Probiotics Terms:

- **Probiotic** – Probiotics are live microorganisms (bacteria or yeasts) which, when administered in adequate amounts, confer a health benefit on the host
- **Prebiotic** - nutritional supplement taken to increase the amounts of beneficial bacterial in the gut or vagina. Example "FOS" (fructose oligosaccharides)
- **Biotherapeutic agent** - microorganism used for specific therapeutic activity in humans
- **Nutriceutical** - food products with beneficial effects in preventing or treating diseases

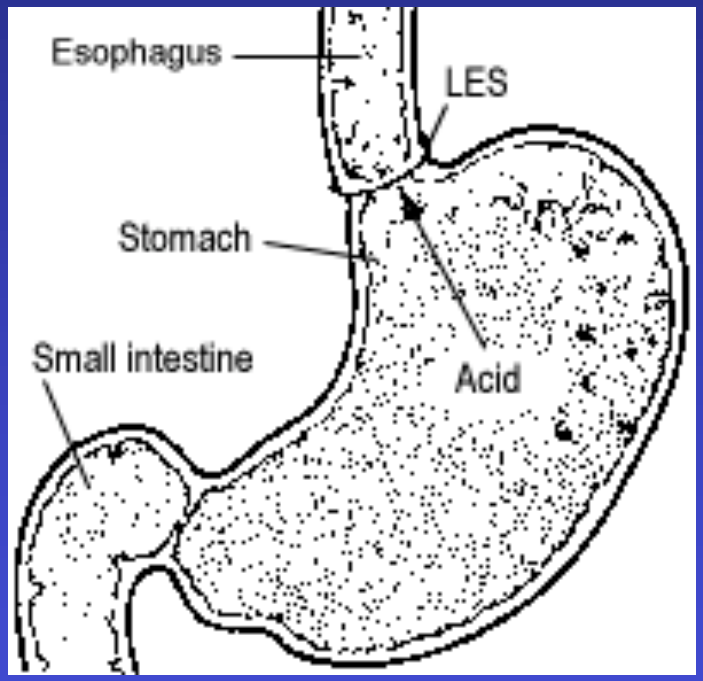
Probiotics- basic definition

- Joint Food and Agriculture Organization/World Health Organization Working Group's definition of probiotics: “Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host”

Historical Prospective- then to now

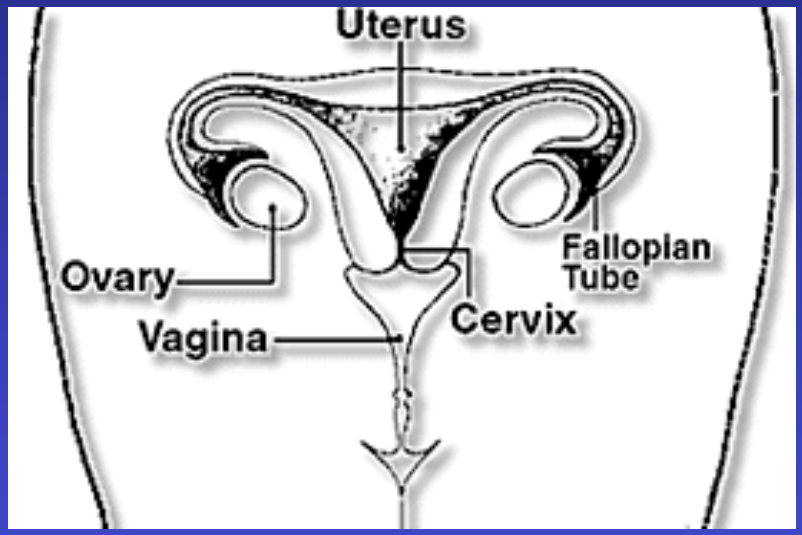
- Yogurts and other fermented dairy products
- General probiotics- Lactobacillus caseii, L. acidophilus, etc
- Specific probiotics selected
- Probiotic yogurts- Activia, Colon health, etc
- Opimization of probiotic therapy

Predominant Flora: Stomach



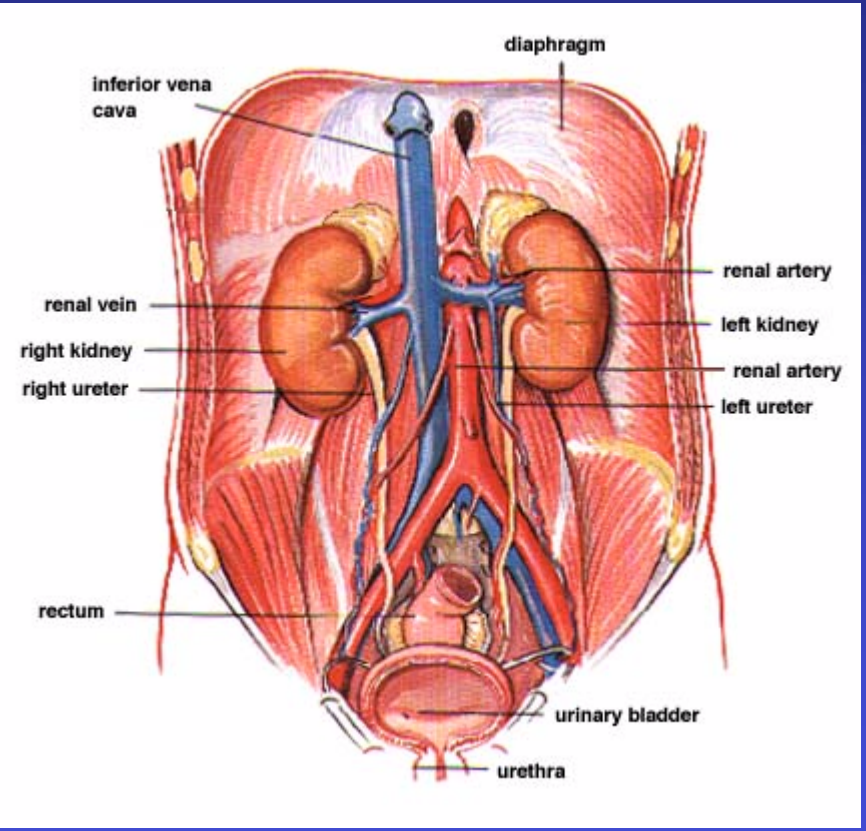
Stomach ($0\text{-}10^3$ cfu/ml):
Gram+ aerobes,
Lactobacillus &
Streptococcus

Predominant Flora: Vagina



Vagina: diverse aerobes & anaerobes including Lactobacillus jensenii, Lactobacillus acidophilus, Lactobacillus casei.

Predominant Flora: Urinary Tract

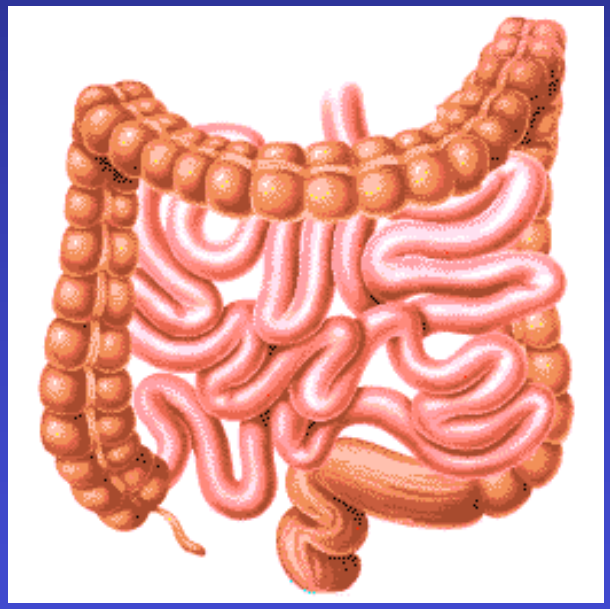


Kidneys: sterile

Bladder: sterile

Urethra: 10^1-10^2 E. coli

Predominant Flora: Intestines



Small intestine:
Proximal ileum (10^3-10^4 cfu/ml)
aerobic Gram+
Distal ileum (10^{11}-10^{12} cfu/ml)
Gram- anaerobes

Colon (10^{11}-10^{12} cfu/ml):
Bacteroides, Eubacteria,
Peptostreptococci, E. coli,
Bifidobacterium, Fusobacteria

Functions of Normal Flora

- Digestion
- Production of vitamins
- Mucosal maturation
- Stimulate Immune System
- Attachment
- Intestinal transit
- Colonization resistance

The US Market for Probiotics (source: SRI Consulting, Menlo Park, CA)

$764M (2005); ↑ 10%/yr

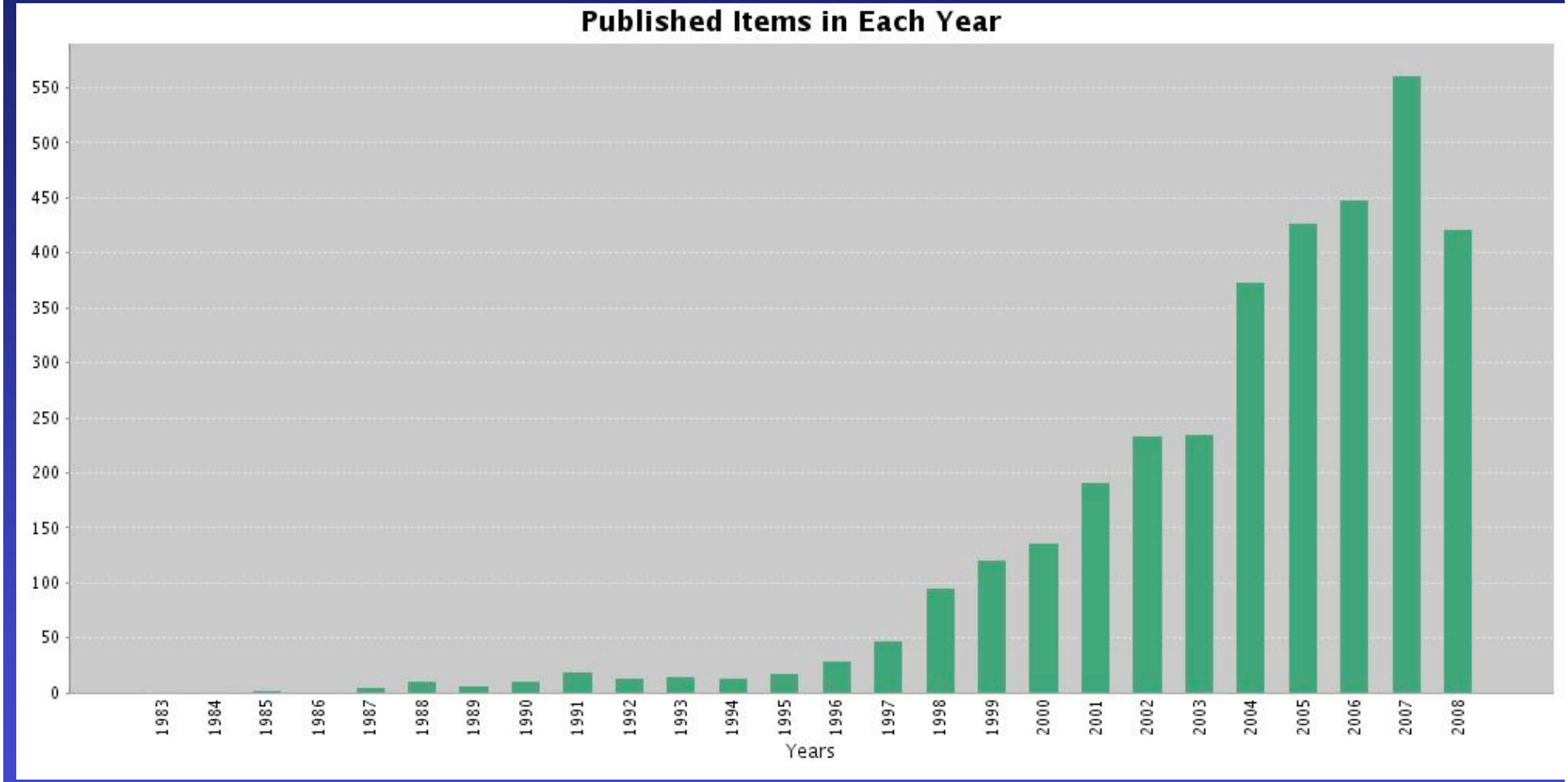
Herbal products $4790 (2007); ↑ 4%/yr

About 2M consumers use probiotics (2006)

- Probiotic foods vs probiotic therapeutics

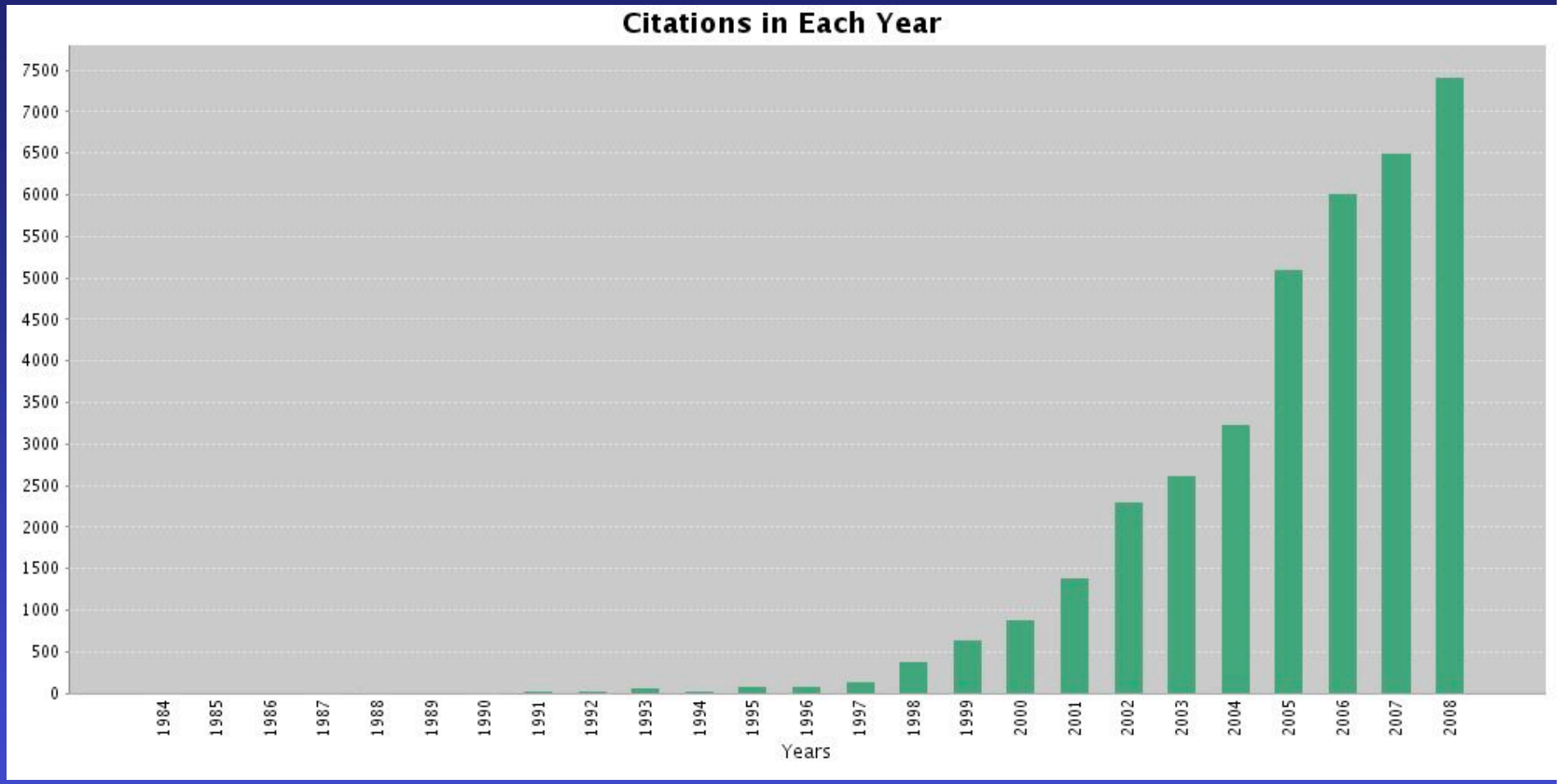
Myths about Probiotics

- Not well studied
- Are narrow spectrum agents for diarrhea only
- Not well regulated (partly true)
- Cultures are all therapeutically similar
- Optimum therapeutic dose is about 1 billion CFU

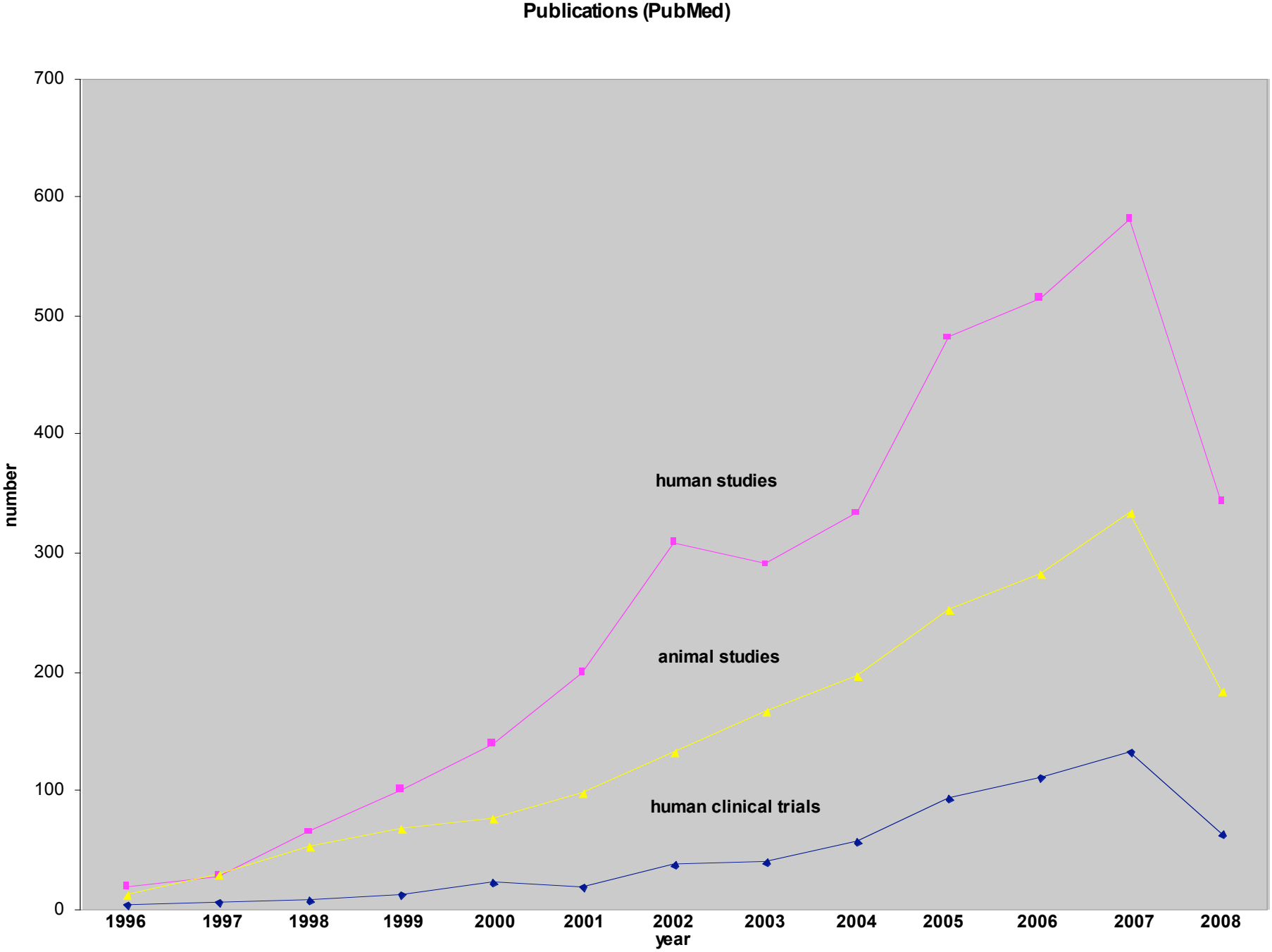


ISI Web of Science

Probiotic (s) in title. Publications by year



ISI Web of Science
Probiotic (s) in title being cited by year


Publications (PubMed)
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600
500
400
300
200
100
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animal studies
human clinical trials
1996
1997
1998
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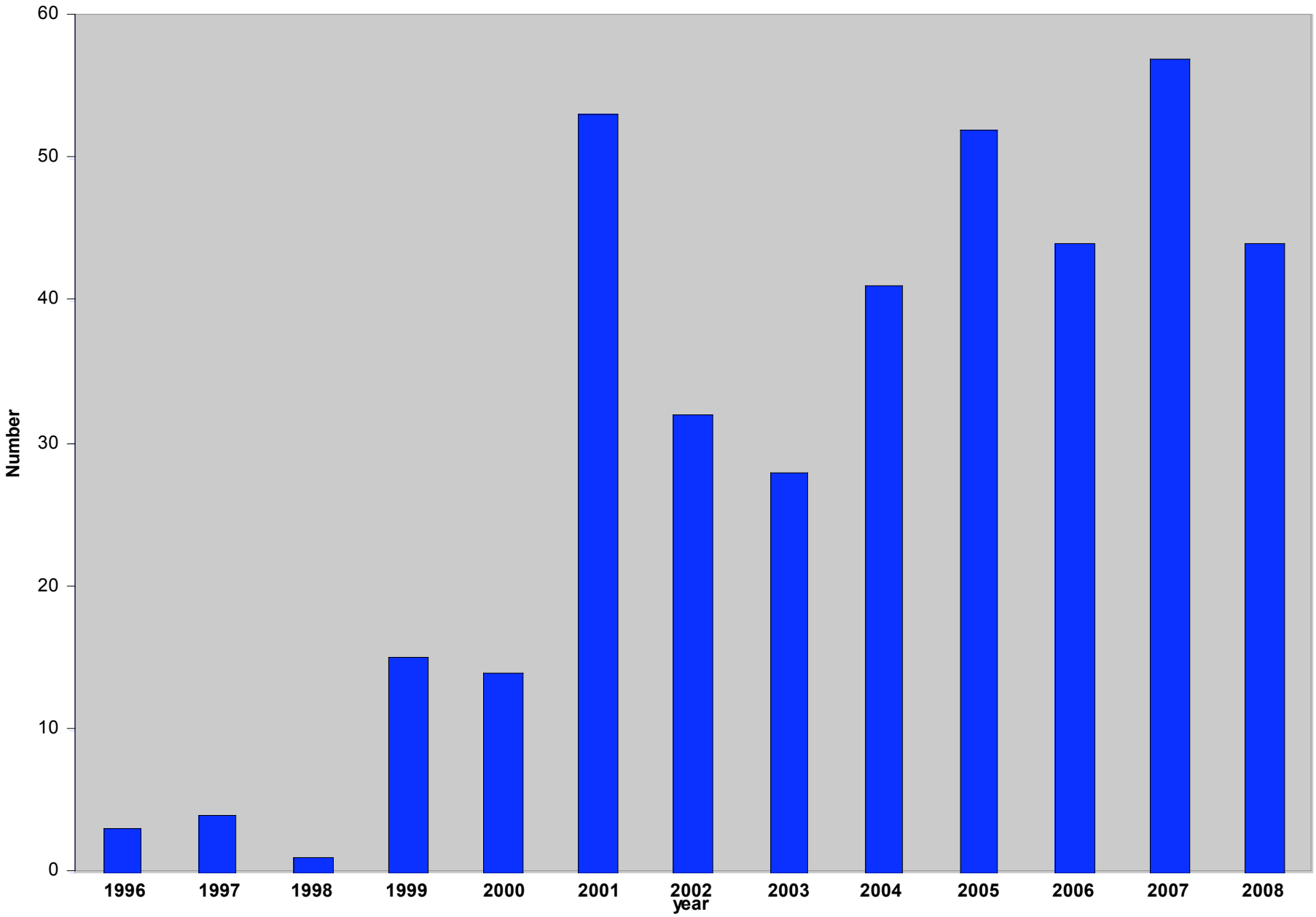
2000
2001
2002
2003
2004
2005
2006
2007
2008
year


Publications in PubMed Core Clinical Journals
Number
60
50
40
30
20
10
0
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
year

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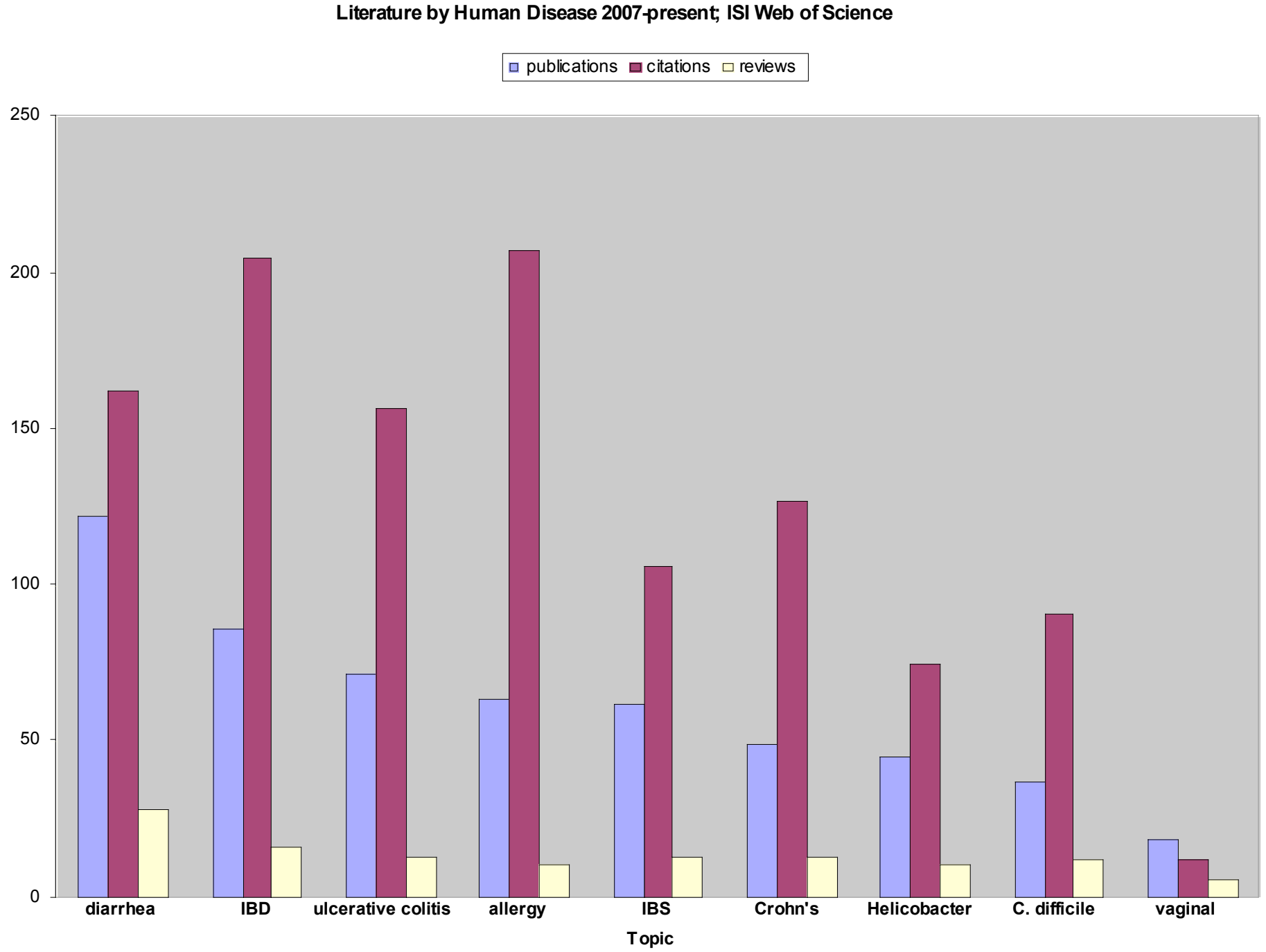
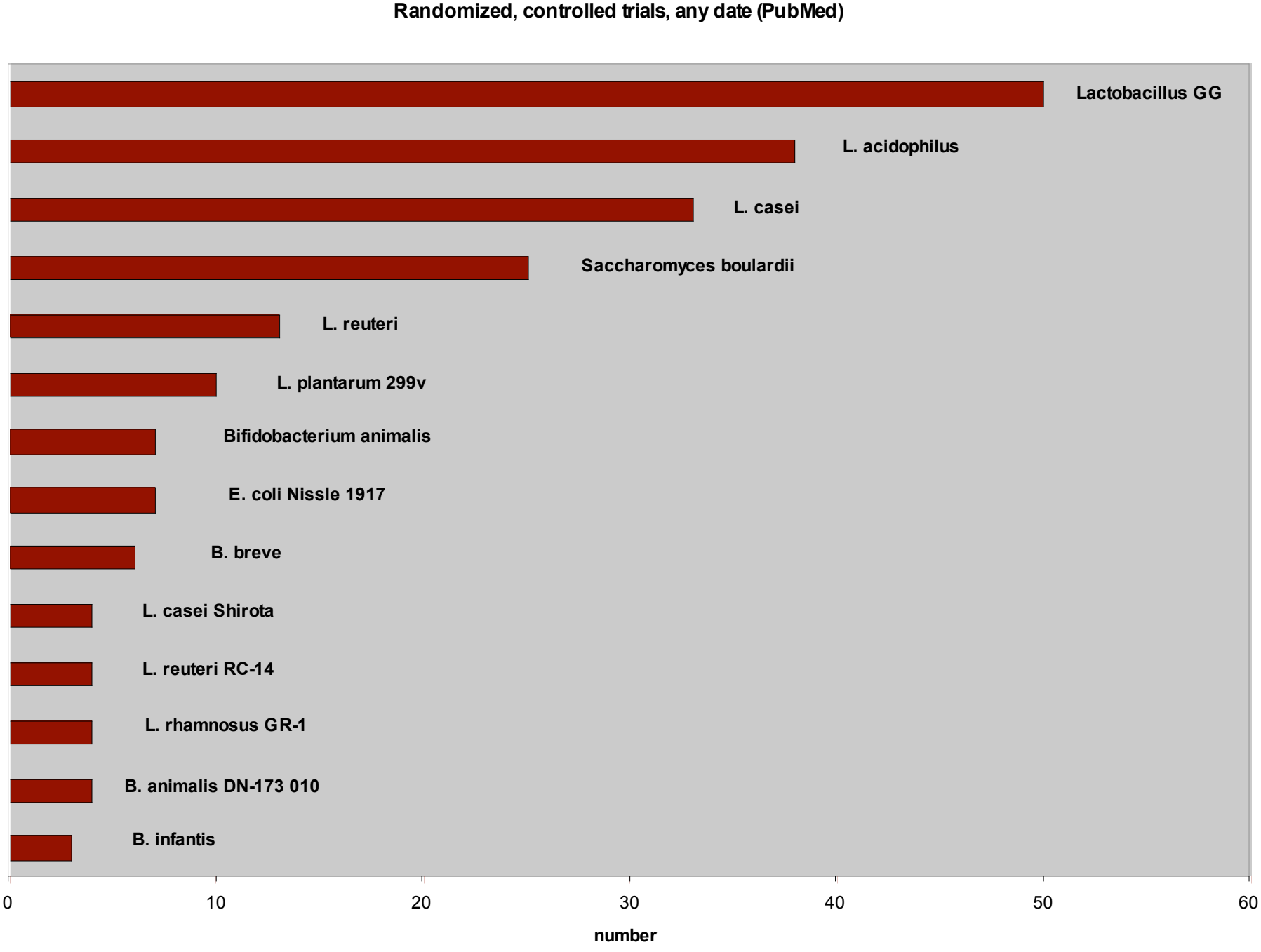

Literature by Human Disease 2007-present; ISI Web of Science
publications
citations
reviews
250
200
150
100
50
0
diarrhea
IBD
ulcerative colitis
allergy
IBS
Crohn's
Helicobacter
C. difficile
vaginal
Topic


Randomized, controlled trials, any date (PubMed)
Lactobacillus GG
L. acidophilus
L. casei
Saccharomyces boulardii
L. reuteri
L. plantarum 299v
Bifidobacterium animalis
E. coli Nissle 1917
B. breve
L. casei Shirota
L. reuteri RC-14
L. rhamnosus GR-1
B. animalis DN-173 010
B. infantis
0
10
20
30
40
50
60
number

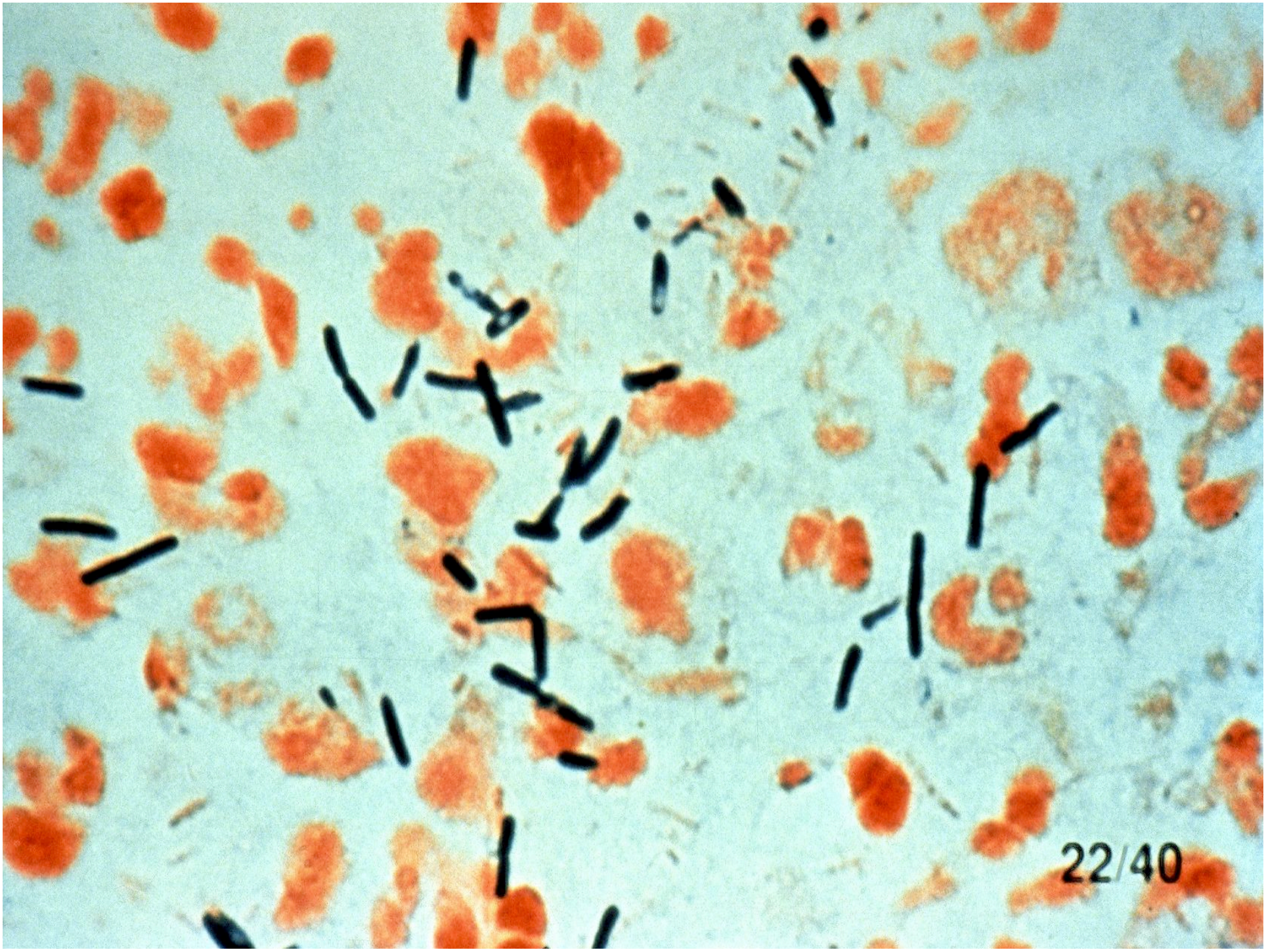
Meta-Analyses of Probiotic Treatments (2005 to present)

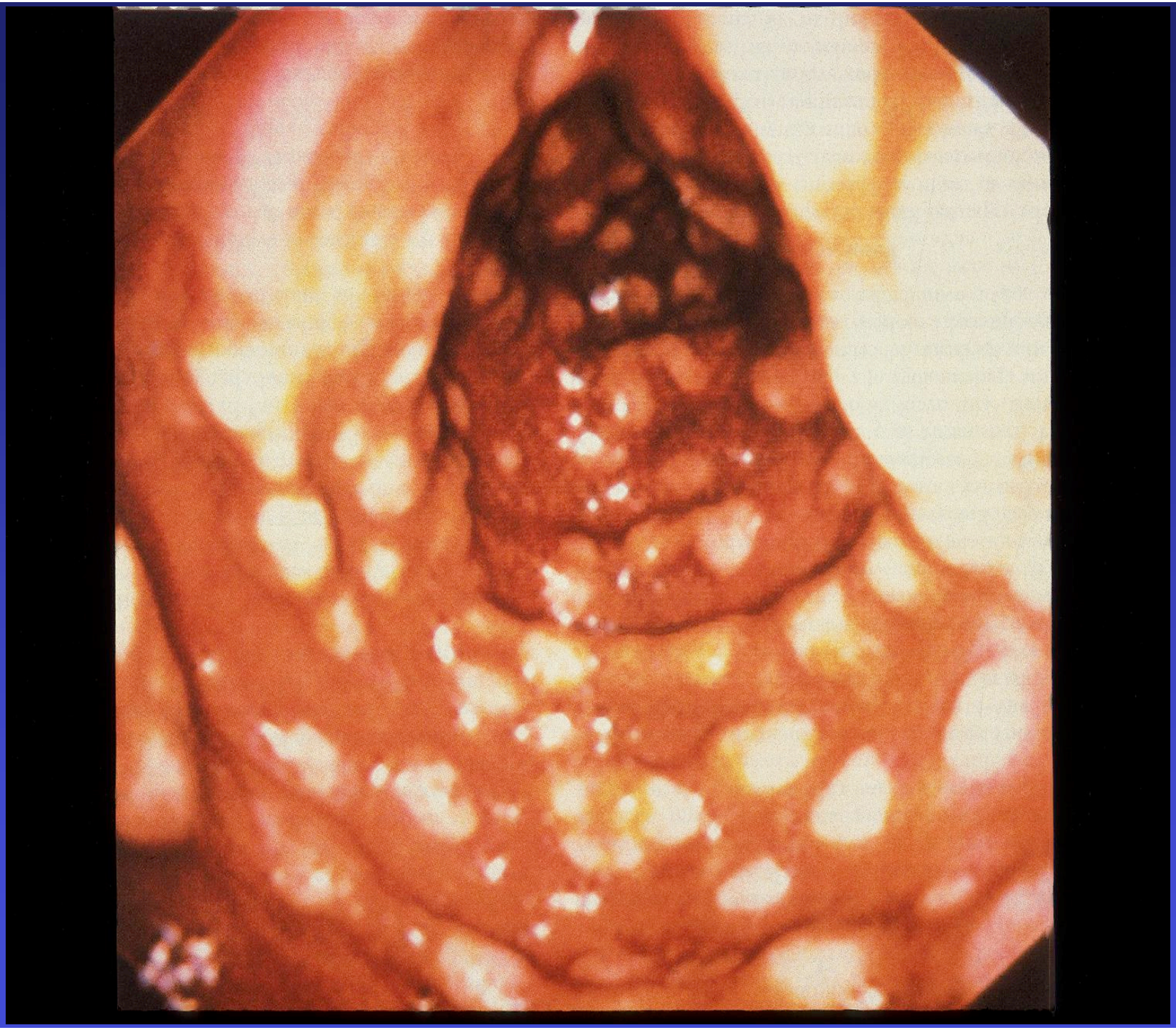
Disease	year	n	result	ref
IBS	2008	20	0.77 (improvement)	1
Nec. enterocolitis	2008	9	0.32 (prevention)	2
Ped. atopic dermatitis	2008	6	0.69 (prevention	3
Pediatric a llergy and food sens.	2008	5	Promise but data lacking	4
Pouchitis	2008	5	0.04	5
Traveler's diarrhea	2007	5	Promise but data lacking	6
Traveler's diarrhea	2007	12	0.85	7
Preterm labor	2007	2	Data lacking [1]	8
H. pylori eradication (+antibiotics)	2007	14	1.84	9
H. pylori eradication adverse effects	2007	14	0.44	9
Antibiotic diarrhea (pediatric)	2006	6	0.43	10
Antibiotic diarrhea (pediatric)	2006	6	0.44	11
Antibiotic diarrhea	2006	25	0.43	12
C. difficile disease	2006	6	0.59	12
Crohn's disease	2006	7	Data lacking [2]	13
Acute diarrhea	2006	28	0.65	14

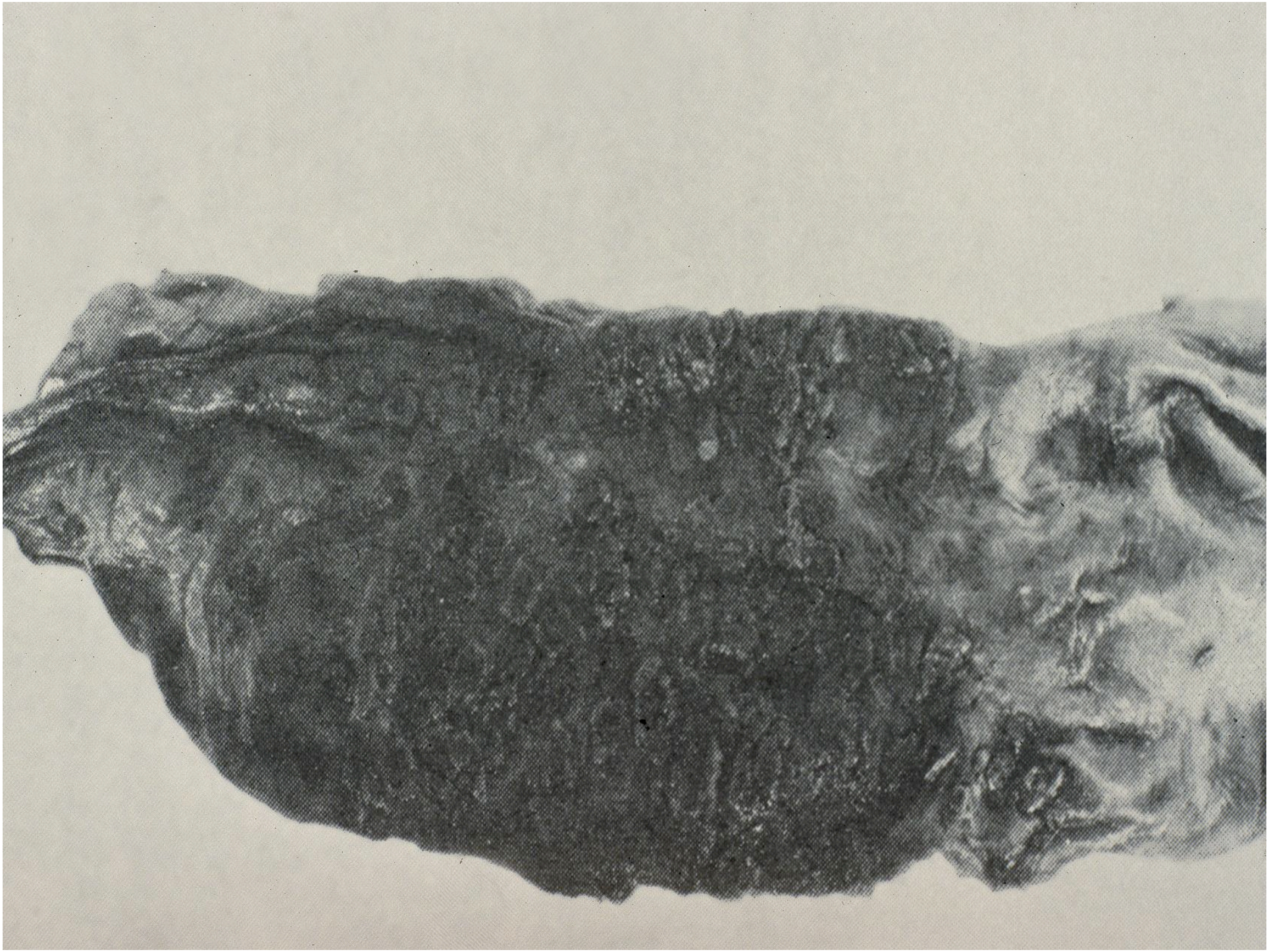
1. Vaginal infections decreased, however (GWE)
2. Results promising for E. coli Nissle and Saccharomyces boulardii, however (GWE)

References for previous slide showing Meta-Analyses of Probiotic Treatments (2005 to present)

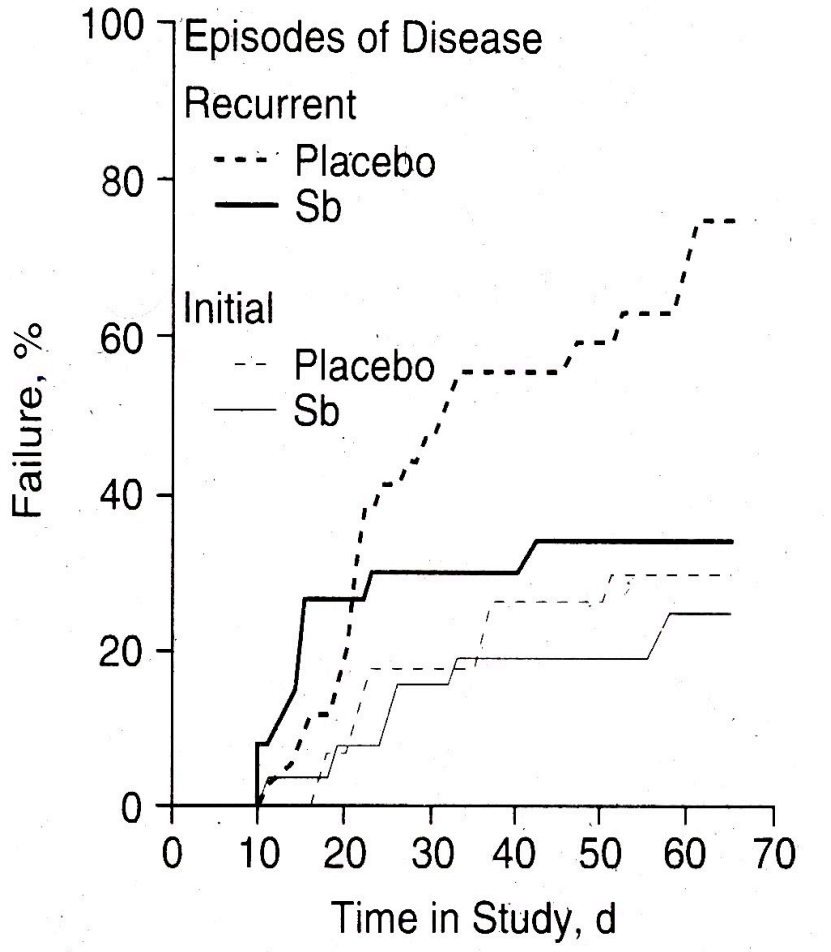
1. McFarland LV, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. World J Gastroenterol. 2008 May 7; 14(17):2650-61.
2. Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2008 Jan 23;(1):CD005496.
3. Lee J, Seto D, Bielory L. Meta-analysis of clinical trials of probiotics for prevention and treatment of pediatric atopic dermatitis. J Allergy Clin Immunol. 2008 Jan;121(1):116-121.
4. Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database Syst Rev. 2007 Oct 17;(4):CD006475.
5. Elahi B, Nikfar S, Derakhshani S, Vafaie M, Abdollahi M. On the benefit of probiotics in the management of pouchitis in patients underwent ileal pouch anal anastomosis: a meta-analysis of controlled clinical trials. Dig Dis Sci. 2008 May; 53(5):1278-84.
6. Takahashi O, Noguchi Y, Omata F, Tokuda Y, Fukui T. Probiotics in the prevention of traveler's diarrhea: meta-analysis. J Clin Gastroenterol. 2007 Mar; 41(3):336-7.
7. McFarland LV. Meta-analysis of probiotics for the prevention of traveler's diarrhea. Travel Med Infect Dis. 2007 Mar; 5(2):97-105.
8. Othman M, Neilson JP, Alfirevic Z. Probiotics for preventing preterm labour. Cochrane Database Syst Rev. 2007 Jan 24;(1):CD005941.
9. Tong JL, Ran ZH, Shen J, Zhang CX, Xiao SD. Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during Helicobacter pylori eradication therapy. Aliment Pharmacol Ther. 2007 Jan 15;25(2):155-68.
10. Johnston BC, Supina AL, Vohra S. Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized placebo-controlled trials. CMAJ. 2006 Aug 15;175(4):377-83.
11. Szajewska H, Ruszczy_ski M, Radzikowski A. Probiotics in the prevention of antibiotic-associated diarrhea in children: a meta-analysis of randomized controlled trials. J Pediatr. 2006 Sep; 149(3):367-372.
12. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am J Gastroenterol. 2006 Apr;101(4):812-22.
13. Rolfe VE, Fortun PJ, Hawkey CJ, Bath-Hextall F. Probiotics for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev. 2006 Oct 18;(4):CD004826.
14. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. Lancet Infect Dis. 2006 Jun; 6(6):374-82.


22/40



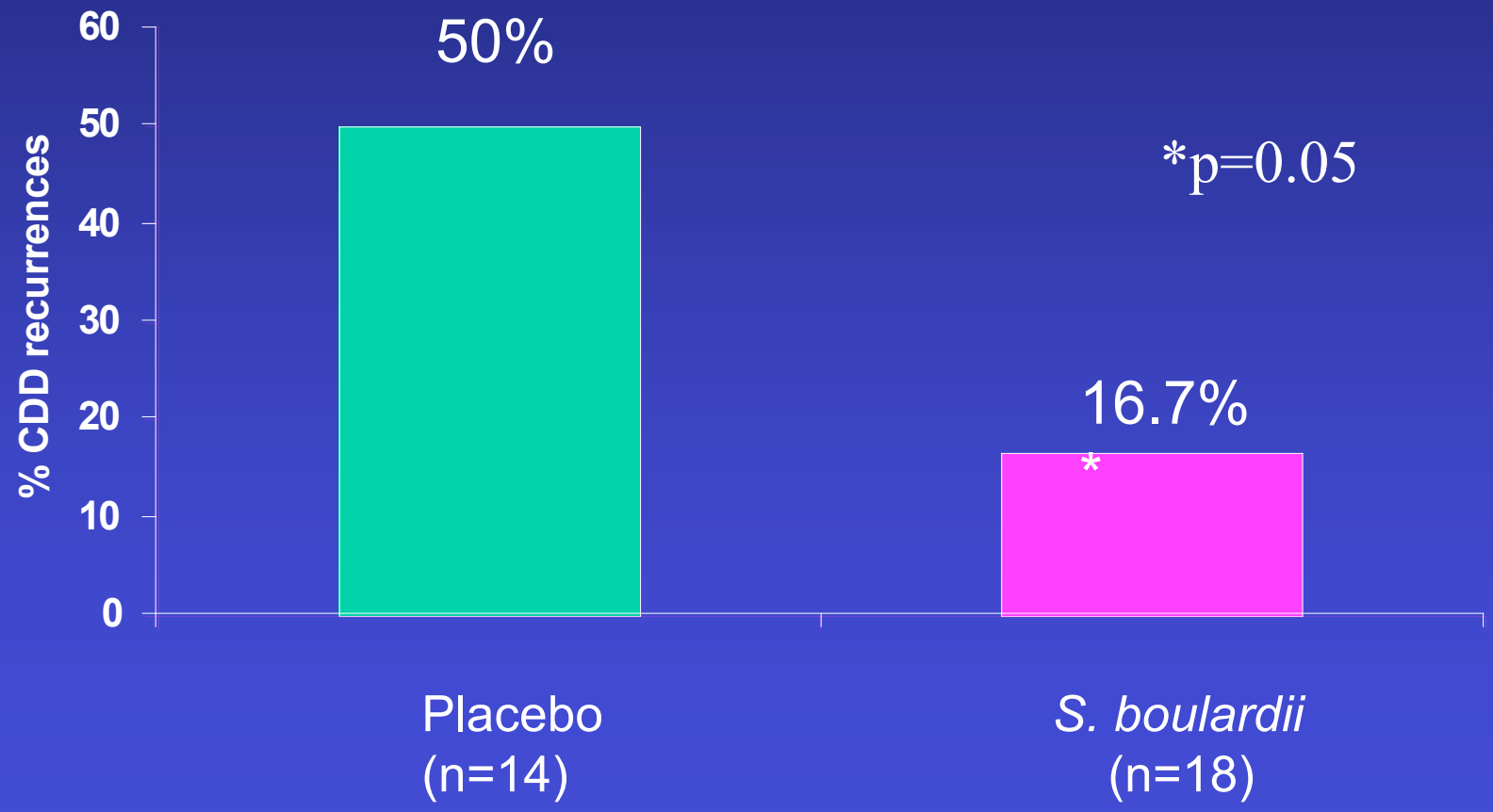


McFarland et al., JAMA; **271**, 1913-1918, (1994).



Kaplan-Meier failure curve for the probability of *Clostridium difficile* disease recurrence. Sb indicates *Saccharomyces boulardii.*

S. boulardii & High Dose Vancomycin for Recurrent *C. difficile* Disease



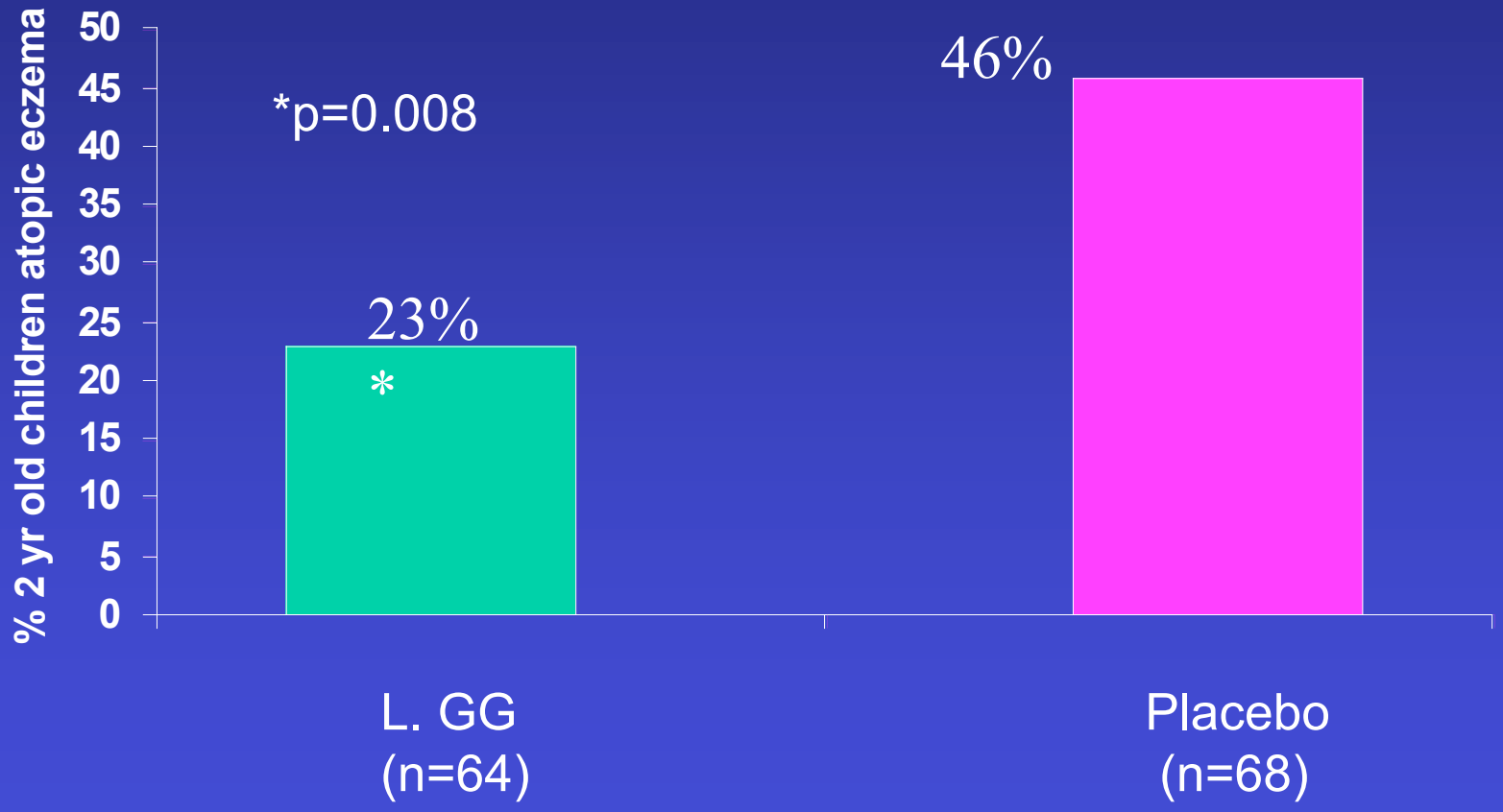
Surawicz CM. Clin Infect Dis 2000;31:1012-7.

Lactobacillus GG to Prevent Infantile Atopic Disease

- DBPC in Finland
- Family history atopic disease (eczema, allergic rhinitis, asthma)
- Mothers randomized:
  - *Lactobacillus* GG (1 x 10^{10} CFU/d)
  - Placebo
- Mothers treated 2-4 weeks before delivery
  Infants treated for 6 months
- Followed for 2 years

Kalliomaki M. Lancet 2001;357:1076-9

Lactobacillus GG and Infantile Atopic Disease [Results]



Kalliomaki M. Lancet 2001;357:1076-9

Myths about Probiotics

- Not well studied
- Are narrow spectrum agents for diarrhea only
- Not well regulated (partly true)
- Cultures are all therapeutically similar
- Optimum therapeutic dose is about 1 billion CFU

Regulation of Probiotics in USA

- Foods- no health claims
- Dietary supplements-no therapeutic claims
  - Structure/function claims only
- GMP- As of Aug 2008 GMPs in effect for larger probiotic companies
- AER- adverse event reporting program mandatory as of 2007
- FTC regulates advertising

Better regulation needed

- Better enforcement of meeting stated potency
- Protection for innovator companies
- Reasonable evidence accepted so OTC status can be obtained
- Enforcement of GMPs
- Better oversight of labeling and advertising

Consumerlab.com findings (5/29/08)

- 5/20 products tested did not meet labeled claim of potency or at least 1 billion CFU per recommended dose
- No findings of contamination with unwanted bacteria or molds

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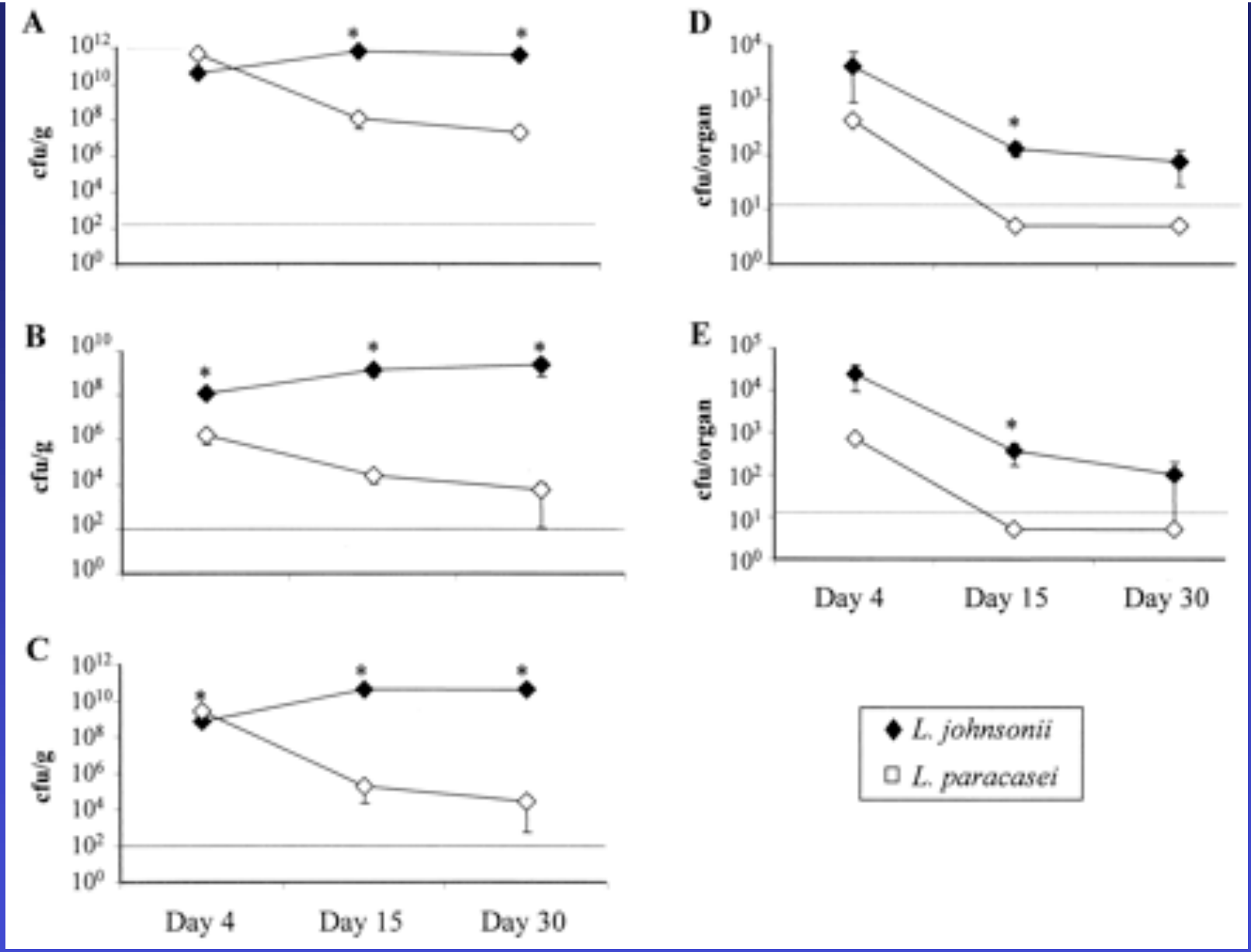
Table 6.1. Controlled clinical trials evaluating probiotics and Crohn's disease (from Elmer et al. The Power of Probiotics, Haworth Press 2007)

Probiotic	N	Result	Ref
L. rhamnosus GG	45	10.5% placebo 16.6% LGG, ns	Prantera [7]
L. rhamnosus GG	11	2/4 relapse placebo 3/5 relapse LGG, ns	Schultz [8]
Saccharomyces boulardii	17	4.6 stools/day placebo 3.3 stools/day in Sb*	Plein [9]
Saccharomyces boulardii	32	6/16 relapse in mesalamine 1/16 relapse in mesalamine/Sb	Guslandi [10]
E. coli Nissle 1917	28	7/12 relapse in prednisone 4/12 relapse in prednisone/Ec	Malchow [11]

* probiotic significantly better than control, $p<0.05$; ns=probiotic not significantly different than control



Germfree C3H/n mice received a single gavage with 109 CFU of *L. johnsonii* or *L. paracasei* at weaning. Bacterial loads were counted in fecal pellets extracted from the rectum (A), the luminal contents of the small intestine (B), and the colon (C). Bacteria were also counted in Peyer's patches (D) and mesenteric lymph nodes (E). Ibnou-Zekri et al. Infec Immun 2003;71:428-436

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Elmer GW and Corthier G. Can J Microbiol 1991;37:315-317.

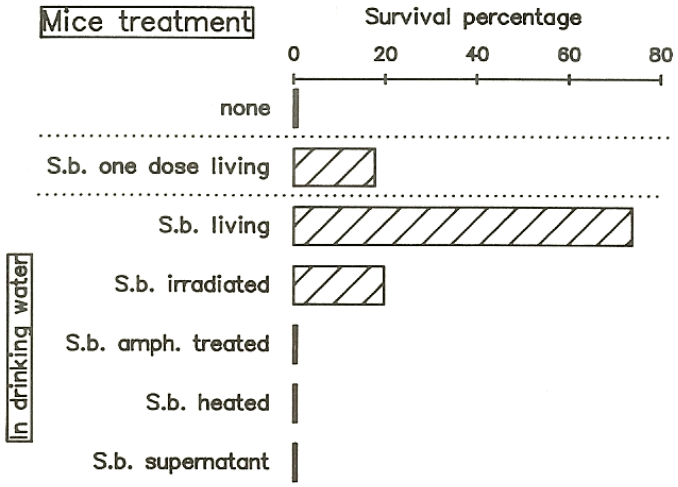


FIG. 1. Effect of viability of *S. boulardii* (S.b.) on the ability to protect *C. difficile* infected gnotobiotic mice. Amph., amphotericin B.

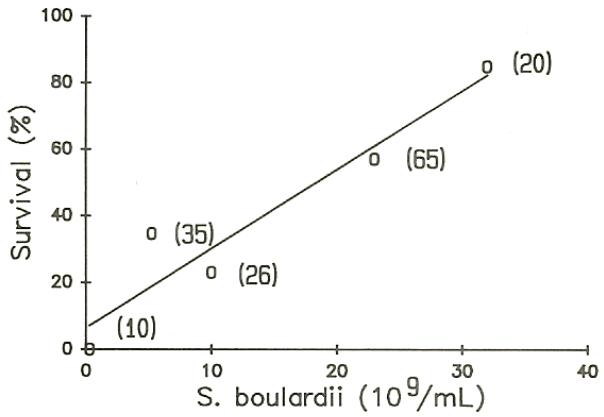
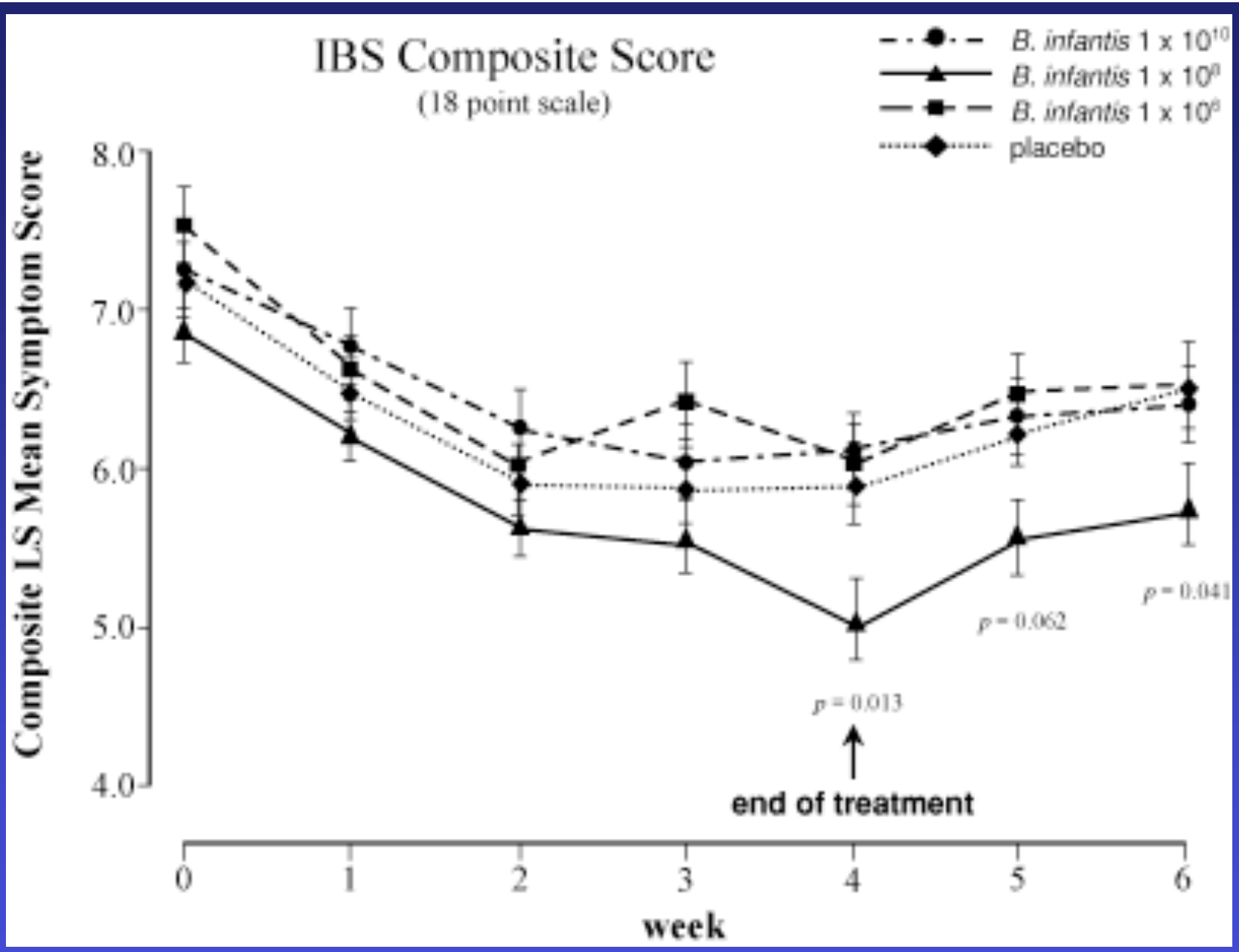


FIG. 2. Dose–response relationship of viable *S. boulardii* treatment on survival of *C. difficile* infected gnotobiotic mice. The numbers in parentheses represent the number of animals tested.

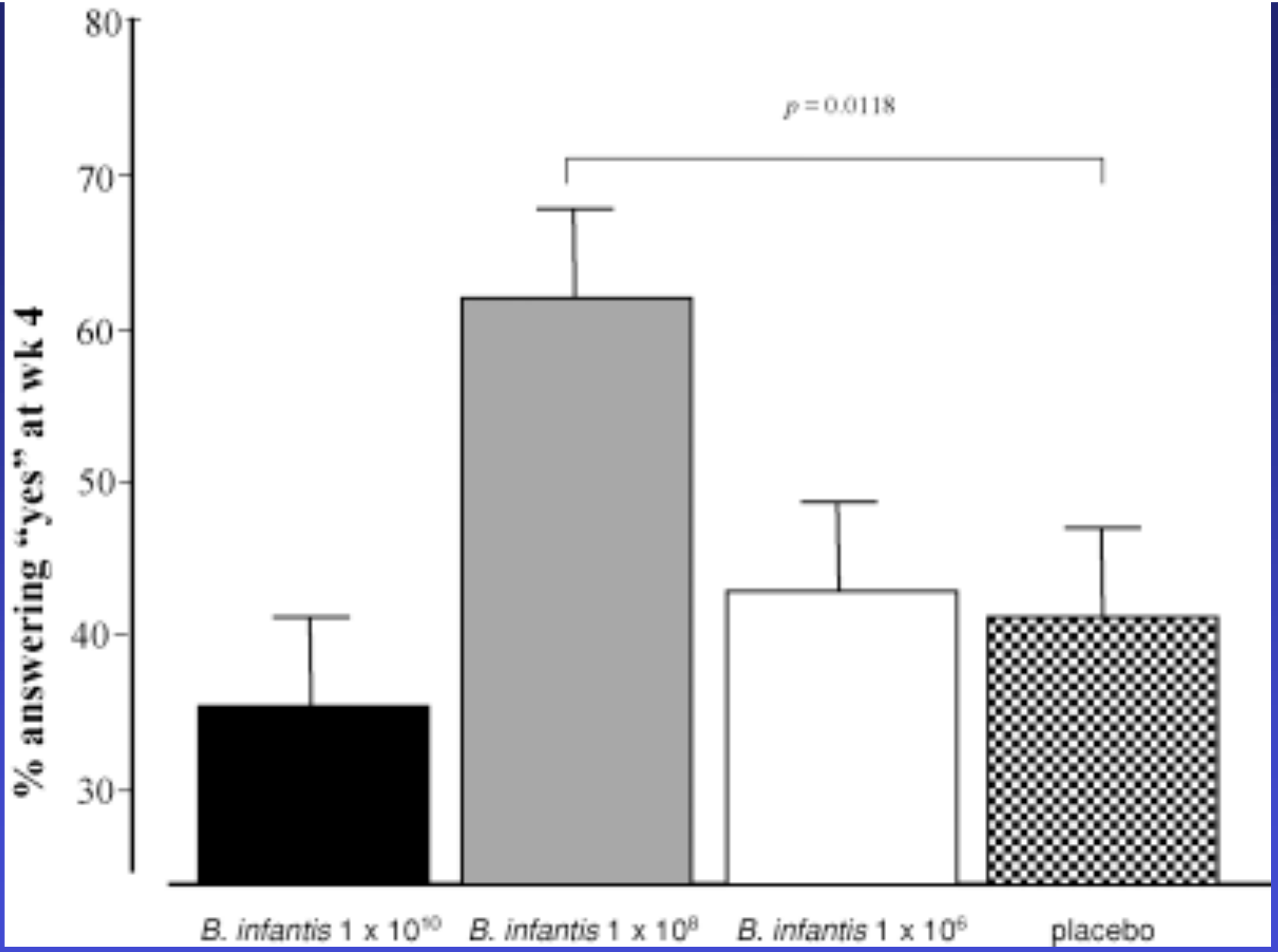
suspension was replaced by a freshly prepared one each day. Supernatants of the yeast fermentation (before and after the fermentation process) were provided by Laboratoires Biocodex, diluted 1:2, and were provided as a source of drinking water as described above. The manufacturer uses a mineral salts, malt extract, inositol medium to produce the yeast. Irradiated *S. boulardii* was prepared by exposing a suspension to 40 kilorads (1 rad = 10 mGy) of gamma rays for 2 h. No viable *S. boulardii* remained after this irradiation procedure. Amphotericin B treated *S. boulardii* was prepared by suspending 100 mL of washed packed cells of the yeast with an equal volume of a 5 mg/mL solution of amphotericin B (Fungizone, Laboratoires Squibb, Paris) followed by incubation for 72 h at ambient temperature. The treated yeast was then washed 4 times with 0.9% NaCl and resuspended in 100 mL of 0.9% NaCl. The viable counts before treatment were 2×10^{10}/mL

boulardii became established in these mice as evidenced by the continued excretion of about 3×10^6/g viable yeast in the feces. However, this was considerably lower than the 5×10^8/g steady-state levels obtained from mice subjected to continuous feeding of *S. boulardii*.

Prior amphotericin B treatment of *S. boulardii* eliminated the protective ability of the yeast. All animals died in this group despite the presence of 2×10^6 *Saccharomyces boulardii* in the cecum at the time of inoculation of *C. difficile*. Also without efficacy was yeast killed by autoclaving. To examine whether some extracellular protective metabolite could be produced by the yeast *in vitro*, supernates from the fermentor used to produce the yeast (before and after the fermentation) were tested and were found to be nonprotective. It appears that either soluble protective products are not responsible for the activity of the yeast or that the yeast may produce some extracellular protective products *in vivo* but not *in vitro*. While no activity was observed with these supernate prepara-



Whorwell et al. Am J Gastroenterol 2006;101:1581-1590. n=362



Whorwell et al. Am J Gastroenterol 2006;101:1581-1590. n=362 Q=relief?

Needed Directions

- Focus on therapeutic uses other than diarrhea
- Mechanisms of action determined
- Better appreciation of strain selection
- Recombinant strains to optimize
- Dose response data needed
- Effect of disease on dose needed
- Effect of diet
- Dose timing optimization needed
- Optimize drug delivery
- Research funding by governments and nonprofits

Recovery of probiotics in healthy humans

Probiotic	dose	% recovery	reference
L. casei Shirota	10^{11}	~0.1	Fujimoto 2008
B. animalis	10^{10}	22	Rochet 2008
S. boulardii	10^{10} single dose	0.12±0.04 (2.77±1.9 with ampicillin)	Klein 1993
S. boulardii	10^{10} steady state	.20±0.08 (.43±0.16 with ampicillin)	Klein 1993
S. cerevisiae	$3x10^{8}$	~0.03	Pequet 1991
L. rhamnosus GG	$4x10^{10}$	<0.01	Golden 1992

Saccharomyces boulardii recoveries

Population	Dose (g/d)	Duration (d)	Log CFU /g Feces at steady state	Ref
Rats				
	0.8 g/kg	14	7.83 ± 0.45	Blehaut 1989
	0.96/kg	8	7.32 ± 0.28	Boddy 1991
Humans (healthy)				
	1	14.5	7.15 ± 0.98	Blehaut 1989
	0.2	7	7.56 ± 0.16	Klein 1993
	1	7	8.12 ± 0.93	
	3	7	8.93 ± 1.87	
Humans (with CDD)				Elmer 1999
asymptomatic	1	28	6.04 ± 1.72	
symtomatic	1	28	4.43 ± 2.79	

1g dose =~10^{10} CFU

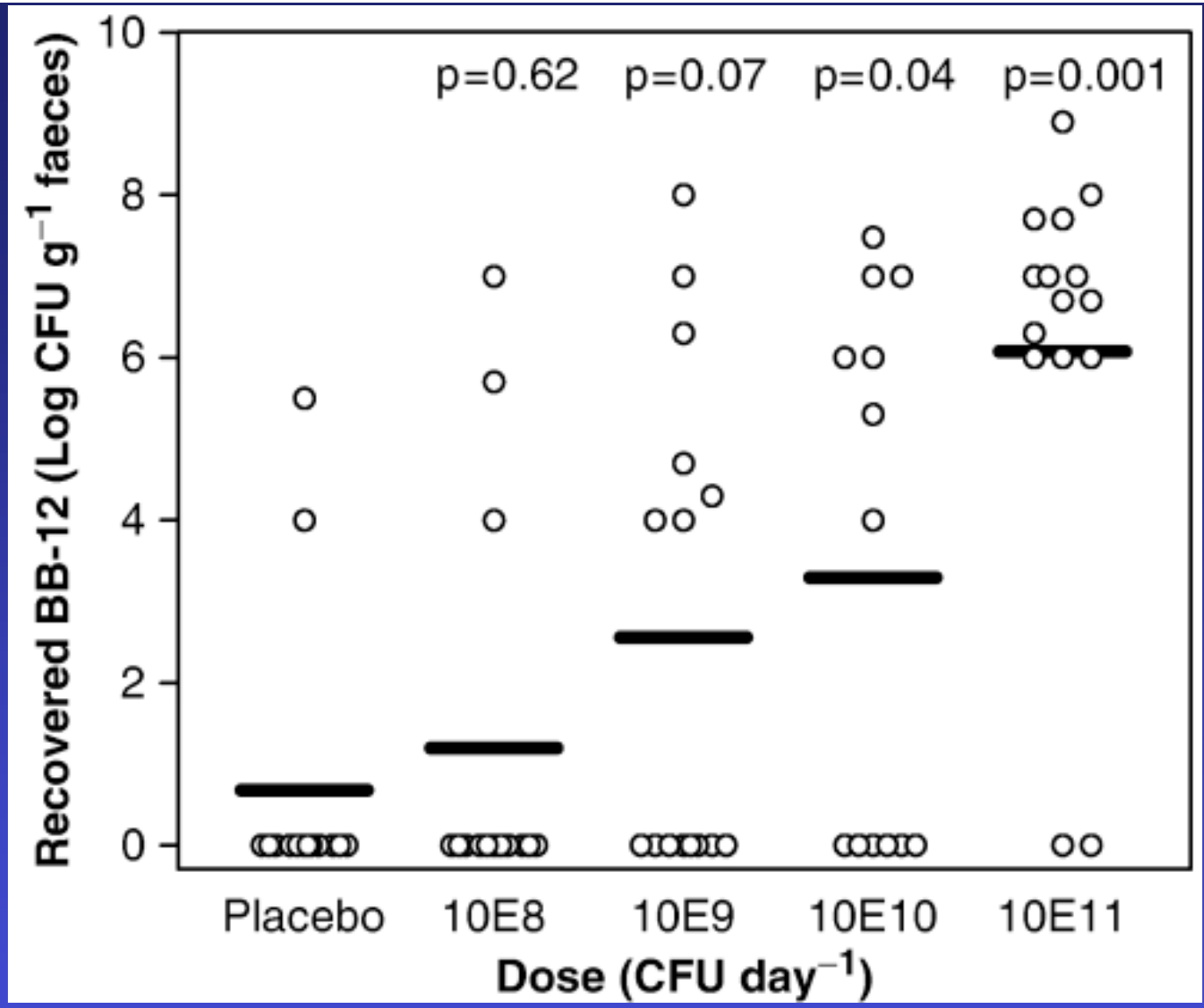
Adapted from Elmer et al. Aliment Pharmacol Ther 1999;13:1663-1668

The effect of ampicillin on the mean CFU/10^6 of S. boulardii at steady state in healthy volunteers

Subject	-amp	+amp
1	105±1.0	57.3±30.4
7	351±117	629±143
8	483±143	1070±199
4	28.9±13	246±48
6	576±57	1347±197
10	6.5±3.6	315±167
recovery	0.20±0.08	0.43±0.16

P<0.05

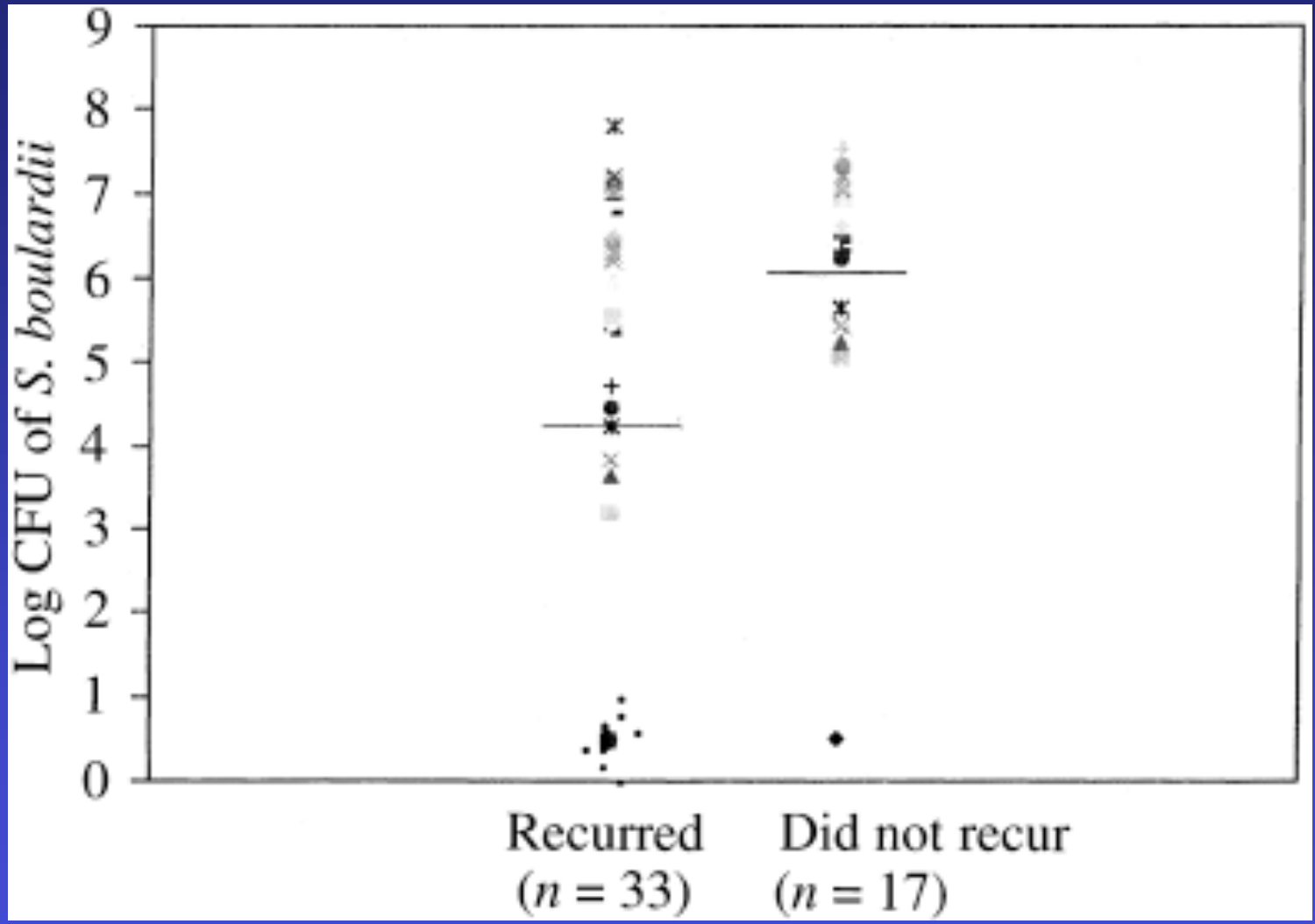
Klein et al. Pharmaceutical Res 1993;10:1615-1619



Christensen et al. FEMS Immunology & Medical Microbiology 2006;47:380-390

B. animalis BB-12; N=71 healthy adults

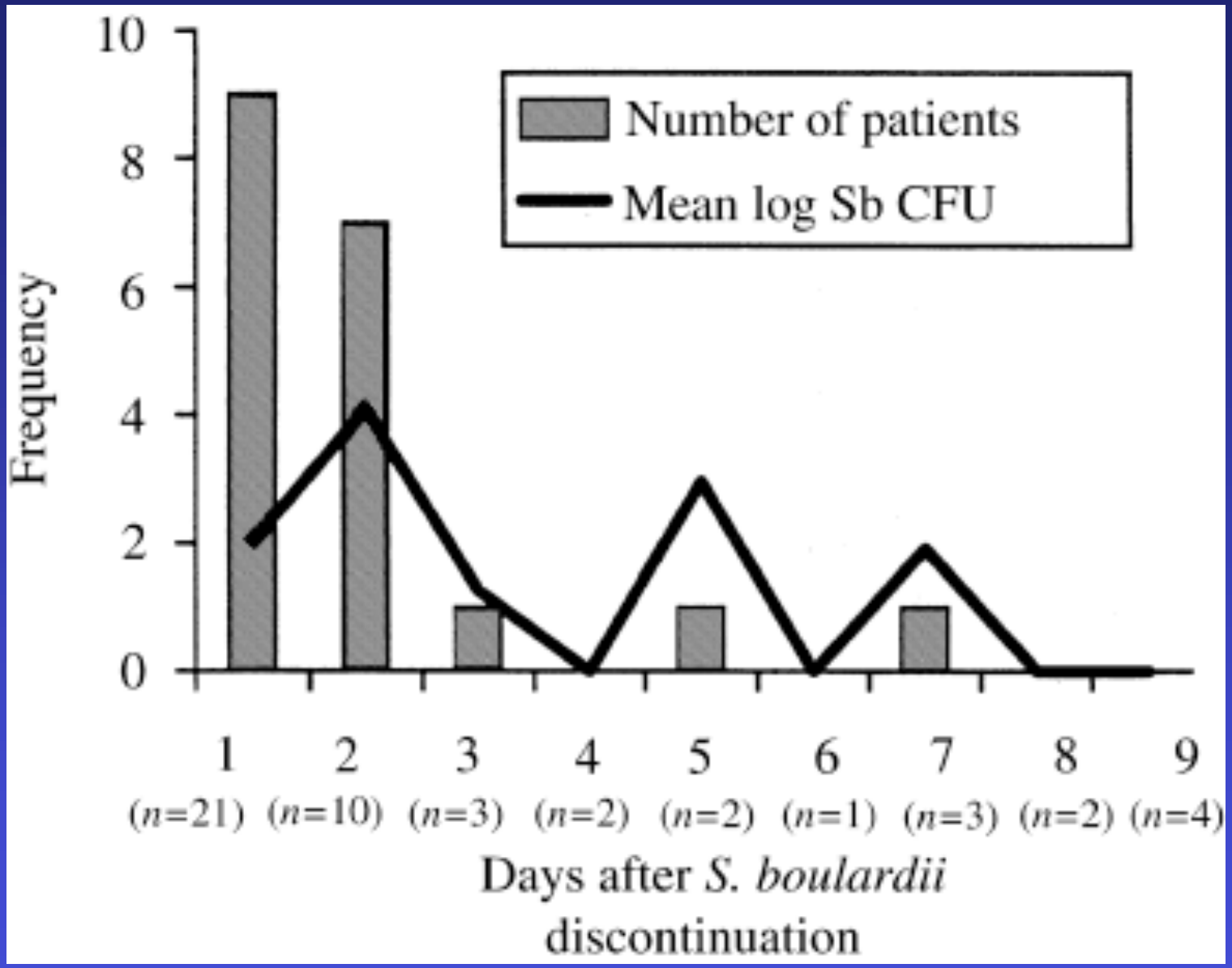
S. boulardii in patients with C. difficile disease



Elmer et al. Aliment Pharmacol Ther 1999;13:1663-1668.

$<10^4$ 14/15 (93%) recurred; $>10^4$ 19/35 (54%)

S. boulardii clearance after dose cessation



Number of positive samples n=48; Elmer et al. Aliment Pharmacol Ther 1999;13:1663-1668

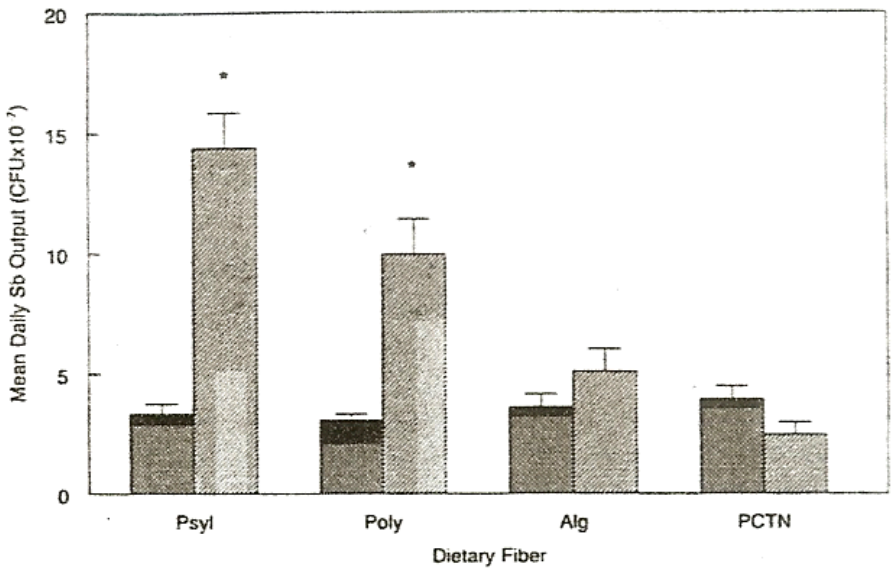


Fig. 1. Effect of dietary fiber on *S. boulardii* steady state levels in the rat. * = $p < 0.001$ compared to fiber-free controls. Hatched = fiber-free controls, striped = fiber (psyl = psyllium hydrocolloid, poly = polycarbophyl, alg = alginic acid, Pctn = pectin). n = 6–8 per group.

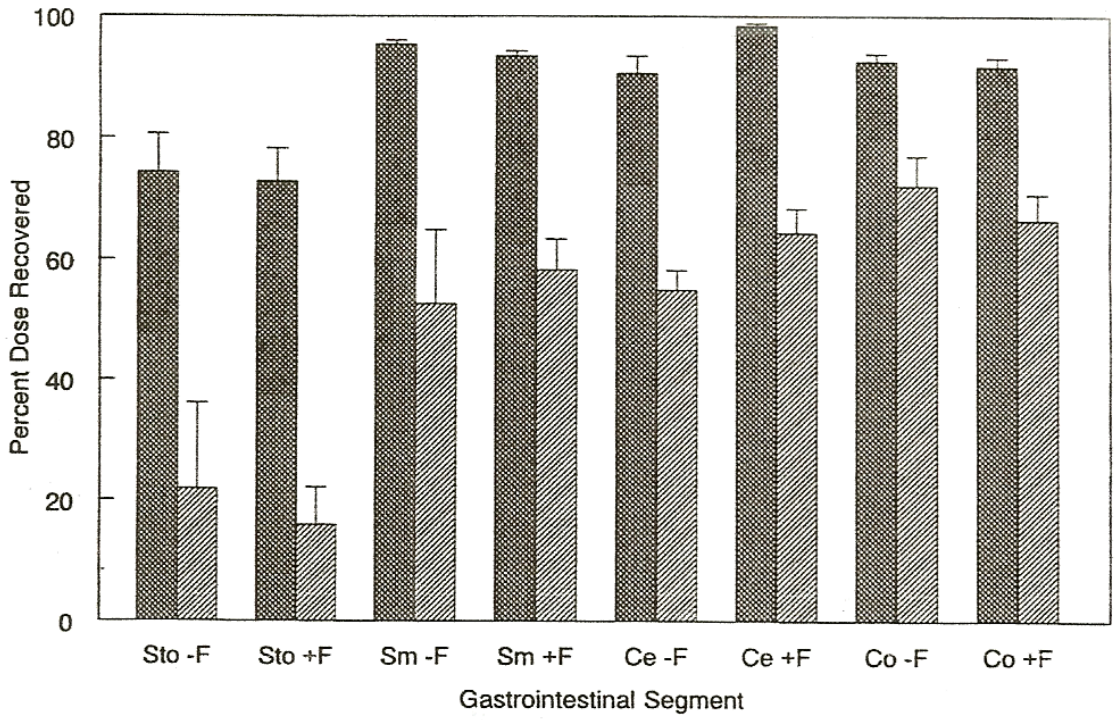


Fig. 2. Percent of *S. boulardii* dose recovered in ligated segments of the gastrointestinal tract as a function of incubation time. Hatched = zero time. Striped = 9 h incubation. − F = fiber-free diet. + F = psyllium containing diet. Sto = stomach, Sm = small intestine, Ce = cecum, Co = colon. n = 3–4 per group.

Elmer et al. Microbial Ecology in Health and Disease1999;11:29-34

Potential Advantages and Disadvantages of Probiotics

Advantages

- Multiple Mechanisms of Action
- Resistance is Infrequent
- Use May Reduce Exposure to Antibiotics
- Delivery of Microbial Enzymes
- Well Tolerated
- Benefit to Risk Ration is Favorable

Disadvantages

- Few Controlled Trials
- Persistence Possible
- Translocation Possible
- Transfer of Resistance Plasmids?
- Infection Possible
- Quality Control Issues
- Regulatory Issues in USA

Evidence supporting commercially available (USA) probiotics*

condition	VSL#3	L. reueri	LGG	Sb
AAD	Uneven	Good	Good	Good
Acute Adult	NA	NA	Good	Good
Acute pediatric	NA	NA	Good	Good
Traveler diarrhea	NA	NA	Fair	Fair
C. dif	NA	NA	Limited	Good
IBS	Fair	NA	None	NA
Crohns	NA	NA	None	Fair
UC	Fair	NA	NA	Fair

NA=not available (no studies), None=negative studies

Conclusions

1. Enhanced funding for basic research on probiotics badly needed
2. It is time for optimization of existing therapies with proven probiotics
3. Exploration needed of new applications for probiotics
4. Improved regulatory oversight of commercial products
5. OTC status granted for some well studied probiotics