Evidence-based Clinical Application of Probiotics

益生菌之實證醫學臨床應用

Shiuh-Bin Fang, MD, PhD
Director, Division of Pediatric Gastroenterology, Hepatology and Nutrition
Department of Pediatrics
Taipei Medical University Shuang Ho Hospital
New Taipei City, Taiwan
Assistant Professor in Pediatrics, School of Medicine, College of Medicine
Taipei Medical University, Taipei, Taiwan

臺北醫學大學部立雙和醫院小兒部 小兒胃腸肝膽營養科主任
臺北醫學大學醫學院醫學系 小兒學科專任助理教授
倫敦大學醫學院 小兒胃腸學醫學博士
方旭彬 醫師

2015 Dr Stanley Ho Medical Development Foundation Symposium
Macau, China
13:15-13:45 18 January 2015

臺北醫學大學 TAIPEI MEDICAL UNIVERSITY

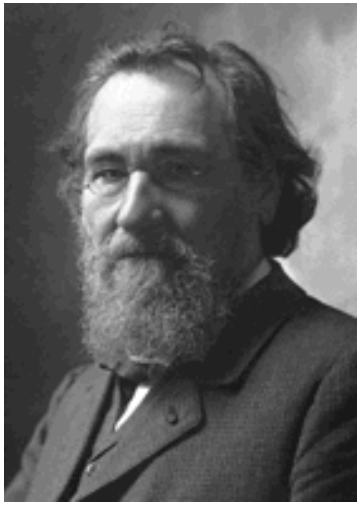
臺北醫學大學・部立雙和醫院 Taipei Medical University - Shuang Ho Hospital

Outlines

- History, definition, and classification of probiotics
- Mechanisms of probiotic actions
- Safety assessment of probiotics
- Clinical indications for probiotics
- Trends of development in probiotics
- Conclusions

History of probiotics (1)

- **Henry Tissier, 1906**
  - French paediatrician at the Pasteur Institute to first isolate *Bifidobacteria* from a breast-fed infant
  - Children with diarrhoea had in their stools a low number of bacteria characterized by a peculiar, Y shaped morphology.
  - These “bifid” bacteria were abundant in healthy children and could be administered to patients with diarrhoea to help restore a healthy gut flora.
- **Elie Metchnikoff, 1907**
  - Russian born Nobel Prize Laureate at the Pasteur Institute
  - “The long life of Bulgarian farmers resulted from their consumption of fermented milk products.”
  - “The **dependence of the intestinal microbes on the food** makes it possible to adopt measures to modify the flora in our bodies and **to replace the harmful microbes by useful microbes**.”
- **Werner Kollath, 1953**
  - The term “**probiotics**” was **first introduced**.



Elie Metchnikoff, 1907

History of probiotics (2)

- **Daniel M. Lilly and Rosalie H. Stillwell, 1965**
  - stimulation *in vitro* of growth of protozoa by other protozoa
  - Probiotics: “**substances** produced by microorganisms which promote the growth of other microorganisms”.
- **Roy Fuller, 1989**
  - redefined probiotics: "A **live** microbial feed supplement which beneficially affects the **host animal** by improving its **intestinal balance**".
- **Havenaar and Huis in 't Veld, 1992**
  - similar definition: "a **viable** mono or mixed culture of bacteria which, when applied to **animal or man**, beneficially affects the host by improving the properties of the **indigenous flora**".
  - broadened definition: microflora of other habitats from the intestinal tract to the **upper respiratory tract** or the **urogenical tract**.
- **Guarner and Schaafsma, 1998**
  - a more recent, but probably not the last definition: "**live microorganisms**, which when consumed **in adequate amounts**, confer a **health effect** on the **host**".

Definition of probiotics

- Probiotic (益生菌): Greek meaning of the term "for life"
  - Live microorganism which when administered in adequate amounts confer a health benefit on the host.

    Guarner F, Schaafsma GJ. *Probiotics.* Int J Food Microbiol. 1998;39:237-8.

- Prebiotic (助生質、益菌生)
  - a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health.

    Roberfroid MB. *Prebiotics: The Concept Revisited.* J Nutr. 2007;137: 830S

- Synbiotics (共生質)
  - synergistic combinations of probiotics and prebiotics.

    de Vrese M. *Probiotics, prebiotics, and synbiotics.* Adv Biochem Eng Biotechnol. 2008;111:1-66.

Health/Functional food vs Biotherapeutic agent (BTA)

Classification of probiotics

- Species of biotherapeutic agents (BTA)
  - Bacterial microorganisms
    - ✓ Lactic acid bacteria:
      - Lactobacilus - *Lactobacilus acidophilus, Lactobacillus casei* GG
      - Streptococcus - *Streptococcus thermophilus*
      - Enterococcus - *Enterococcus faecium* SF68
    - ✓ Bifidobacterium - *Bifidobacterium longum, Bifidobacterium bifidum*
    - ✓ Propionibacterium
    - ✓ Bacillus
    - ✓ *Escherichia coli* - *Escherichia coli* Nissle 1917
  - Yeast species
    - ✓ Saccharomyces - *Saccharomyces boulardii, Saccharomyces cerevisiae*

Mechanisms of probiotic action

- **Evidence-based studies for probiotics**: from anecdotal evidence
  - *in vitro*: cell cultures
  - *in vivo*: animal studies
  - clinical trials: randomized double-blind placebo-controlled studies

- ***In vitro* screening of microorganisms with a probiotic value**
  1. Human origin (safety for human use)
  2. Resistance to gastric acidity & bile toxicity (good survival during GI transit)
  3. Adhesion to gut epithelial cells (successful colonization *in vivo*)
  4. Production of antimicrobial substances or bacteriocins (for pathogen antagonism)
  5. Ability to modulate immune responses and to influence metabolic activities of faeces (for prevention of colon cancer)

Guarner F *et al* 2005 Br J Nutri 93:783-6

Lactobacillus rhamnosus inhibits inflammation and enhances barrier integrity of epithelium

Journal of Medical Microbiology (2010), **59**, 573–579 DOI 10.1099/jmm.0.009662-0

Inhibitory effects of *Lactobacillus casei* subsp. *rhamnosus* on *Salmonella* lipopolysaccharide-induced inflammation and epithelial barrier dysfunction in a co-culture model using Caco-2/peripheral blood mononuclear cells

Hsu-Wei Fang,[1,2]† Shiuh-Bin Fang,[3]† Jen-Shiu Chiang Chiau,[4] Chun-Yan Yeung,[5,6] Wai-Tao Chan,[5] Chuen-Bin Jiang,[5] Mei-Lien Cheng[4] and Hung-Chang Lee[5,7]

Correspondence
Hung-Chang Lee
ped2435@ms2.mmh.org.tw

[1]Department of Chemical Engineering and Biotechnology, National Taipei University Technology, Taipei, Taiwan, ROC

[2]Division of Medical Engineering Research, National Health Research Institutes, Miaoli, Taiwan, ROC

[3]Centre for Paediatric Gastroenterology, Royal Free Campus, University College London Medical School, London, UK

[4]Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, ROC

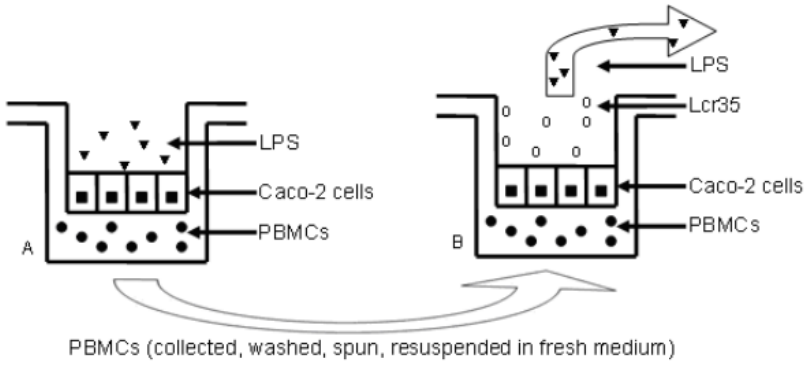
[5]Department of Paediatrics, Mackay Memorial Hospital, Taipei, Taiwan, ROC

[6]Mackay Medicine, Nursing and Management College, Taipei, Taiwan, ROC

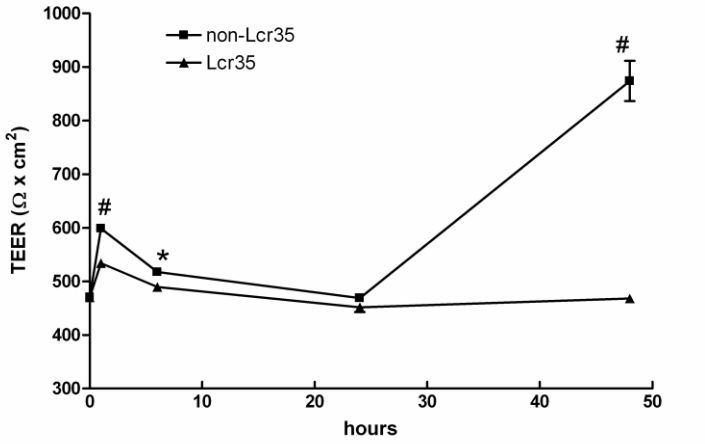
[7]Department of Paediatrics, Taipei Medical University, Taipei, Taiwan, ROC

Lactobacillus rhamnosus inhibits inflammation and enhances barrier integrity of epithelium

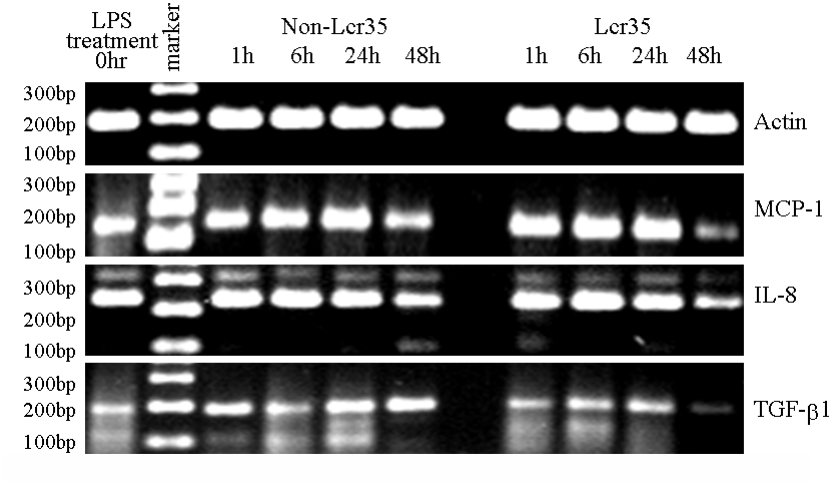
Caco-2/PBMC co-culture model



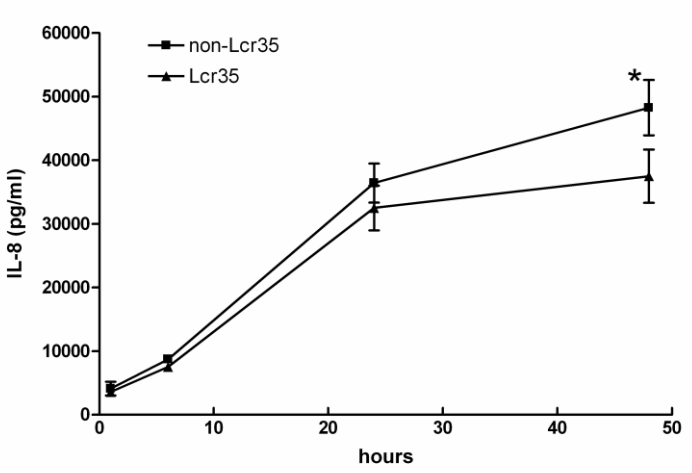
Lcr35 increased transepithelial electrical resistance (TEER)



Lcr35 decreased mRNA expression of 3 cytokines



Lcr35 decreased IL-8 secretion



Safety assessment of probiotics (1)

- **Use of probiotics in otherwise healthy: no reports of adverse effects**
- **Use of probiotics in the critically ill:**
  1. Risk factors for serious adverse events of probiotic treatment:
     - Immune compromised state (stressed or aged people, newborns, pregnant women)
     - Impaired intestinal barrier function (e.g. multiple organ failure, severe acute pancreatitis)
     - Central venous catheter
  2. Safer application as preventive treatment than therapeutic treatment: e.g. before surgery or an oxidative assault like acute pancreatitis

Sanders ME *et al* 2010 gut Microbes 1(3):164-85

- **Use of probiotics in patients with inflammatory bowel disease**
  1. Increased risk of bacterial translocation and secondary bacteraemia in cases of active disease with mucosal ulceration and exposure of submucosa
  2. Sepsis with a *Lactobacillus rhamnosus* strain in a patient with severe ulcerative colitis

Farina C *et al* (2001) J Clin Gastroenterol 33:251-2

Safety assessment of probiotics (2)

- **Use of probiotics in healthy, term infants**

  1. Use of infant formula containing Lactobacillus, Bifidobacterium and/or *S. thermophilus*: allowable in parts of Asia, Europe and US.
  2. No short-term, serious adverse events: long-term effects rarely measured
  3. Newborn infants are microbiologically and immunologically naïve, a vulnerable population
     - Safety studies should be done for specific strains in infants and not extrapolated from data on taxonomically related strains

- **Use of probiotics in premature infants**: whether or not it's save to give probiotics ⇨ whether it's safe not to give probiotics

  1. Prophylactic administration of certain Lactobacillus, Bifidobacterium, and *S. thermophilus* probiotics in >2,000 premature infants reduces both the incidence and severity of NEC ⇨ no adverse short term effects
  2. Long term effects: no cohorts have been followed long-term

Sanders ME *et al* 2010 gut Microbes 1(3):164-85

Probiotic may be harmful (1)

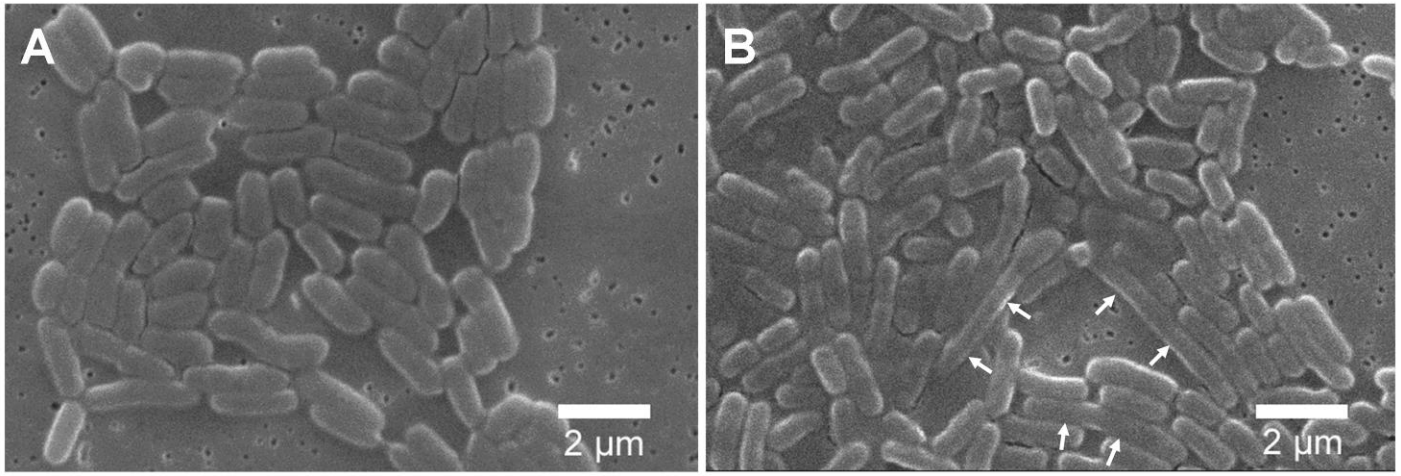
Support Care Cancer (2014) 22:1647–1654
DOI 10.1007/s00520-014-2137-z

ORIGINAL ARTICLE

Live and heat-killed *Lactobacillus rhamnosus* GG upregulate gene expression of pro-inflammatory cytokines in 5-fluorouracil-pretreated Caco-2 cells

Shiuh-Bin Fang · Hsin-Yu Shih · Chih-Hung Huang · Li-Ting Li · Chia-Chun Chen · Hsu-Wei Fang

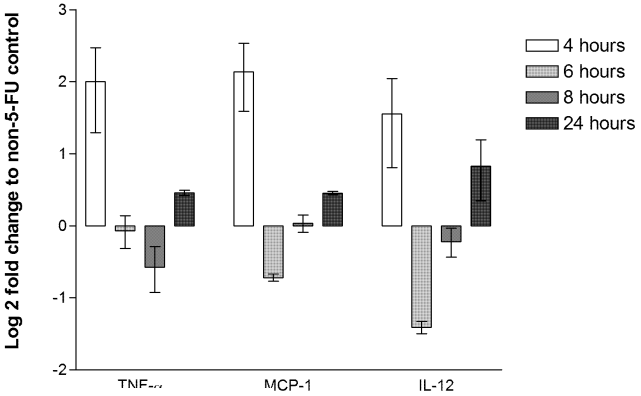
Received: 13 July 2013 / Accepted: 13 January 2014 / Published online: 6 February 2014

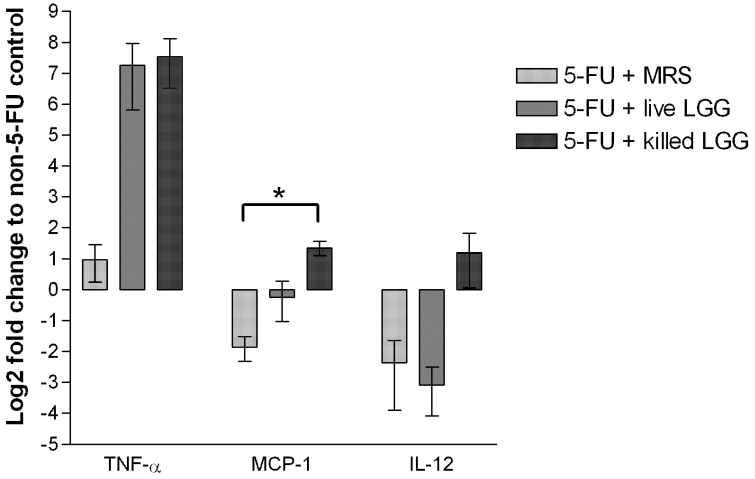


Live *L*GG

Killed *L*GG (85˚C x 20 min)

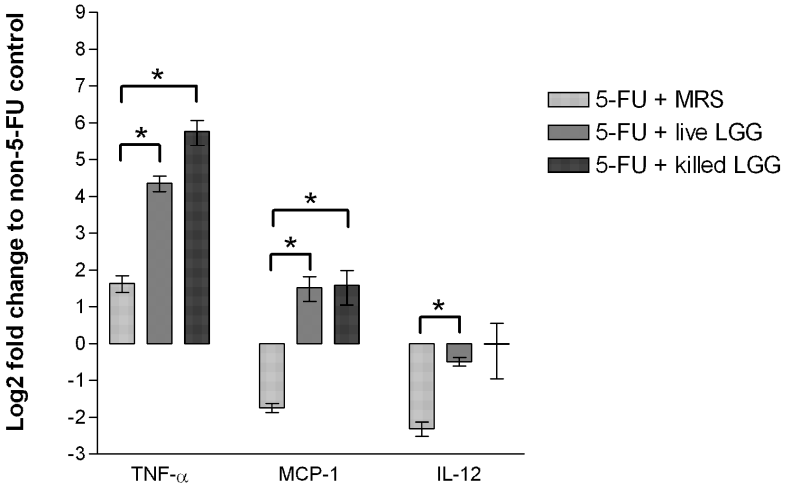
Probiotic may be harmful (2)





5-FU x4h →

MRS/live *L*GG/killed *L*GG x2h

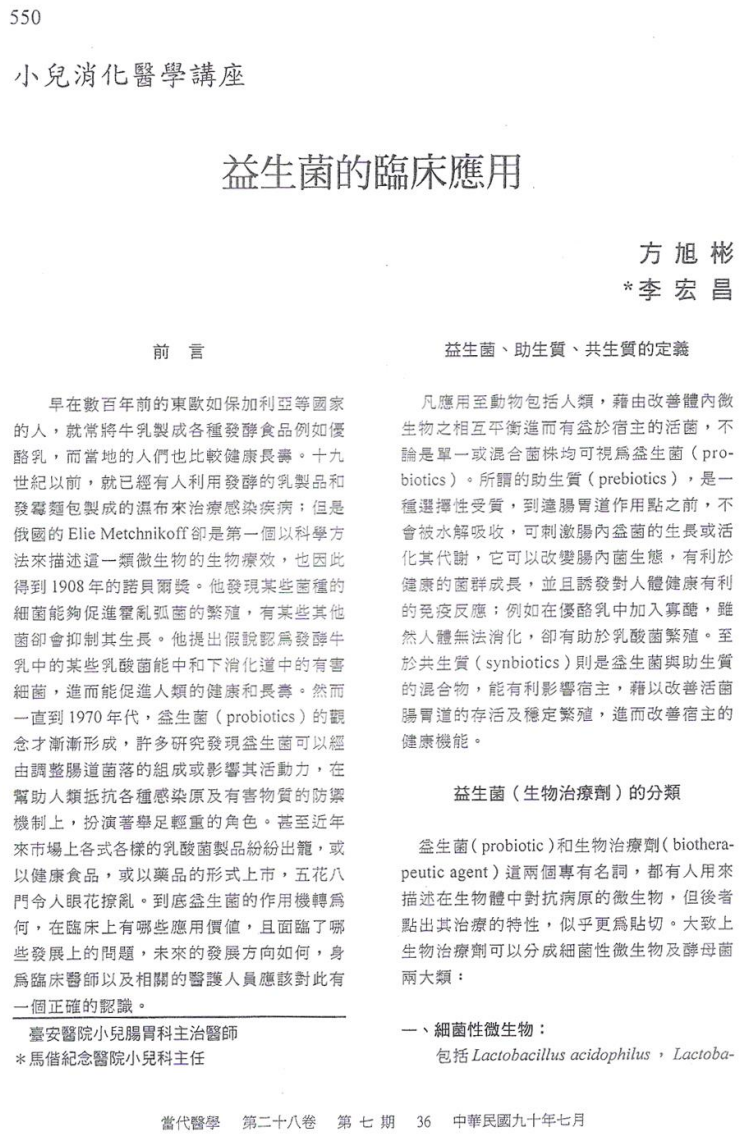


5-FU x4h →

MRS/live *L*GG/killed *L*GG x4h

Human intestinal epithelium may be vulnerable to the postchemotherapeutic use of *L. rhamnosus* GG in 5-FU-induced mucositis.

Clinical applications of probiotics (1)



550

小兒消化醫學講座

益生菌的臨床應用

方旭彬
*李宏昌

前言

早在數百年前的東歐如保加利亞等國家的人，就常將牛乳製成各種發酵食品例如優酪乳，而當地的人們也比較健康長壽。十九世紀以前，就已經有人利用發酵的乳製品和發霉麵包製成的濕布來治療感染疾病；但是俄國的 Elie Metchnikoff 卻是第一個以科學方法來描述這一類微生物的生物療效，也因此得到 1908 年的諾貝爾獎。他發現某些菌種的細菌能夠促進霍亂弧菌的繁殖，有某些其他菌卻會抑制其生長。他提出假說認爲發酵牛乳中的某些乳酸菌能中和下消化道中的有害細菌，進而能促進人類的健康和長壽。然而一直到 1970 年代，益生菌（probiotics）的觀念才漸漸形成，許多研究發現益生菌可以經由調整腸道菌落的組成或影響其活動力，在幫助人類抵抗各種感染原及有害物質的防禦機制上，扮演著舉足輕重的角色。甚至近年來市場上各式各樣的乳酸菌製品紛紛出籠，或以健康食品，或以藥品的形式上市，五花八門令人眼花撩亂。到底益生菌的作用機轉爲何，在臨床上有哪些應用價值，且面臨了哪些發展上的問題，未來的發展方向如何，身爲臨床醫師以及相關的醫護人員應該對此有一個正確的認識。

臺安醫院小兒腸胃科主治醫師
＊馬偕紀念醫院小兒科主任

益生菌、助生質、共生質的定義

凡應用至動物包括人類，藉由改善體內微生物之相互平衡進而有益於宿主的活菌，不論是單一或混合菌株均可視爲益生菌（probiotics）。所謂的助生質（prebiotics），是一種選擇性受質，到達腸胃道作用點之前，不會被水解吸收，可刺激腸內益菌的生長或活化其代謝，它可以改變腸內菌生態，有利於健康的菌群成長，並且誘發對人體健康有利的免疫反應；例如在優酪乳中加入寡醣，雖然人體無法消化，卻有助於乳酸菌繁殖。至於共生質（synbiotics）則是益生菌與助生質的混合物，能有利影響宿主，藉以改善活菌腸胃道的存活及穩定繁殖，進而改善宿主的健康機能。

益生菌（生物治療劑）的分類

益生菌（probiotic）和生物治療劑（biotherapeutic agent）這兩個專有名詞，都有人用來描述在生物體中對抗病原的微生物，但後者點出其治療的特性，似乎更爲貼切。大致上生物治療劑可以分成細菌性微生物及酵母菌兩大類：

一、細菌性微生物：
包括 *Lactobacillus acidophilus*，*Lactoba-*

1. 改善雙醣的消化
2. 預防與抗生素相關之腹瀉
3. 預防旅行者腹瀉
4. 治療與*Clostridium difficile*相關的疾病
5. 預防與放射治療相關之腹瀉與腸道併發症
6. 治療與預防嬰幼兒腹瀉
7. 治療小腸細菌過度增生
8. 治療陰道炎
9. 預防泌尿道感染（特別是女性膀胱炎）
10. 降低齲齒的發生
11. 加速牛奶蛋白過敏的復原
12. 減少異位性溼疹的發生率

益生菌的臨床應用（當代醫學）Fang SB, Lee HC. **Clinical application of probiotics.** Medicine Today [Chinese][Review Article]. 2001;28(7):550-7.

Clinical applications of probiotics (2)

Table 2. Present and future clinical applications of probiotics, by level of evidence of efficacy.

Applications with strong evidence
Gastroenteritis
Acute
Antibiotic associated
Applications with substantial evidence of efficacy
Allergic reactions, specifically atopic dermatitis
Applications that have shown promise
Childhood respiratory infection
Dental caries
Nasal pathogens
Relapsing *Clostridium dificile*–induced gastroenteritis (prevention)
Inflammatory bowel disease
Potential future applications
Rheumatoid arthritis
Irritable bowel syndrome
Cancer (prevention)
Ethanol-induced liver disease
Diabetes
Graft-versus-host disease

Goldin BR, Gorbach SL. Clinical Infectious Diseases 2008;46:S96-100.

Clinical applications of probiotics (3)

- Acute gastroenteritis: prevention & tx
- Antibiotic-associated diarrhea (AAD)
- Lactose intolerance
- Traveller's diarrhoea
- Atopic dermatitis
- Neonatal necrotizing enterocolitis (NEC)
- Dental caries
- Childhood respiratory tract infection
- Inflammatory bowel disease (IBD)
- Small bowel bacterial overgrowth
- Rheumatoid arthritis
- *Helicobacter pylori* infection
- Post-operative bacterial infections
- Non-alcoholic fatty liver disease (NSFLD)
- Maternal GBS vaginal/rectal colonisaiton
- Prevention of urinary tract infection
- Ethanol-induced liver disease
- Celiac disease
- Cow's milk protein allergy?
- Constipation?
- Cancer (prevention)?
- Obesity/weight loss?
- HIV infection?
- Irritable bowel syndrome (IBS)?
- Radiotherapy-induced diarrhea?
- Diabetes?
- Vaginitis?
- Hypercholesterolemia? (VLDL)
- Burn?
- Hepatic encephalopathy?

Acute gastroenteritis & traveller's diarrhoea

- **Treatment and prevention for acute gastroenteritis**
  - Mostly in infants, toddlers, and children
  - Either rotavirus or unknown etiologic agent
  - *Lactobacillus rhamnosus* GG, *Lactobacillus reuteri*, *Saccharomyces boulardii*, etc.
  - Treatment vs Prevention
    - ✓ Treatment of moderate-to-severe diarrhea in children: decreased severity, shorter duration of illness, shorter hospital day, decreased likelihood of persistent diarrheal illness
    - ✓ Prevention: decrease in the rate of incidence of diarrhea among children who received LGG and whom were not breast fed.
  - Preparations: powders, capsules, in ORS, in formula, etc.
- **Prevention for traveller's diarrhoea**
  - *Lactobacillus rhamnosus* GG, *Saccharomyces boulardii*
  - Preparations in warm climate situations

Goldin BR *et al* 2008 CID 46(supple2):S96-100
Chapman CM *et al* 2011 Eur J Nutr 50:1-17
Vandenplas Y *et al* 2013 Curr Infect Dis Rep 15(3):251-62

Lactobacillus rhamnosus (Lcr35) on fecal rotavirus shedding

Dose-dependent effect of *Lactobacillus rhamnosus* on quantitative reduction of faecal rotavirus shedding in children

by Shiuh-Bin Fang,[a,b] Hung-Chang Lee,[c,d] Jen-Jan Hu,[a] Shao-Yi Hou,[e] Hsuan-Liang Liu,[e] *and* Hsu-Wei Fang[e]

[a]*Department of Paediatrics, Taiwan Adventist Hospital, Taipei, Taiwan*

[b]*Centre for Paediatric Gastroenterology, Royal Free and University College Medical School, London, UK*

[c]*Department of Paediatrics, Mackay Memorial Hospital*

[d]*Department of Paediatrics, Taipei Medical University*

[e]*Institute of Biotechnology, National Taipei University of Technology, Taipei, Taiwan*

Summary

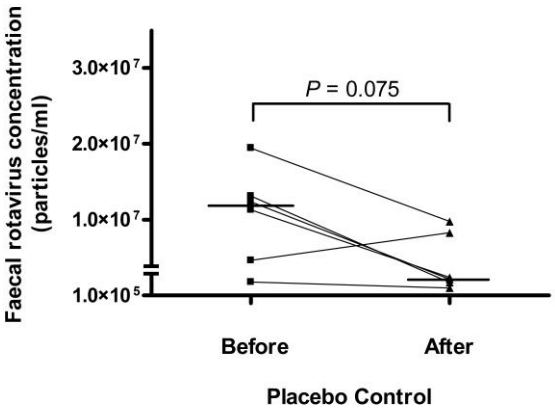
Beneficial effects of probiotics in acute infectious diarrhoea in children are mainly seen in watery diarrhoea and viral gastroenteritis. *Lactobacillus rhamnosus*, one the most extensively studied probiotic strains, is effective in shortening courses of acute diarrhoea in children. However, the dose-dependent effect of *Lactobacillus* upon quantification of faecal rotavirus shedding in humans remains little known. Thus, an open-label randomized trial in 23 children with acute rotaviral gastroenteritis was undertaken by randomly allocating patients to receive one of the three regimens for 3 days: daily *Lactobacillus rhamnosus* 35 (Lcr35) with 0 CFU/day to six patients in the control group, 2×10^8 CFU/day to nine patients in the low-dose group, and 6×10^8 CFU/day to eight patients in the high-dose group. Faecal samples were collected before and after the 3-day regimen for measurements of rotavirus concentrations by ELISA. There was no statistically significant change in faecal rotavirus concentrations in either the control group (119.2×10^5 particles/ml vs. 23.7×10^5 particles/ml, $p = 0.075$) or the low-dose group (36.1×10^5 particles/ml vs. 73.5×10^5 particles/ml, $p = 0.859$). However, the high-dose group had a significant reduction of faecal rotavirus concentration (64.2×10^5 particles/ml vs. 9.0×10^5 particles/ml, $p = 0.012$). Without any exception, the faecal rotavirus concentrations of all eight patients in the high-dose Lcr35 group declined by 86% after 3 days when compared with those before Lcr35 administration. In conclusion, this is the first report to provide quantitative evidence of the dose-dependent effect of *Lactobacillus rhamnosus*, a minimal effective dose of 6×10^8 CFU for 3 days, upon the faecal rotavirus shedding in paediatric patients.

Key words: Dose-dependent, *Lactobacillus rhamnosus*, rotavirus, virus shedding, ELISA.

Fang SB *et al* 2009 J Trop Pediatr 55(5):297-301

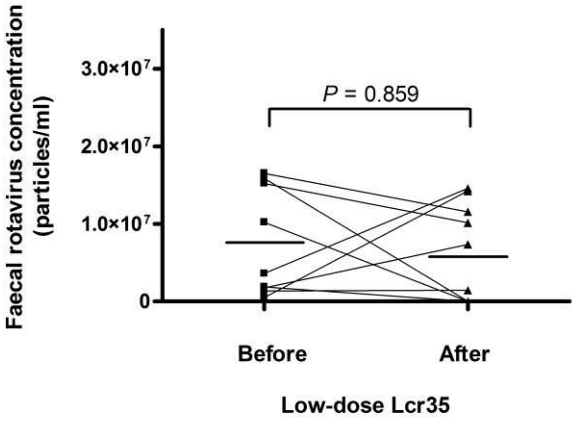
Lactobacillus rhamnosus (Lcr35) on fecal rotavirus shedding

0 CFU/day



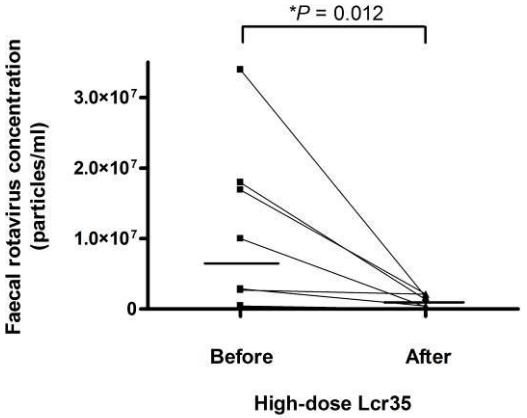
N = 6

2×10^8 CFU/day



N = 9

6×10^8 CFU/day



N = 8

Fang SB *et al* 2009 J Trop Pediatr 55(5):297-301

Only **5 nucleotide sequences** specific to Lcr35 were revealed by performing substractive hybridisation between genomic DNAs from this strain and from *Lactobacillus rhamnosus* GG.

Coudeyras S *et al.* Appl Environ Microbiol. 2008;74(9):2679-89

Lactose intolerance

- Improve lactose digestion and eliminate symptoms of lactose intolerance
  - due to microbial ß-galactosidase — de Vrese M *et al* Am J Clin Nutr 2001;73:421S-429S
  - In yogurt: *Lactobacillus bulgaricus & Streptococcus thermophilus* — Goldin BR *et al* CID 2008;46(2):S96-100

TABLE 2 Studies on the influence on hydrogen exhalation of native and heated yogurt compared with milk in lactose-intolerant persons

Reference	Product	Lactose[1]	Hydrogen exhalation[2,4]
		g	
Kolars et al (22) (*n* = 10)	400 g milk	18	293 ppm/h
	270 g yogurt	11	72 ppm/h
	440 g yogurt	18	108 ppm/h
Savaiano et al (23) (*n* = 9)	410 g milk	20	≈180 ppm/h
	500 g yogurt	20	≈50 ppm/h
	500 g pasteurized yogurt	20	≈170 ppm/h
Martini et al (24) (*n* = 9)	415 g milk	20	185 Δ ppm/h
	455 g yogurt	20	37 Δ ppm/h
McDonough et al (25) (*n* = 14)	250 g milk	15.7	28.7 ppm
	250 g yogurt + lactose	15.7	15.5 ppm
	250 g yogurt	12	5.4 ppm
	250 g heated yogurt	12	14.9 ppm
Dewit et al (26) (*n* = 8)	Milk	18	12.5 ppm
	Lactose	18	17.0 ppm
	Yogurt	18	2.2 ppm
	Heated yogurt	18	12.4 ppm
Martini et al (27) (*n* = 7)	315 g milk	18	≈350 Δ ppm/h
	425 g yogurt 1[4]	18	≈60 Δ ppm/h
	425 g yogurt 2[4]	18	≈30 Δ ppm/h
	450 g yogurt 3[4]	18	≈20 Δ ppm/h
Rosado et al (28) (*n* = 14)	360 g milk	18	220 ppm/h
	454 g low-fat yogurt	20.4	76 Δ ppm/h
	454 g yogurt 1	18.6	36 Δ ppm/h
	454 g yogurt 2	18.6	26 Δ ppm/h
	454 g lactose-reduced yogurt	3.6	0.5 Δ ppm/h
Murao et al (29) (*n* = 30)	300 g milk	14	150 ppm_{max}
	500 g yogurt	14	32 ppm_{max}
Martini et al (30) (*n* = 12)	480 g milk	20	437 Δ ppm/h
	480 g yogurt	20	133 Δ ppm/h
	480 g breakfast	20	68 Δ ppm/h
	435 g yogurt + breakfast	20	62 Δ ppm/h
Gilliland and Kim (31) (*n* = 6)	Yogurt	—	9.9 ppm_{max}
	Heated yogurt	—	22.8 ppm_{max}
Varela-Moreiras et al (32) (*n* = 19)	200 g milk	11	135 ppm/h
	200 g yogurt	11	55 ppm/h
	200 g pasteurized yogurt	11	85 ppm/h
Marteau et al (33) (*n* = 8)	450 g milk	—	439 ppm/h
	450 g yogurt	—	103 ppm/h
	450 g heated yogurt	—	191 ppm/h
Lerebours et al (34) (*n* = 24)	125 g milk	18	34.7-39.0 ppm/h
	125 g yogurt	18	11.4 ppm/h
	125 g pasteurized yogurt	18	27.4 ppm/h

TABLE 3 Studies on the influence on gastrointestinal symptoms of yogurt or nonyogurt fermented milk products compared with milk in lactose-intolerant subjects

Reference	Product	Symptoms
Martini et al (27) (*n* = 7)	315 g milk [18][1]	2.0 ± 0.8[2,3]
	425 g yogurt[4] [18]	1.3 ± 0.7
	425 g yogurt[5] [18]	0.4 ± 0.2
	450 g yogurt[6] [18]	0.6 ± 0.3
Rosado et al (28) (n = 14)	360 g milk [18.0]	3.8 ± 0.7[7]
	454 g low-fat yogurt [20.4]	1.5 ± 0.5
	454 g yogurt 1 [18.6]	1.6 ± 0.5
	454 g yogurt 2 [18.6]	1.3 ± 0.5
	454 g lactose-reduced yogurt [3.6]	1.4 ± 0.6
Montes et al (35) (*n* = 20 children)	250 g low-fat milk [11.6]	4.0[7]
	250 g low-fat acidophilus (10^{10} CFU/g NCFM) milk [11.6][8,9]	1.8
	250 g low-fat *thermophilus*+ *Lactobacillus lactis* (10^{10} CFU/g) milk [11.6]	1.0
Gaon et al (36) (*n* = 18)	480 g fermented *Lactobacillus casei* + *Lactobacillus acidophilus* milk [25]	Fewer symptoms than with milk
Savaiano et al (23) (*n* = 9)	410 g milk [20][10]	11/33[11]
	500 g yogurt [20]	0/0
	465 g buttermilk [20]	44/90
	420 g acidophilus (NCFM) milk [20][8]	0/44
Jiang et al (37) (*n* = 15)	400 g low-fat milk [16][10]	47/60[11]
	400 g *Bifidobacterium longum* (B6, L + G)[12,13] milk [16]	27/87
	400 g *B. longum* (B6, L)[12,13] milk [16]	40/47
	400 g *B. longum* (15708)[14] milk [16]	27/80

Antibiotic-associated diarrhoea (AAD) and *Clostridium difficile* infection

- AAD: in 5-25% of patients receiving antibiotic treatment
- One of the major causes of AAD is infection with *Clostridium difficile*:
  - responsible for 15-25% of AAD cases

Table 7 Human studies: antibiotic-associated diarrhoea (AAD)

Organisms/dose	Study type	End points	Outcome	Reference
Group 1: lactinex mix: *L. acidophilus, L. bulgaricus.* Dose not stated, but each Lactinex packet contains 10^8 CFU (www.bd.com) Group 2: placebo (not stated)	RDBPCT, $n = 79$	Prevention of antibiotic-associated diarrhoea (AAD) by *lactobacillus* blend or placebo	Reduced incidence of AAD in experimental group (8.3% vs. 21%)	Gotz V *et al* 1979; 36(6):754-7
Group 1: 97 ml yoghurt drink containing: *L. casei:* 10^8 CFU/ml, *L. bulgaricus:* 10^8 CFU/ml, *S. thermophilus:* 10^7CFU/ml, Total dose: 2.04×10^{10} CFU twice daily Group p2: placebo (sterile milkshake)	RDBPCT, $n = 135$	Prevention of *C. difficile*-associated diarrhoea (CDCAD) and AAD	Reduced incidence of both forms of diarrhoea in experimental group (12% ≠ 34%); 75% reduction in relative risk for probiotic group	Hickson M *et al* 2007; 335(7610):80
Group 1: mixture of *L. acidophilus* and *B .bifidum,* total dose: 2×10^{10} per capsule (proportions not stated), one capsule/day for 20 days Group 2: placebo (sterile capsule)	RDBPCT, $n = 150$	Probiotic administration in the prevention of CDAD in the elderly	No difference in incidence between groups; more *C. difficile* carriers but, fewer toxins, recorded in probiotic group	Plummer S *et al* 2004; 7(1):59-62

Chapman CM *et al* 2011 Eur J Nutr 50:1-17

- Treatment of relapsing gastroenteritis induced by *Clostridium difficile* toxin:
  - 60% relapse rate after therapy with metronidazole or vancomycin
  - 16% of patients receiving *LGG* experienced a relapse

Goldin BR *et al* 2008 CID 46(supple2):S96-100

Traveller's diarrhoea

- Prevention of traveller's diarrhoea
  - *Lactobacillus* GG: 11.8%-47% protection
  - *Saccharomyces boulardii*: clinically modest but statistically significant protection, with marked regional differences (highest benefit in North Africa and Turkey)
  - *Enterococcus faecium*: No
- Treatment of traveller's diarrhoea
  - Probiotics: not studied in travellers

Ericsson DC 2005 Clinical Infectious Diseases 41:S557-63

Atopic dermatitis (1)

Table 6 Human studies: atopic dermatitis

Organisms	Study type	End points	Outcome	Reference
Group 1: mixture of *L. reuteri, L. rhamnosus*, 10^{10} CFU each strain Group 2: placebo (skimmed milk powder)	RDBPCT, $n = 43$	Amelioration of AD symptoms in children; plasma sECP levels	(Non-significant) improvement of symptoms, decreased in sECP	Rosenfeldt V *et al* 2003;111(2):389-95
Group 1: *L. rhamnosus GG* 5×10^9 CFU; Group 2: mixture of *L. rhamnosus GG* 5×10^9 CFU, *L. rhamnosus LC705* 5×10^9 CFU, *B. breve Bbi99* 2×10^8 CFU, P. *freudenrechii* ssp. *Shermanii JS* 2×10^9 CFU Group 3: placebo (microcrystalline cellulose)	RDBPCT, $n = 230$	Changes to levels of inflammatory markers	Reduction of IgA and AT, no change in TNF-α	Viljanen M *et al* 2005;16(1):65-71
Group 1: *L. rhamnosus GG* 5×10^9 CFU; Group 2: mixture of *L. rhamnosus GG* 5×10^9 CFU, *L. rhamnosus LC705* 5×10^9 CFU, *B. breve Bbi99* 2×10^8 CFU, P. *freudenrechii* ssp. *Shermanii JS* 2×10^9 CFU Group 3: placebo (microcrystalline cellulose)	RDBPCT, $n = 230$	Amelioration of symptoms	Symptomatic improvement in both probiotic groups and placebo group For the subgroup of IgE-sensitized children, the SCORAD ratings were significantly reduced in the LGG group, with no reduction in the mixture group.	Viljanen M *et al* 2005;60(4):494-500

Chapman CM *et al* 2011 Eur J Nutr 50:1-17

■ Overall, the evidence for a probiotic mixture as a treatment for atopic dermatitis appears contradictory.

Neonatal necrotizing enterocolitis (NEC)

TABLE 5. *Probiotics in prevention of necrotizing enterocolitis in premature infants: randomized controlled trials*

Study	N	Population	Probiotic(s) (dose)	RR (95% CI)	NTT (95% CI)
Dani et al. (74)	585	Birth weight <1500 g or <33 wk of gestation	LGG (6×10^9 CFUs once daily; with milk formula)	0.5 (0.15–1.6)	Not significant
Lin et al. (75)	367	Birth weight <1500 g	*L. acidophilus* + *B. infantis* (125 mg/kg, per dose twice daily; with breast milk)	0.2 (0.05–0.8)	24 (12–142)
Bin-Nun et al. (76)	145	Birth weight ≤1500 g	*B. infantis* + *S. thermophilus* + *B. bifidum* (10^9 CFUs/d; with breast milk and/or formula)	0.3 (0.07–0.8)	9 (5–39)

NNT, number needed to be treated; RR, relative risk.

- Prospective randomized trials during the past decade have evaluated the effects of various probiotics to prevent necrotizing enterocolitis.
  - the probiotic approach decreased the incidence of NEC but did not decrease mortality from NEC.

    Lin HC *et al* Pediatrics 2005;115:1-4; Bin-Nun A *et al* J Pediatr 2005;147:192-6; Dani *et al* Biol Neonate 2002;82:103-8
  - a higher incidence of sepsis among infants receiving probiotics, especially birth weight <750 g

    Lin HC *et al* Pediatrics 2008;122:693-700
- Use of *Lactobacillus acidophilus/Bifidobacterium infantis* probiotics reduced the risk for gastrointestinal morbidity but not sepsis in very low birth weight infants. (observation cohort)

Hartel C *et al* J Pediatr 2014 Au;165(2):285-9.

Oral health

- Prevention and treatment of oral infections
  - **Dental caries**
    - ✓ Probiotic strains: *Streptotoccus thermophilus*, *Lactobacillus lactis* ssp. *lactis*, *Lactobacillus rhamnosus, Lactobacillus bulgaricus, Lactobacillus reuteri*
    - ✓ Against: cariogenic species *Streptococcus mutans* and *Streptococcus sobrinus*
  - **Periodontal disease (gingivitis, periodontitis)**
    - ✓ *Lactobacillus reuteri*
    - ✓ *Lactobacillus brevis*
    - ✓ *Lactobacillus helveticus*
  - **Halitosis**
    - ✓ *W. cibaria, Streptotoccus salivarius*
    - ✓ Against:
      - *F. nucleatum*
      - *Atopobium parvulum*
      - *Eubaterium sulci*
      - *Solobacterium moorei*

Bonifait L *et al* 2009 JCDA 75(8):585-90

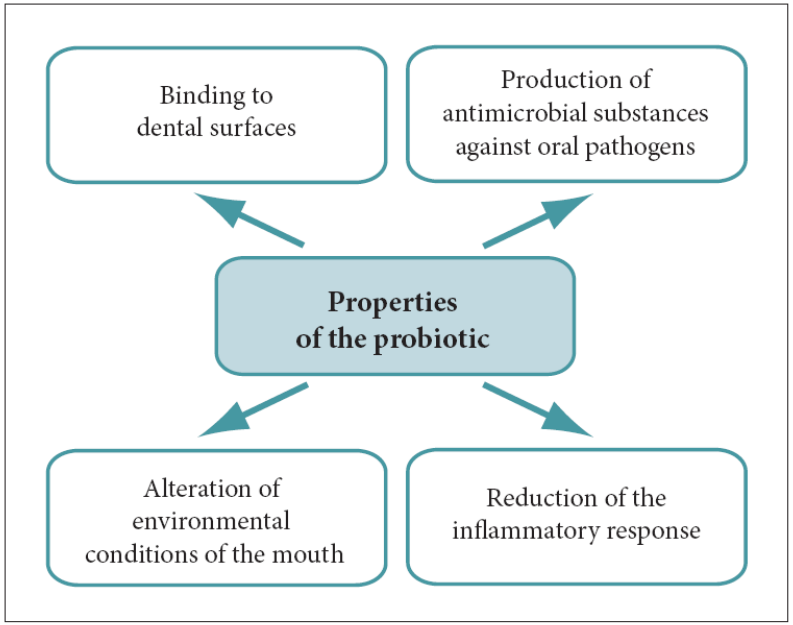


Figure 1: Ideal properties of a probiotic intended for use in disorders of the mouth.

Dental caries

- Children in a day care center who were given *Lactobacillus* GG for 7 months were examined for dental caries
  - The children in the 3-4 y/o age group had:
    - ✓ Significantly lower rates of dental caries
    - ✓ A reduced oral count of *Streptococcus mutans* compared with before the treatment

Nase L *et al* Caries Res 2001;35:412-20

- *L. salivarius*-containing tablets increased resistance to caries risk factors. (randomised open-label clinical trial)

Nishihara T *et al* BMC Oral Heath 2014 Sep 2;14:110.

- Daily supplementation with *Lactobacillus reuteri* from birth and during the first year of life is associated with reduced caries prevalence and gingivitis score in the primary dentition at 9 years of age. (single-blind RCT)

Stensson M *et al* Caries Res 2014;48(2):111-7.

Respiratory tract infection

Table 8 Human studies: immune function, incidence and duration of respiratory tract infections (RTI)

Organisms/dose	Study type	End points	Outcome	Reference
Group 1: multivitamin/mineral plus *L. gasseri*: 4×10^7 CFU, *B. longum*: 5×10^6 CFU, *B. bifidum*: 5×10 CFU, 5×10^7 CFU in total Group 2: placebo (multivitamin/mineral only)	RDBPCT, $n = 479$	Incidence and severity of common cold over a 3-month period in adults treated with probiotic and/or vitamin and mineral supplements, assessed by total symptom scoring, duration, cellular immune response and faecal probiotic bacteria counts	Reduced number of days with fever duration of cold episodes (mean 2 days), total symptom score; increased T-suppressor cells in probiotic group compared to vitamin/mineral only group; increased numbers faecal *lactobacilli* and *bifidobacteria* in probiotic group from day 1–14 of study. No reduction in incidence of RTIs in either group	Plummer S *et al* 2004;7(1):59-62
Group 1: multivitamin/mineral plus *L. gasseri*: 4×10^8 CFU, *B. longum*: 5×10^7 CFU, *B. bifidum*: 5×10^7 CFU, 5×10^8 CFU in total Group 2: placebo (multivitamin/mineral only)	RDBPCT, $n = 477$	Incidence, duration and severity of common cold in adults given probiotic multivitamin and mineral supplement or placebo over 3-month period	Incidence 13.6% lower, symptoms an number of days with fever reduced in probiotic group; 9.3% shorter duration in probiotic group. Greate increase in immune function (CD4+ , CD8+ , T-lymphocytes, monocytes) in probiotic group	Winkler P *et al* 2005;43(7):318-26
Group 1: fermented milk drink containing *Lactobacillus GG:* 7.1×10^9 CFU, *Bifidobacterium B420:* 8.4×10^9 CFU, *L. acidophilus:* 3.2×10^9 CFU, *S. thermophilus:* 27×10^9 CFU. Total: 4.57×10^{10} Group 2: placebo (standard yoghurt drink)	Open trial, $n = 209$	Colonisation of nasal pathogens over a 28-day period in patients supplemented with a probiotic mixture or standard yoghurt	19% reduction of colonisation in probiotic group; between days 1 an 21 pathogenic bacteria eliminated 13/108 probiotic-fed subjects	Gluck U *et al* 2003;77(2):517-20
Group 1: capsule containing *Lactobacillus GG, Bifidobacterium breve, Propionibacterium freudenreichii;* $8–9 \times 10^9$ CFU of each strain per capsule, 1 capsule per day Group 2: placebo (capsule containing microcrystalline cellulose)	RDBPCT, $n = 269$	Occurrence and duration of otitis and upper respiratory infections in susceptible children (10 m–6y) treated with probiotic combination or placebo over a 24-week period; assessed by clinical examination and bacteriological screening of nasal swab	No effect on occurrence or recurrenc no reduction in carriage of potentia pathogens	Hatakka K *et al* 2007;26(3):314-321
Group 1: *L. casei:* 6×10^8 CFU/day, 5 days/week Group 2: *L. rhamnosus:* 3.42×10^{10} CFU/day, 5 days/week Group 3: mixture of *B. bifidum, B. infantis, B. longum, L. casei, L. acidophilus, L. salivarius, L. brevis, L. plantarum, L. helveticus, L. rhamnosus, S. thermophilus, E. faecium*: 10^{11} CFU/day, 5 days/week Group 4: control group—no probiotic supplementation	RDBCT, $n = 1062$	Prevention of paediatric bacterial, viral, gastrointestinal and respiratory diseases in children under 5 years old by supplementation with single- or multi-strain probiotic of placebo	Reduced incidence of respiratory infections (bacterial and viral) as well as physician visits in all group no significant differences between groups; reduction in GI infections only in multi-strain group	Lin JS *et al* 2009;27(7):1073-9
Group 1: yoghurt containing *S. thermophilus*: 4×10^8 CFU, *L. bulgaricus*: 4×10^9 CFU Group 2: mixture of *S. thermophilus* 4×10^8 CFU, *L. gasserii* 4×10^9 CFU, *L. corniformis*: 4×10^9 CFU	RDBPCT, $n = 30$	Multiple immune responses in adults (23–43) after probiotic supplementation	Increase in neutrophils in both group only maintained by probiotic grou increased natural killer (NK) cells and interleukin-4 and -10, decreased immunoglobulin E in probiotic group; greater increase in phagocyte activity in probiotic group	Olivares M *et al* 2006;9(1):47-52

- Supplementation with certain multi-strain probiotics can reduce severity, duration, and possibly incidence of RTIs.
- Further work should be done to determine the relative efficacy of single- and multi-strain probiotics in this area.

Chapman CM *et al* 2011 Eur J Nutr 50:1-17

Inflammatory bowel disease (IBD)

- **Crohn's disease (CD)**
  - No benefit of various probiotic preparations on induction of remission, prevention of recurrent of CD following active disease, or for prevention of post-operative recurrence
- **Pouchitis**
  - Extending remission
- **Ulcerative colitis (UC)**
  - A slight decrease in severity of active UC observed in 4 studies using a single Bifidobacterium strain, an *E. coli* strain and 2 multiple strain probiotic products.

Sanders ME *et al* Gut Microbes 2010;1(3):164-85

Small bowel bacterial overgrowth

- Small bowel bacterial overgrowth (SBBO)
  - A common clinical condition due to an increase in the level of microorganisms, >10^6 cfu/ml of intestinal aspirate, and/or colonic-type bacteria within the small intestine.
- Probiotic strains
  - *Bacillus clausii*: normalization of hydrogen glucose breath tests in 20-75% of SIBO patients

Gabrielli M *et al* Am J Gastroenterol 2009;104:1327-8

  - *Lactobacillus plantarum* 299V and *Lactobacillus* GG: successful treatment in 6 patients with short bowel syndrome and SBBO, who did not respond to antimicrobial therapy.

Vanderhoof JA *et al* JPGN 1998;27(2):155-60

Rheumatoid arthritis

- 46 patients with RA (double-blind RCT)
  - Probiotic group: 10^8 CFU of *Lactobacillus casei* 01 for 8 wk
- Results
  - Significantly decreased disease activity score ($P < 0.01$).
  - significantly decreased 3 serum proinflammatory cytokines (TNF-α, IL-6, and IL-12) in the probiotic group ($P < 0.05$)
  - Increased serum level of regulatory cytokine (IL-10) and the IL-10/IL-12 ratio by the supplementation ($P < 0.05$).
- *L. casei 01* supplementation improved the disease activity and inflammatory status of patients with rheumatoid arthritis in which the gut microbiota is altered. (double-blind RCT)

Vaghef-Mehrabany E et al Nutrition 2014 Apr;30(4):430-5.

Helicobacter pylori infection

- As an adjuvant therapy
- Eradication of *H. pylori* by triple therapy
  - better in multi-strain group (*L. acidophilus, B. lactis*) but non-significant

    Cremonini F *et al* 2002;97(11):2744-9
  - better in probiotic group (milk-based fruit drink containing *L. GG, L. rhamnoss, B. breve, Propionibacterium shermanii*)

    Myllyluoma E *et al* 2005;21(10):1263-72
  - better in *L. reuteri* combination (*Lactobacillus reuteri* DSM 17938 and *L. reuteri* ATCC PTA 6475) but non-significant

    Francavilla R *et al* J Clin Gastroenterol 2014;48(5):407-13.
  - better in *L. acidophilus* and *B. bifidum* supplementation

    Wang YH *et al* World J Microbiol Biotechnol 2014 Mar;30(3):847-53.
- Reduced incidence of diarrhea and side-effects of antibiotic treatment

  Chapman CM *et al* 2011 Eur J Nutr 50:1-17

Post-operative bacterial infections

- Reduce post-operative bacterial infections
  - pre-op probiotic tx > post-op probiotic tx
  - pre-op + post-op probiotic tx > post-op probiotic tx alone

Sanders ME *et al* 2010 gut Microbes 1(3):164-85

Table 1. Effects of probiotic treatment in 7 randomized controlled trials in surgical patients with a high risk of post operative bacterial infections

	Category	Length of therapy (days)	Treatment	n	Control group	n	Infection rate (probiotic vs. control)
Post-operative treatment							
203	Major abdominal surgery (liver gastric, pancreas colon)	5 post	10^9 *L. plantarum* 299 + oat fiber	30	10^9 heat killed *L. plantarum* 299 + oat fiber	30	10% vs. 10% (n.s.)
134	Liver transplantation	12 post	10^9 *L. plantarum* 299 + oat fiber	31	10^9 heat killed *L. plantarum* 299 + oat fiber	32	13% vs. 34% (n.s.)
204	Liver transplantation	14 post	Synbiotic 2000 (10^{10} of 4 different LAB and 4 fibers)	33	Fibers only	33	3% vs. 48% ($p = <0.0001$)
205	Hepatectomy	14 post	10^8 *B. breve*, 10^8 *L. casei* + enteral feeding	21	Enteral feeding	23	19% vs. 52% ($p = 0.03$)
Peri-operative treatment							
206	PPPD	1 pre 8 post	Synbiotic 2000 (10^{10} of 4 different LAB and 4 fibers)	40	Fibers only	40	12.5% vs. 40% ($p = 0.01$)
207	PPPD	3–15 pre 10 post	*E. faecalis, C. butyricum, Bacillus mesentericus*	30	No treatment	34	23% vs. 53% ($p = 0.02$)
38	Hepatectomy	14 pre 14 post	10^{10} *B. breve*, 10^{10} *L. casei* (perioperatively)	41	10^{10} *B. breve*, 10^{10} *L. casei* (post operatively only)	40	12.1% vs. 30% ($p = 0.049$)

PPPD, pylorus-preserving pancreaticoduodenectomy; post, post-operatively; pre, pre-operatively; L, Lactobacillus; B, Bifidobacterium; E, Enterococcus; C, Clostridium. p values were calculated using Fisher's exact test.

Non-alcoholic liver disease (NAFLD)

- A 4-month supplement of **VSL#3** (*Lactobacillus casei, L. plantarum, L. acidophilus*, *L. delbrueckii subsp. bulgaricus*, *Bifidobacterium longum*, *B. breve, B. infantis*, and *Streptococcus salivarius subsp. thermophilus*) significantly improves non-alcoholic fatty liver disease (NAFLD) in children.
- The VSL#3-dependent glucagon-like peptide-1 increase could be responsible for these beneficial effects. (double-blind RCT)

Alisi A *et al* 2014 Jun;39(11):1276-85.

Maternal GBS colonisation

Table 4: Quantitative GBS Results in Colony Counts at Three Study Visits Compared with 36±2 Weeks Qualitative GBS Prenatal Culture

Study group	Subject number	29±2 weeks (study baseline) Vaginal	29±2 weeks (study baseline) Rectal	32 ±2 weeks Vaginal	32 ±2 weeks Rectal	36 ±2 weeks Vaginal	36 ±2 weeks Rectal	36 ±2 weeks qualitative prenatal culture
Probiotic	1							*
	2							
	3							
	4	1.0×10^3	2.0×10^4		2.0×10^2		2.0×10^2	Positive
	5							
	6			2.0×10^5		2.0×10^5		Positive
	7							
	8		1.63×10^5		3.5×10^5		3.5×10^5	
	9							
	10							
Control	11							
	12	5.5×10^4	3.3×10^4		7.0×10^2		7.0×10^2	Positive
	13							
	14							
	15							
	16							
	17	3.4×10^4	9.0×10^2		2.07×10^5		2.07×10^5	Positive
	18			7.0×10^2		7.0×10^2		
	19				1.6×10^4		1.6×10^4	
	20							

Note. *Blank cell = Negative at 10^2 Colony Forming Units/Swab

- Oral prenatal probiotic (**Florajen3**: *Lactobacillus acidophilus*, *Bifidobacterium lactis, Bifidobacterium longum)* therapy potentially reduced maternal group B *Streptococcus* vaginal and rectal colonization. (an open-label, two-group quasi-experiment)

Hanson L *et al* J Obstet Gynecol Neonatal Nurs 2014;43(3):294-304.

Prevention of recurrent urinary tract infection

- 100 young women with a history of recurrent UTI received antimicrobials for acute UTI
  - Recurrent UTI occurred in 7/48 15% of women receiving **Lactin-V** (*Lactobacillus crispatus* CTV-05) QD x 5d & QW x 10wk compared with 13/48 27% of women receiving placebo (relative risk, 0.5; 95% confidence interval, 0.2-1.2).
  - High-level vaginal colonization with *L. crispatus* (≥10^6 16S RNA gene copies per swab) throughout follow-up was associated with a significant reduction in recurrent UTI only for Lactin-V

Stapleton AE et at Clin Infect Dis 2011 May;52(10):1212-7.

- 128 infants with primary vesicoureteral reflux (VUR) were prospectively randomized into a probiotic (n = 64, *Lactobacillus acidophilus*, 1.0×10^8 CFU/g) or antibiotic (n = 64, trimethoprim/sulfamethoxazole, 2/10 mg/kg) group.
  - The **incidence of recurrent UTI** in the probiotic group was **slightly lower** than in the antibiotic group without statistical significance (32.8 % [21 out of 64] vs 40.6 % [26/64]) (P = 0.348).
  - The **incidences of antibiotic resistance** of causative organisms in recurrent UTI were **significantly lower** in the probiotic group than in the antibiotic group.

Lee SJ et al Pediatr Nephrol 2014 Oct 30. [Epub ahead of print]

Cow's milk protein allergy?

- **Probiotics: a novel approach in the management of food allergy.**
  - 31 infants with atopic eczema who were removed from exposure to cow milk and were given either *Lactobacillus* GG or a placebo
  - Treatment with *Lactobacillus* GG resulted in a significant improvement in their conditions that was not observed in the placebo group.

Majamaa H *et al* J Allergy Clin Immunol 1997;99(2):179-85

- Supplementation of *Lactobacillus casei* CRL431 and *Bifidobacterium lactis* Bb-12 to extensively hydrolyzed formula **does not** accelerate cow's milk tolerance in infants with cow's milk protein allergy.

Hol J *et al* J Allergy Clin Immunol 2008;121:1448-54

Constipation?

- No evidence to recommend the use of probiotics in the children with constipation.

Szajewska W *et al* JPGN 2006;42:454-75

- *Lactobacillus casei rhamnosus* Lcr35 were effective in treating children with chronic constipation (vs placebo group, n = 9), with no significant difference in the efficacy between MgO (n = 18) and Lcr35 (n = 18), but less abdominal pain when using Lcr35.

Nu LN *et al* Pediatrics International 2007;49:485-90

- Probiotics may improve whole gut transit time, stool frequency, and stool consistency, with subgroup analysis indicating beneficial effects of *Bifidobacteria lactis* in particular.

Dimidi E *et al* Am J Clin Nutr 2014 Oct;100(4):1075-84

- Compared with lactulose alone, lactulose plus **Protexin** (7 probiotic bacteria including *Lactobacillus casei* PXN 37, *Lactobacillus rhamnosus* PXN 54, *Streptococcus thermophiles* PXN 66, *Bifidobacterium breve* PXN 25, *Lactobacillus acidophilus* PXN 35, *Bifidobacterium infantis* (child specific) PXN 27, and *Lactobacillus bulgaricus* PXN 39) for 4 weeks increased the stool frequency and improved stool consistency at the end of 4th week. (48 children aged 4–12 years with constipation)

Sadeghzadeh M *et al* Int J Pediatr 2014; 2014:937212.

Colon cancer?

- Administration of *Lactobacillus casei* was tested as a method to prevent the occurrence of colorectal tumours.

Ishikawa H *et al* 2005 Int J Cancer 116:762-7

- In a 12-week clinical trial, polypectomized patients were treated with *Lactobacillus rhamnosus* GG (*LGG*) and *Bifidobacteria lactis Bb12* (*BB12*) and oligofructose-enriched inulin.
  - significantly reduced colorectal proliferation in the patients

Rafter J *et al* 2007 Am J Clin Nutr 85:488-96

- A cohort study with 12 years of follow-up on 45,241 volunteers
  - High yogurt intake was significantly associated with decreased colorectal cancer risk

Pala V *et al* 2011 Int J Cancer 129(11):2712-9

Obesity?

- Potential mechanisms might allow gut microbes to interact with the host's tissues and to regulate energy metabolism.
  - Increase in LPS levels (metabolic endotoxemia) occurs in individuals with obesity: specific components of the gut microbes could trigger metabolic disorders
  - Gut microbiota influences energy metabolisms of the host: certain molecular targets (ANGPTL4, GPR43/41, GLP-2 and the intestinal endocannabinoid system) might be involved in the control of obesity and obesity-related disorders.

Delzenne NM *et al* Nat Rev Endocrinol 2011 Aug 9;7(11):639-46

- *Lactobacillus rhamnosus* CGMCC1.3724 formulation helps obese women to achieve sustainable weight loss. (double-blind RCT)

Sanchez M et al Br J Nutr 2014 Aoru 28;111(8):1507-19.

HIV infection (1)

- GI tract: a site of early HIV replication and CD4+ cell destruction
  - The intestinal microbiota of HIV patients contain higher levels of pathogens (e.g. *Pseudomonas aeruginosa*, *Candida albicans*) and reduced or undetectable levels of *Bifidobacterium* and *Lactobacillus* species.

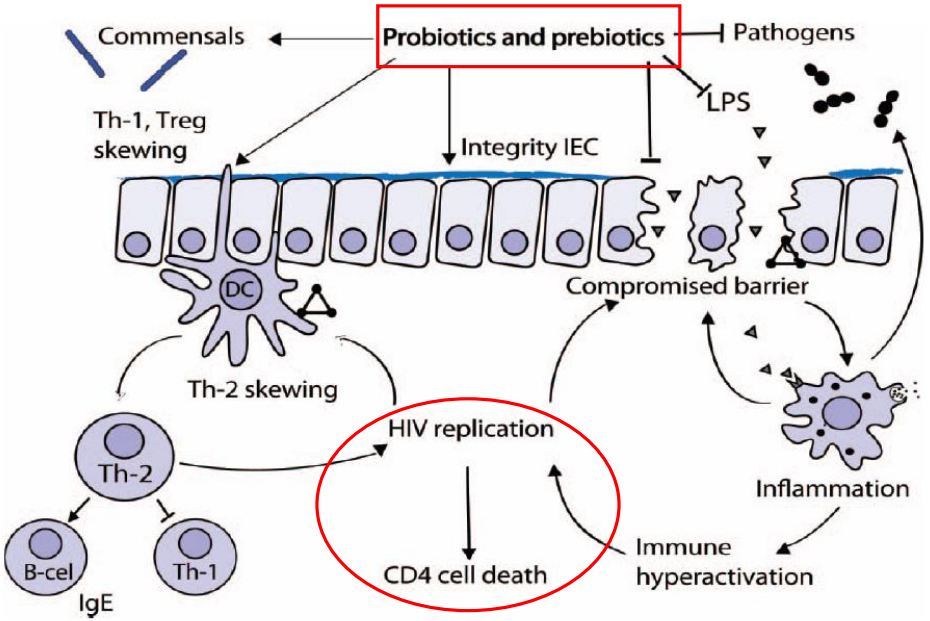


FIGURE 2 Potential benefits of probiotics and prebiotics in HIV-induced intestinal pathogenesis: HIV infection induces effects and positive feedback mechanisms that induce a loss of intestinal homeostasis and promote replication of the virus (triangles). Pro- and prebiotics may ameliorate the HIV-induced intestinal problems through effects on the microbiota and its metabolism, on various cells of the immune system (as represented by the arrow pointing at the sampling DC), and on intestinal epithelial cells.

Hummelen R *et al* Int Rev Immunol 2010;29:485-513

HIV infection (2)

- In human clinical studies, probiotics have been applied to reduce bacterial translocation
  - RCT of 77 children in Brazil ⇨ *B. bifidum* + *S. thermophilus* x 2 mo vs placebo: an increase of 118 $CD4^+$ cells/μl vs a decrease of 42 $CD4^+$ cells/μl

    Trois L *et al* J Trop Pediatr 2008;54:19-24
  - RCT of 24 HIV patients in Nigeria ⇨ *L. rhamnosus* GR-1 + *L. reuteri* RC-14 x 4 wk vs placebo: an increase of 6.7 $CD4^+$ cells/μl vs a decrease of 2.2 $CD4^+$ cells/μl

    Anukam KC *et al* J Clin gastroenterol 2008;42:239-43
  - A large RCT of 795 children in Malawi ⇨ synbiotic 2000 Forte (10^{10} CFU of each of *Pediococcus pentoseceus* 5–33:3, *Leuconostoc mesenteroides* 32–77:1, *L. paracasei* ssp paracasei 19 and *L. plantarum* 2362) on malnutrition: included 361 HIV-infected children; no improved nutritional cure, but an overall reduction in outpatient mortality

    Kerac M *et al* Lancet 2009;374:136-44
- Safety
  - To date: 5 cases of lactobacillemia in end-stage AIDS p'ts ⇨ 3 with central venous catheters, 1 with pneumonia; all had extremely low CD4 counts (<55 $CD4^+$ cells/μl)
  - No indication exists to avoid oral probiotic use in HIV populations, but close monitoring of safety parameters is recommended.

    Hummelen R *et al* Int Rev Immunol 2010;29:485-513

Trends of development in probiotics

- Principal treatment or adjuvant therapy
- Strains
  - Strain specificity
- Preparations
  - How to stabilize the strain(s) in the preparations? Which mode of administration is better?
  - Rectal enema, fermented milks/yogurt, dried powder in capsules
- Dosage, frequency, and duration
- Mixtures or single strains
  - Mixtures of probiotics had beneficial effects on irritable bowel syndrome (IBS), gut function, diarrhoea, atopic disease, immune function, and respiratory tract infections, gut microbiota modulation, and treatment of *H. pylori* infection
  - Unclear whether due to synergistic interactions between strains or a consequence of higher dose

Chapman CM *et al* 2011 Eur J Nutr 50:1-17

- Safety assessment

Conclusions

- More and more clinical indications for probiotics are being proposed, but
  - most of them require further large double-blind randomized controlled clinical trials following *in vitro* and animal studies.
- When applied as biotherapeutic agents, probiotics should be more specifically categorised for different clinical indications according to:
  - strains
  - doses (usually >10^8 CFU/day, better 10^9-10^{10} CFU/day particularly for treatment of acute gastroenteritis)
  - preparations (yogurt, capsules, powders, enema, etc)
  - timing of supplementation (for prevention, or for treatment)
  - co-treatment with probiotic strains (mixed strains) or other medications (as an adjuvant).

Thank you for your attention

臺北醫學大學部立雙和醫院 (臺灣新北市中和區)