Gut Microbiota and Health:
Food Fad or Bacterial Therapy ?

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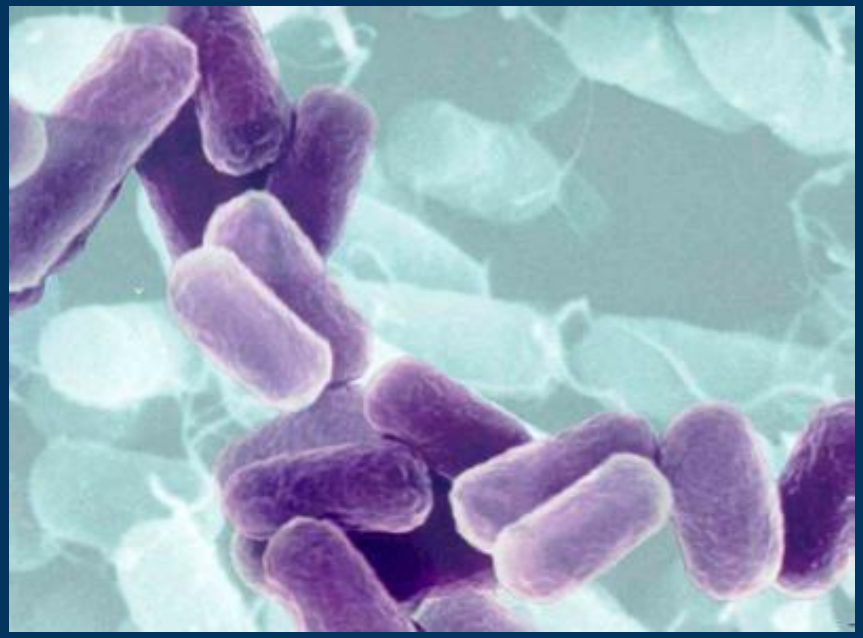
Have We Now Moved From Disease to Dessert ?



VRE growing on mitral valve

Frozen yogurt *L. acidophilus*

Probiotics in Clinical Medicine: Two Schools of Thought !

Definition: " Microorganisms normally present in the human body that when delivered in adequate amounts, confer a health benefit on the host"

Quakery

- Claims to cure "everything"
- Why so many strains ?
- How much is needed ?
- Review articles vs original articles
- Inconsistent studies
- Few ITT studies with tangible outcomes
- Marginal statistics
- Growing suspicion of "holoistic" medicine

Therapy

- Differential support based on cultural and educational background

  (yogurt, kurd, kefir, kombucha)
- Aware of the differences in strains etc
- Understand the differences in study design

Probiotic literature ?
Science or Quackery

- **Professional Literature**
  - Few ITT studies available
  - Widely variable, heterogeneous groups
  - Meta-analysis not consistent
    - Questions of outcome
  - 2009 analysis of what is published probiotics vs antibiotics in PubMed Search
    - Probiotics > 5000 papers ----- 28% were review articles
    - Antibiotics > 500,000 papers----8% review articles
- **Lay literature**
  - Recent lead articles NY Times 2012
  - Wall Street Journal 2012
  - Economist 2012

Some say we should be killing our bacterial !!

Gastroenterologist Survey of Probiotics

- Evaluate MD opinions regarding probiotics
- Large metropolitan area in midwest
- Results:
  - Safe for most patients 100%
  - 98% felt probiotics had a role in treating GI disease
  - 93% had patients currently taking probiotics
  - Most common bacteria used
    - Yogurt based, B.infantis (Align®), VSL#3,
  - Most common clinical diagnosis used
    - IBS, AAD, C.difficile
  - Most believed their practice was ***not supported by scientific data***

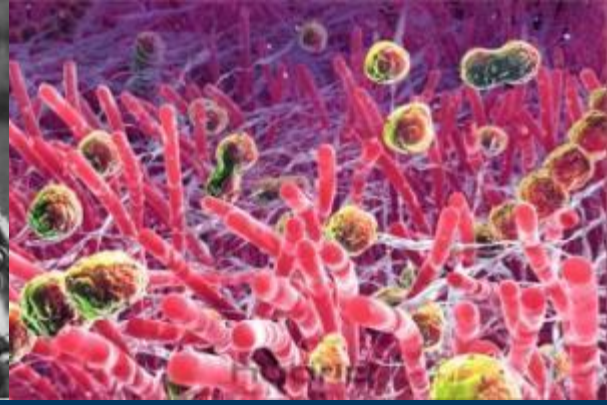
Williams MD J Clin Gastro 2010

Nutritional Goals in Clinical Medicine Have Changed From Adjunctive Care to a "Therapeutic" Strategy

- Previous goals
  - Attempt to preserve lean body mass
  - Avoid metabolic complications
- Current Goals: "Therapy not support"
  - Attenuate metabolic response
  - Reverse loss of lean body tissue
  - Prevent oxidant stress
  - Favorably modulate immune response
    - Enteral feeding (GALT)
    - Appropriate macro and micronutrients
      - » Glutamine, arginine, omega-3-FA, antioxidants
  - Maintaining "normal " commensal flora
  - Manipulating flora to host benefit
      - » SCFA, anti-inflammatory changes, decrease sepsis

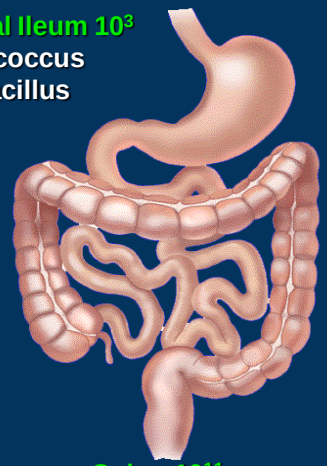
Where “man meets microbe” a dynamic interplay


Metchnikoff 1906



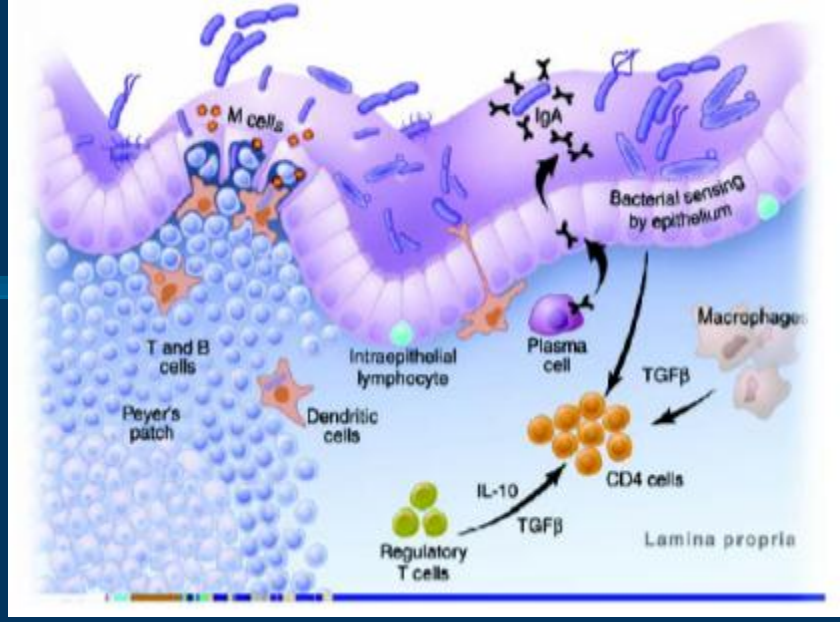
- Concepts are not new
  - Biblical references,
- 300 to 400 sq meter surface area
  - Surface area of a tennis court
- > 2 million genes in the bacterial genome vs 35,000 in the human
  - 100 trillion living bacteria in the human intestine
  - Over 500 species in human colon
  - Each individual with own bacterial fingerprint
- Significant “cross-talk” between bacteria and host
  - One bacteria species can turn on > 100 genes
  - Toll receptors on dendritic cells / macrophages
  - Gut contains complex neuroendocrine system
- Quorum sensing
  - Molecules secreted by bacteria: they partially explain bacterial community behavior and activation of virulence genes etc

Proximal Ileum 10^3
Streptococcus
Lactobacillus



Colon 10^{11}
Bacteroides
Bifidobacterium
Clostridium coccoides
Clostridium lepium/Fusobacterium

Does the Mucosal Surface Environment Alter Function or Clinical Outcomes ?



- Inflammatory changes
- Bacterial interrelationships
- Bacterial changes with host stress situations
  - Bacterial use environmental clues
    - pH, temperature, redox potential, osmolality
  - When energy supply is limited genes “switch on” virulence factors
  - Ex: E.coli can rapidly become virulent with host stress (epinephrine, cortisol etc)

•Alverdy J, CCM 31:598-607,2003

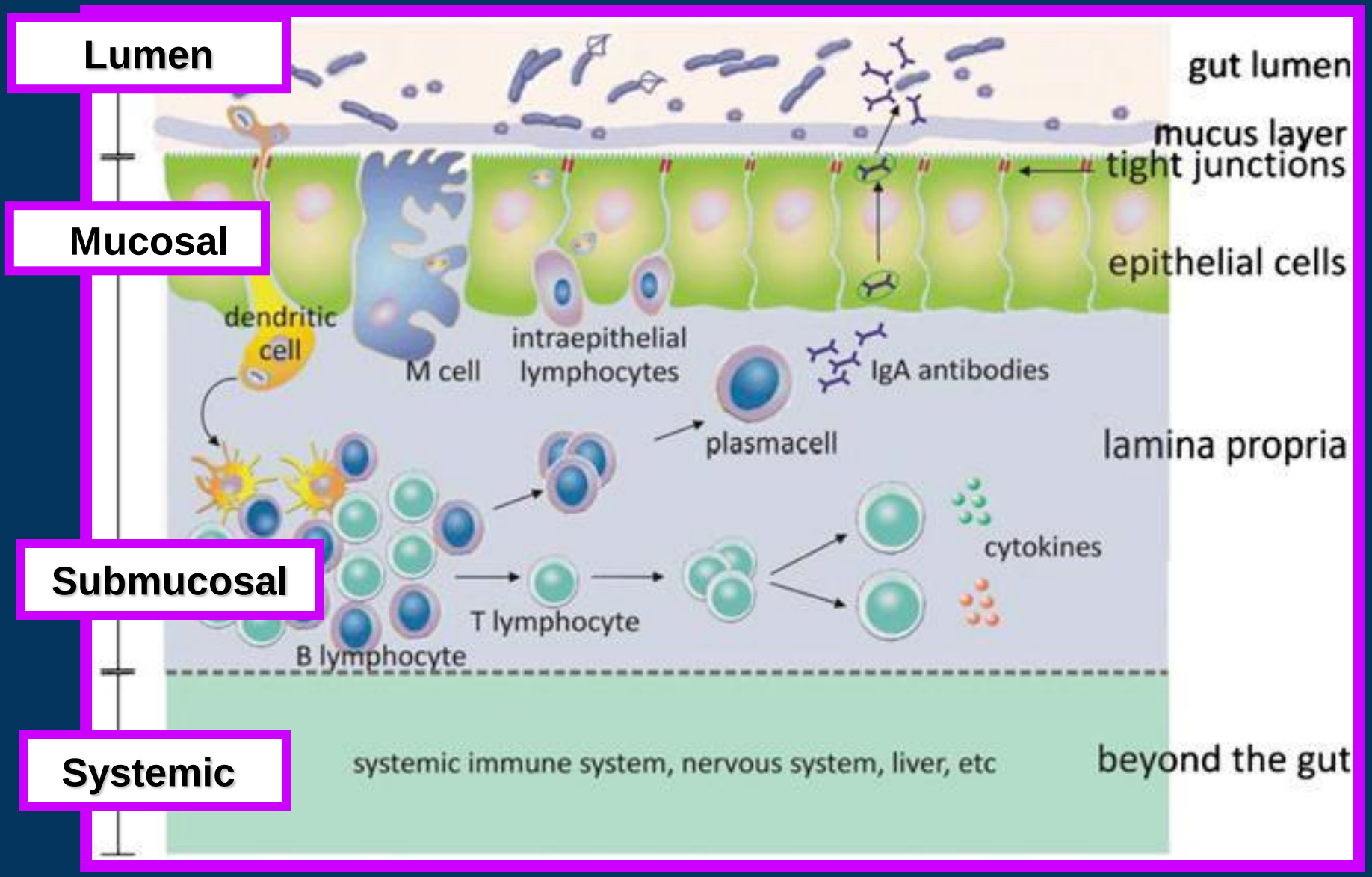
Probiotics can *prevent, mitigate* and *treat* many of the current health crisis facing the western world

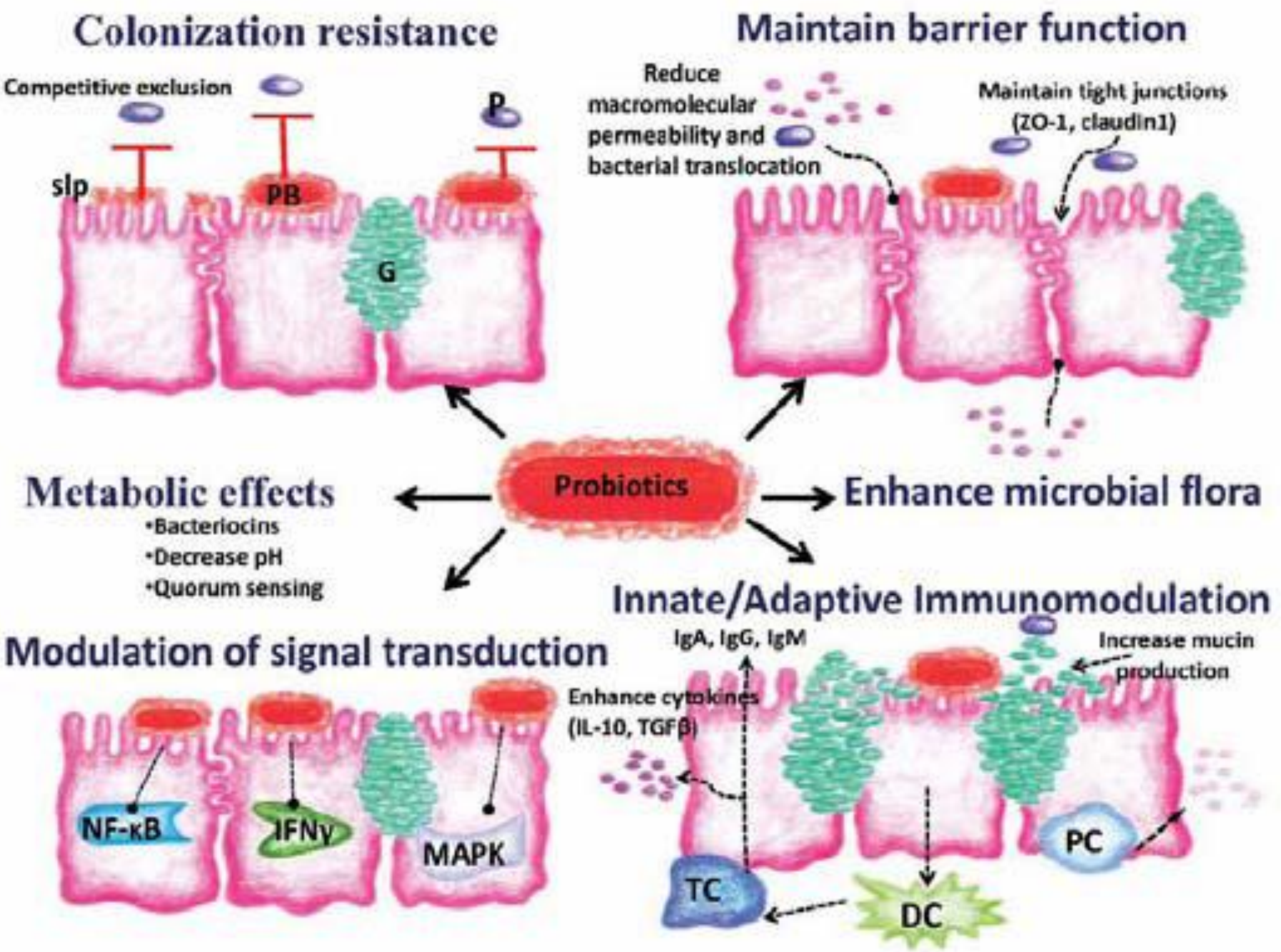
- Cancer
  - Multiple mechanisms
- Heart disease
  - Metabolic syndrome
  - atherosclerosis
- Depression
- Hepatic diseases
  - NASH
- Infectious disease
- Diarrheal diseases
  - AAD
  - Bacterial
  - C.diff
  - Viral
- Inflammatory diseases
  - IBD
  - Allergy
  - Asthma
- Autoimmune diseases
- Aging
- Obesity
- Critical Care / Surgery
  - Trauma
  - Pancreatitis +/-
  - Transplantation
  - Sepsis
  - VAP prevention
  - C.difficile

Potential applications for probiotics

- Metabolism
- Metabolism of dietary compounds
- in the gut lumen:
  - Lactose digestion
  - Lipid metabolism
  - Oxalate metabolism
- Composition and metabolic markers of the gut microbiota
  - Xenobiotics, phytochemicals
  - Indigestible dietary components
- Metabolic activity of gastrointestinal mucosa and liver
  - IBD and IBS
  - Inflammatory bowel diseases:
  - Crohn's disease
  - Ulcerative colitis
  - Pouchitis
  - IBS
- Allergic diseases
  - Eczema, atopic eczema
  - Allergic rhinitis
  - Asthma
- Reduction of risk factors of infection
  - Infectious diarrhea (acute and antibiotic-associated)
  - Traveler's diarrhea
  - Necrotozing enterocolitis (infants)
- Helicobacter pylori
- Respiratory tract infections (adults and children)
  - Ear, nose, and throat infections
- Infectious complications in surgical critically ill patients

Probiotics: Levels of Action

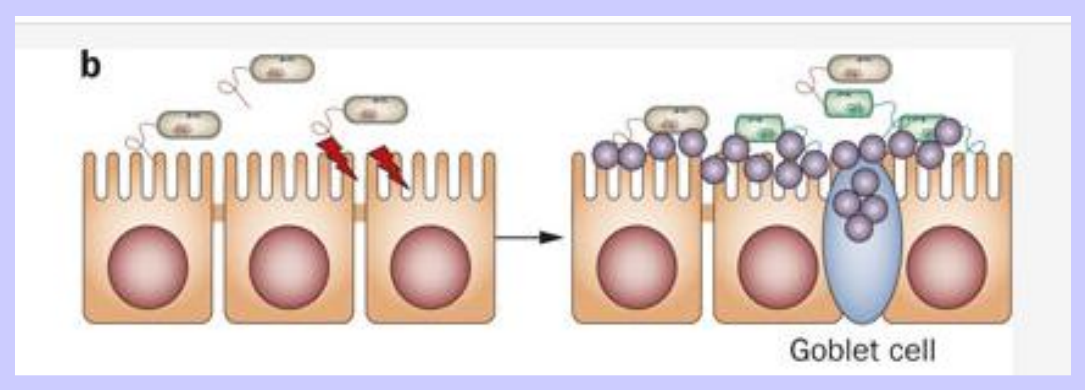




Sherman NCP 2009 , Wallace TC 2011

Mechanisms:

Colonization Resistance
Antimicrobial Factors



Mechanisms

- Competitive inhibition
- Physical barrier (mucous)
- ↓ Adherence, attachment
- Produce bacteriocins
  - Defensins, Trefoil
  - Bind pathogens
- ↓ pH reduces growth
- Interfers quorum sensing
  - ↓ Virulence expression
- Breaks up biofilms

L. reuteri* inhibits *H. pylori

PM Sherman (NCP 2009;24:10)

L. reuteri* inhibits *Staph aureus

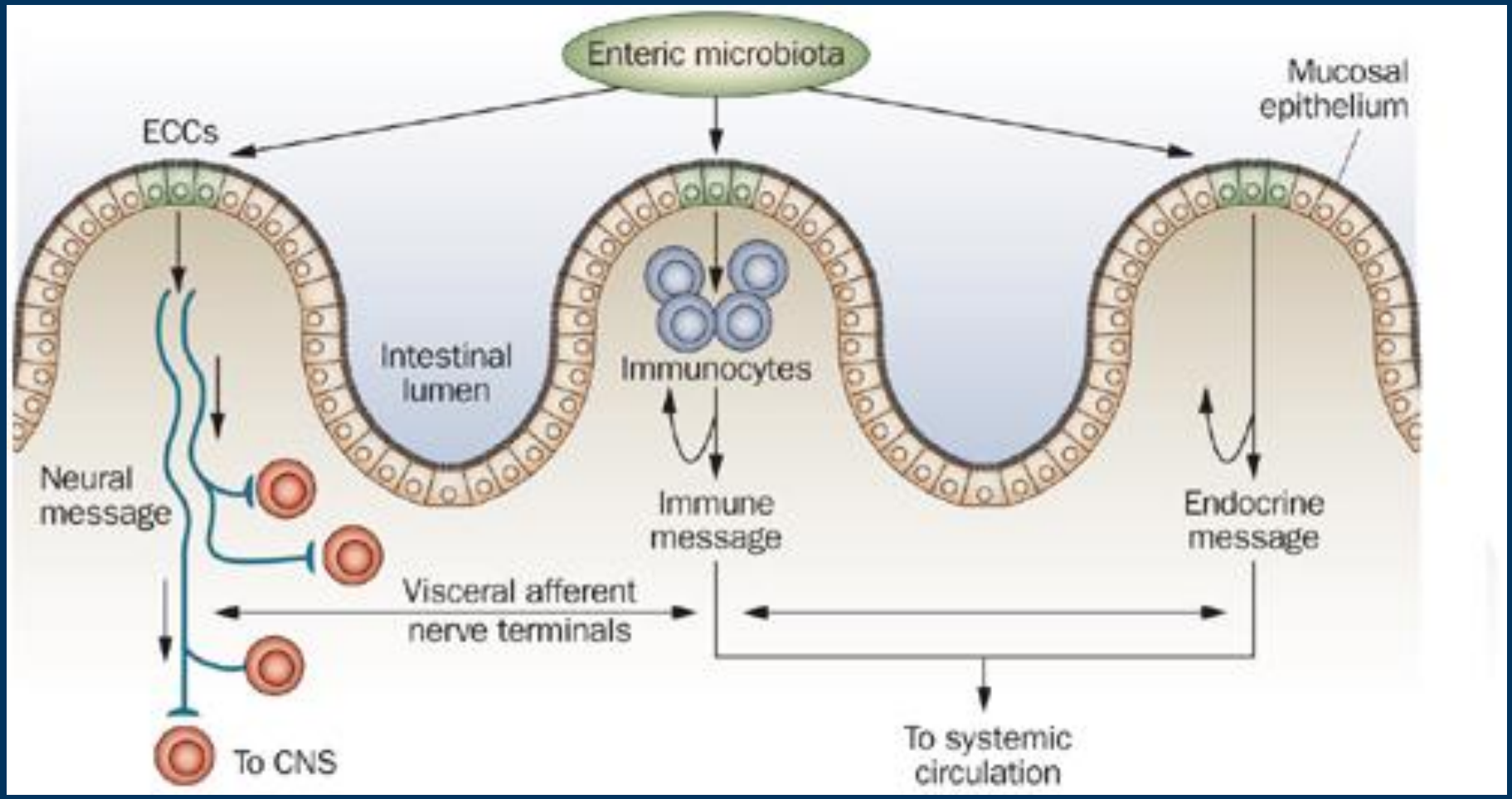
Bacteria

- *Escherichia coli (pathogenic)*
- *Salmonella typhimurium*
- *Shigella spp.*
- *Campylobacter jejuni*
- *Streptococcus mutans*
- *Bacillus subtilis*
- *Clostridium perfringens*
- *Helicobacter pylori*
- *Staphylococcus aureus*
- *Listeria monocytogenes*
- *Pseudomonas fluorescens*

Fungi

Candida albicans
Aspergillus flavus

Schematic representation of endocrine cell-mediated signaling from enteric microbiota to host



Nature Rev 2010

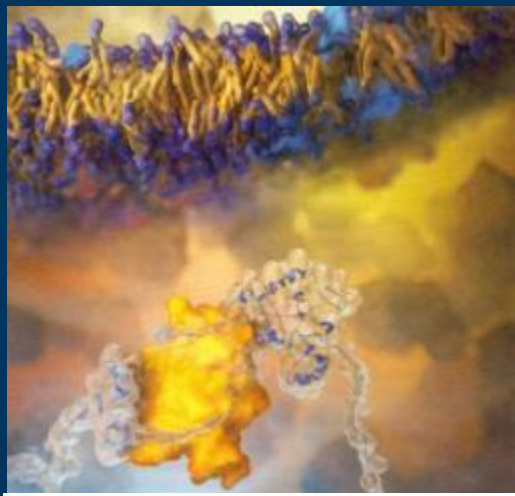
nature REVIEWS GASTROENTEROLOGY & HEPATOLOGY

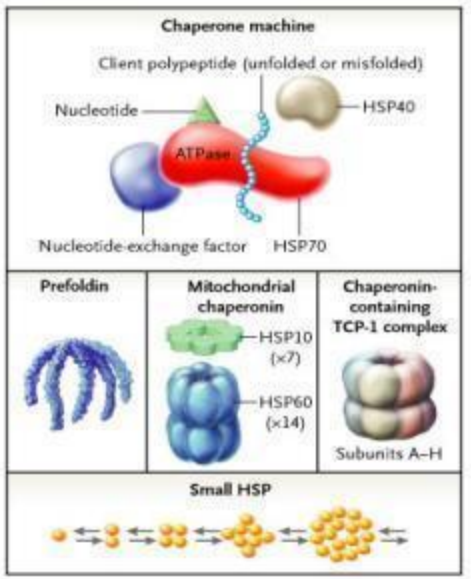
Protecting the mucosal lining:

"Soluble factors for *Lactobacillus rhamnosus GG* activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells"

- Cell culture model
- DNA microarray methods, real-time PCR and electrophoretic mobility shifts studied

- Studies confirm:
  - L. GG modulates signaling pathways
  - Activates via MAP kinase
  - L.GG protects mucosa from oxidant stress via expressing HSP

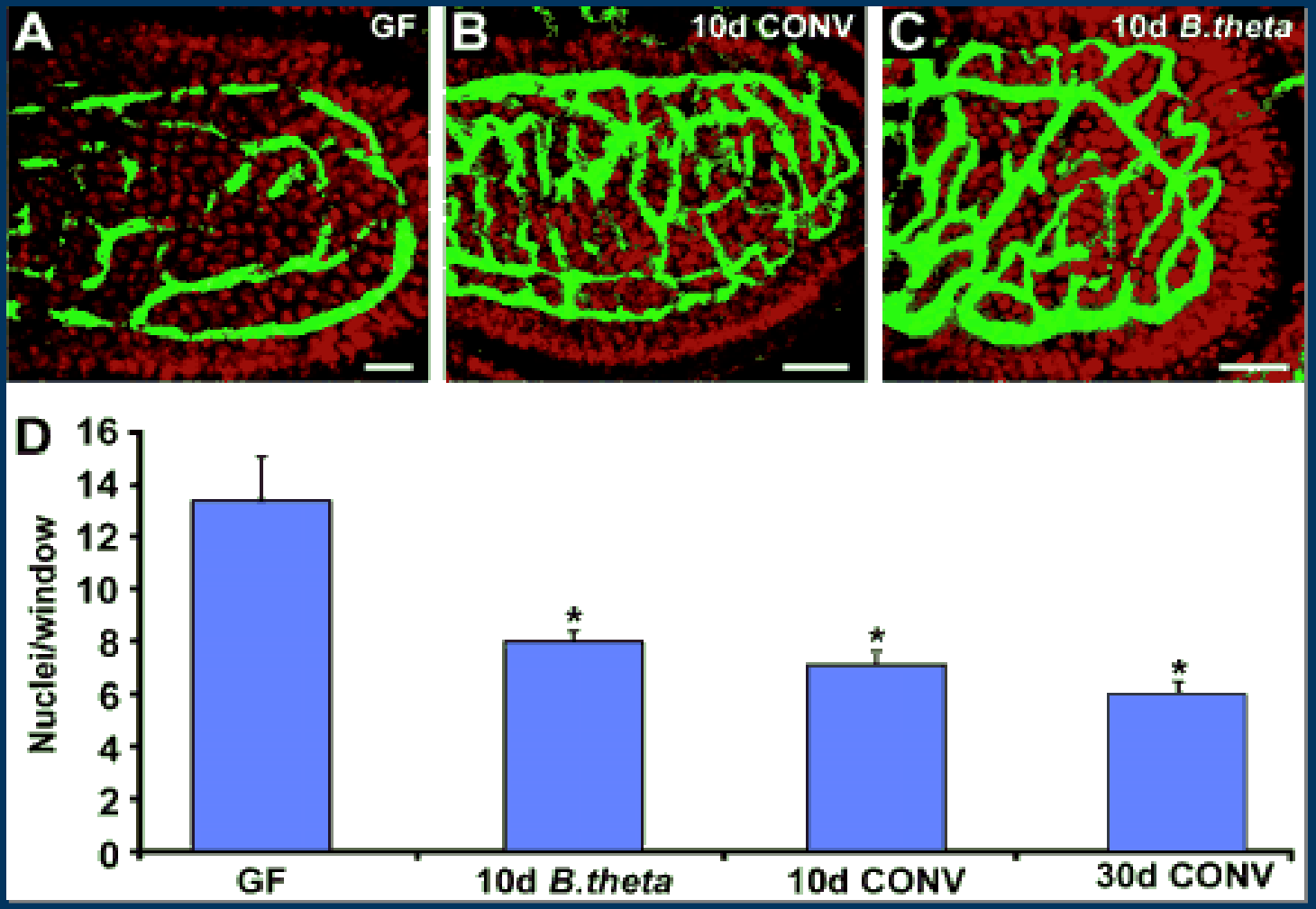




Tao K , Drabik K, Waypa T

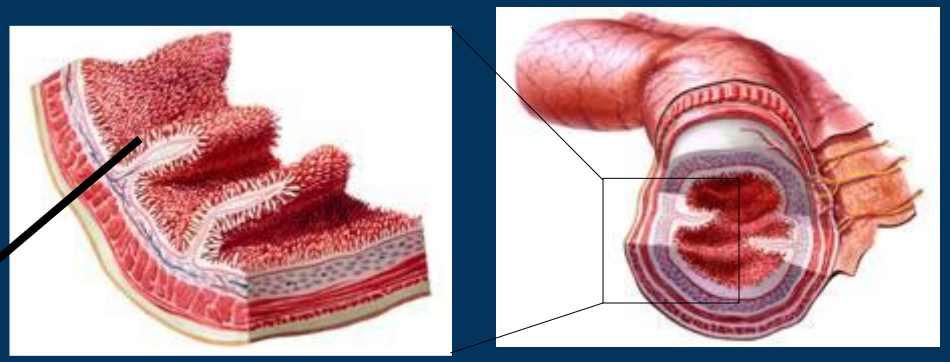
Am J Physiol Cell Physiol 290;1018-1030,2006

Mechanisms:
Enhancing mucosal blood flow



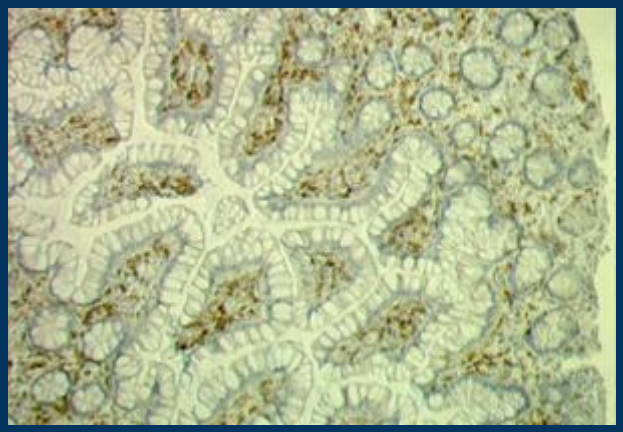
- Stappenbeck TS, Hooper LV, Gordon JI.
  Proc Natl Acad Sci U S A. 99: 15451-15455, 2002

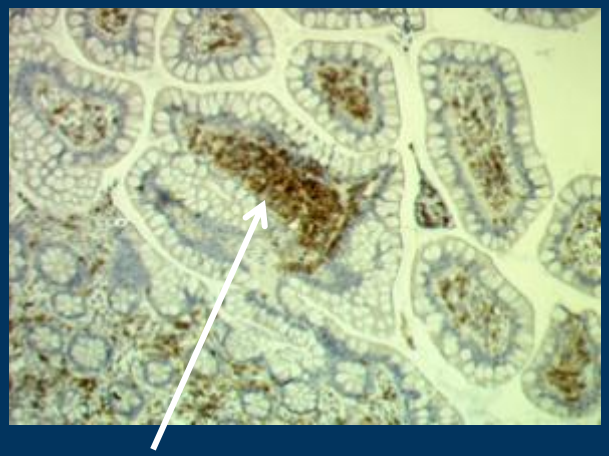
Mechanisms: stimulation the immune system in the small intestine of healthy subjects



Before Reuteri intake

After Reuteri intake



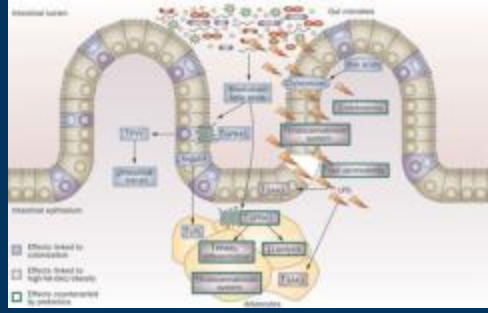


Resting CD4+ T-helper cells

Activated CD4+ T-helper cells

•*Valeur et al., Appl Environ Microbiol 70 1176-1181 (2004)*

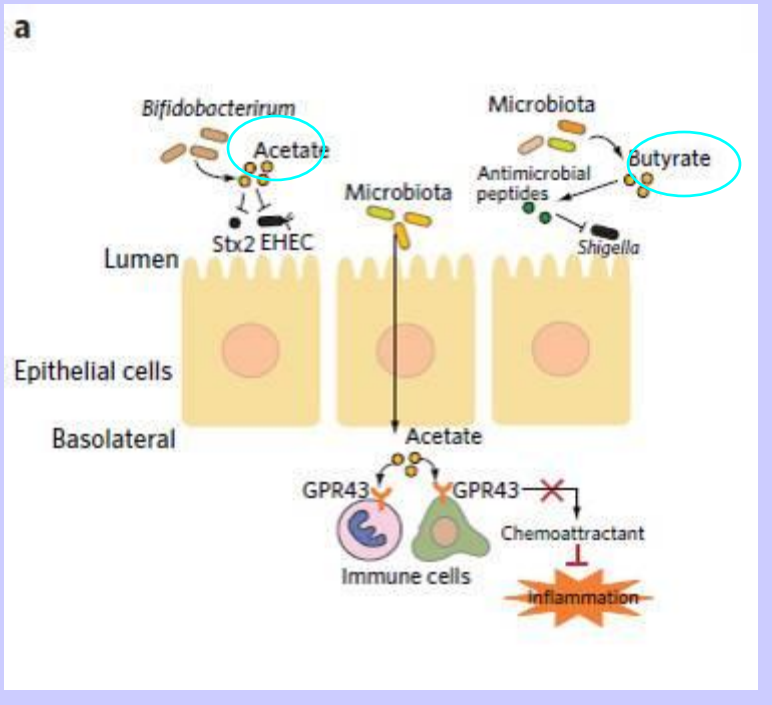
SCFA = Fermentation end product of some probiotics (from prebiotics)

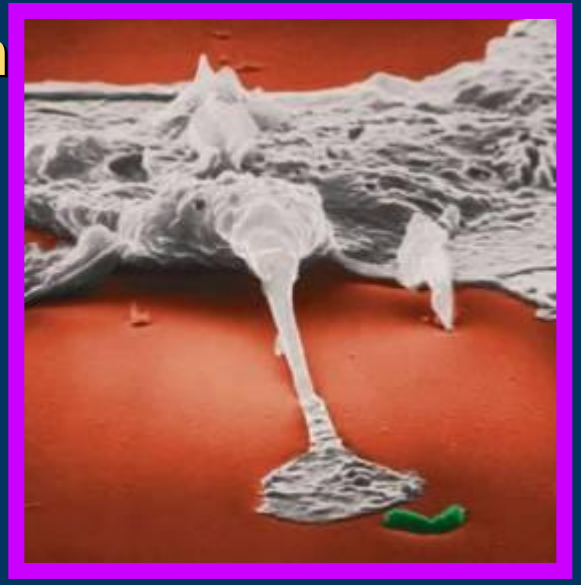


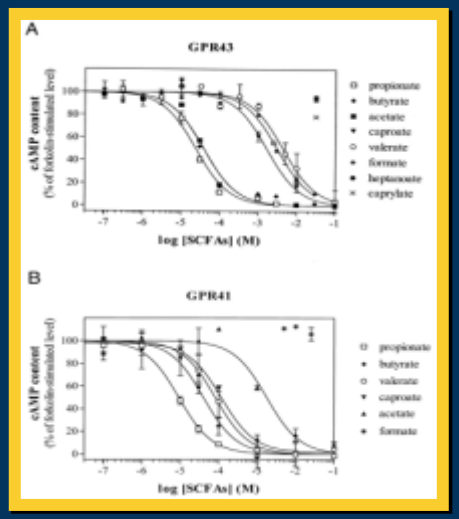
- Energy source;
  - **colonic mucosa;**
    - **Stimulates cell proliferation, Promotes sodium and water absorption**
  - **Cardiac, Skel Mus , Brain**
    - **Acetate;**
  - **Proprionate; gluceneogenesis**
- Regulation of gene expression for ICAM-1 and E-Selectin on endothelial cells
- Decrease COX-2 expression
    - (butyrate and proprionate)
- Prevention of neoplastic transformation
    - Inhibits histone deactylase by DNA hypermethylation to promote differentiation in cancer cell lines
- Enhances Leptin secretion
- Inhibition of pathogen overgrowth in gut lumen
- ROS scavenger
    - Pyruvate is anti-inflammatory and decrease NFKB expression
- Activation of polymorphonuclear cells
    - **Both local and systemic**
    - **G-protein receptors on circulating PMN's**

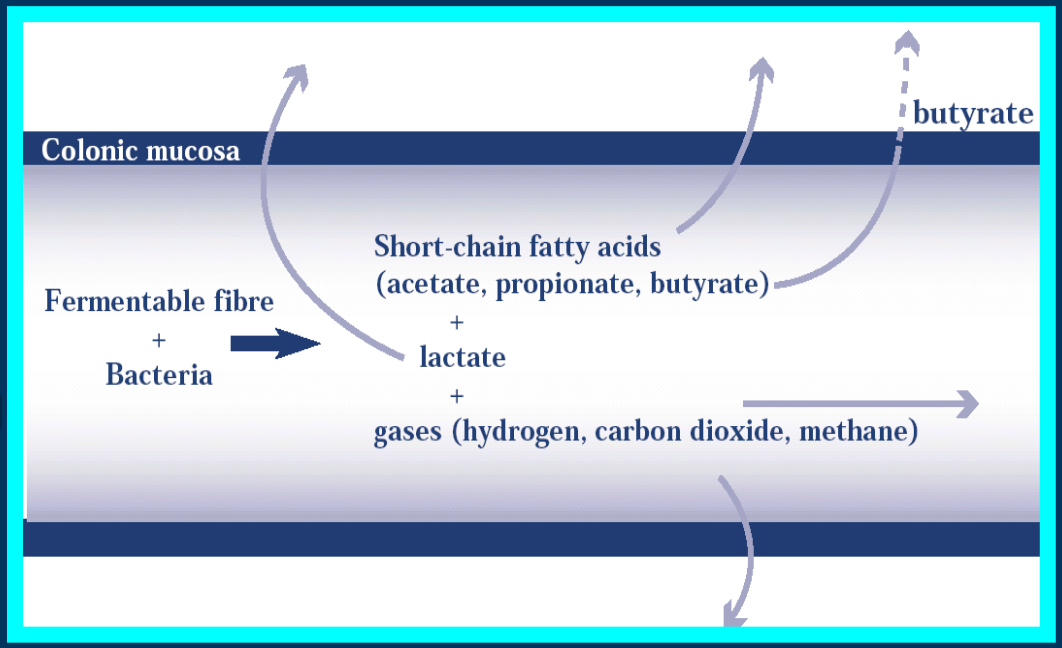
Thangaraju M et al J GI Surg 2008

SCFAs, Fiber Fermentation and Butyrate Receptors









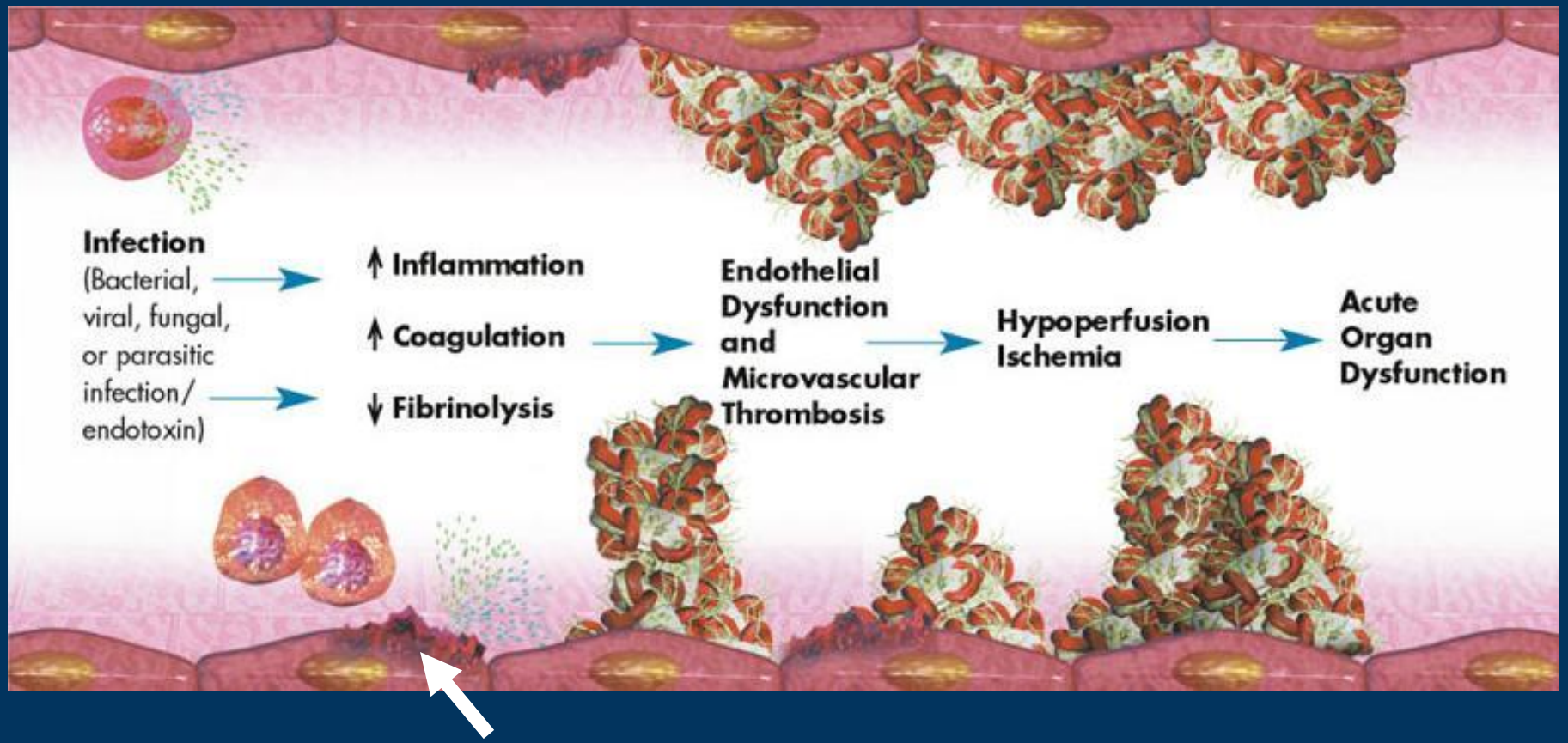
- Trophic effect, colonocyte fuel
- Anti-inflammatory
- Enhance WBCs, macrophage
- ↓Adhesion molecules
- (↓microvasc thrombosis)

Thangaraju M et al J GI Surg 2008

Ganapathy V 2011

Preventing Microvascular Thrombosis:

Regulation of gene expression for ICAM-1 and E-Selectin on endothelial cells



Probiotics (via SCFA) shown to decrease ICAM-1 and E-Selectin expression on endothelial cells

Clinical Use of Probiotics in the ICU

Where does the rubber meet the road?

Clinical Use: Sorting evidence from myths !

- **Specific effects can be strain specific !**

- **Level I evidence in:**
  - **Infectious diarrhea (L GG)**
  - **Prevention of traveller's diarrhea**
  - **Prevention of pouchitis after total colectomy for UC**
  - **Prevention of Ventilator Associated Pneumonia (VAP)**
  - **Prevention of Necrotizing fasciitis in neonates**
  - **Prevention of anti-biotic diarrhea**

- **Level 2 evidence in:**
  - **S.boulardii (with vanc) in preventing recurrent C.difficile**
  - **Prevention of post op infections in liver transplant**
  - **Prevention of post op infections in abdominal surgery**

Can Probiotics be used for prevention of disease in “Healthy People”

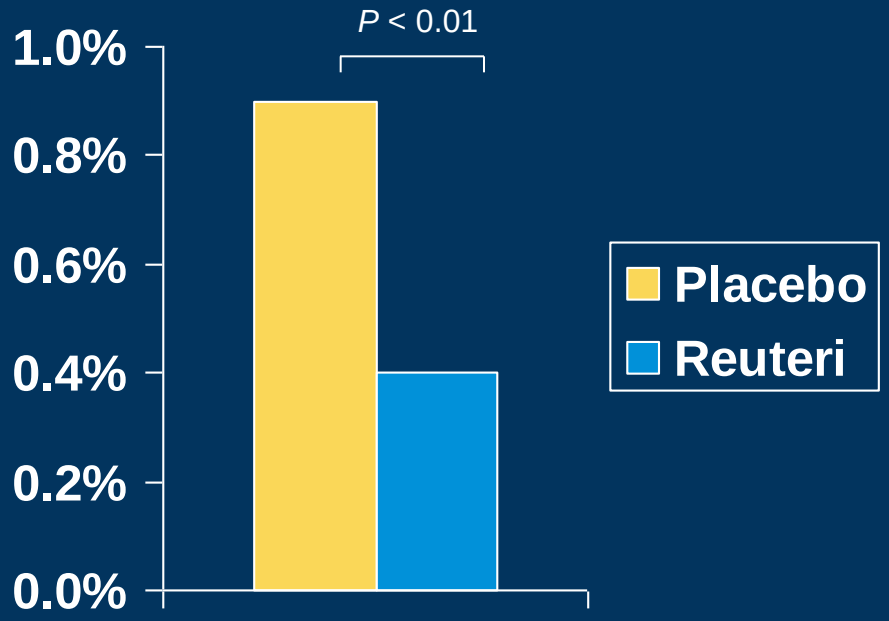
Sick days at home with short term gastro-intestinal or respiratory illness

Placebo: 0.9 % sick days
2 days per individual and year

Reuteri: 0.4 % sick days
<**1** day per individual and year **

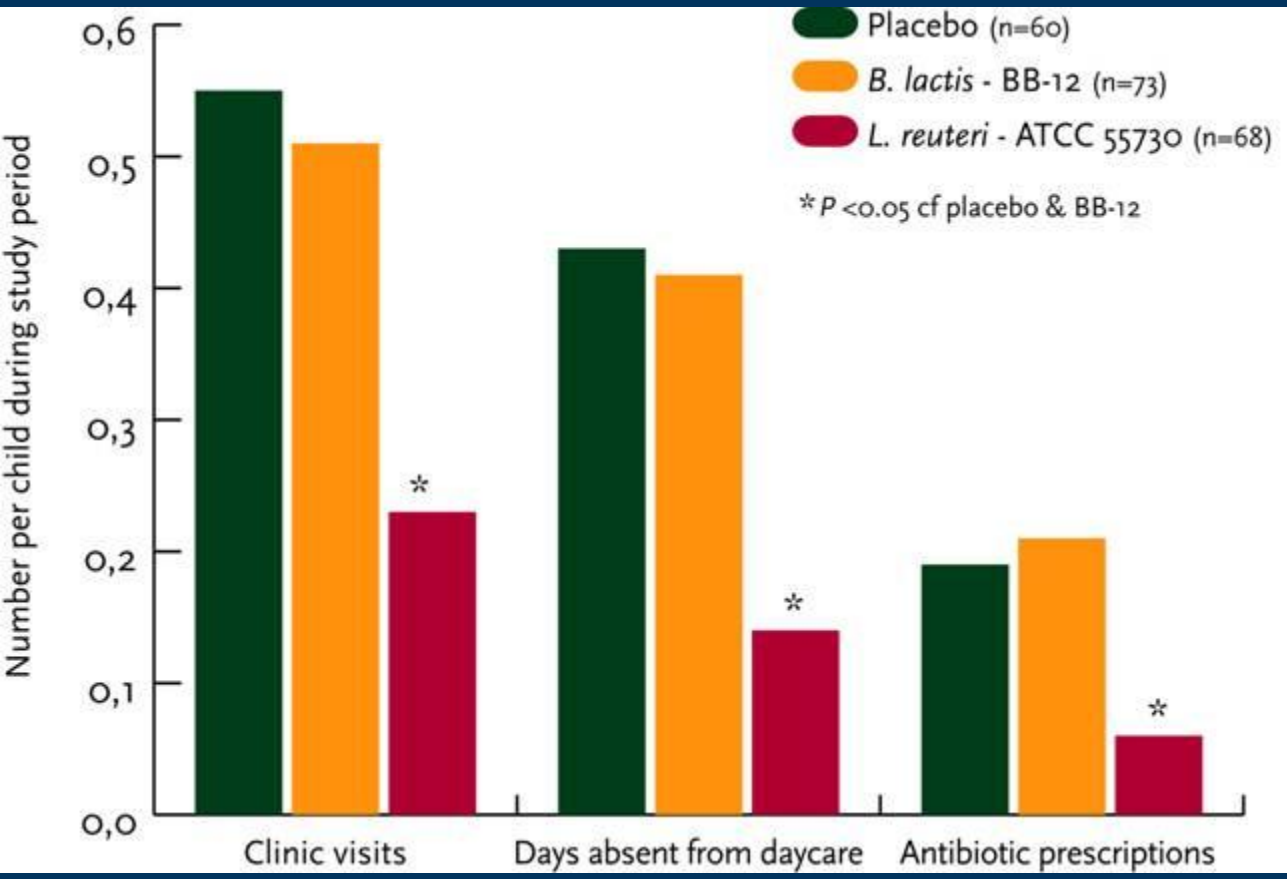
Number of people sick

26% on placebo (23 persons)
11% on Reuteri (10 persons) p<.01**



Tubelius P et al., Environ Health 2005

Probiotics use in healthy nursery school children



- *Children (4-10m) with increased risk for infection*

12 weeks supplementation in baby formula

Weizman et al., Pediatr 115; 5-9 (2005)

Gestational Diabetes

- Finland N=256 (3 groups)
- Strict definition of Gestational diabetes (GTT)
- Control, placebo, probiotics

- Results:
  - Control 36%
  - Placebo 34%
  - Probiotics 13%
  - No change in pregnancy outcome
  - No change in children at two years

Luoto R British J Nutrition 2010

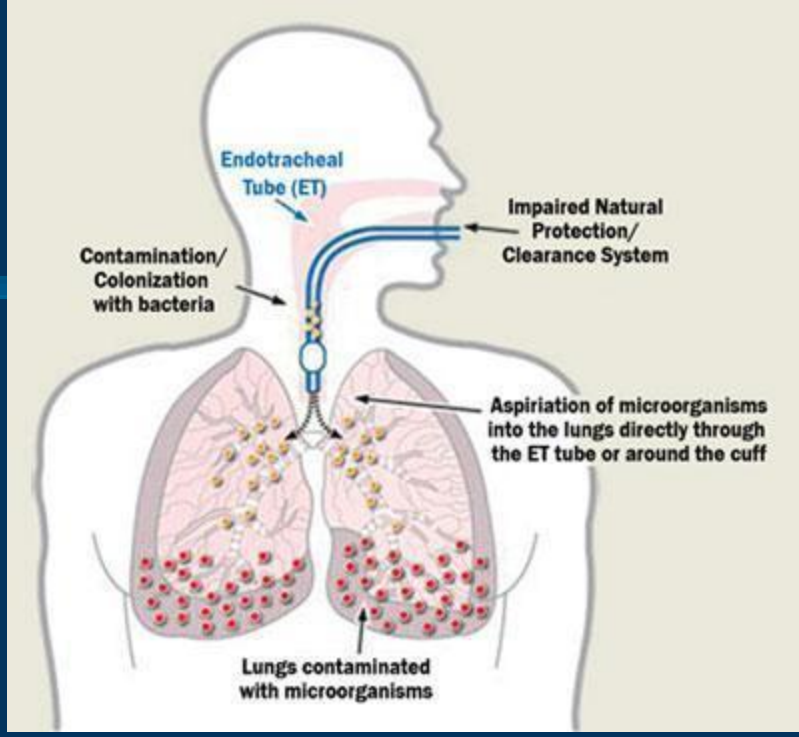
Areas of Critical Care Where Probiotics Have Reported Benefit

- **Treatment:**
- Trauma
- Pancreatitis +/-
- Transplantation
- Sepsis
- NASH

- **Prevention:**
- VAP
- Antibiotic associated diarrhea
  - C.difficile
- VRE colonization

Ventilator Associated Pneumonia

- One of most frequently occurring nosocomial infections in the ICU
- Current strategy not working
  - Antibiotics – increases resistant flora
  - Ventilator adjustments – variable success
  - Prokinetic agents – no influence
  - Medications – no influence
  - Surfactants – no influence
  - Mouth wash – variable
  - Etc etc etc



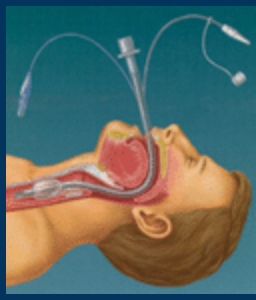
"Oral probiotics and prevention of *P. aeruginosa* infections: a randomized, double-blind, placebo-controlled pilot study in intensive care unit patients"

- Hypothesis: oral application of probiotics will prevent the secondary colonization with pathogens
- PRDBPC trial
  - Inclusion criteria
    - patients in ICU >48 hours
  - 807 eligible:106 placebo vs 102 probiotic completed
  - 10^9 L.casei BID started day 3 until discharge
  - Monitored gastric and oral bacteria cultures
- Results:
  - Delayed colonization of *P.aeruginosa* in respiratory tract

•**Forestier C Critical Care 2008,12:1-10**

Use of Probiotics to Prevent Ventilator Associated Pneumonia

- *Lactobacillus GG* vs placebo (DBPCT)
  - (2871 patients screened 146 met criteria)
  - On vent > 72 hours
  - Oral *and* via feeding tube
  - 1.0 x 10^{10} BID to each site
- Evaluated
  - Oral flora pathogen vs normal flora
  - Gastric flora pathogen vs normal flora
  - Incidence of VAP
- Results
  - Less antibiotics used
  - Less C.difficile 5.8% vs 18.6% ($p<.05$)
  - Clinical VAP 35% vs 47% ($p<.05$)
  - Microbiologic VAP 19% vs 40% ($p<.05$)
  - Mortality 14% vs 24% (NS)



•**Morrow S, Kollef M et al 2010 AJRCCM**

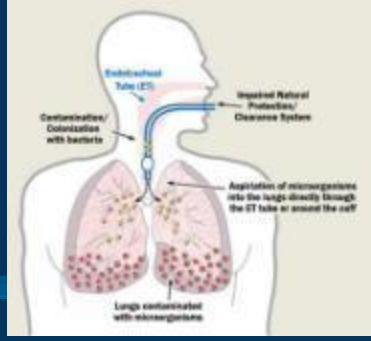
Not all Probiotics VAP studies positive:

- N = 259 ICU Mechanical ventilation > 72 h
- Probiotics delivered to GI via tube
  - With soluble fiber
- Results:
  - VAP w/ probiotics 9% vs 13 % in control (NS)
  - Mortality 27% in probiotics vs 33% in control (NS)
- Conclusion:
  - No significant improve in VAP or mortality

- (note: probiotics only given enterally, no oral / pharnyx delivery)

Knight DJ Int Care Medicine 2009

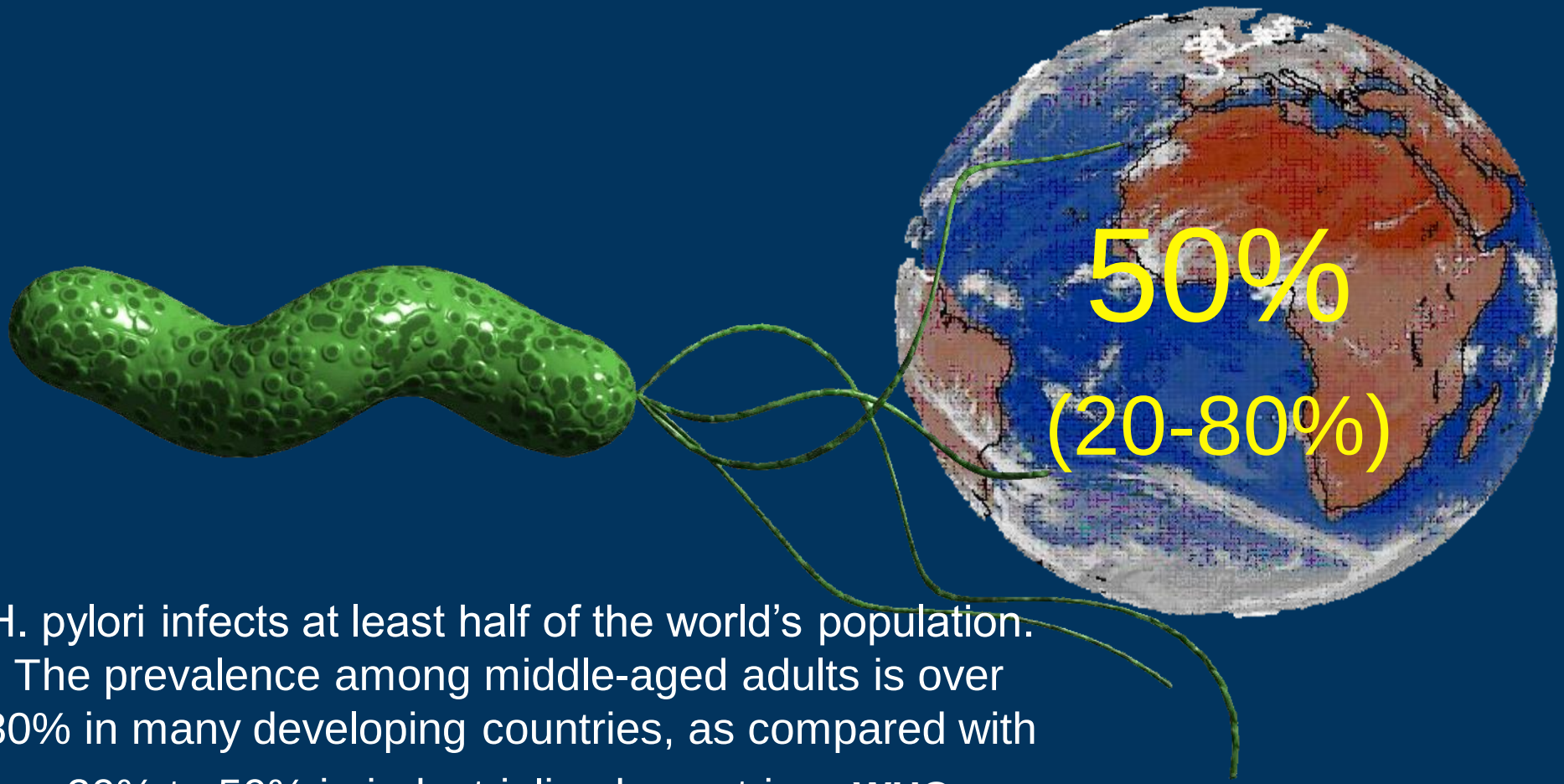
Impact of administration of probiotics on VAP: Meta-analysis



- RCT with mechanical ventilation
- 5 RCT included
- Results:
  - Probiotics decrease VAP
  - Decrease in Pseudomonas colonization
  - No change in mortality
  - No change in ventilator days

I Siempos et al Crit Care Med 2010

Probiotic based control of *H. pylori* infection



H. pylori infects at least half of the world's population. The prevalence among middle-aged adults is over 80% in many developing countries, as compared with 20% to 50% in industrialized countries. **WHO classifies H. pylori as class one carcinogen**

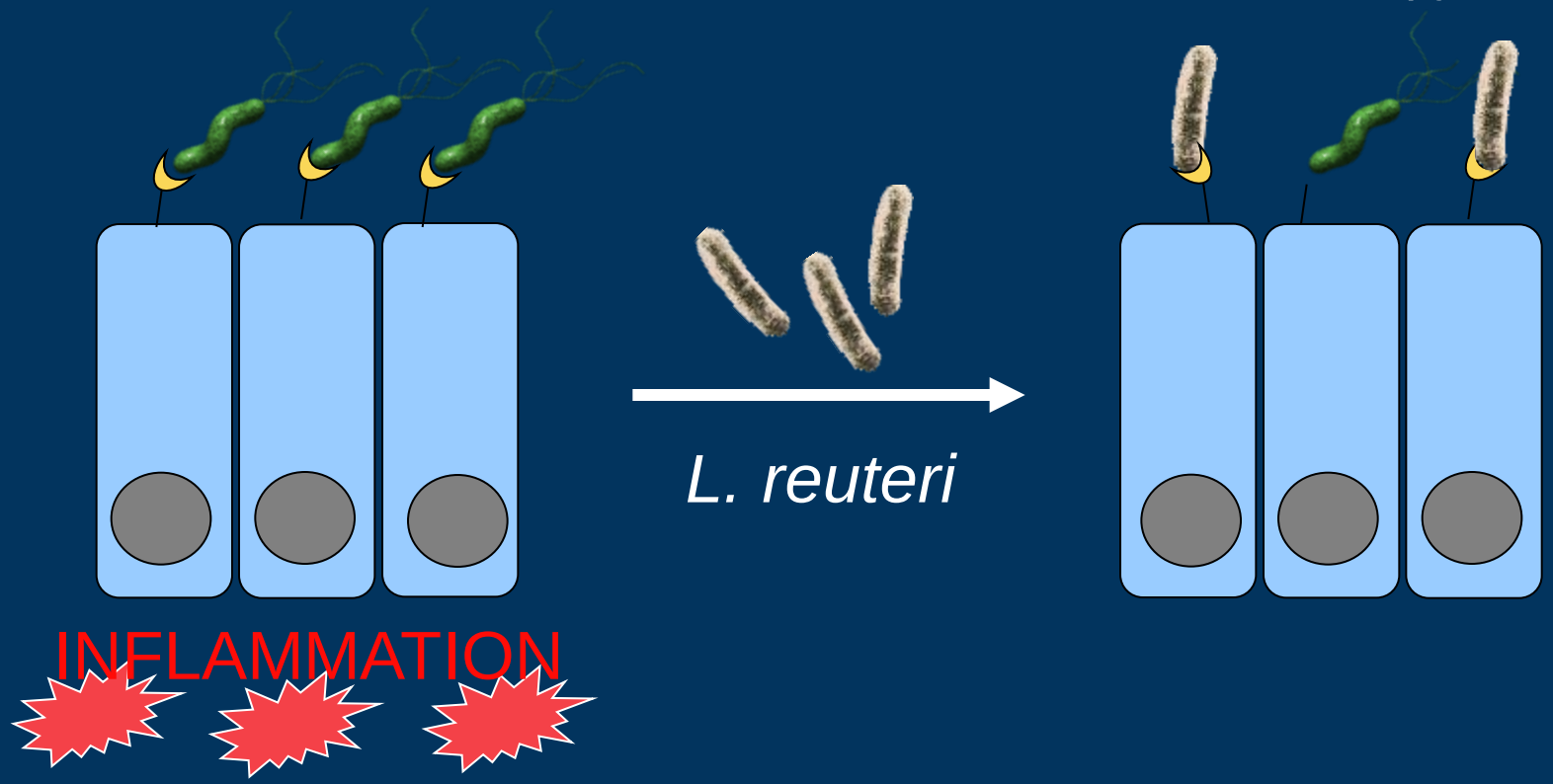
Suerbaum & Michetti NEJM 2002; 347:1175

Morowitz MJ Ann Surg 2011; 253:1094-1101

Specific probiotics have surface proteins that inhibit the binding of *H. pylori* in the stomach

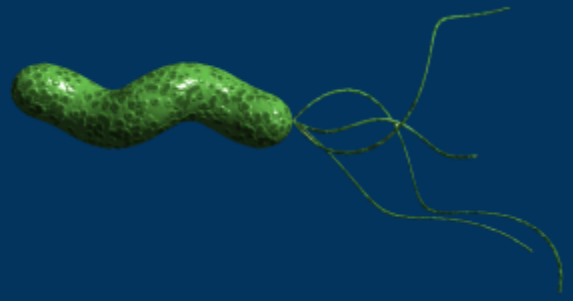
H. pylori attached to gastric cells

L. reuteri inhibits *H. pylori* binding



•*Mukai et al. FEMS 32:105 (2002)*

HP Eradication Therapy with and without Probiotics- Meta-analysis



Outcomes	# Trials / (n)	with	w/o	NNT
Eradication Rates	11(1074)	85%	75%	11
Total Side Effects	7(625)	22%	38%	6
Diarrhea	8(997)	6.1%	16%	11
Epigastric Pain	7(608)	16%	23%	14
Nausea	7(608)	16%	25%	12
Taste Disturbance	5(418)	14%	25%	5

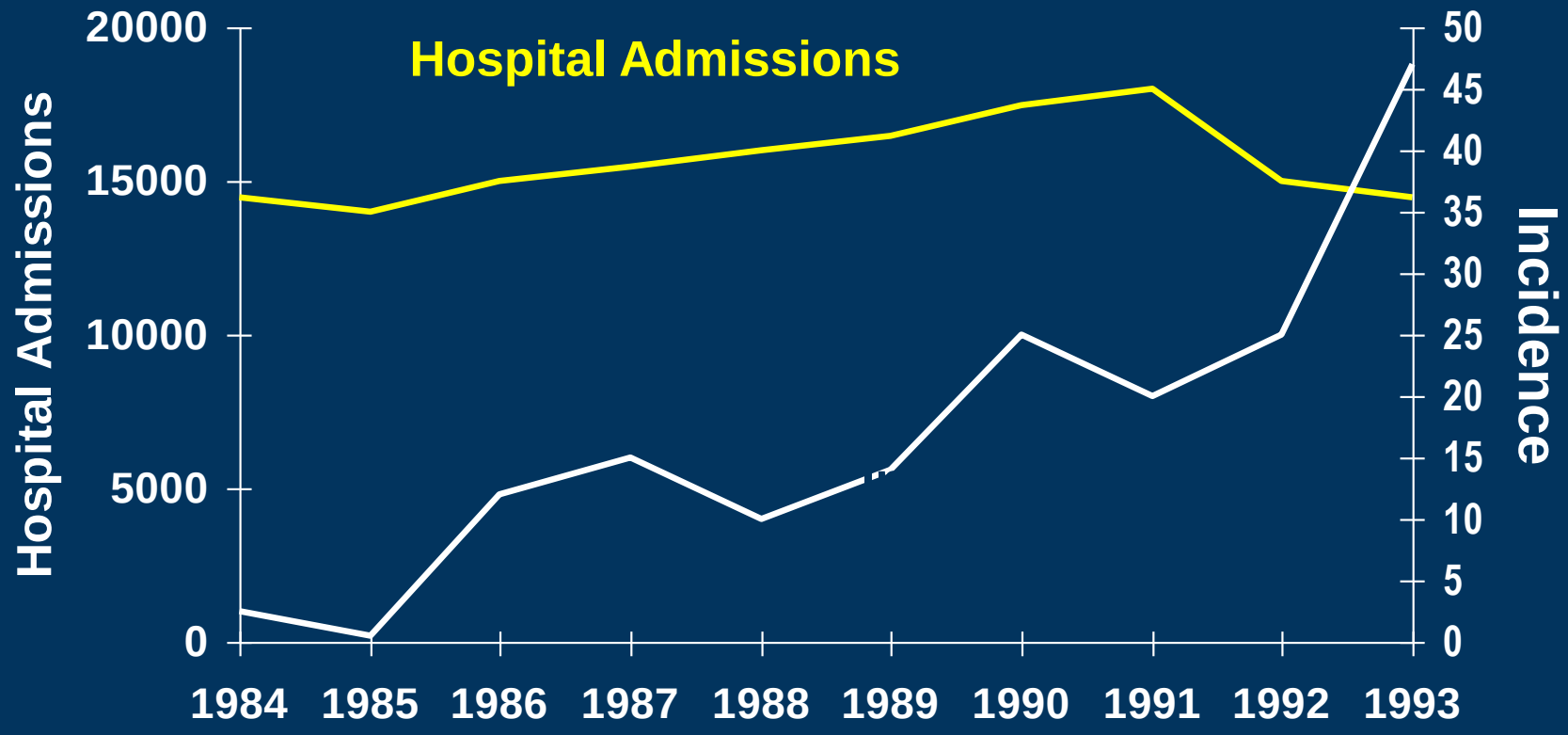
Tong, A Pharm Therap 2007

Antibiotic Associated Diarrhea: preventable or inevitable ?

- Hempel S et al JAMA 2012
- Meta-analysis 82 RCT met criteria for inclusion
- Probiotics strains were poorly documented
- N=11,811 participants (pooled data)
- Conclusion:
  - Probiotics confer significant decrease in AAD ($p<.001$)
  - # needed to treat N=13

Hempel S et al JAMA May 9, 2012

Rising Incidence of C.difficile



- Incidence of C.difficile by year

Adapted from Jobe BA et al. *Am J Surg.* 1995;169:480-483.

Pathogenesis of CDAD

Antibiotic therapy

↓

Alteration in colonic microflora

↓

C. difficile exposure and colonization

↓

Release of toxin A and Toxin B

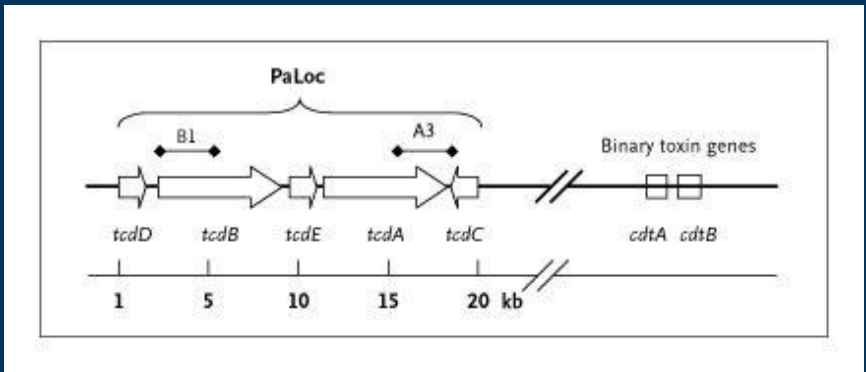
↓

Colonic mucosal injury and inflammation

- Adapted from Kelly CP et al Ann Rev Med 1998;48:375-390
- Badger, VO et al JPEN 2012

Emergence of B1/NAP1 Strain

- Produces 16-23 times C. diff. toxins A and B in vitro, represented 50% of isolated strains between 2001-2003
  - Produces a 3rd binary toxin
- Increased risk of relapse
- Less responsive to standard therapies



Major Genes in the Pathogenicity Locus (PaLoc) of Clostridium difficile and Relation to the Genes for Binary Toxin

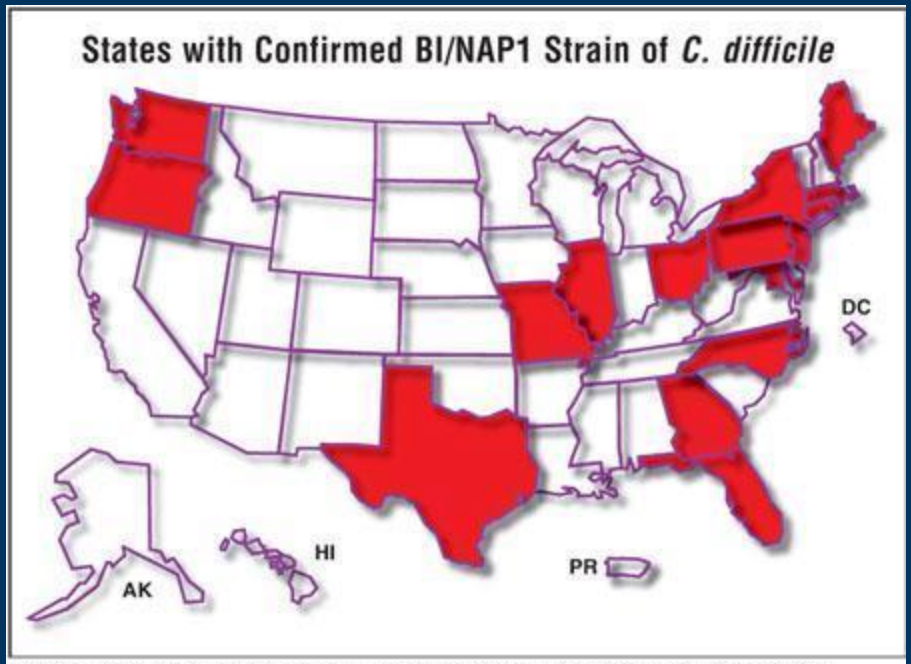
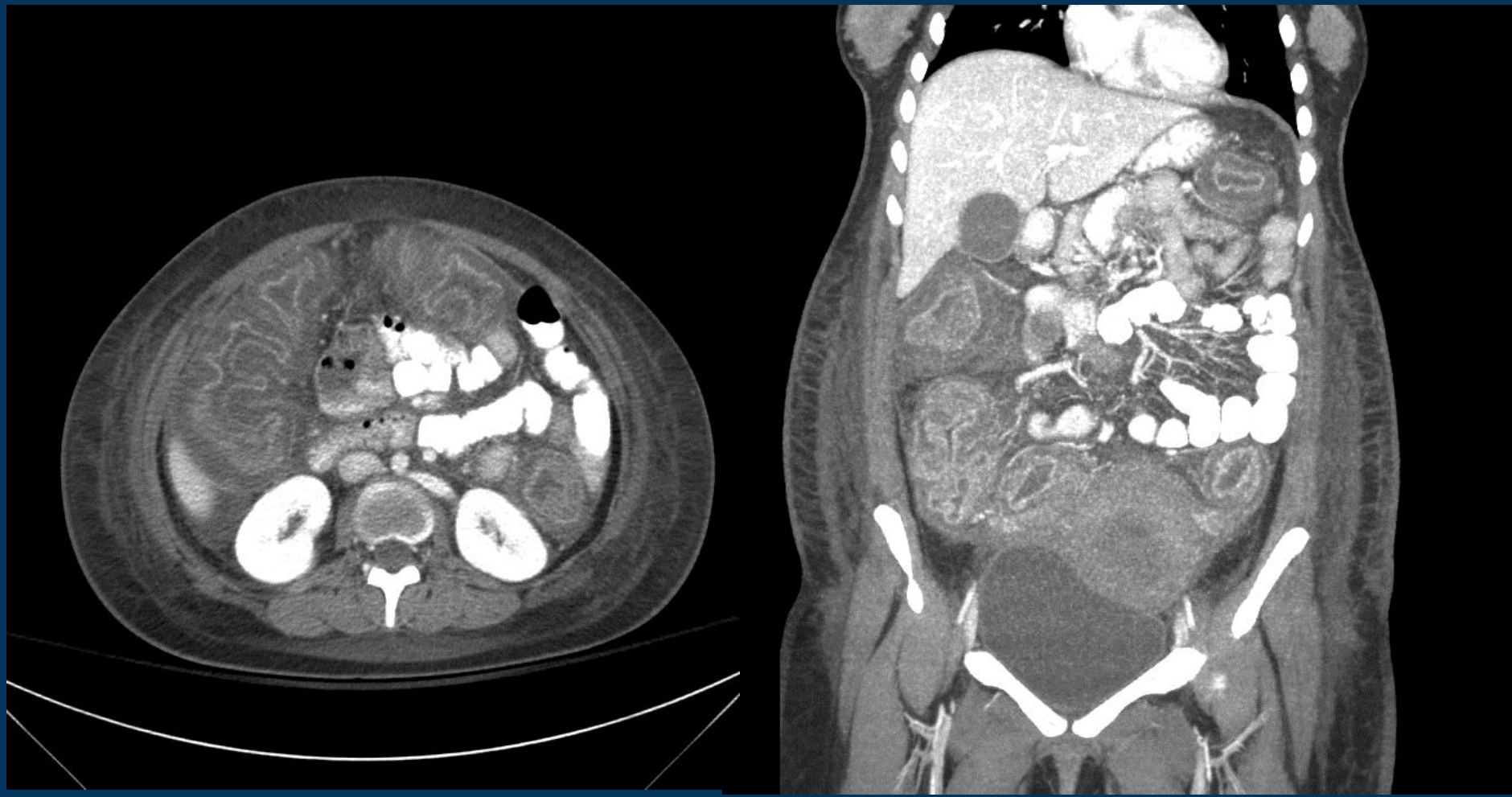
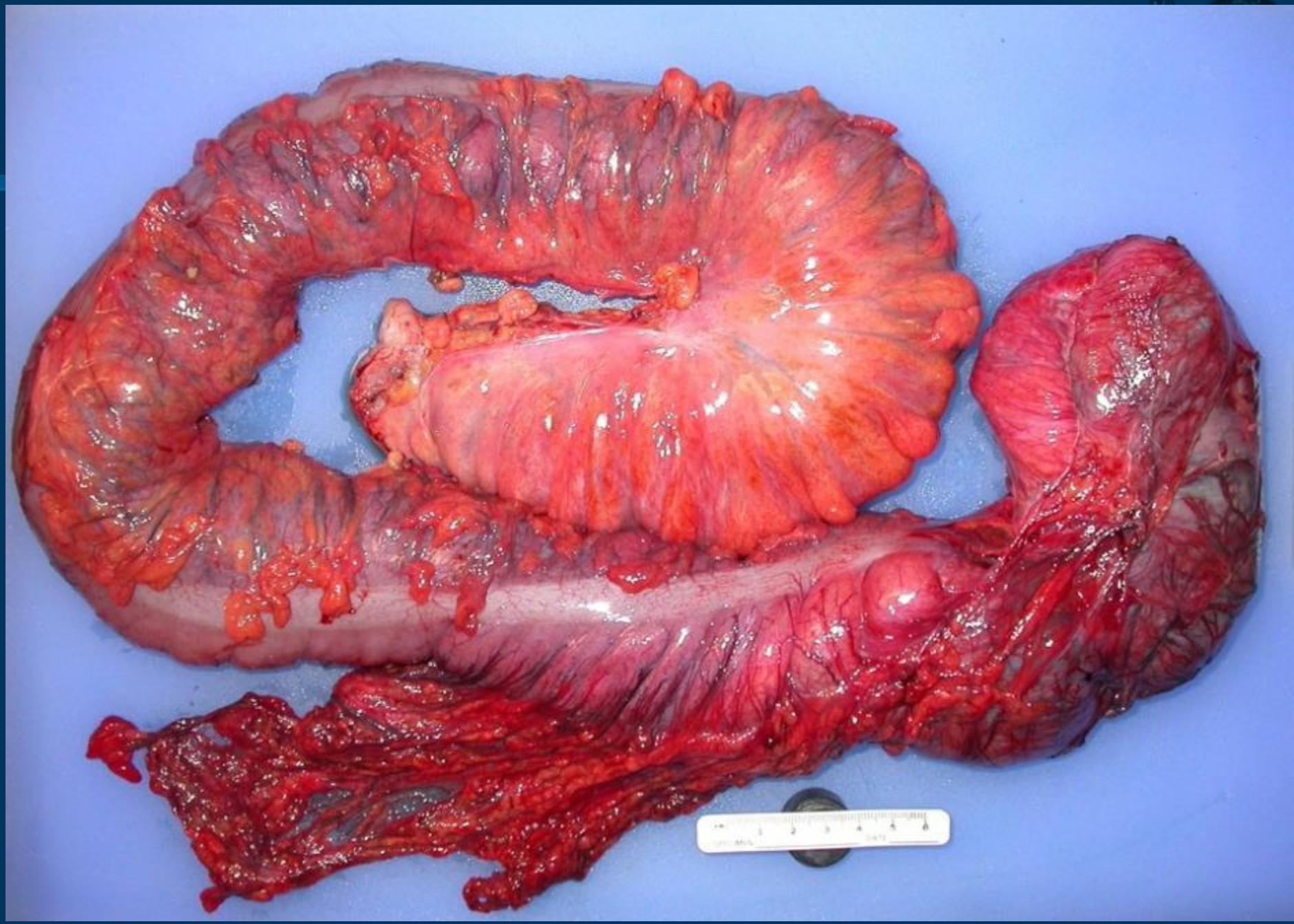


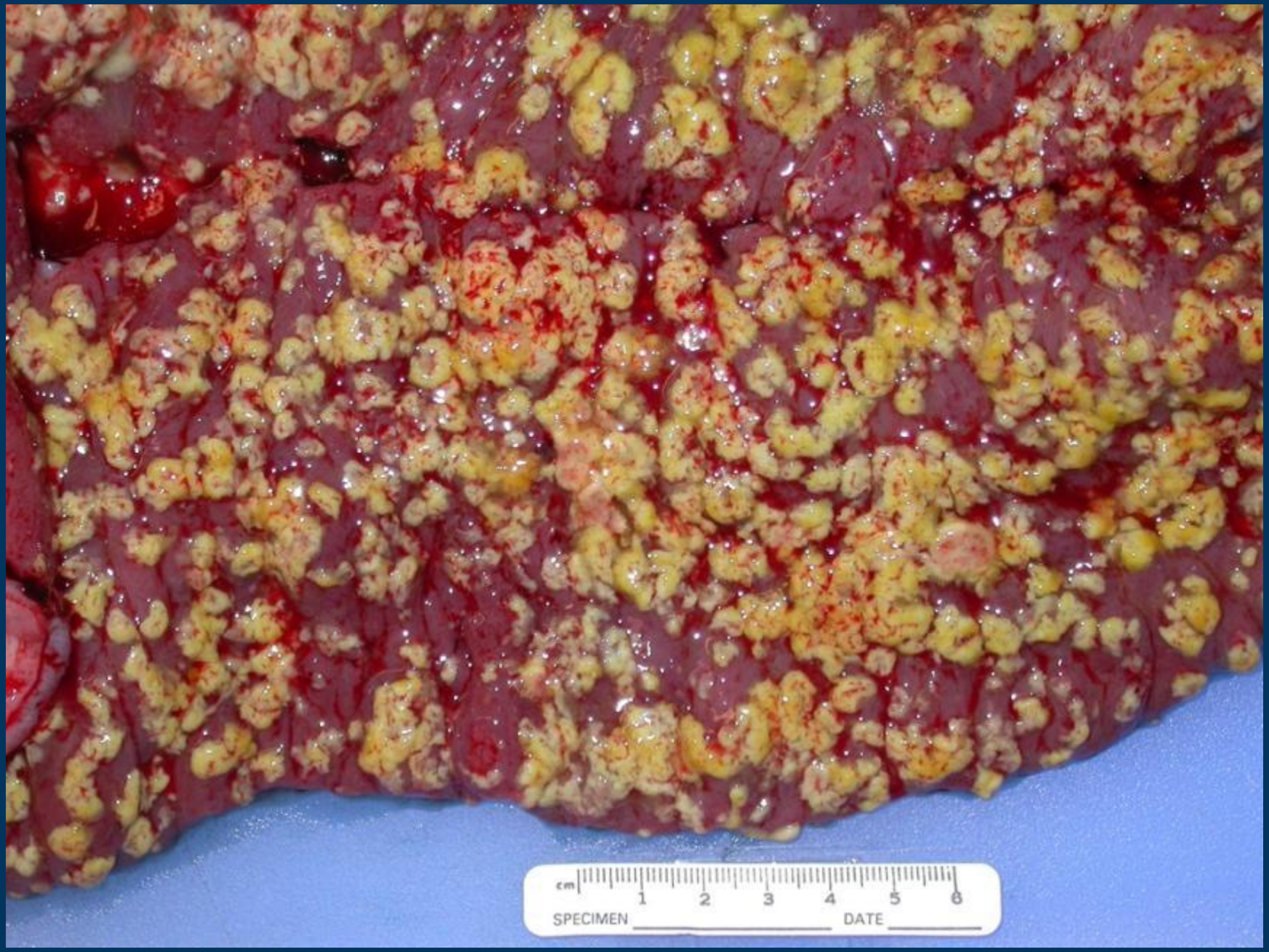
Figure 2. States with the North American Pulsed Field Type 1 (BI/NAP1) strain of C. difficile confirmed by CDC as of November 15, 2005 (N=16).

- **McDonald NEJM 2005**

The changing face of Clostridium difficile !






cm
1
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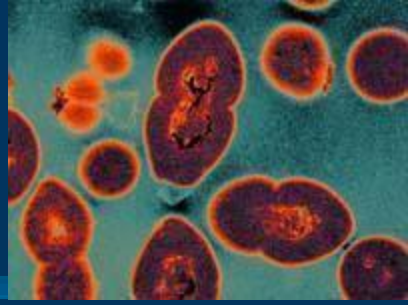
SPECIMEN
DATE

Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics:

- RDBPCT N=135
- Age 64 all taking antibiotics
- 100 gm BID L. casei as drink
- Results:
  - Diarrhea: 7/57 (12%) vs 19/56 (34%)
  - 21% relative risk reduction, NNT 5
  - C.diff 0/57 vs 9/53 (17%)

Hickson M, et al . BMJ June 29 2007

"Probiotic treatment of VRE: Randomized Controlled Trial."



- PRPCBT 27 VRE positive patients
- Yogurt (containing live Lactobacillus GG vs Pasteurized yogurt)
- 100 gm daily x 4 weeks
- Primary outcome measure: clearance of VRE
- Results:
  - L.GG group: 11/11 cleared VRE at 4 weeks, 3/11 reconverted + at 4 weeks
  - Control: 1/12 cleared
    - » Allowed to crossover at 4 weeks 8/11 crossed over
    - » 8/8 of the crossover group cleared in 4 weeks

Manley KJ, Fraenkel MB et al Med J Australia 2007;186:454-457
PRPCBT = Prospective Randomized Placebo Control Blinded Trial

Pre and Probiotics in the Surgery and ICU Setting

Author	Year	Population	Design	Outcome
Olah	2002	N = 45 pancreatitis	Oat fiber +/- L.Plantarum	Dec infection 4.5 vs 30%
McNaught	2002	N = 129 surgery	+/- L.plantarum	No change
Rayes	2002	N = 60 Abdominal surgery	Oat fiber +/- L.plantarum	Dec infection 10 v 30%
Rayes	2005	N = 66 Hepatic transplant	Fibers +/- 4 strains probiotics	Dec infection 3 vs 48%
Katsumpasi	2007	N=65 Vent, multiple trauma	Synbiotics	Dec infection, SIRS, Sepsis, mortality
Rayes	2007	N=67 Whipple	Synbiotics	Decrease infections
Alberda	2007	N=28 ICU	Probiotics VSL # 3	Enhance immune func
Springer-vessel	2007	N=113 Trauma	4 groups, Synbiotics	Decrease infection, perm
Chunmao	2007 (in press)	N = 45 Post op GI cancer	Syn / pre/ TPN	Dec infection 47 v 20 v 7 %

Pre and Probiotics in the ICU Setting

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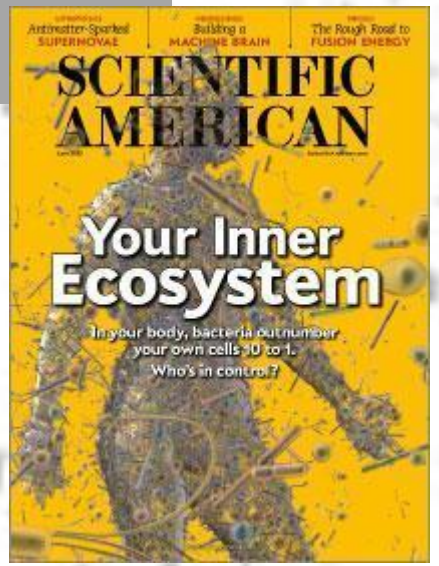
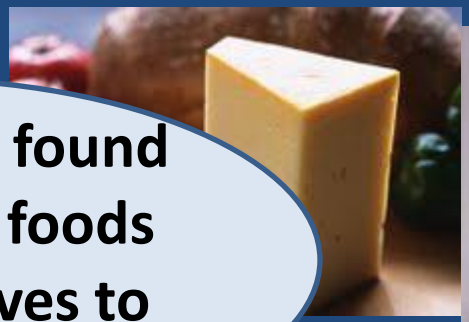
Prebiotics – Probiotics or Synbiotics

Bringing the Science to Practice



Scientific American 2012



Probiotics are found in fermented foods and as additives to many foods

Prebiotics can be found naturally in foods

Probiotics + Prebiotics = Synbiotics

Probiotics and Prebiotics

- Probiotics
  - Food sources (most often in dairy products)
  - Capsules, tablets, powder or liquid form
  - Infant formulas
- Prebiotics
  - Occur naturally in food: Honey, wheat, onions, bananas, leeks, garlic
  - added as dietary ingredients: Fructo oligosacharides (FOS), inulin, galacto oligosaccharides, sugar alcohols
  - Enteral formulas containing fiber: Jevity with fiber, Replete with fiber, Specialized formulas (DM, ICU)

Probiotic Beverages

Chilled dairy

Yakult

Danactive / Actimel

Stonyfield

BioQ

Chilled non-dairy

ProViva

Good belly

Kombucha

Bravo Friscus

Shelf stable

Cocobiotic, Dong Quai, Innergy Biotic

Better probiotic delivery systems

- **Keep it away from the liquid until ready to use!**
  - **Micro encapsulation**
  - **Packaging solutions**
    - **Bottle closures**

    - **Drinking straws**

Common Products

NCP 2012

Table 1. Common Probiotic Preparations Available in the United States

Trade Name	Active Ingredient	Form
Florastor	*Saccharomyces boulardii* 250 mg	Capsules
Florastor Kids	*S boulardii* 250 mg	Powder
Align	*Bifidobacterium infantis* 35264 (1×10^9 CFU)	Capsules
DanActive	*Lactobacillus casei* DN-114 001	Fermented milk
Activia	*Bifidobacterium lactis* DN-173 010	Yogurt
Fem-Dophilus	*Lactobacillus reuteri* RC-14, *Lactobacillus rhamnosus* GR-1	Capsules
Culturelle	*L rhamnosus GG* (1×10^{10} CFU)	Capsules
Culturelle for Kids	*L rhamnosus GG* (1×10^9 CFU)	Packets
Sustenex	*Bacillus coagulans* GBI-30, 6086 (BC30)	Capsules, chewies, and gummies
Floranex	*Lactobacillus acidophilus* (2×10^6 CFU)	Capsules
Lactinex	*L acidophilus* and *Lactobacillus helveticus* (*bulgaricus*)	Capsules and packets
Phillips Colon Health	*Lactobacillus gasseri*, *Bifidobacterium bifidum*, and *Bifidobacterium longum*	Capsules

CFU, colony-forming units.

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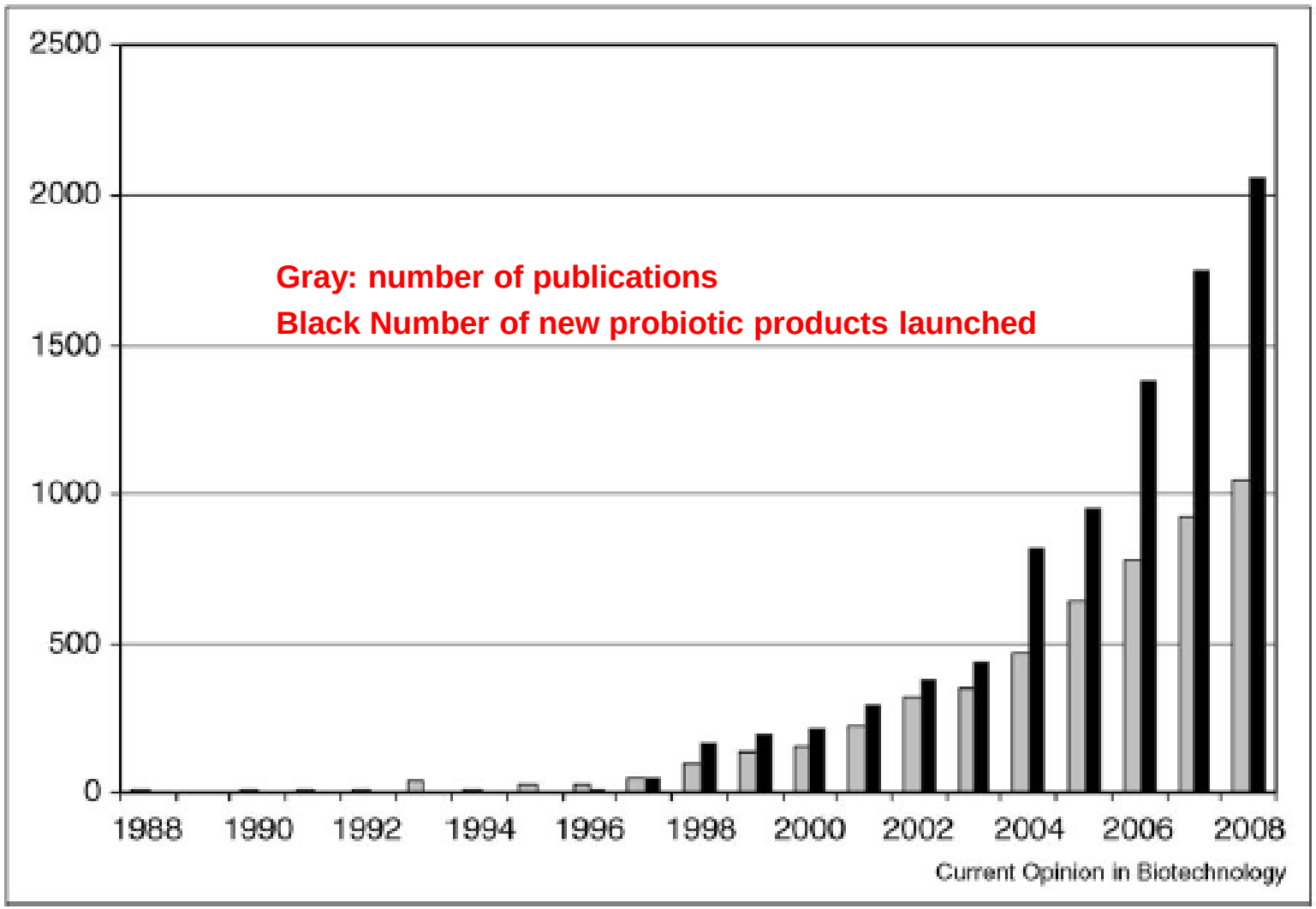
CFU, colony-forming units.

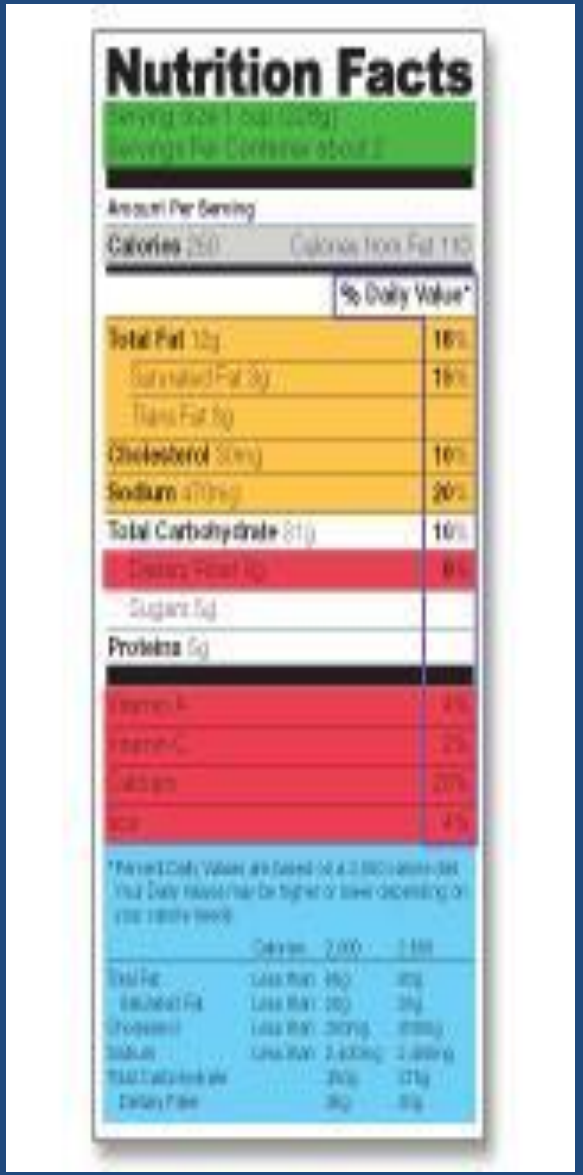
NOT a true probiotic

15.4 Billion US Market in 2008



What's in a label?

- **Marcobal et al tested 14 US commercial probiotic products:**
  - **93% incorrectly labeled**
  - **57% had contaminants**
  - **36% did not list strains on the label**
- **Masco et al tested 58 different products from EU, UK, Asia, Japan, Canada:**
  - **Only 38% had the dose stated on the label**
  - **29% did not contain strains on the label**



Marcobal 2008 J of Pediatr Gastroenterol Nutr

Masco 2005 Int J Food Microbiol

Not all *lactobacilli* survive in the GI tract

12 dairy products off the shelf in UK stores

↓

8 with the “correct” bacteria

↓

35 strains of mainly *Lactobacillus* and *Bifidobacterium* isolated

↓

Stomach (pH, enzymes)
Duodenum (enzymes)
Ileum (bile)
Colon (competition)

Gibson & Brostoff (2004) www.foodstandards.gov.uk

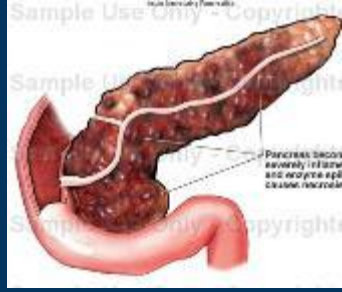
It is all about “Risk vs. Benefit”

Probiotic Safety:
Generally Recognized as Safe (GRAS) USA
Qualified Perception of Safety (QPS) EU

- Can probiotic species transfer resistance genes ?
- Lactobacillus bacteremia
  - 180 cases in 30 years
  - 69 cases of endocarditis in 30 years
    - (majority of L. rhamnosus)
  - Several cases of liver abscess in immunocompromised hosts
  - Hepatic Lactobacillus abscess in transplanted liver
- *S. Boulardii*
  - Recent data showing several outbreaks of S. Cervesiae fungemia when giving S.Boulardii
  - ***S.boulardii* not true probiotic ?**
- Host risk factors
  - Immunocompromised
    - » This is theoretical, clinical data would support use
  - Recent major dental work (theoretical anecdotal reports)
- Caution in severe pancreatitis (Lancet Feb 2008)

Sanders ME Ann NY Acad Science 2011

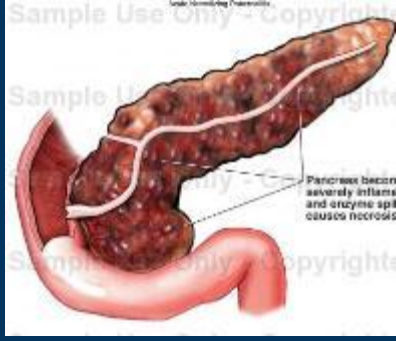
Probiotics in Pancreatitis: Randomized Prospective Multicenter Trial



- Multicenter RDBPC Trial 298 patients ITT analysis
- APACHE > 8, Imrie >3 or CRP > 150
- Assigned within 72 hours of symptoms
- Control N=145 Multispecies probiotic N=153
- 2 weeks of therapy
- Endpoints: Inf nec, BSI, pneumonia, urosepsis etc
- Results:
  - Infectious complications 30% vs 28%
  - Mortality 16% probiotic vs 6% in control
-

Besselink M, et al Lancet 2008

Probiotics in Pancreatitis ?



- Majority of deaths were from bowel necrosis
  - No bacteremia with probiotic species
  - Necrosis patchy, not just at site of probiotic delivery

- What happened ?
  - More organ failure in exp group at start (13% vs 4% in control)
  - Large number of bacteria (>10 billion)
  - Location of delivery D3 –D4
  - Bowel dysmotility “ileus”
  - Insoluble and soluble fiber in formula

  - ? Localized fermentation, acidosis, necrotic bowel, poor randomization ????

Ritchie, M et al PLoS One 2012– Met Analysis of Probiotic Efficacy of GI Disease

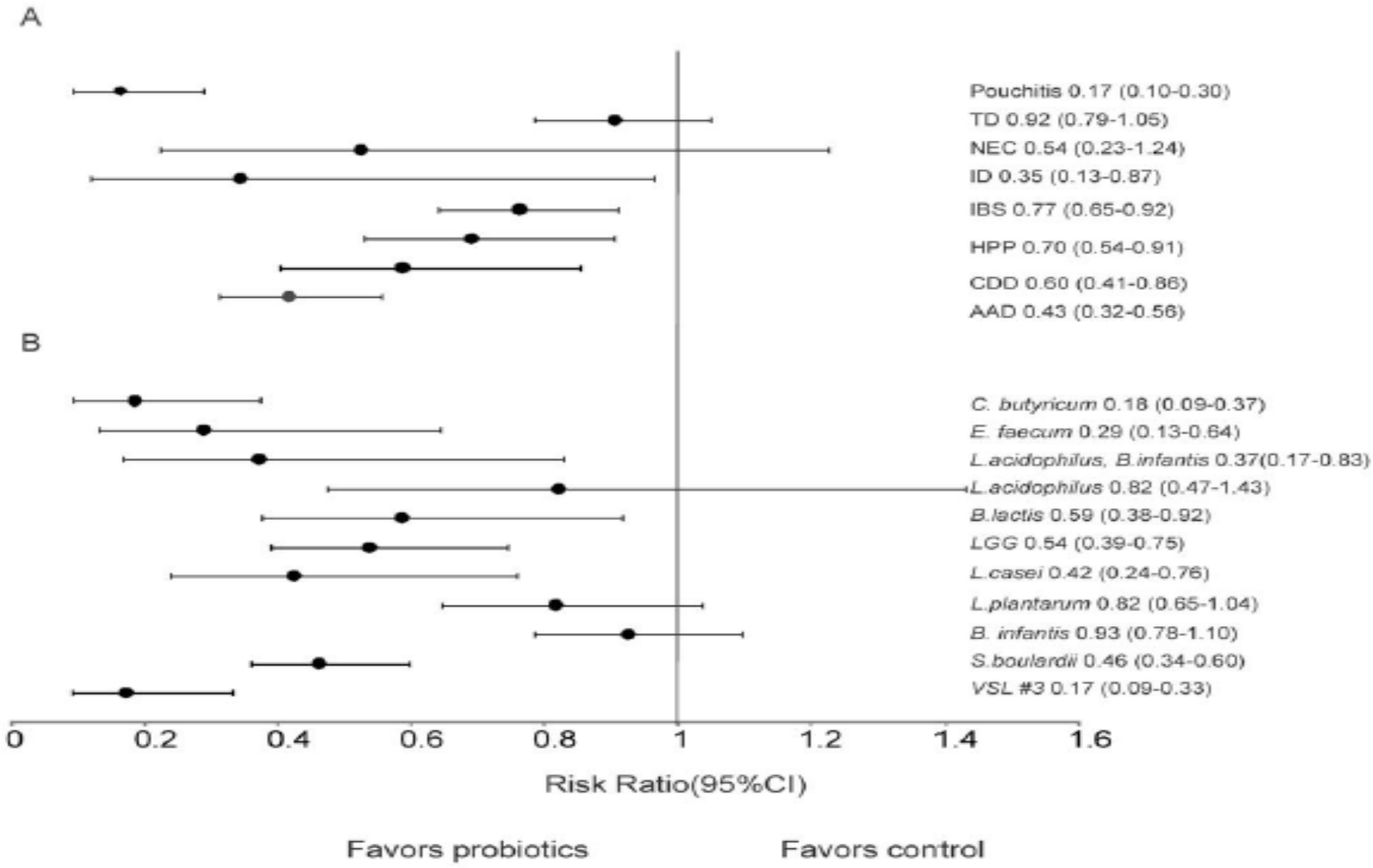


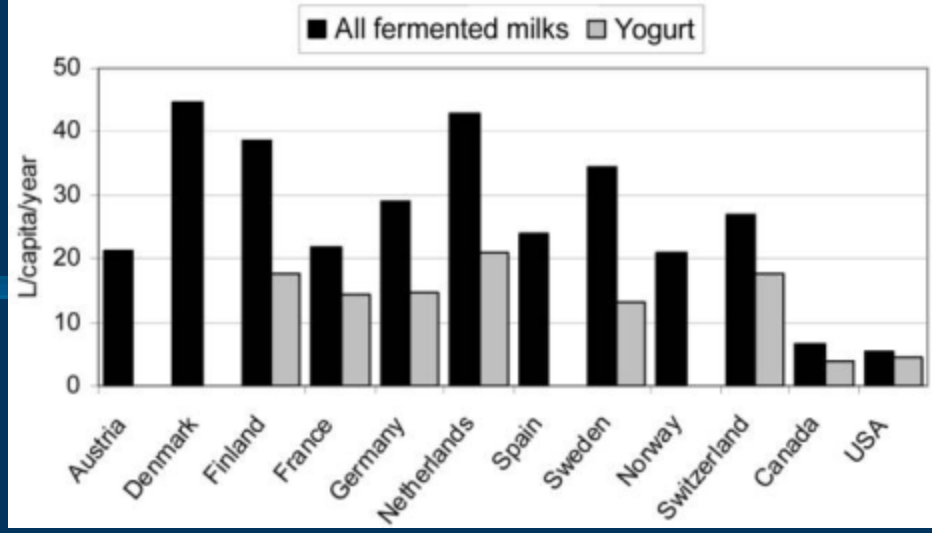
Figure 3. The effect size (risk ratio) for gastrointenstinal diseases and for probiotic species. (A) The effect size including the 95% confidence intervals for the total events of Antibiotic-associated diarrhea (AAD), *Clostridium difficile* disease (CDD), *Helicobacter pylori* positive (HPP)

Current Problems with “Probiotic”

- Extravagant claims without research
  - Still perceived as “quackery” by many
- ? of good manufacturing practice
  - Quality assurance
    - » Additional species and devoid of label common
  - Label vs content
  - Viabilty of bacterial species
    - » Strain variation, SNP changes ?
- Validate biomarkers for assessing function and activity
- Improve the reliability and ease of taxonomic classification of pre and probiotic
  - Fermentation index
  - FISH (fluorescent in situ hybridization)
  - 16S rRNA
  - Pulse-field gel electrophoresis
  - Amplified fragment-length typing
  - Multilocus sequence typing
- No specific guidelines currently
  - USA far behind EU in regulation

Sanders ME Ann NY Acad Sci 2011

Probiotics : So many questions, so few answers !!!



Saxelin MJ CID 2009

- Monostrain vs multistrain ?
- Pre, pro or synbiotic ?
- Will cell free extracts work ?
- Quantity and quality of probiotic needed for desired effect ?
  - Most studies “doses” range from 10^7 to 10^{12}
  - What dose in Peds ?
- How best to assess the activity / viability ?
- Probiotic safety ?
- Which Probiotics remain viable in GI tract ?
- When are probiotics contraindicated ?
- Resistant patterns ?
- Immunocompromised host ?

OREGON HEALTH & SCIENCE UNIVERSITY

Probiotic Protocols

OHSU Protocol for Synbiotic Use in Hospitalized Adult Patients	
Indications	**Patients at risk for developing AAD, CDI (broad spectrum antibiotics, ex: fluoroquinones)**
Contraindications	**Immunosuppressed patients (ex: BMT) (neutrophil count <500)**
Route & Dosage	
Oral	**4 ounces Nancy's Yogurt or Kefir BID 1 pack Benefiber QID**
Feeding Tube	**80 ml Nancy's Kefir + 1 pack Benefiber + 60ml sterile water TID**

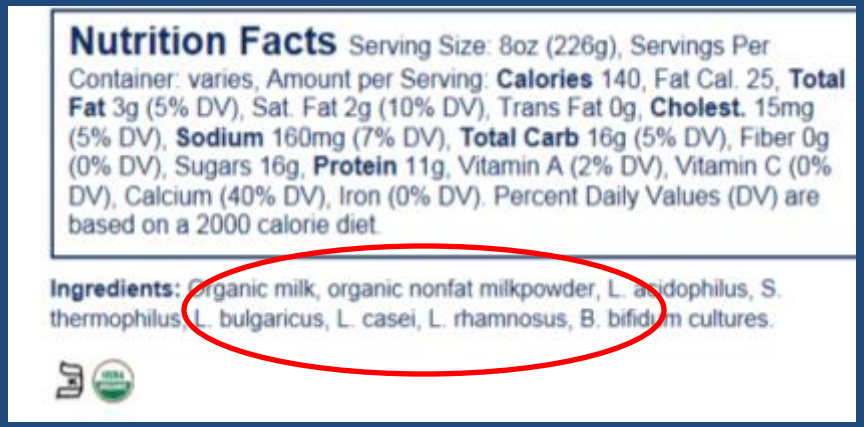
OREGON HEALTH & SCIENCE UNIVERSITY

Probiotic Protocols

OHSU VAP Prevention Protocol for Adults	
Indications	**Ventilated patients**
Contraindications	**Immunosuppressed patients (neutrophil count <500)**
Route & Dosage	
Oropharyngeal	**Swabbed with Nancy's Kefir BID (following oral care)**
Feeding Tube	**80 ml Nancy's Kefir + 1 pack Benefiber + 60ml sterile water TID**

Nancy's cultured dairy and soy

Springfield Creamery Est. 1960



Probiotics Protocols

Legacy Health Probiotic Protocol for Prevention of AAD	
Indications * Critically ill patients will be assessed by RD for appropriateness	**Patients at risk for developing AAD (broad spectrum antibiotics, ex:fluoroquinones)**
Contraindications	**Pancreatitis, Neutropenic precautions, AIDS (T-Cell count <200)**
Route & Dosage	
Oral Feeding	**8 ounces Nancy's Kefir daily**
Feeding Tube – gastric only	**80 ml Nancy's Plain Yogurt + 200ml water daily**
For patients with dairy intolerance ??	**Culturelle LGG 1 pill BID taken 1 hr before or after antibiotics**

Probiotic Protocols

Portland VAMC NFS Probiotic Protocol for Hospitalized Patients	
Indications	**Patients at risk for developing AAD, CDI (broad spectrum antibiotics, ex:fluoroquinones)**
Contraindications	**Neutropenic precautions**
Route & Dosage	
Oral Feeding	**100 ml container DanActive® BID**
Feeding Tube	**100 ml DanActive® + 60 ml water BID**

Calcium 10%

Not a significant source of Dietary Fiber, Vitamin A, Vitamin C and Iron.

*Percent Daily Values are based on a 2,000 calorie diet.

INGREDIENTS: CULTURED GRADE A LOW FAT MILK, WATER, SUGAR, WHEY PROTEIN CONCENTRATE, BLUEBERRY PUREE, CONTAINS LESS THAN 1% OF DEXTROSE, NATURAL FLAVOR, MODIFIED CORN STARCH, PECTIN, BLACK CARROT JUICE CONCENTRATE (FOR COLOR), CALCIUM CITRATE, MICROCRYSTALLINE CELLULOSE, MALIC ACID, SODIUM CITRATE.

CONTAINS THE ACTIVE CULTURES ***L.BULGARICUS, S.THERMOPHILUS*** AND ***L.CASEI IMMUNITAS®*** (*Lactobacillus casei* DN-114 001)

Product Comparison

Product	Type of Bacteria	Estimate CFU/g	Recommended dosage	Estimate of Cost
Nancy's Yogurt	*L. acidophilus* *L. casei,* *B. bifidum,* *L. rhamnosus*,	~9.6 billion per cup	8 ounces per day	$0.90
Nancy's Kefir	*L. acidophilus* *L. casei,* *B. bifidum,* *L. rhamnosus*, Prebiotic - inulin	~74 billion per cup	8 ounces per day	$1.00
Culturelle LGG	*L. GG*	~10 billion/ capsule	2 capsules per day	$1.70
Danactive	*L. casei*	~100 million /g	2, 100 ml bottles per day	$0.66

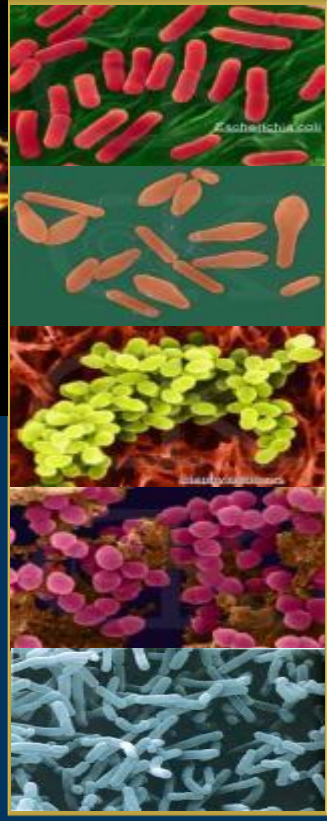
General Guidelines for Use of Probiotics

- Critically evaluate and use only when data supports
  - Base choice on molecular typing, metabolic characteristics and interaction in the environment
  - Caution with meta-analysis, heterogeneity is key
- Do not extrapolate from one strain to another
- Identify optimal strain, insoluble fiber and commercially available product
  - ~Probiotic: $10^{9\text{-}11}$ viable cells per day ?
  - ~Prebiotic: 20-30 gm/day ?
- Continued intake of probiotic be required to maintain benefits
- Prebiotic are an excellent option to modify flora on long term basis
  - Persistent levels require continuous intake !

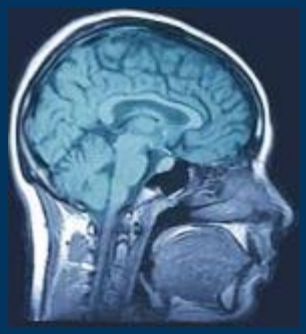
Concepts the clinical team need to understand regarding probiotics !

- ***NO*** **single probiotic meets the need in all patients**
  - **Effects are often strain specific**
- **Consider the disease process: prevention vs treatment**
- **Decision should depend upon:**
  - **Metabolic insult or expected insult**
  - **Timing of delivery; pre, post, or both**
  - **Severity of condition**
  - **Expected duration of need**
  - **Tolerance**
  - **Function of GI tract remaining**
  - **Strain by strain assessment**
- **Base decision on scientific evaluation of the data**

Future Trends:
Probiotics in Clinical Medicine



- Understanding of inflammation relationship
- More data on specific strains of probiotics
- Better acceptance by “public and scientific community”
- New attention to gut / microbe mutualism
- Probiotics as drug delivery tools genetically engineered
  - “Designer probiotics”

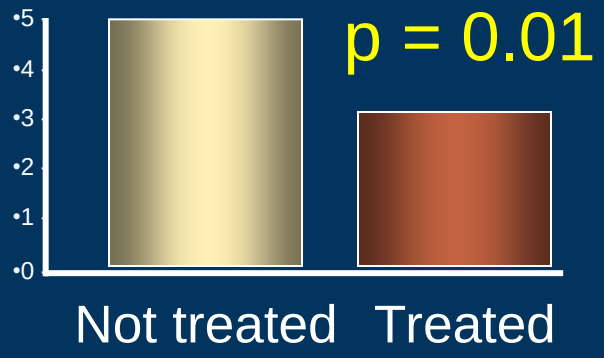


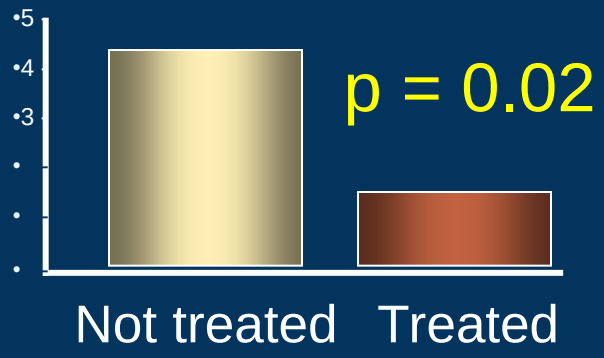
Probiotics as drug delivery tools !

DSS

IL-10 knockout

Histologic Score

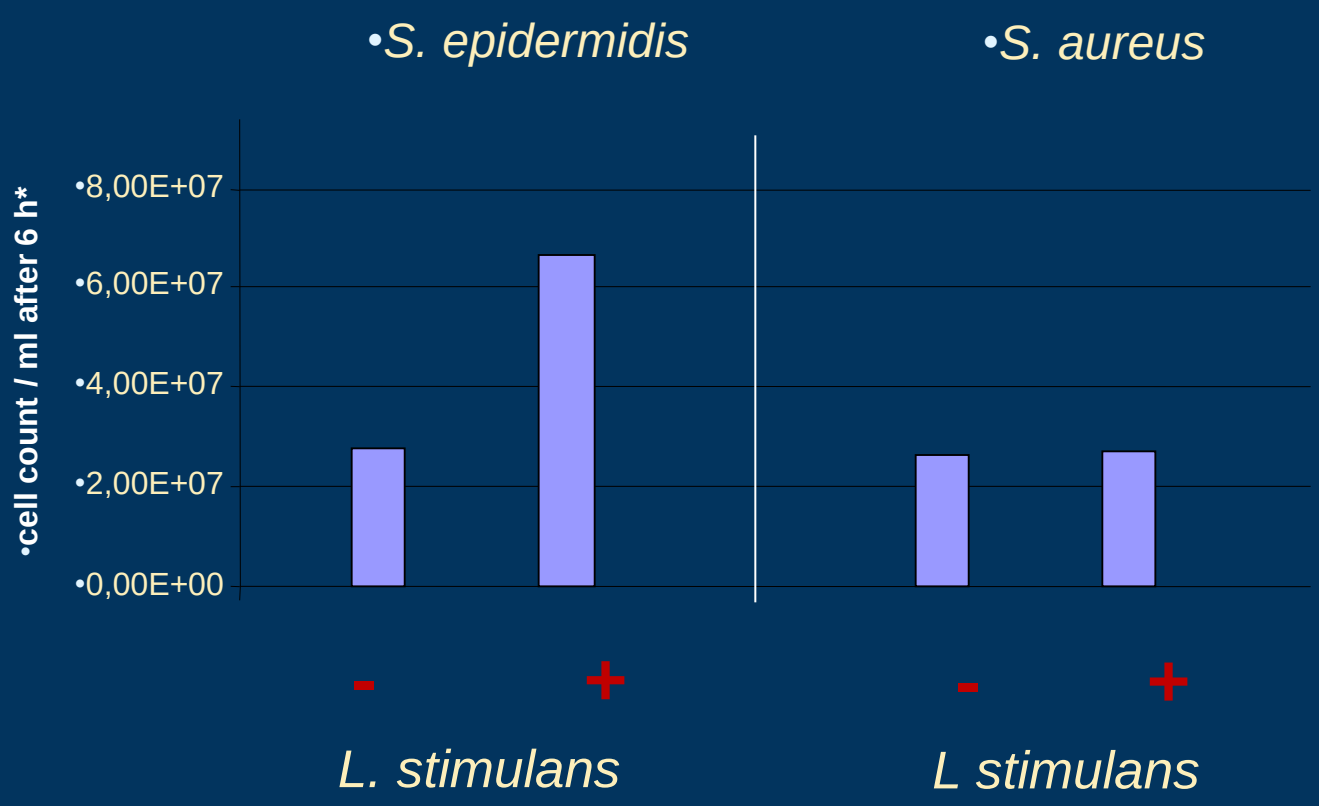




Science 2000; 289:1352-5 (mice)
Clin Gastroenterol Hepatol 2006;4:754-759 (humans)

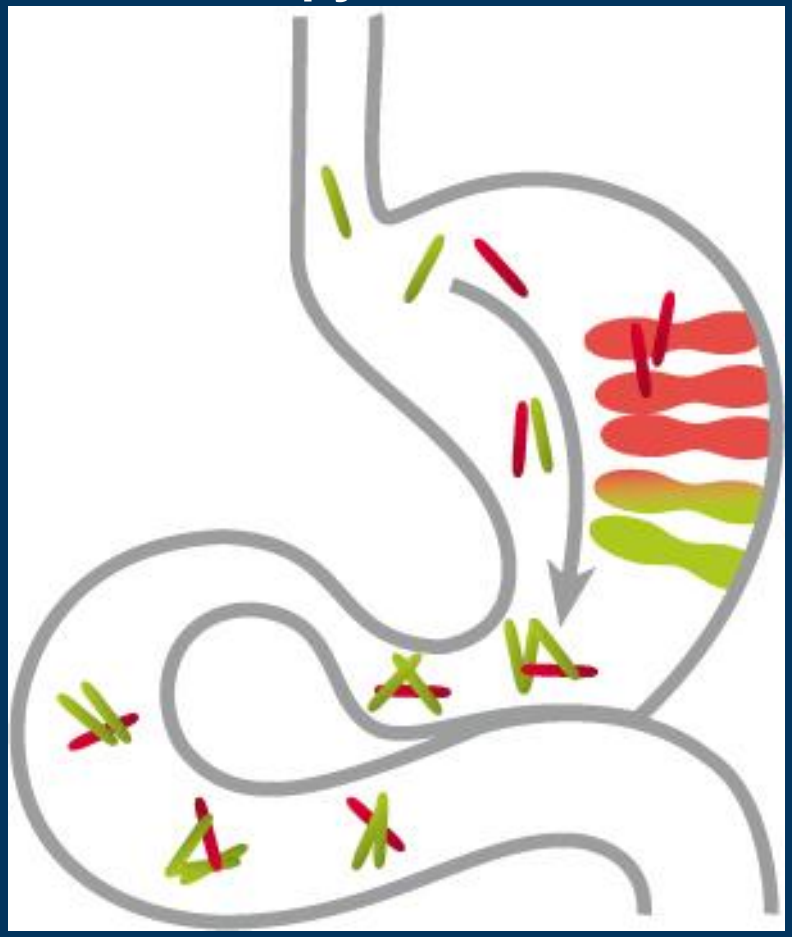
L. stimulans Probiotic – balancing skin microflora

Stimulation of *S. epidermidis*, but not *S. aureus* is seen after 6 h of co-culture



L. anti-pylori
Probiotic - the gentle alternative

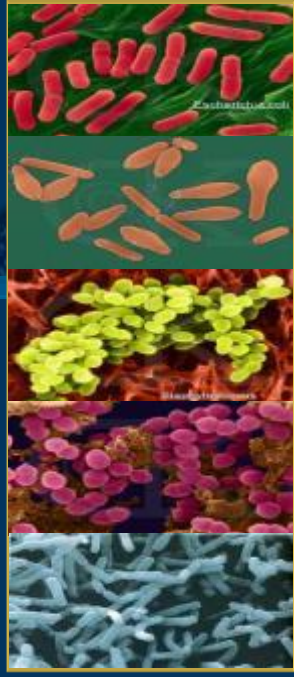
LB anti-pylori is a natural isolate which acts as a “probiotic“



- ***H. pylori*** **loses mobility by co-aggregation with**
- ***L. anti-pylori***
- ***H. pylori*** **no longer adheres to the mucosa**
- **Aggregated *H. pylori* are carried out of the stomach**

Lang et al 2010

Ongoing Trials : Probiotics



- Neurologic disorders
  - Pain control, ADHD, Tourette syndrome
- Inflammatory diseases
  - Aging, IBD, arthritis, asthma, diabetes
- Use on non-GI surfaces
  - Burns, tracheostomy sites, skin in ICU, wounds, STSG, Vagina, respiratory tree
- AIDS prevention
  - Changing the pH of the vagina alters HIV receptors
  - Gene transfer HIV receptor into probiotics
    - » Already done for L. jensenii
- Cancer prevention
  - Multiple mechanisms
    - » Dietary procarcinogens by commensal bacteria
    - » Histone deacetylase inhibitor
-

Is it time for a paradigm shift regarding bacteria ?

Are we making a leap of faith ?

Supply viable beneficial bacteria or a substrate which enhances these specific beneficial bacteria instead of trying to eliminate the pathogen ?

“Bioecological control”