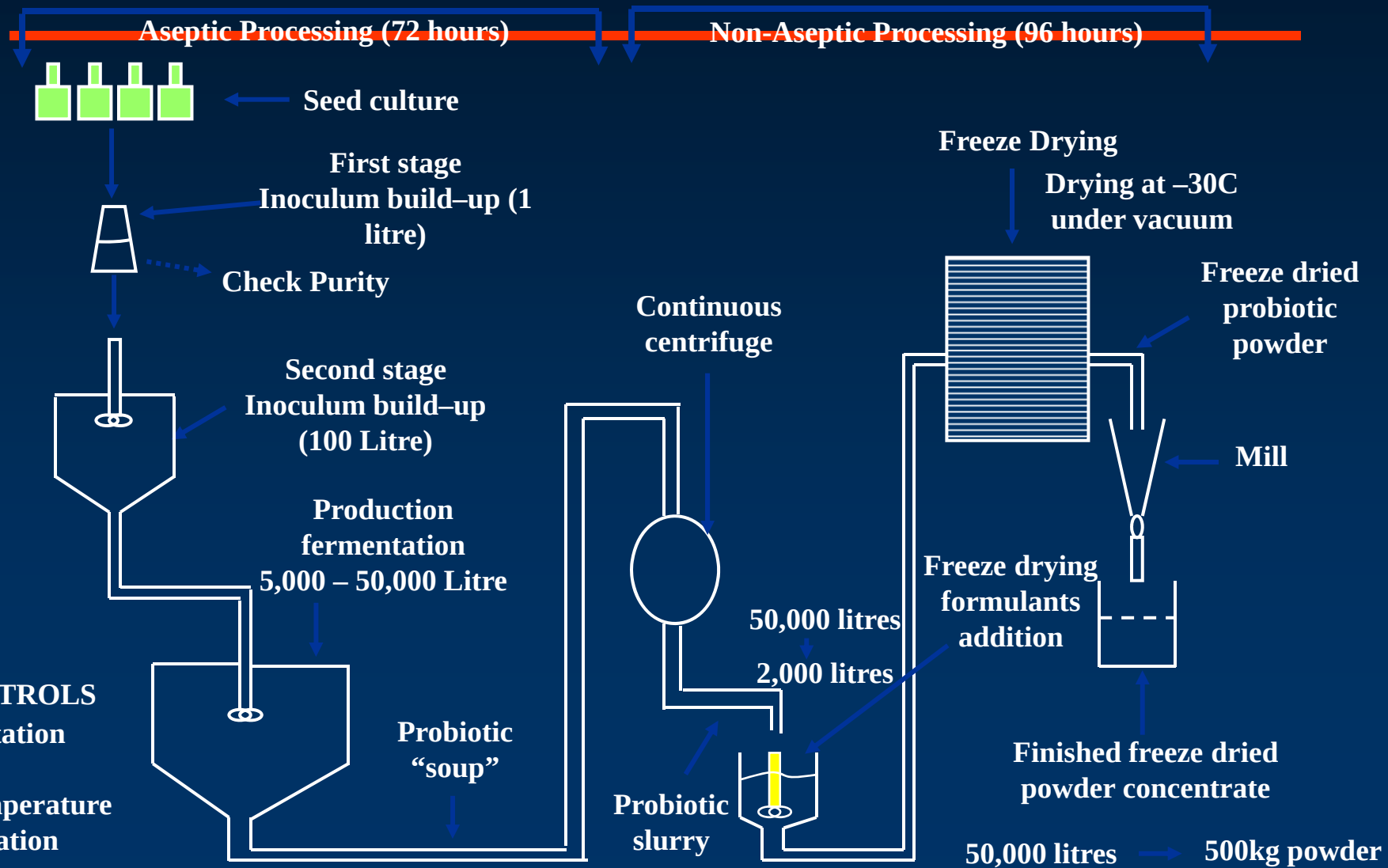


Strain 1 – CLT 21
Strain 2 – CLT 60

Strain 3 – CLT 20
Strain 4 = CLT 89 *L.salivarius*
Strain 4 = CLT 34

Stability and Quality Assurance

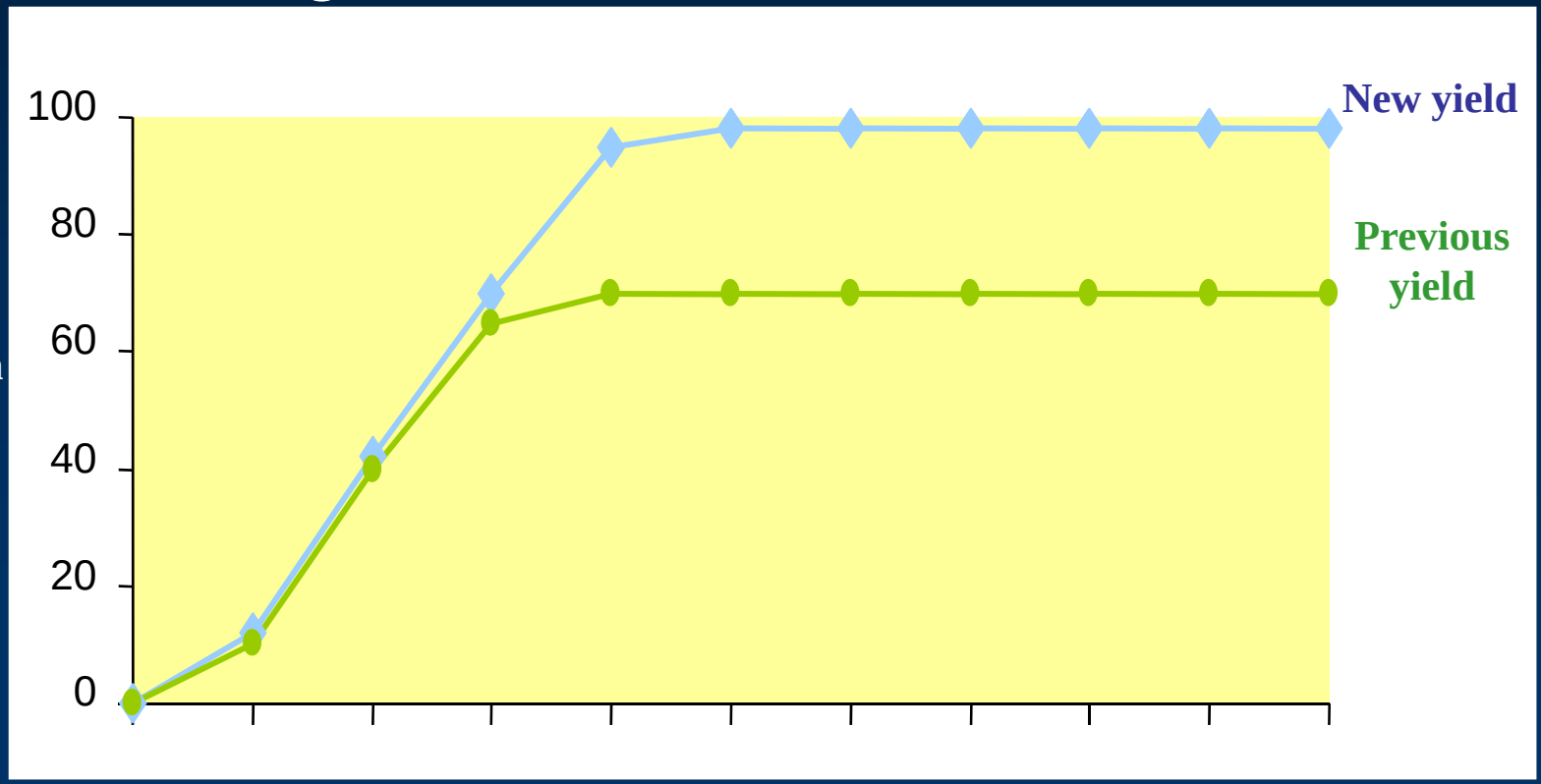
Flow Diagram for Manufacture of Probiotic Cultures



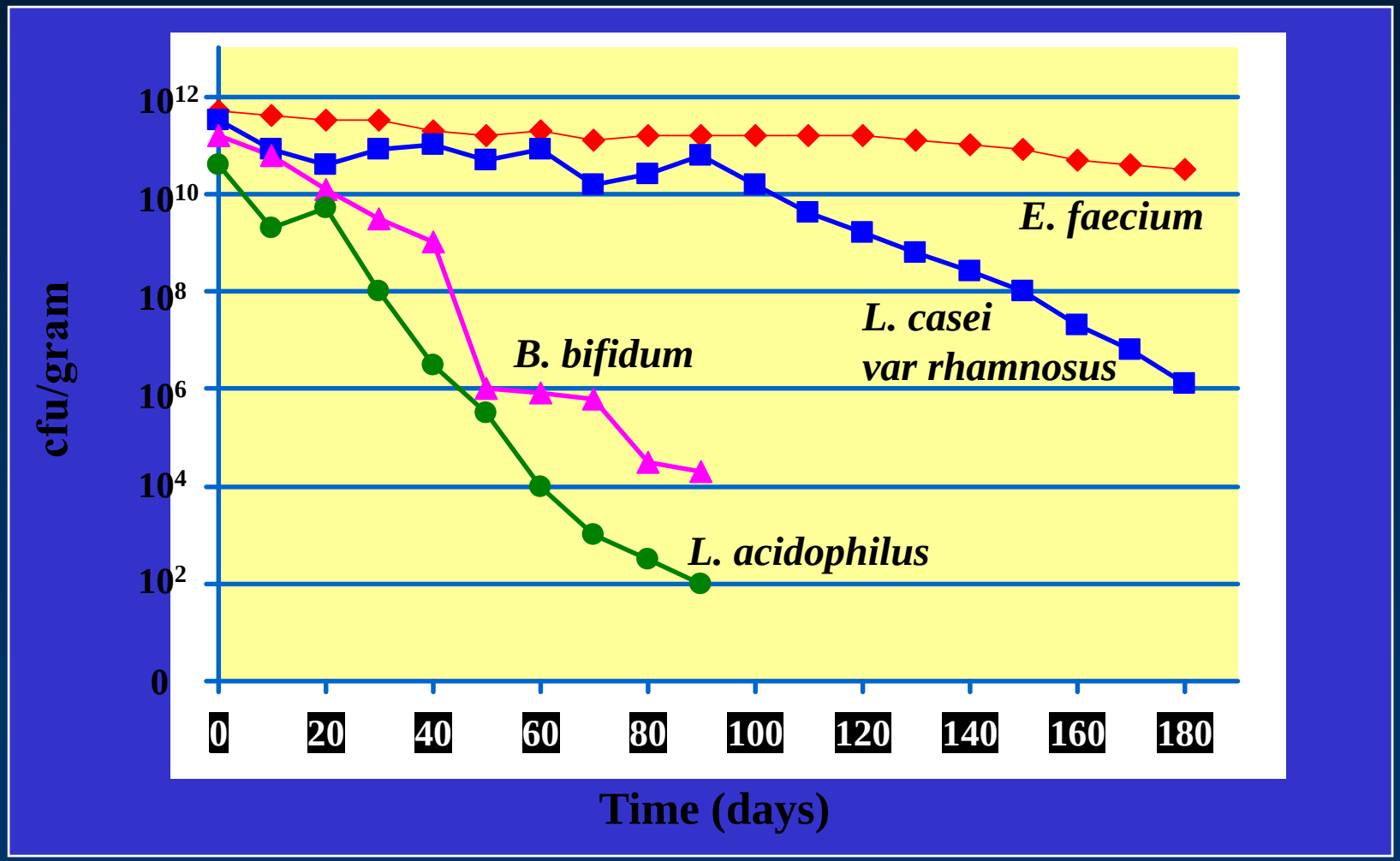
Manufacturing – Technical Breakthrough

group continuously looking to improve:

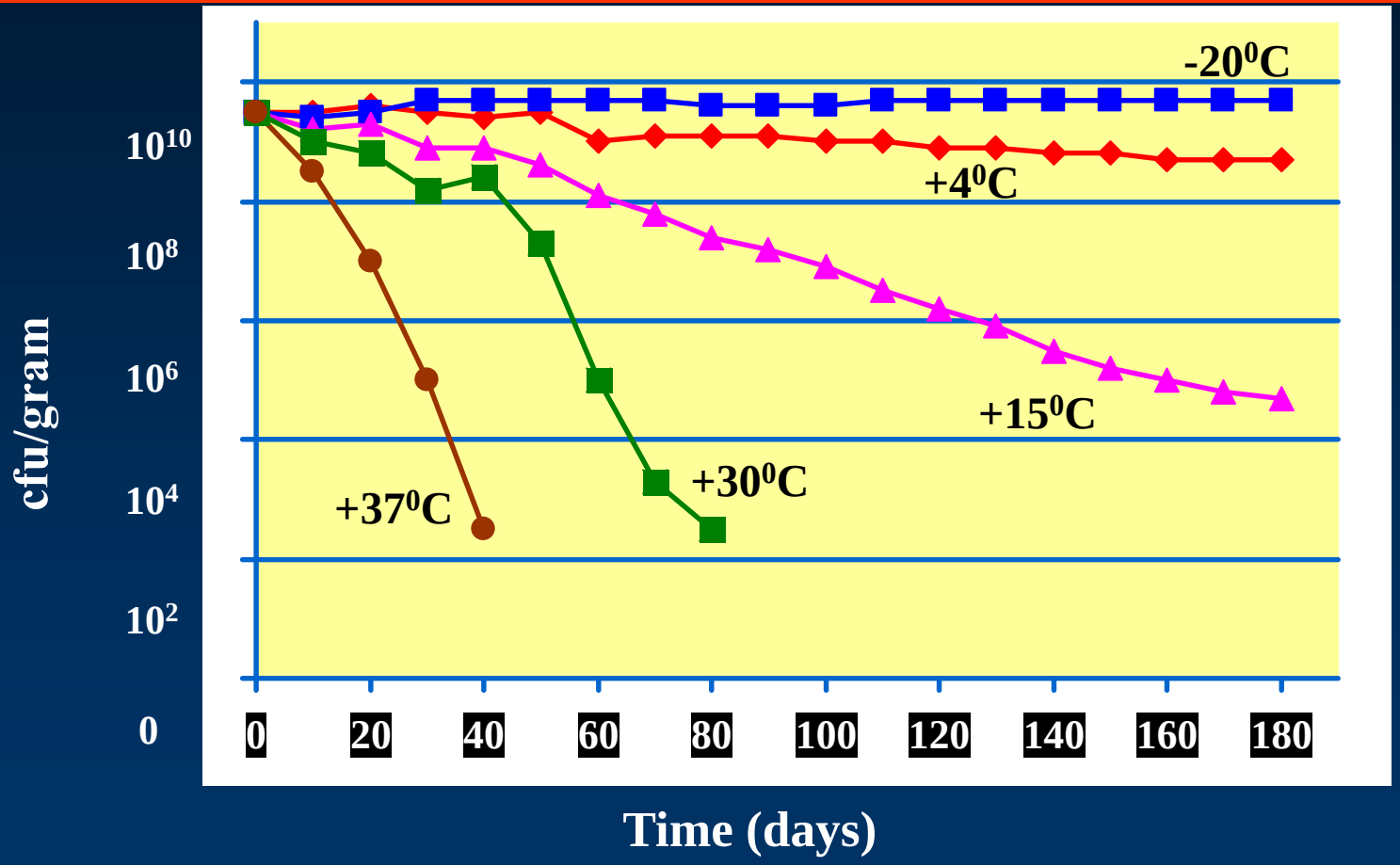
1. **Yield of LAB 4 organisms**



Effect on Different Freeze-dried Lactic Acid Bacteria of Long Term Storage at 30^{0}C



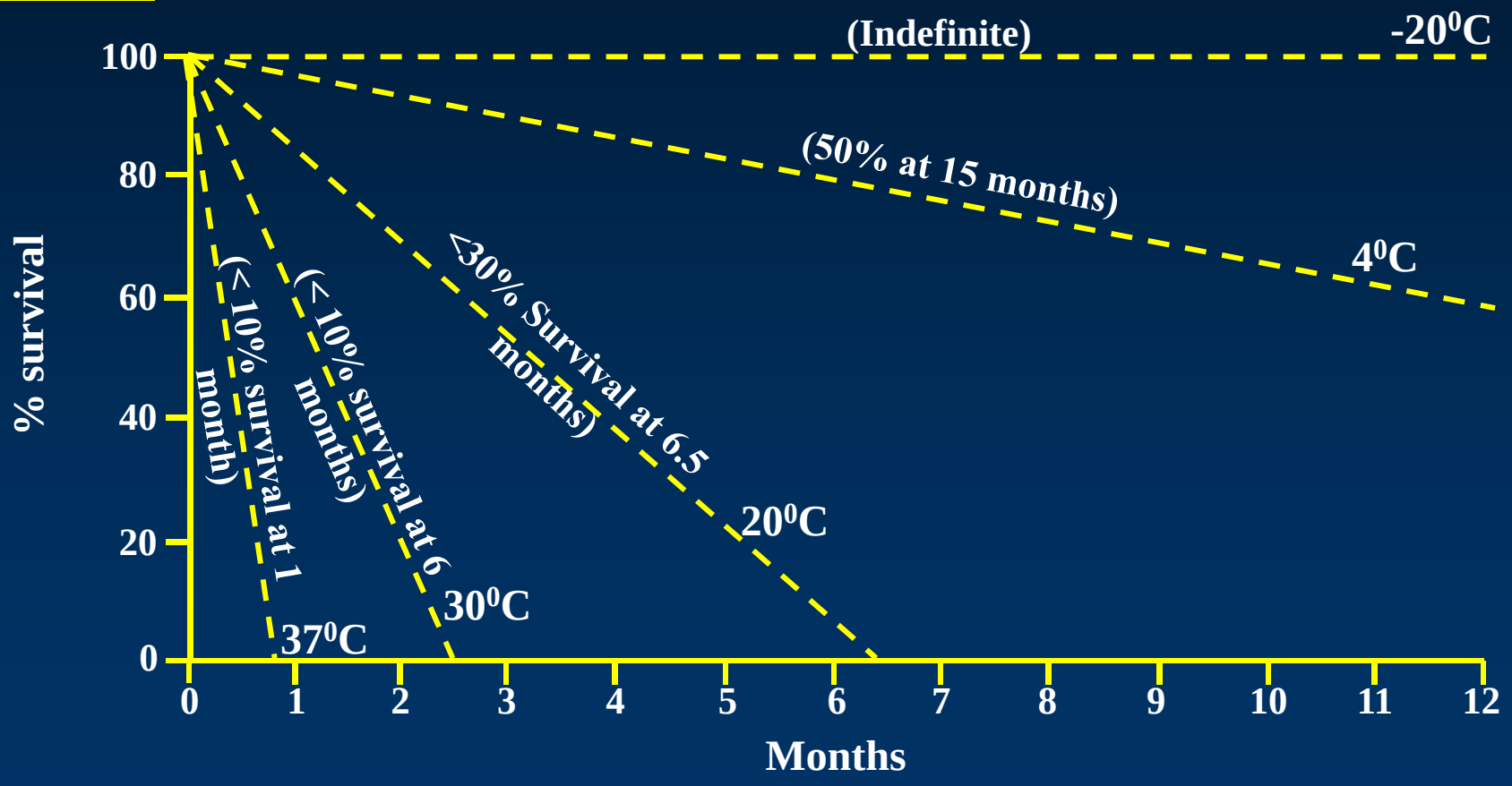
Effect of Variable Temperature Storage on Freeze-Dried *L. acidophilus*



All bacteria freeze-dried under same conditions in same formulation

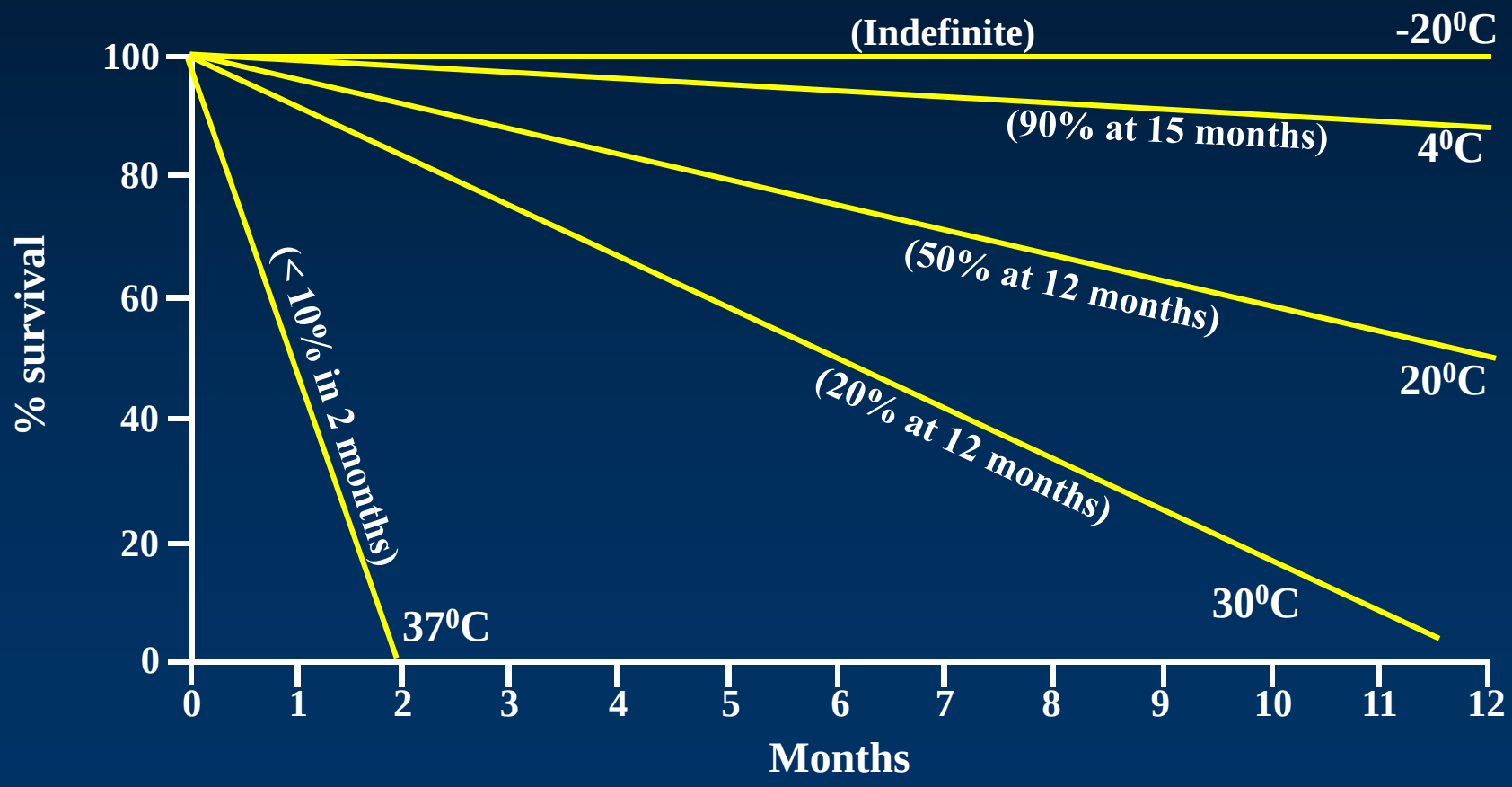
Improvement in Stability of Probiotics

Previous

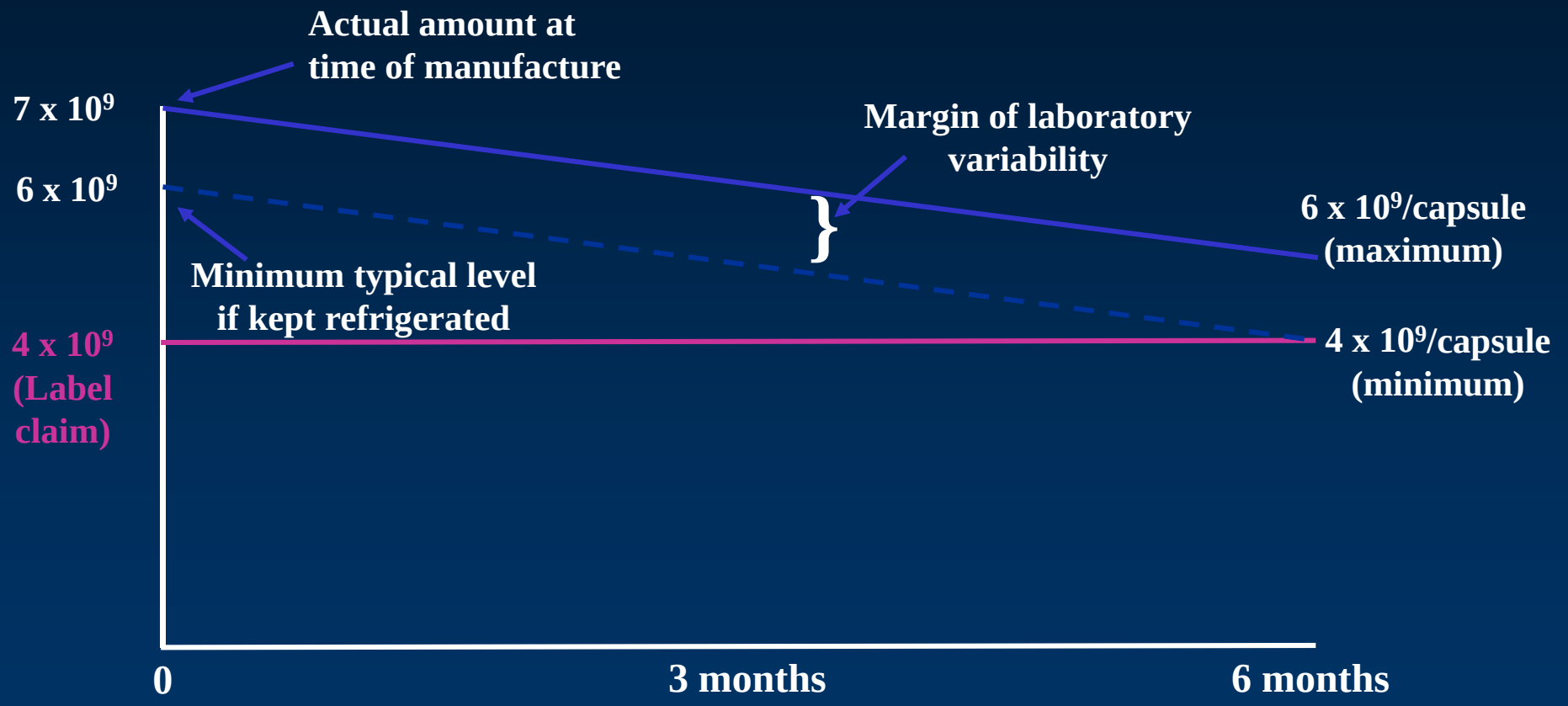


Improvement in Stability of Probiotics (Cont..)

Now



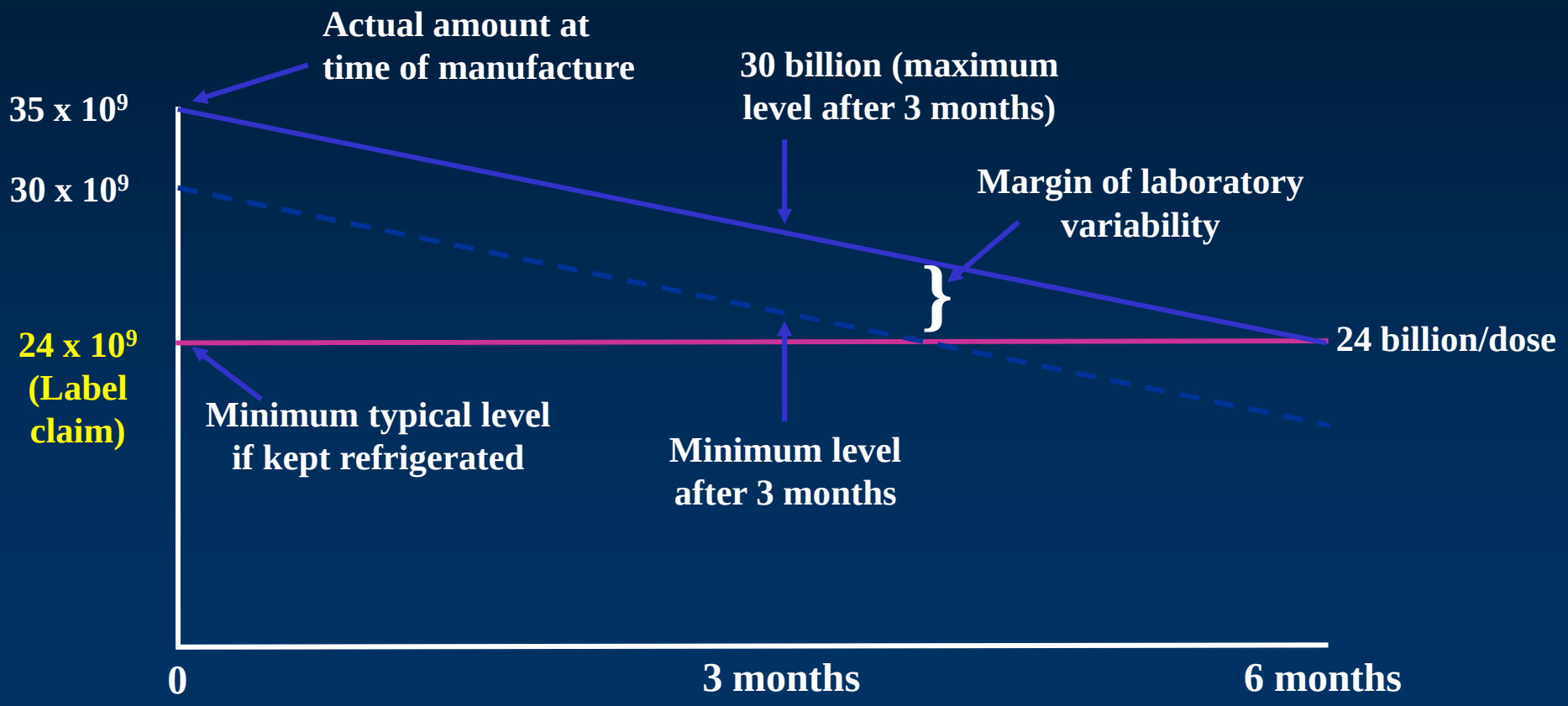
Bioacidophilus Shelf Life at Ambient 15-30 Celcius



Bioacidophilus (capsules/powders);

Neonate

Bioacidophilus Forte Shelf Life at Ambient 15-30 Celcius



Improved Stability

Use of overage and improved stability now means that the range of probiotics has:

- **Indefinite shelf life at –20^{0}C**
- **12 months shelf life at 4^{0}C and still has 90% overage**
- **3 – 6 months shelf life at ambient 30^{0}C → still reach label claim!**

Parallel Development Pathway both Produce Remarkable Improvements

Clinical trials

Demonstrable clinical effectiveness in human trials

PLUS

Manufacturing technology

Improved yield and stability

Prebiotics – Good? Bad? Or Just Another Source of Fibre?

Definition

“ Non-digestible food ingredients that beneficially affect the gut by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve host health” (*Gibson & Roberfroid 1995*)

But in Practical Terms:

The function of prebiotics is to specifically promote the growth and/or activity of the desirable types ie. Bifidobacteria, Lactobacilli and others, which consequently produces a relative reduction in undesirable types such as E coli, Klebsiella, Clostridium and Candida.

Types of Prebiotic

Classification	Origin/*Manufacturing Procedure*
Disaccharides	
Lactulose	**From Lactose** *Synthetic*
Lacticol	**From Lactose** *Synthetic*
Oligosaccharides	
Fructooligosaccharides (FOS)	**Legumes, vegetables, cereals** *Extraction/hydrolysis*
Soybean oligosaccharides	**Soybean** *Extraction/hydrolysis*
(Trans) Galactooligosaccharides	**From lactose** *Synthetic*
Polysaccharides	
Inulin	**Legumes, vegetables, cereals** *Extraction*
Resistant starches	**Legumes, vegetables, cereals** *Extraction*

Fructo-oligosaccharides – Basic Facts

CONTENT OF FOS IN RAW COMMON FOODSTUFFS

Banana	**0.3 – 0.7g/100g**
Asparagus	**2.0 – 3.0g/100g**
Garlic	**3.6 – 6.4g/100g**
Leeks	**2.4 – 8.0g/100g**
Onions	**1.1 – 7.5g/100g**
Chicory	**19.6 – 26.2g/100g**
Wheat Flour	**1.0 – 3.8g/100g**

(Figures for Inulin are similar)

Moshfegh *et a1*, 1999

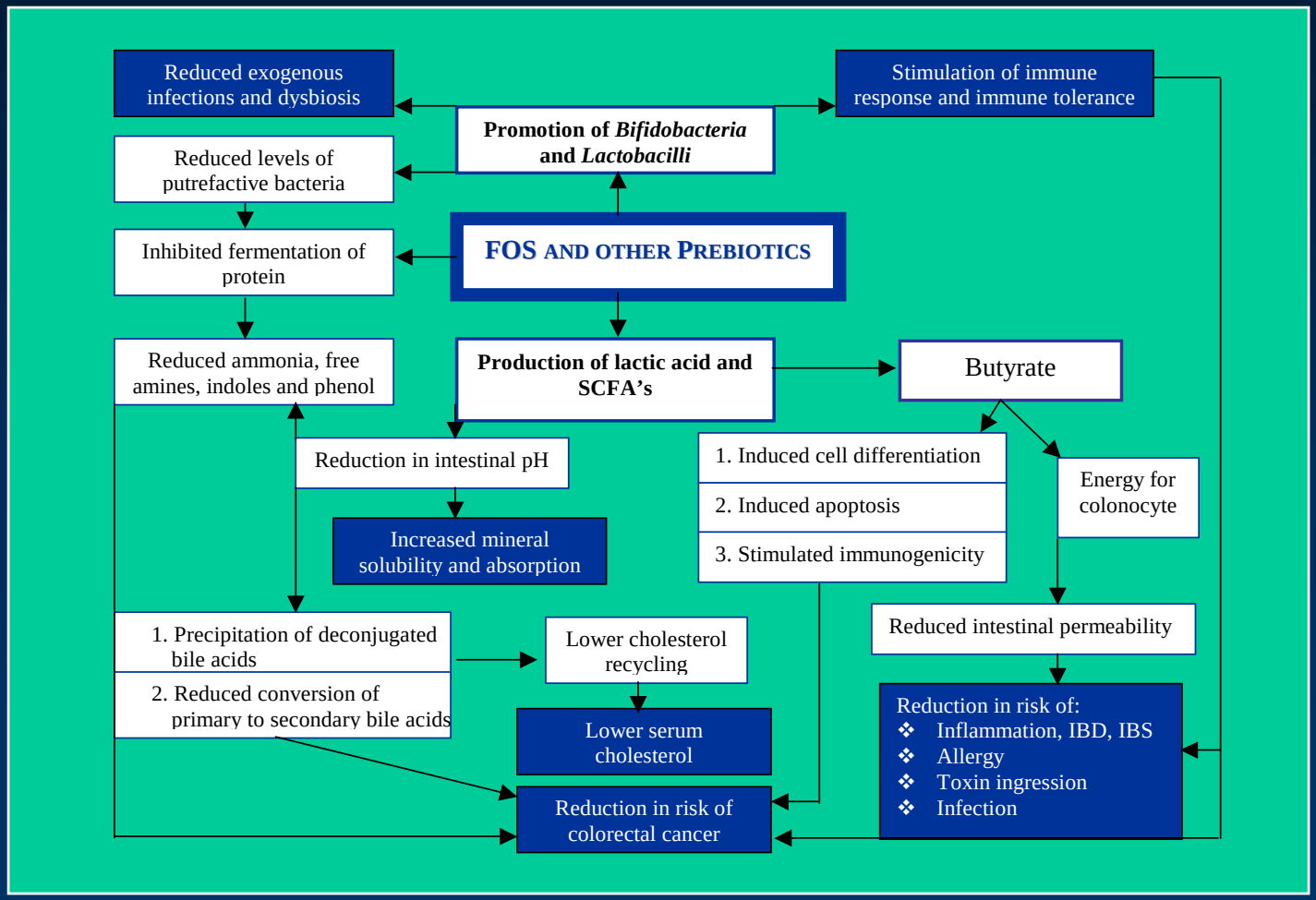
- **US intake range of FOS and Inulin 1-5g/day**
- **European intake range of FOS and Inulin 5-18g/day**
- **Northern Europe typically at low range Mediterranean Europe at high range**

Physiological Effects of FOS

- **The specific stimulation of the "probiotic" component of the normal microflora, ie. *Bifidobacteria* and *Lactobacilli*, together with a concomitant reduction in less desirable microflora components, eg. *Clostridia* and *Coliforms***

- **The stimulation of production of lactate and short chain fatty acids, notably butyrate.**

Physiological Effects and Mechanism of Action of FOS



Differential Effects on the Human Microflora
The Bifodogenic Effect

- In Vitro Experiment Action
  and
  Animal In-Vivo Experimentation

Organism	Effect of FOS
Bifidobacteria	Increase
Lactobacilli	Increase
Clostridia	Decrease
E.coli	Decrease
Other Coliforms	Decrease

Wang & Gibson 1993
Davidson et al 1998
Rycroft et al 2001
Djouze&Andrieux 1997
Hussein et al 1999

Differentiation Effects on Human Microflora (Cont'd)

- **Human Trials – Randomised, Blinded**

Amount FOS Fed	Duration	Significant Increase	Significant Decrease	No Change	Reference
15g/day	15 days	*Bifidobacteria*	*Bacteroides* *Fusobacteria* *Clostridia*	*Lactobacilli* Gram positive cocci *Coliforms*	(*Gibson et al, 1995*)
8g/day	35 days	*Bifidobacteria*	*Bacteroides*		(*Roberfroid et al, 1998*)
10g/litre	28 days	*Bifidobacteria*		*Bacteroides* *Clostridia* *E. coli* *Klebsiella* *Citrobacter*	(*Boehm et al, 2002*) Pre-term infants
20-40g/day		*Bifidobacteria*	*Enterococci*	*Candida albicans*	(*Kleessen et al, 1997*)
18g/day	12 days	*Bifido/Lactob*			(*Bouhnik et al, 1996*)
8g/day		*Bifidobacteria*	*Bacteroides*		(*Menne et al, 1997*)
5-20g	8 days	*Bifidobacteria*			(*Bornet et al, 2002*)

The Effects of FOS on Short Chain Fatty Acid (SCFA) Production

- **Fermentation of FOS and other Prebiotics result in the production of Lactate, Biomass, Gas and SCFA**
- **Ratio of SCFA production is relatively constant.**

	Acetate	***Propionate***	***Butyrate***
Ratio	**3**	**: 1 :**	**1**
Typical amount/day	**18g**	**6g**	**6g**

Butyrate – A Metabolic Powerhouse

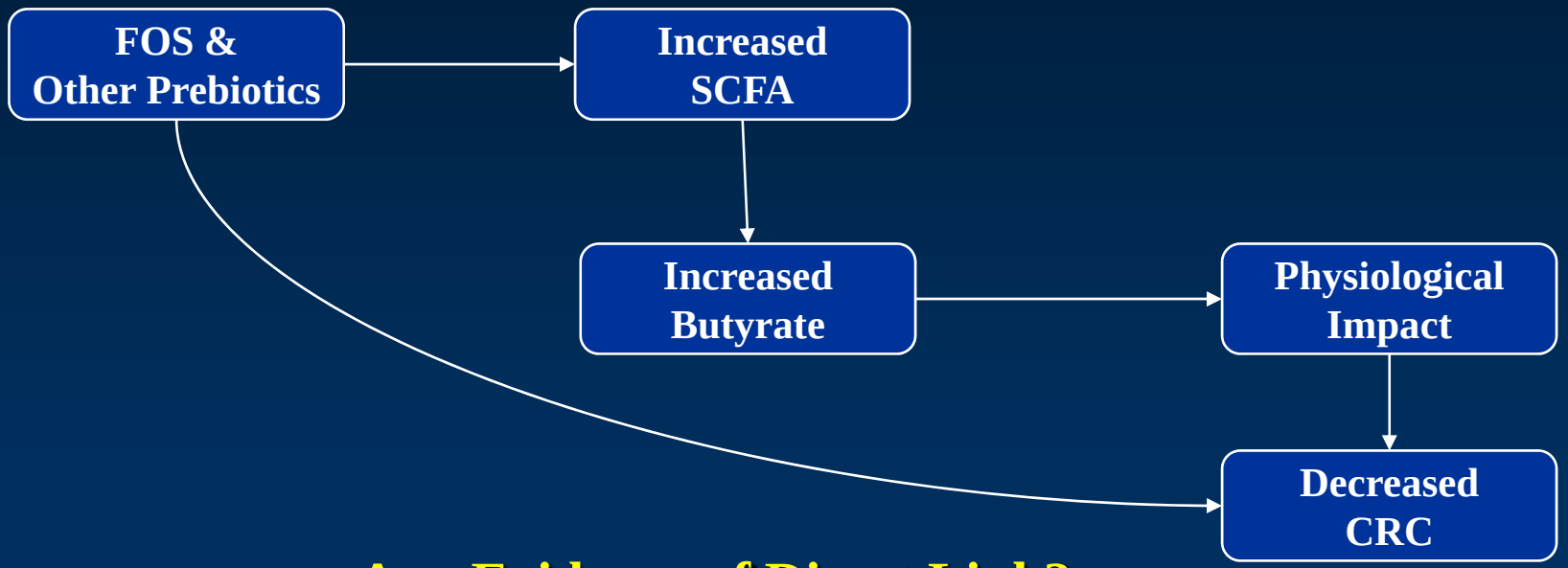
- Over the past 15 years the importance of butyrate to human metabolism has become clear, and is summarized below:
- Butyrate provides 70% of the energy for the colonic epithelial cells, or coloncytes (Roediger, 1980; Cummings & Macfarlane, 1997)
- Buyrate controls the turnover and differentiation of the colonic epithelial cells and is capable of inducing differentiation in colon carcinoma cells (Smith *et al,* 1998; Velzquez *et al,* 1996)
- Butyrate induces apoptosis (programmed cell death) in normal growing colonic cells and reverses resistance to apoptosis in colonic cancer cells (Bornet, 2002)
- Butyrate increases immunogenicity (susceptibility to immune cell policing eg. by NK cells) of cancer cells. Indeed, butyrate in combination with interleukin 2 caused complete clearance of induced colon carcinoma in rats (Perrin *et al,* 1994; Bornet *et al,* 2002)

Supplementation with FOS Increases Production of SCFA

- In vitro models of colonic fermentation show 4-fold increase in butyrate production within 24 hours feeding FOS (Rycroft *et al,* 2001)
- Numerous studies in rats have shown substantial increases in SCFA concentration and decreased faecal pH, following administration of prebiotic oligosaccharides (Djouze & Andrieux, 1997; Campbell *et al,* 1997; Younes *et al,* 1995)
- In human studies the demonstration or significant levels of SCFA in the human colon was demonstrated using autopsy of sudden death victims (Macfarlane *et al,* 1992)
- Also increase in SCFA production following supplementation of FOS has been demonstrated in several human studies:

  Gibson *et al,* 1995
  Stowe *et al,* 1987
  Rumessen *et al,* 1990

Prebiotics Decrease Risk of Colecterol Cancer



Any Evidence of Direct Link?

FOS & Mineral Absorption

- Increasing acidity in large intestine increases mineral solubility – magnesium, calcium, iron & zinc (Crittenden 1999; Trinidad *et al,* 1999)
- Dietary calcium absorption increases significantly by 26% from 47.8% to 60.1% in human trials where adolescents fed 5g/day FOS (Van de Heuval, 1999)
- In separate trial dietary calcium absorption increased 20% with adolescents fed 8g/day FOS (Van Poppel 2000)
- Dietary magnesium absorption and plasma magnesium levels showed significant increase of 12% from 30.2% to 33.9% when human subjects supplemented with 10g/day FOS (Tahiri *et al,* 2001)

FOS and Other Prebiotics Reduces Risk of Colorectal Cancer

- Studies in rats & mice show significant reduction in formation of abberant crypt foci (ACF) when FOS or inulin is fed

  (Reddy *et al* 1997; Rowland *et al,* 1998)

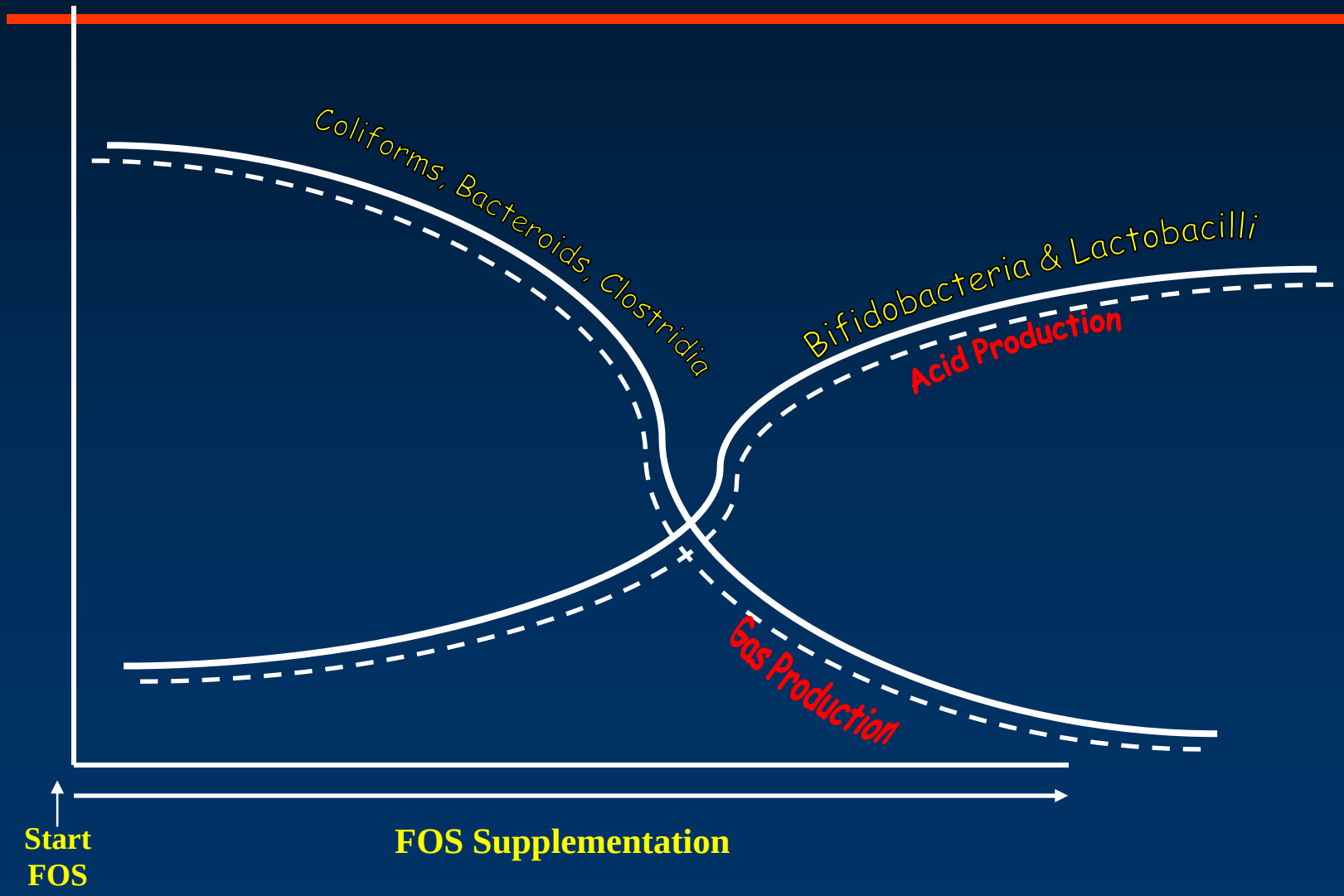
- Combining use of FOS with Bifidobacterium longum gave greater effect (in above) than either individual component.
- In human trials 35.9% of patients with colonic adenomas removed has re-occurrence within 12 months, use of lactulose reduced this to 14.7% - a significant reduction.
- Only butyrate generating prebiotics produce reduction in ACF, insoluble fibre such as starch free wheat bran does not reduce ACF

  (Perrin *et al,* 2001)

Flatulence & Bloating

- Consumption of FOS and other prebiotics causes increase in gas production in most people
- This is temporary and in a high majority of people subsides in 2-3 weeks, to level associated with relatively high fibre diet
- Lactobacilli & Bifidobacteria – High acid producers and low gas producers
- Coliforms, Bacteroids etc – Low acid producers and high gas producers
- Good regime is to start supplementation at 2g/day and build to 10g/day over a 2-3 week period

Flatulence & Bloating



Probiotics + Prebiotics ≈ Synbiotics (Combined Benefits)

- Conceptually, the desirable state or optimising intestinal flora can be achieved by either probiotics or prebiotics.
- Prebiotics help probiotics become established.
- Probiotics provide the most physiological beneficial strains.
- Prebiotics help maintain high populations of probiotics.
- Combination of prebiotics and probiotics will provide synergistic benefits

(Gibson, 1998)

Thank You