World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i10.1527

World J Gastroenterol 2013 March 14; 19(10): 1527-1540
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Actual concept of "probiotics": Is it more functional to science or business?

Michele Caselli, Francesca Cassol, Girolamo Calò, John Holton, Giovanni Zuliani, Antonio Gasbarrini

Michele Caselli, School of Gastroenterology, University of Ferrara, I-44121 Ferrara, Italy
Francesca Cassol, Department of Gastroenterology, S Anna Hospital, I-44121 Ferrara, Italy
Girolamo Calò, Department of Experimental and Clinical Medicine, Section of Pharmacology, University of Ferrara, I-44121 Ferrara, Italy
John Holton, Department of Health and Social Science, University of Middlesex, London, NW4 4BT, United Kingdom
Giovanni Zuliani, Department of Clinical and Experimental Medicine, Section of Internal Medicine, Gerontology and Geriatrics, University of Ferrara, I-44121 Ferrara, Italy
Antonio Gasbarrini, Division of Internal Medicine and Gastroenterology, Catholic University of Rome, I-00168 Rome, Italy
Author contributions: Caselli M and Cassol F conceived the study and drafted the article; Holton J revised the article and revised the microbiological aspects; Calò G contributed to acquisition and analysis of data; Zuliani G and Gasbarrini A revised the article and critically revised the clinical aspects.
Correspondence to: Francesca Cassol, MD, Department of Gastroenterology, S Anna Hospital, 44121 Ferrara, Italy. cassol.francesca@libero.it
Telephone: +39-348-4121382 Fax: +39-532-762096
Received: November 10, 2012 Revised: December 18, 2012
Accepted: January 11, 2013
Published online: March 14, 2013

Abstract

It is our contention that the concept of a probiotic as a living bacterium providing unspecified health benefits is inhibiting the development and establishment of an evidence base for the growing field of pharmacobiotics. We believe this is due in part to the current regulatory framework, lack of a clear definition of a probiotic, the ease with which currently defined probiotics can be positioned in the market place, and the enormous profits earned for minimum investment in research. To avoid this, we believe the following two actions are mandatory: international guidelines by a forum of stakeholders made available to scientists and clinicians, patient organizations, and governments; public research funds made available to the scientific community for performing independent rigorous studies both at the preclinical and clinical levels.

Key words: Probiotics; Market; Regulations; Guidelines; Metanalysis

Caselli M, Cassol F, Calò G, Holton J, Zuliani G, Gasbarrini A. Actual concept of "probiotics": Is it more functional to science or business? *World J Gastroenterol* 2013; 19(10): 1527-1540 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i10/1527.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i10.1527

INTRODUCTION

Probiotics are generally defined as live microorganisms, preferentially of human origin, that upon ingestion in specific and sufficient numbers confer unspecified health benefits to the host. During the last twenty years the therapeutic potential of probiotic bacteria has been evaluated in a large number of basic, experimental and clinical studies[1-3] and their use in different clinical conditions has received considerable scientific and commercial attention.

Today probiotics represent a very big business. The global functional food market has been recently estimated at up to $50 billion annual share[4], while the world probiotic market is estimated at $15 billion. Today, this market is growing at a pace of 5%-30% depending on the country and product type[5]. The marketing agency Frost and Sullivan believes that the possibility to use salutistic indications on the label of the products containing probiotics, as permitted according to CE 1924/2006 rules, can further increase the consumer interest. Proper communication paired with effective marketing strategies will prove to be useful to this aim. Consumer acceptance varies greatly across Europe, with the most developed

market in Northern European and Scandinavian countries, having a long traditional consumption of probiotic products[5]. The existing consumer confusion over the different probiotic strains as well as skepticism about their efficacy donot seem sufficient to counteract the salutistic propaganda of the media advertisements.

The regulatory status for probiotic products is not well established at international level yet. The United States Food and Drug Administration apply a conceptual distinction among "medical foods", "dietary supplements", "drugs" and "biological products" to probiotic products. The regulatory consequences that accompany a probiotic product that is categorized as a dietary supplement obviously dramatically differ from those that accompany a probiotic product categorized as a drug. If the probiotic product meets the definition of dietary supplement, the manufacturers may place the probiotic product on the market without any pre-market approval and may market the product with claims concerning the effect that the product has on the structure or function of the body. The European Commission has recognized probiotic bacteria as having the status of nutrients; in addition probiotics in powder, capsule or tablet form are in most European countries regarded as "food supplements" but with important differences: according to Bianca Herr of the Leatherhead Food International, in Italy and in Hungary probiotic products are widely accepted as food supplements, in Germany these products are accepted as food supplements in some cases but their acceptance as drugs depends on their concentration, while in Spain there is no specific legislation or guidance for probiotic products. Thus, in most cases, these products reach the market without being tested in the expensive three phases required for approval of a new drug. For these reasons not only big pharma and manufacturers of probiotics but also national pharmaceutical industries and even family farms are involved in this market. Also, the work of the European Food Safety Authority regarding claims made on food labeling and advertising concerning nutrition and health provides an important but very partial solution to the problem.

One would expect that the available scientific evidence is comparable to the size of this market; however, this is certainly not the case. Food And Agriculture Organization and World Health Organization defined the following characteristics of probiotic microorganisms: (1) probiotics should be taxonomically classified and deposited in an internationally recognized culture collection; (2) they have to remain viable and stable after culture, manipulation, and storage before consumption; (3) they have to survive to gastric acid and biliary and pancreatic digestion; (4) they have to induce a host response once ingested by adhering to gut epithelium or by other mechanisms; (5) they have to yield a functional or clinical benefit to the host when consumed; and (6) finally they have to be safe, not only regarding the assessment of side effects, but also in relation to antibiotic resistance patterns. In fact beneficial bacterial populations may play a role in the transfer of antibiotic resistance to pathogenic and opportunistic bacteria. These general rules are certainly meaningful but not sufficient as guidelines for this field. Although there are few international organizations that purport to be independent of industry, such as International Scientific Association for Probiotics and Prebiotics (ISAPP), whose mission is to engender and disseminate information on high quality, multidisciplinary, scientific investigation in the field of probiotics, in actual fact there is no organization, agency or scientific network able to (1) reduce the incredible confusion related to every aspect of probiotics; (2) direct the rudder of basic and experimental research on probiotics and, in the future, on pharmacobiotics (a fundamental goal is to move away from the restrictive and perhaps outdated term "probiotics" and over to the more inclusive term "pharmacobiotic or pharmabiotic"); and (3) propose well accepted guidelines for evaluating these products in controlled clinical trials. To date variability is the keyword and includes every aspect of probiotics: strain, dose, route of administration, trial methodology, endpoints and outcomes. A very large number of probiotic strains have been used in clinical studies for the treatment of the same clinical condition, and the same strain of probiotics has been used to treat very different disease states. In addition an incredible large range of doses [from 4.5×10^2 colony-forming units (CFUs) to 3.6×10^{12} CFUs] of probiotics has been assayed in clinical trials. Furthermore, in different studies probiotics were administrated in a great variety of ways: capsules, powders, tablets, drops or yogurts. An equally great variability exists in methodology, endpoints and outcomes of clinical trials carried out so far, even limiting the analysis to a single clinical condition. As an example we summarized in Tables 1-3 the number of patients, duration of treatment, probiotic strains used, dose used and outcomes of clinical trials carried out on three adult clinical conditions in which probiotics have widely tested: irritable bowel syndrome, ulcerative colitis and Crohn's disease. The tables end with the indication of published meta-analyses. Despite the existence of guidance[6] and recommendations[7] for probiotic use in these intestinal diseases, it seems clear from the tables that the lack of homogeneity of the published studies does not allow to draw final conclusions and to generate, through an evidence-based approach, true guidelines useful for adult patients. This is corroborated by meta-analysis studies that recognize the variety of species, strains and doses of probiotic used associated to an evident heterogeneity of study methodologies as main limitations in the field.

This would not be accepted in clinical pharmacology. No drug can be approved for the market with a defined clinical indication without sufficient knowledge of its mode of action, pharmacokinetic parameters, toxicological features, tolerability and effectiveness. In addition this knowledge will be substantially increased by post-marketing surveillance. By contrast, probiotics are commonly commercialized and prescribed for spe-

Table 1 Results of clinical trials with probiotics in irritable bowel syndrome

Ref.	Patients (*n*)	Duration of therapy	Probiotic strains	Dose (CFU/d)	Outcomes
Maupas *et al*[88]	34	1 mo	*Saccharomyces boulardii*	9×10^9	Improved stool number and consistency
Gade *et al*[89]	54	1 mo	Paraghurt (*Streptococcus faecium*)	1×10^{12}	Improved symptoms
Halpern *et al*[90]	18	4 mo	*Lactobacillus acidophilus*	2×10^{10}	Improved symptoms
O'Sullivan *et al*[91]	25	1 mo	*Lactobacillus GG*	1×10^{10}	No benefit
Nobaek *et al*[92]	60	1 mo	*Lactobacillus plantarum 299V* Pro-Viva®	5×10^7	Improved global symptoms
Niedzielin *et al*[93]	40	1 mo	*Lactobacillus plantarum 299V* Pro-Viva®	2×10^{10}	Improved global symptoms
Kim *et al*[94]	25	2 d-IBS	VSL3®	9×10^{11}	Reduced bloating
Tsuchiya *et al*[95]	68	3 mo	*Lactobacillus acidophilus* *Lactobacillus helveticus* *Bifidobacterium*	1.5×10^6 1.3×10^9 4.95×10^9	Improved symptoms
O'Mahony *et al*[96]	80	2 mo	*Bifidobacterium longum subspecies infantis vs Lactobacillus salivarius*	1×10^{10}	*B. infantis*: improved global symptoms and anti-inflammatory cytokine profile *Lactobacillus salivarius*: no benefit
Kajander *et al*[97]	103	6 mo	Mixture (2 strains of *Lactobacillus rhamnosus*, *Bifidobacterium breve*, *Propionibacterium freudenreichil*)	$8\text{-}9 \times 10^9$	Improved global symptoms
Bittner *et al*[98]	25	0.5 mo	29 bacteria + prebiotic Prescript-Assist®	2.6×10^8	Improved wellbeing
Sen *et al*[99]	12	1 mo	*Lactobacillus plantarum 299V* Pro-Viva®	5×10^7	No benefit; Study design flawed
Bausserman *et al*[100]	50	1.5 mo	*Lactobacillus GG*	2×10^{10}	No benefit
Niv *et al*[101]	39	6 mo	*Lactobacillus GG*	2×10^8	No benefit Francis severity IBS score
Kim *et al*[102]	48	1 or 2 mo	VSL3®	8×10^9	Reduced flatulence, retarded colonic transit
Whorwell *et al*[103]	362	1 mo	*Bifidobacterium longum* *subspecies infantis* 35 624 in 3 doses	1×10^6 1×10^8 1×10^{10}	Improved global symptoms
Long *et al*[104]	60	0.5 mo	*Bifidobacterium*	2×10^8	Symptoms resolved
Gawrońska *et al*[105]	104	1 mo	*Lactobacillus GG*	6×10^9	Reduced frequency of pain
Moon *et al*[106]	34	1 mo	*Bifidobacterium subtilis*, *Streptococcus faecium*	750 mL/d, CFU/d not given	Reduced frequency pain
Marteau *et al*[107]	116	1 mo	Lactibiane® (4 strains of *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus lactis*, *Streptococcus thermophilus*)	1×10^{10}	Reduced pain Increased colonic transit in those with constipation
Simrén *et al*[108]	76	1.5 mo	*Lactobacillus plantarum 299V*	2×10^9	No benefit
Simrén *et al*[109]	118	2 mo	*Lactobacillus paracasei ssp paracasei*	2×10^{10}	No benefit
Guyonnet *et al*[110]	274	1.5 mo	*Bifidobacterium animalis*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*	1.25×10^{10} 1.2×10^9	Improved bloating and constipation
Drouault-Holowacz *et al*[111]	116	1 mo	*Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus lactis*, *Streptococcus thermophilus*	1×10^{10}	Not significant in relieving symptoms
Sinn *et al*[112]	40	1 mo	*Lactobacillus acidophilus*	2×10^8	Improved abdominal pain and discomfort
Enck *et al*[113]	297	1 mo	*Escherichia coli*, *Enterococcus faecalis*	4.5×10^2	Improvement in pain
Hun *et al*[114]	44	2 mo	*Bacillus coagulans*	8×10^8	Improvement abdominal pain and bloating
Dolin *et al*[115]	61	2 mo	*Bacillus coagulans*	2×10^9	Diminution of diarrhea
Ligaarden *et al*[116]	16	1 mo	*Lactobacillus plantarum*	10^{10}/L	Worsening of symptoms
Moayyedi *et al*[117]	19 randomised controlled trials in 1650 patients				Probiotics appear to be efficacious but the magnitude of benefit and the most effective strains are uncertain

CFU: Colony-forming unit; IBS: Irritable bowel syndrome.

cific clinical indications in the absence of any conclusive proof concerning their putative pharmacological properties. Finally, it should be remembered that the safety of probiotics should not be considered a foregone datum: in abdominal surgery, translocation of bacteria from the gastrointestinal tract through the mucosa could occur[8], and probiotic treatment has been associated with increased mortality in patients with acute pancreatitis[9].

Only a few cases based on studies regarding pediatric population formal meta-analyses have been used to generate clinical guidelines. These studies demonstrated beneficial effects of probiotics in acute diarrhea of children. These effects are strain- and dose-dependent, being generally greater with doses > 10^{10} CFUs, highly significant for watery diarrhea and viral gastroenteritis but less so for invasive bacterial diarrhea, more evident when the treatment is started early in the course of disease, and more evident in children living in developed than in developing countries[10]. In May 2008, probiotics were for the first time included in a guideline document named "the guidelines for the management of acute gastroenteritis" and produced by a Committee of the European

Table 2 Results of clinical trials with probiotics in ulcerative colitis

Ref.	Patients (*n*)	Duration of therapy	Probiotic strains	Dose (CFU/d)	Outcomes
Kruis *et al*[118]	120	12 wk	*Escherichia coli* Nissle 1917	50×10^{10}	Maintaining the remission (similar to 5-ASA)
Rembacken *et al*[119]	116	1 yr	*Escherichia coli* Nissle 1917	5×10^{10}	Induction of remission (similar to 5-ASA); maintaining of relapses (similar to 5-ASA)
Venturi *et al*[120]	20	1 yr	VSL3®	5×10^{11}	Maintaining the remission
Ishikawa *et al*[121]	21	1 yr	Milk with bifidobacteria	10×10^{8}	Maintaining the remission
Guslandi *et al*[122]	25	4 wk	*Saccharomyces boulardii*	250 mg × 3	Induction of remission
Kruis *et al*[123]	327	1 yr	*Escherichia coli* Nissle 1917	$2.5\text{-}25 \times 10^{9}$	Induction of remission (5-ASA better than probiotic)
Tursi *et al*[124]	90	8 wk	Balsalazide/VSL3®	900×10^{8}	Induction of remission
Cui *et al*[125]	30	8 wk	Bifidobacteria	1.26 g/d	Maintaining of remission
Kato *et al*[126]	20	12 wk	*Bifidobacterium*-fermented milk *vs* placebo	10^{9}	CDAI lower in Bifidobacterium fermented milk that in placebo
Furrie *et al*[127]	18	4 wk	*Bifidobacterium longum* + prebiotic (Synergy 1)	4×10^{11}	Induction of remission
Bibiloni *et al*[128]	32	6 wk	VSL3®	1800 billion × 2	Induction of remission
Zocco *et al*[129]	187	12 mo	*Lactobacillus GG vs* mesalazina	18×10^{9}	No difference between the treatment groups
Henker *et al*[130]	34	12 mo	*Escherichia coli* Nissle 1917	5×10^{10}	Maintenance of remission
Miele *et al*[131]	29	12 mo	VSL3®	$450\text{-}1800 \times 10^{9}$	Induction of remission (92.8% in treated with VSL3® and 36.4% in the placebo group)
Sood *et al*[132]	147	12 wk	VSL3®	3.6×10^{12}	Induction of remission (42.9% against 15.7% in the placebo group)
Matthes *et al*[133]	57	4 wk	*Escherichia coli* Nissle 1917	$10\text{-}40 \times 10^{8}$	Induction of remission
Sang *et al*[134]	13 RCTs				Heterogenity between the studies in their methodology and results

5-ASA: 5-aminosalicylic acid; CDAI: Crohn's disease activity index; CFU: Colony-forming unit; RCTs: Randomised controlled trials.

Table 3 Results of clinical trials with probiotics in patients with Crohn's disease

Ref.	Patients (*n*)	Duration of therapy	Probiotic strains	Dose (CFU/d)	Outcomes
Malchow *et al*[135]	24	3 mo	*Escherichia coli* Nissle 1917	2.5×10^{10}	Maintaining the remission
Guslandi *et al*[136]	32	6 mo	*Saccharomyces boulardii*	1 g	Postsurgical prevention of CD recurrence (relapse rate probiotic+ 5-ASA *vs* 5-ASA alone)
Prantera *et al*[137]	45	1 yr	*Lactobacillus GG*	12×10^{9}	Postsurgical prevention of CD recurrence (no effects)
Schultz *al*[138]	11	6 mo	*Lactobacillus GG*	2×10^{9}	Probiotics are not superior to placebo in maintaining remission
Bousvaros *et al*[139]	75	1 yr	*Lactobacillus GG*	2×10^{10}	Probiotics are not superior to placebo in maintaining remission
Marteau *et al*[140]	98	6 mo	*Lactobacillus johnsonii*	4×10^{9}	Postsurgical prevention of CD recurrence (recurrence rate decreased *vs* placebo)
Chermesh *et al*[141]	30	24 mo	Synbiotic 2000 (*Pediococcus pentoseceus*, *Lactobacillus raffinolactis*, *Lactobacillus paracasi* susp paracsei, *Lactobacillusplantarum 2362*) and 4 fermentable fibers *vs* placebo	10^{11}	Postsurgical prevention of CD recurrence (NS)
Van Gossum *et al*[142]	70	12 wk	*Lactobacillus johnsonii* or placebo	10^{10}	Postsurgical prevention of CD recurrence (NS)
Rolfe *et al*[143]	7 RCTs				No benefit of probiotics in the maintenance of remission of CD
Rahimi *et al*[144]	8 RCTs				None benefit for probiotic treatment in the maintenance of clinical remission of CD

RCTs: Randomised controlled trials; CD: Crohn's disease; 5-ASA: 5-aminosalicylic acid; CFU: Colony-forming unit; NS: Not significant.

Society for Pediatric Infectious Diseases[11]; this guideline document was developed through an evidence based systematic review approach that incorporates tables of evidence with their grading. The guidelines state that only the use of probiotic strains with proven efficacy and in appropriate doses is suggested for the management of acute diarrhea in European children as an adjunct to rehydration therapy. The evidence of efficacy regards only two strains: *Lactobacillus rhamnosus GG* was rated as 1A and *Saccharomyces boulardii* was rated as 2B, corresponding to the level of evidence based respectively on meta-analysis of randomised controlled trials (RCTs) and properly designed RCTs of appropriate size. This evidence is actually confined only to the prevention/treatment of childhood acute gastroenteritis and of antibiotic-associated diarrhea. In the few conditions in which selected probiotic bacteria have shown a proven efficacy competitive mechanisms or mechanisms of restoration of bacteria flora seem to be involved. No final evidence is available in other conditions or diseases in

which probiotic agents are largely used. It appears evident that the tremendous dichotomy between the huge market of probiotic products and the insufficient knowledge of probiotic-based therapies. This would be unacceptable for any other pharmacological treatment.

We believe that some important fields of research exist that should be encouraged due to the possibility of getting information of incommensurate value in the near future. These fields of investigation will possibly permit development of a new concept of "probiotic agents"[12,13], and should be adequately investigated.

A NEW CONCEPT OF "PROBIOTICS"

The relationship between probiotic agents and innate immune system

In recent years there have been tremendous advances in our understanding of the structure and function of signal receptors, and the pivotal role of pattern recognition receptors (PRRs) and cells of the innate immune system in processing bacterial and food components is now well established[14-17]. PRRs include trans-membrane Toll-like receptors (TLRs) and Dectin- I ; endosomal PRRs (TLR 3, 7/8 and 9); and cytosolic nucleotide oligomerization domain (NOD)-like receptors: (NOD1 and NOD2), Rig-1-like RNA helicase receptor (retinoic acid-inducible gene-1 and iron-regulated surface determinant sensors). The cells involved are dendritic cells (DC), intraepithelial lymphocytes, macrophages, neutrophils and enterocytes. At this level microorganisms are recognized only as microorganism-associated molecular patterns (MAMPs). MAMPs are first recognized by a PRR, and activation of the receptor by binding of the MAMP sequentially activates intracellular molecules such as the cytoplasmic adapter molecule MyD88, leading to the activation of transcription factors including nuclear factor-κ B (NFκB) and activator protein-1 (AP-1), which are required for gene transcription and cytokine synthesis. The different receptors of the innate immune system are obviously only able to process specific molecular components of microorganisms and foods, whereas the recognition of a whole bacterium or food does not appear possible although simultaneous activation of several PRR's may be characteristic of a specific organism or food and lead to a different outcome than activation by single PRR. For example, studies on host mucosal gene expression following exposure to different whole bacteria have demonstrated up-regulation of different gene networks for each organism. Networks stimulated by these probiotic bacteria included cell proliferation, Th-1/Th2 balance, control of blood pressure, tissue development, water and ion regulation and wound healing. Major host differences were noted in the stimulated transcriptosome. The pathways stimulated by the whole organism corresponded to pathways stimulated by known pharmacological preparations. However, the specific molecules of the bacteria that caused these effects are currently unknown[18]. Further, whether the bacterium is alive or dead does not seem relevant for the recognition of a molecular pattern by specific PRRs. The accessibility of MAMPs for PRRs and the presence of other microbial effector molecules, such as toxins produced by pathogens, have a pivotal role in the modulation of host immune response. Other important factors determining the host response are host-derived direct or indirect negative regulators of PRR signaling.

To date pathogenic, probiotic and commensal bacteria are considered to induce different levels of immune response: a strong host response stimulated by pathogens, an intermediate response induced by probiotics and finally a homeostatic control of the response is triggered by commensal bacteria. An important exception to this concerns a restricted number of commensal bacteria, the prototype of which is the segmented *Filamentous Bacteria* (SFB), which could largely recapitulate and orchestrate a broad spectrum of B and T cell responses[19,20]. SFB colonized mice had low levels of ATP, suggesting that host sensing of SFB does not involve TLR or NOD receptors[21]. We have recently showed that the progressive penetration of the holdfast segments of these bacteria within the specialized epithelial cells of the terminal ileum could permit an impressive presentation of bacterial antigens directly to the lymphocytes contained in the lymphoid packets characteristic of the M cells and to antigen presenting cells[22].

It should also to be remembered that interactions between PRR and ligands are not as specific as those between antigens and antibodies, and ligands for PRR such as TLRs are generally present in repetitive structures to increase avidity.

Therefore, some very important and specific questions concerning immune-mediated probiotic activity are: (1) Are whole live bacteria essential to promote biological effects on the immune system? (2) Can the concept of probiotics be extended to include bacterial-derived molecular bioactive components? (3) Moreover, can probiotic molecules be also produced by non-probiotic bacteria? and (4) Finally, can probiotics be genetically manipulated to synthesize specific bioactive molecules?

Probiotic molecules

Bacterial DNA: Bacterial genomic DNA of probiotics in VSL-3™ induced a remarkable strain-specific immune response in humans as evaluated by the release of interleukin (IL)-1β, IL-6 and IL-10. Total bacterial DNA from faeces increased the Th-1 cytokine IL-1β more than IL-10 compared to DNA from the probiotic bacteria which had the reverse effect. However, total DNA from faeces, after being given a course of the probiotic bacteria, produced a greater stimulation of IL-10 compared to IL-1[23]. Notably, the respective role of IL-1β and IL-6 in the beginning and maintenance of a Th17 response is well known[24,25]. An important and provocative study[26] showed that in a mouse irritable bowel disease model the protective effects of probiotics contained in VSL-3 are mediated by their DNA rather than by their ability

Table 4 Reference studies concerning the probiotic role of bacterial DNA

Ref.	Outcomes
Lammers *et al*[23]	Bacterial DNA from faeces collected after VSL-3 administration modulated a decrease of IL-1β and an increase of IL-10
Rachmilewitz *et al*[26]	Study in a mouse IBD model: protective effects of probiotics contained in VSL-3 are mediated by their DNA and TLR9 signaling mediates anti-inflammatory effect
Iliev *et al*[27]	*Lactobacillus rhamnosus GG* DNA induces B-cell proliferation and activate DC
Ghadimi *et al*[28]	Bacterial DNA inhibited IL-4 and IL-5 secretion in different *Lactobacilli*
Ménard *et al*[30]	Study from 5 bifidobacterial strains: unmethylated CpG motifs are specific to bacterial DNA by activating TLR9

IL: Interleukin; IBD: Irritable bowel disease; TLR: Toll-like receptor.

to colonize the gut mucosa. TLR 9 signaling is essential in mediating the anti-inflammatory effects of probiotics. TLR-9 is an endosomal TLR which is known to interact with bacterial DNA upon bacterial lysis. The authors suggested that DNA-TLR9 signaling resulted in the differentiation of naive cluster of differentiation-4 (CD4) T lymphocytes into regulatory T cells, mediating the protective action. Another example of the immunomodulatory capacity of probiotic DNA is represented by DNA of *Lactobacillus rhamnosus GG* that induces B-cell proliferation and activates DCs[27]. More recently, the effects on the Th1/Th2 balance by genomic DNA of different probiotic bacteria (*Lactobacillus rhamnosus GG*, *Lactobacillus gasseri*, *Bifidobacterium bifidum*, *Bifidobacterium longum*) were compared with the effects of live bacteria by using peripheral blood mononuclear cells from healthy subjects and from patients with an allergy against the house dust mite[28]. Compared with live *Lactobacilli*, bacterial DNA inhibited IL-4 and IL-5 secretion in a similar way, and based on the maximal effects achieved with *Lactobacilli* and their DNA, more than 50% of these effects seem to be due to their DNA (Table 4).

The immunomodulatory activity of DNA is characterized by unmethylated CpG motifs which can activate innate immune responses through binding to TLR9 and triggers the translocation of NFκB and AP-1 from the cytoplasm to the nucleus thereby up-regulating gene expression pathways. Stimulatory oligodeoxynucleosides contain the CpG within a flanking region to give a motif of Pur-p-Pur-p-CpG-p-Pyr-p-Pyr. Typically more than one CpG is present in the immunostimulatory oligodeoxynucleoside and maximal effect occurs if they are separated by 1-2 base pairs. A 5' TpC and pyrimidine rich 3' ends also increases the immunostimulatory effects. In terms of a potential therapeutic, the *in-vivo* degradation can be decreased by synthesizing a phosporthiorate backbone which increases the stimulatory activity of the motif[29]. A very recent study based on entire genome sequences from five bifidobacterial strains[30] showed that *Bifidobacterium* genomes contained several CpG motifs and biologically active sequences previously identified in *Lactobacilli*. These bioactive sequences induced the production of monocyte chemotactic protein-1 and tumor necrosis factor-α (TNF-α) through a pattern of TLR-9 stimulation of macrophages. An inter- and intra-species investigation of 71 strains of *Bifidobacteria* of various origins showed that these bioactive DNA sequences were highly conserved in the genus. The results of these studies clearly suggest the necessity of further investigation.

Table 5 Reference studies concerning the probiotic role of molecules presented at the bacterial surface

Ref.	Outcomes
Mazmanian *et al*[33]	Bacterial capsular PSA elaborated by *Bacteroides fragilis* activates CD4+ and elicits cytokine production
Mazmanian *et al*[35]	Purified PSA suppress pro-inflammatory IL-17 production and protects from inflammatory disease by induction of IL-10
Ryu *et al*[36]	Purified LTA from Gram-positive bacteria has lower potency in the stimulation of Toll-like receptor-2 pathway to induce pro-inflammatory molecules.
Grangette *et al*[37]	Modified LTA is able to induce secretion of anti-inflammatory IL-10
Benz *et al*[39]	Lipoproteins and glycoproteins at the cell surface are attractive candidates as probiotic molecules
Schlee *et al*[40]	Flagellins of the *Escherichia coli* Nissle 1917 induces expression of human β-defensin 2
Matsumoto *et al*[83]	Purified PSPG- I from *Lactobacillus casei Shirota* has anti-inflammatory actions in chronic intestinal inflammatory disorders

PSA: Polysaccharide A; IL: Interleukin; LTA: Lipotheichoic acid; PSPG: Polysaccharide-peptidoglycan.

MOLECULAR PRESENT AT THE BACTERIAL SURFACE

Bacterial cells wall molecules are potential probiotic ligands that can interact with PRRs and induce signaling pathways resulting in probiotic effects (Table 5).

The immune system is able of recognizing any biological polymer constituting the bacterial cell wall and presenting it to T cells. Most probiotics are typically *Gram-positive bacteria*, in which the cell wall is composed of a thick peptidoglycan layer with proteins, theicoic acids and polysaccharides[31]. However few *Gram-negative probiotics* exist, such as *Escherichia coli* strain Nissle 1917; in this case the cell wall is composed of a thin peptidoglycan layer and an outer membrane which contains lipopolysaccharides (LPS) that is further decorated with the proteins and polysaccharides[32].

Although adaptive immune responses have been considered the territory of antigenic proteins or glycoproteins, whereas carbohydrates were considered as not recognized by the adaptive immune system, recent studies have revolutionized this assumption. Bacterial wall polysaccharides and glycolipids are now considered perhaps the more attractive targets in the research for immunomodulatory molecules. Interestingly, the bacterial capsular polysaccharide A (PSA), the most immunodominant among the zwitterionic polysaccharides elaborated by *Bacteroides*

fragilis, a commensal *Gram-negative anaerobe* that colonizes the mammalian lower gastrointestinal tract, has been demonstrated to be the archetypal bacterial molecule capable of mediating development of the host immune system[33]. PSA presented by intestinal DCs activates CD4+ T cells and elicits appropriate cytokine production. *Bacteroides* species are among the earliest colonizing and most represented microorganisms of the gut microbiota[34], and they are not considered probiotic species. More recently Mazmanian *et al*[35] showed that the *Bacteroides fragilis*-produced PSA protects mice from experimental colitis induced by *Helicobacter hepaticus*: purified PSA is required to suppress pro-inflammatory IL-17 production by intestinal T cells, and it also protects from inflammatory disease by induction of IL-10-producing CD4+ T cells. Therefore, although bacteria may have developed polysaccharide capsules known to be not recognized by the immune system, it may be that the host not only tolerates but also has evolved to require cooperation by commensal bacteria for its health. Strikingly, the finding that PSA from *Bacteroides fragilis* is a natural anti-inflammatory molecule that promotes health, so clearly performing important probiotic activities, is not produced by a probiotic bacteria, provides a fundamental platform for the discovery of new biomolecules having important probiotic effects independently from their bacterial derivation.

Polysaccharides synthesized extracellularly (exopolysaccharides, EPSO) also represent attractive candidates as probiotic effector molecules interacting witch PRRs. EPSO are produced by both probiotic and symbiotic bacteria, and also potentially pathogenic bacteria, but they have not yet been studied in detail.

On the other hand, lipoteichoic acid (LTA) is considered the major immunostimulating component of the cell wall of *Gram-positive bacteria via* TLR 2 (most of the known probiotics, *Lactobacilli* and *Bifidobacteria*, are *Gram-positive bacteria*), in the same way as LPS is the major immunostimulating component in the cell wall of Gram-negative bacteria *via* TLR 4. Two important concepts concerning LTA have emerged in recent years: the first concerns the much lower potency in the stimulation of TLR 2 pathway to induce pro-inflammatory molecules by using purified LTA from a probiotic strains of *Lactobacillus plantarum* in comparison with a pathogenic strain of *Staphylococcus aureus*[36]; the second very important concept is related to the possible modification of LTA molecules to induce a substantial reduction in *D*-alanine content with a marked increase in glucose substitutions[37]. These modified LTA may be candidates as probiotic effector molecules able to induce secretion of anti-inflammatory IL-10.

On the other hand, LPS synthesized by *Gram-negative bacteria* of the gut microbiota have been recently involved in the development of inflammation, obesity and type 2 diabetes induced by a high fat diet[38]. If confirmed, these findings open up a new possible role in this field not only for a direct bacterial competition by live probiotics, but also for research into non-immunostimulating molecules competing with LPS for the TLR 4 pathway.

Finally, both lipoproteins and glycoproteins present at the cell surface are also attractive candidates as probiotic molecules for their interactions with TLR 2 receptors, but to date their role is unexplored even in pathogenic bacteria[39]. However flagellins of the *Escherichia coli* Nissle 1917 induce the expression of human β-defensin 2, an inducible antimicrobial peptide[40].

Recombinant probiotics: Colonizing (*e.g.*, *Streptococcus gondii*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus acidophilus*) as well as non-colonizing (*e.g.*, *Lactobacillus lactis*) bacterial species have been investigated both as live vaccine vehicles (acting as carriers for protective antigens) and as active producers of molecules with known pharmacological properties.

In respect to the use of these microorganisms as carriers for antigens, the most complete studies have been carried out with the 50 kDa carboxy-terminal fragment of tetanus toxin[41]; this approach has now been extended to additional antigens eg the B subunit of cholera toxin[1-3].

Transfected bacteria have also been used to deliver cytokines, but this technique was recently used to investigate other biological properties. Steidler *et al*[42] chose to construct recombinant *Lactobacillus lactis* strains secreting murine IL-10. These authors demonstrated that these recombinant strains were able to prevent and treat inflammation in two murine models of colitis. Significantly, the same effects were obtained with much lower doses of IL-10 than those required when IL-10 itself was used as a drug. The same authors further constructed a safe (no antibiotic resistance markers and a chromosomally integrated transgene) strain of *Lactobacillus lactis* secreting IL-10 of human origin[43]. Authorization by a local ethical committee to carry out a phase 1 clinical study on voluntary patients has been obtained in the Netherlands[44]. In this study, Crohn's disease patients were treated with recombinant *Lactobacillus lactis* (LL-THY 12) in which the thymidylate synthase gene was replaced with a synthetic sequence encoding mature human IL-10. The oral administration of this strain was safe and a decrease in disease activity was observed. The authors concluded that the use of genetically modified bacteria for mucosal delivery of therapeutic proteins is a feasible strategy in human beings. This strategy avoids systemic side effects and appears suitable as maintenance treatment for chronic intestinal diseases. Novel therapeutic strategies for acute and chronic colitis based on recombinant probiotics were also assessed by the generation and *in vivo* evaluation of *Lactobacillus lactis* strains secreting bioactive murine trefoil factors (TFF)[45]. The authors demonstrated that intragastric administration of this bacterial strain, but not of purified TFF, led to prevention and healing in the acute dextran sodium sulfate (DSS)-induced murine model of colitis, and was similarly effective in reducing established chronic DSS colitis. It has also to be remem-

bered that production and mucosal delivery of different bioactive molecules such as allergens, digestive enzymes and single-chain Fw antibodies have been achieved using lactic acid bacteria[3]. Targeted diseases included vaginal candidiosis[46], dental caries[47], allergies[48-50], autoimmune diseases[51,52], human papillomaviruses-induced tumors[53] and pancreatic insufficiency[54]. More recently, Rosberg-Cody *et al*[55] investigated whether a recombinant strain of *Lactobacillus paracasei*, previously isolated from the human gastrointestinal tract, expressing conjugated linoleic acid (CLA) isomerase from *Propionibacterium acnes,* could influence the fatty acid composition of different tissues in the mouse. Ingestion of the *Lactobacillus paracasei* strain expressing CLA isomerase was associated with a 4-fold increase ($P > 0.001$) in t10c12 CLA in adipose tissues of the mice when compared with animals that received the non-CLA producing isogenic strain. These data show that a single gene encoding CLA isomerase expressed by an intestinal bacterium can influence the fatty acid composition of the host adipose tissue. This t10c12 CLA isomer is also associated with decreased body fat and increased lean body mass in various animal species[56-60] and, to some extents, human beings[61-65]. It is also well known that t10c12 CLA isomer is the most potent isomer in terms of potential to prevent cell proliferation and induce apoptosis in cancer cells[66-69]; notably, when the microbially derived t10c12 CLA was incubated with SW480 colon cancer cells for 5 d, cell viability was decreased by 92%[70], and it is possible that a CLA-producing probiotic will be able to keep colon cancer cells in check. Although commensal *Bifidobacterium* and *Lactobacillus* species from the gastrointestinal tract have been shown to produce CLA *in vitro*[71-73], the majority of these studies have demonstrated the production of c9t11 CLA from linoleic acid, while only a few bacteria such as *Propionibacterium acnes*[74] , the rumen bacterium *Megasphera elsodenii*[75], and the human derived *Lactobacillus rhamnosus* PL60 and *Lactobacillus plantarum* PL62[76,77] have been reported to produce t10c12 CLA. Modulation of fatty acid production by bacteria may represent very important probiotic activity and recombinant probiotics may become useful for this in the near future.

Recombinant probiotics may be linked not only to the addition of one or more genes but also to the deletion of one or more genes. In fact, to study the molecular mechanisms involved in the induction and repression of intestinal inflammation, Mohamadzadeh *et al*[78] have recently deleted the phosphoglycerol transferase gene that plays a key role in LTA biosynthesis in *Lactobacillus acidophilus* NCK 56.

The results of these authors show that the *Lactobacillus acidophilus* LTA$^-$ not only down regulated IL-12 and TNF-α, which are known pro-inflammatory cytokines, but also significantly enhanced IL-10 production by DC and controlled the regulation of co-stimulatory DC functions, resulting in their inability to induce CD 4+ T cell activation. The treatment of mice with *Lactobacillus acidophilus* LTA$^-$, compared with *Lactobacillus acidophilus* LTA$^+$, significantly counteracted DSS-induced colitis. These authors concluded that directed alteration of cell-surface components of *Lactobacillus acidophilus* represents a potential new strategy for the treatment of inflammatory intestinal disorders.

Moreover, efforts have been devoted to improve the efficacy of probiotic bacteria as delivery systems; in this context cell wall mutants of *Lactobacillus plantarum* and *Lactobacillus lactis* defective in alanine racemase (*alr* gene) were constructed[79,80]: each of these mutants behaved as a substantially improved antigen delivery system compared with its wild-type counterpart[81]. The potency of the *Lactobacillus plantarum* Alr$^-$ mutant was further confirmed using a weak immunogen, such as *Helicobacter pylori* urease B, as a protective antigen; a significant reduction of the *Helicobacter pylori* load in the mouse stomach was achieved after immunization with the recombinant mutant *Lactobacillus plantarum* strain in contrast to results obtained with its wild-type counterpart[82].

Any gene coding for an active molecule, potentially useful for human health, may be used to generate recombinant probiotic bacteria; in this context, an impressive number of options are available to be investigated in *in vitro* and *in vivo* studies. It is worthy of note, however, that several gene products need glycosylation, phosphorylation or other more complex chemical changes; these may require the enzymatic machinery of eukaryotic cells. Thus, although current available genomic information should greatly facilitate the generation of useful recombinant probiotics, several technical issues and biologically limiting factors have to be taken attentively in consideration. In any case, the use of rapidly evolving genomic technology will surely help to evolve this intriguing and fascinating field and we can expect that from the present pioneering status we will soon progress to the generation of innovative therapeutic tools.

CONCLUSION

We are convinced that, even if as mentioned above there is a very large amount of work to be performed in this field, the available evidence is already enough to move from the actual concept of probiotics to novel and very promising pharmacobiotic strategies. In fact, probiotic molecules and recombinant probiotics may represent an unlimited resource for innovative therapeutics. The following questions arise from the present analysis of available knowledge: (1) Why the therapeutic potential of probiotic molecules and recombinant probiotics has been neglected so far? (2) Why important studies showing that whole live bacteria are not needed for probiotic activity have not received adequate attention by the scientific community? (3) Why molecules such as polysaccharide-peptidoglycan (PSPG)- I from *Lactobacillus shirota*, which have demonstrated to be able to suppress inflammation in chronic intestinal inflammatory disorders *via* inhibition of IL-6, have not been extensively investigated yet? IL-6 plays a pivotal role both in activation and sustainment

of Th 17 response as well as a crucial role as a proinflammatory IL in Th 17 and Th1 cell responses. Thus the dose dependent pharmacological inhibition of IL-6 levels could have a crucial clinical impact, as suggested by animal studies[83] . Based on these considerations, (4) Why, after identification of adequate drug delivery strategies (in fact, there may be major challenges with formulation and delivery in single cases), the clinical effectiveness of PSPG- I has not been assessed yet? and (5) Why has only a phase I study has been performed with recombinant probiotics? These are crucial questions with important implications. Thus these questions should be discussed at international level by a forum involving different players including, basic researchers, clinicians (gastroenterologists, pediatrics, allergologists, pneumologists, *etc*.), lawgivers and regulatory agencies, and probiotics pharma. Although there are already international organizations that declare to be independent of the industry, such as the aforementioned ISAPP, which tackle these issues, these have within them industry advisory committee members and have not been able until now to pull the current outdated concept of probiotics to the more inclusive concept of pharmabiotics.

Although guidelines and recommendations substantiating the evidence for beneficial effects of probiotics in different clinical conditions of adult patients have been published[6,7,84-87], the only clinical conditions in which strains of whole live probiotics have been shown to be effective thus far are acute gastroenteritis and antibiotic-associated diarrhoea. It seems therefore that live probiotics can exert a competitive action and can have a role in restoration of intestinal flora. However, a specific role in the cure of chronic and/or autoimmune diseases has not been conclusively demonstrated. Despite this, an entire world involving probiotic molecules and recombinant probiotics is ready to be investigated. In any case, if specific live probiotic strains have been or will be found effective in specific concentrations for specific disease conditions should they still categorized as food supplements? To what extent does the market influence the national regulatory laws in this area? We think that gut microbiota and probiotic bacteria represent an inexhaustible mine from which countless molecules of potential value for human health can be obtained and investigated. If this does not happen, we risk going on discussing whether a live strain is better than another without ever reaching any definitive conclusion for many years. Even if single RCTs demonstrate a level of evidence 1a, but the findings are not confirmed by other authors in order to remove any doubt about the therapeutic role of that strain in the given clinical condition at that specific doses and route of administration, it does not solve the problem and continues to maintain doubts about the role of probiotic therapy. In addition, it should be underlined that clinical studies are almost always sponsored by companies and results of rigorous RCTs are restricted to the strains of company interest. Who needs to maintain the "status quo" without moving the research to a plot of real pharmacobiotic strategies? Is the huge market based on "easy" trade of live microorganisms involved? We do not want to be unpleasant to anybody, but we think that opening an international forum on this important issue would be of great benefit to both physicians and patients. If to tell the story of salutistic products through well-made advertisements in the media induces big gains without big expenses, we fear that hardly anyone will decide to invest in this area. This way the birth of the pharmacobiotic era will turn away more and more. The resources that are available to us are often sacrificed by humans on the altar of interests and market strategies: among the chief concerns of the scientific community is the need to denounce all those situations in which scientific rigor is sacrificed to commercial interests. To avoid this, the following two actions are mandatory: (1) international guidelines by the forum of players made available to scientists and clinicians, patient organizations, and lawgivers; and (2) public research funds made available to the scientific community for performing independent rigorous studies both at preclinical and clinical levels.

REFERENCES

1 **Seegers JF**. Lactobacilli as live vaccine delivery vectors: progress and prospects. *Trends Biotechnol* 2002; **20**: 508-515 [PMID: 12443872]

2 **Wells JM**, Mercenier A. Mucosal delivery of therapeutic and prophylactic molecules using lactic acid bacteria. *Nat Rev Microbiol* 2008; **6**: 349-362 [PMID: 18345021 DOI: 10.1038/nrmicro1840]

3 **Hanniffy S**, Wiedermann U, Repa A, Mercenier A, Daniel C, Fioramonti J, Tlaskolova H, Kozakova H, Israelsen H, Madsen S, Vrang A, Hols P, Delcour J, Bron P, Kleerebezem M, Wells J. Potential and opportunities for use of recombinant lactic acid bacteria in human health. *Adv Appl Microbiol* 2004; **56**: 1-64 [PMID: 15566975 DOI: 10.1016/S0065-2164(04)56001-X]

4 **Pineiro M**, Stanton C. Probiotic bacteria: legislative framework - requirements to evidence basis. *J Nutr* 2007; **137**: 850S-853S [PMID: 17311986]

5 **Bhadoria PBS,** Mahapatra SC. Prospects, technological aspects and limitations of probiotics – a worldwide review. *Eur J Food Res* 2011; **1:** 23-42

6 **Haller D**, Antoine JM, Bengmark S, Enck P, Rijkers GT, Lenoir-Wijnkoop I. Guidance for substantiating the evidence for beneficial effects of probiotics: probiotics in chronic inflammatory bowel disease and the functional disorder irritable bowel syndrome. *J Nutr* 2010; **140**: 690S-697S [PMID: 20107148 DOI: 10.3945/jn.109.113746]

7 **Floch MH**, Walker WA, Madsen K, Sanders ME, Macfarlane GT, Flint HJ, Dieleman LA, Ringel Y, Guandalini S, Kelly CP, Brandt LJ. Recommendations for probiotic use-2011 update. *J Clin Gastroenterol* 2011; **45** Suppl: S168-S171 [PMID: 21992958 DOI: 10.1097/MCG.0b013e318230928b]

8 **Lundell L**. Use of probiotics in abdominal surgery. *Dig Dis* 2011; **29**: 570-573 [PMID: 22179213 DOI: 10.1159/000332984]

9 **Bjarnason A**, Adler SN, Bjarnason I. Probiotic prophylaxis in predicted severe acute pancreatitis. *Lancet* 2008; **372**: 114-115 [PMID: 18620944 DOI: 10.1016/s0140-6736(08)61028-4]

10 **Guarino A**, Lo Vecchio A, Canani RB. Probiotics as prevention and treatment for diarrhea. *Curr Opin Gastroenterol* 2009; **25**: 18-23 [PMID: 19114770 DOI: 10.1097/MOG.0b013e32831b4455]

11 **Guarino A**, Albano F, Ashkenazi S, Gendrel D, Hoekstra JH, Shamir R, Szajewska H. European Society for Paediatric

Gastroenterology, Hepatology, and Nutrition/European Society for Paediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: executive summary. *J Pediatr Gastroenterol Nutr* 2008; **46**: 619-621 [PMID: 18493225 DOI: 10.1097/MPG.0b013e31816e219e]
12 **Caselli M**, Vaira G, Calo G, Papini F, Holton J, Vaira D. Structural bacterial molecules as potential candidates for an evolution of the classical concept of probiotics. *Adv Nutr* 2011; **2**: 372-376 [PMID: 22332079 DOI: 10.3945/an.111.000604]
13 **Caselli M,** Vaira D,Cassol F, Calò G, Vaira G, Papini F, Holton J. Recombinant probiotics and their potential in human health. *Int J Probiotics Prebiotics* 2012; **7:** 53-58
14 **Kang JY**, Lee JO. Structural biology of the Toll-like receptor family. *Annu Rev Biochem* 2011; **80**: 917-941 [PMID: 21548780 DOI: 10.1146/annurev-biochem-052909-141507]
15 **Elinav E**, Strowig T, Henao-Mejia J, Flavell RA. Regulation of the antimicrobial response by NLR proteins. *Immunity* 2011; **34**: 665-679 [PMID: 21616436 DOI: 10.1016/j.immuni.2011.05.007]
16 **Zhao L**, Lee JY, Hwang DH. Inhibition of pattern recognition receptor-mediated inflammation by bioactive phytochemicals. *Nutr Rev* 2011; **69**: 310-320 [PMID: 21631512 DOI: 10.1111/j.1753-4887.2011.00394.x]
17 **Gómez-Llorente C**, Muñoz S, Gil A. Role of Toll-like receptors in the development of immunotolerance mediated by probiotics. *Proc Nutr Soc* 2010; **69**: 381-389 [PMID: 20416121 DOI: 10.1017/s0029665110001527]
18 **van Baarlen P**, Troost F, van der Meer C, Hooiveld G, Boekschoten M, Brummer RJ, Kleerebezem M. Human mucosal in vivo transcriptome responses to three lactobacilli indicate how probiotics may modulate human cellular pathways. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4562-4569 [PMID: 20823239 DOI: 10.1073/pnas.1000079107]
19 **Gaboriau-Routhiau V**, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 2009; **31**: 677-689 [PMID: 19833089 DOI: 10.1016/j.immuni.2009.08.020]
20 **Ivanov II**, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009; **139**: 485-498 [PMID: 19836068 DOI: 10.1016/j.cell.2009.09.033]
21 **Denning TL**, Sitaraman SV. Segmented filamentous bacteria shape intestinal immunity. *Gastroenterology* 2010; **139**: 351-353 [PMID: 20510226 DOI: 10.1053/j.gastro.2010.05.032]
22 **Caselli M**, Holton J, Boldrini P, Vaira D, Calò G. Morphology of segmented filamentous bacteria and their patterns of contact with the follicle-associated epithelium of the mouse terminal ileum: implications for the relationship with the immune system. *Gut Microbes* 2010; **1**: 367-372 [PMID: 21468217 DOI: 10.4161/gmic.1.6.14390]
23 **Lammers KM**, Brigidi P, Vitali B, Gionchetti P, Rizzello F, Caramelli E, Matteuzzi D, Campieri M. Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. *FEMS Immunol Med Microbiol* 2003; **38**: 165-172 [PMID: 13129651]
24 **Bettelli E**, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; **441**: 235-238 [PMID: 16648838 DOI: 10.1038/nature04753]
25 **Iwakura Y**, Ishigame H. The IL-23/IL-17 axis in inflammation. *J Clin Invest* 2006; **116**: 1218-1222 [PMID: 16670765 DOI: 10.1172/jci28508]
26 **Rachmilewitz D**, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, Akira S, Takeda K, Lee J, Takabayashi K, Raz E. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. *Gastroenterology* 2004; **126**: 520-528 [PMID: 14762789]
27 **Iliev ID**, Kitazawa H, Shimosato T, Katoh S, Morita H, He F, Hosoda M, Saito T. Strong immunostimulation in murine immune cells by Lactobacillus rhamnosus GG DNA containing novel oligodeoxynucleotide pattern. *Cell Microbiol* 2005; **7**: 403-414 [PMID: 15679843 DOI: 10.1111/j.1462-5822.2004.00470.x]
28 **Ghadimi D**, Fölster-Holst R, de Vrese M, Winkler P, Heller KJ, Schrezenmeir J. Effects of probiotic bacteria and their genomic DNA on TH1/TH2-cytokine production by peripheral blood mononuclear cells (PBMCs) of healthy and allergic subjects. *Immunobiology* 2008; **213**: 677-692 [PMID: 18950596 DOI: 10.1016/j.imbio.2008.02.001]
29 **Krieg AM**. CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol* 2002; **20**: 709-760 [PMID: 11861616 DOI: 10.1146/annurev.immunol.20.100301.064842]
30 **Ménard O**, Gafa V, Kapel N, Rodriguez B, Butel MJ, Waligora-Dupriet AJ. Characterization of immunostimulatory CpG-rich sequences from different Bifidobacterium species. *Appl Environ Microbiol* 2010; **76**: 2846-2855 [PMID: 20208019 DOI: 10.1128/aem.01714-09]
31 **Delcour J**, Ferain T, Deghorain M, Palumbo E, Hols P. The biosynthesis and functionality of the cell-wall of lactic acid bacteria. *Antonie Van Leeuwenhoek* 1999; **76**: 159-184 [PMID: 10532377]
32 **Erridge C**, Bennett-Guerrero E, Poxton IR. Structure and function of lipopolysaccharides. *Microbes Infect* 2002; **4**: 837-851 [PMID: 12270731]
33 **Mazmanian SK**, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; **122**: 107-118 [PMID: 16009137 DOI: 10.1016/j.cell.2005.05.007]
34 **Könönen E**, Jousimies-Somer H, Asikainen S. Relationship between oral gram-negative anaerobic bacteria in saliva of the mother and the colonization of her edentulous infant. *Oral Microbiol Immunol* 1992; **7**: 273-276 [PMID: 1494450]
35 **Mazmanian SK**, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 2008; **453**: 620-625 [PMID: 18509436 DOI: 10.1038/nature07008]
36 **Ryu YH**, Baik JE, Yang JS, Kang SS, Im J, Yun CH, Kim DW, Lee K, Chung DK, Ju HR, Han SH. Differential immunostimulatory effects of Gram-positive bacteria due to their lipoteichoic acids. *Int Immunopharmacol* 2009; **9**: 127-133 [PMID: 19013542 DOI: 10.1016/j.intimp.2008.10.014]
37 **Grangette C**, Nutten S, Palumbo E, Morath S, Hermann C, Dewulf J, Pot B, Hartung T, Hols P, Mercenier A. Enhanced antiinflammatory capacity of a Lactobacillus plantarum mutant synthesizing modified teichoic acids. *Proc Natl Acad Sci USA* 2005; **102**: 10321-10326 [PMID: 15985548 DOI: 10.1073/pnas.0504084102]
38 **Cani PD**, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis. *Curr Opin Clin Nutr Metab Care* 2007; **10**: 729-734 [PMID: 18089955 DOI: 10.1097/MCO.0b013e3282efdebb]
39 **Benz I**, Schmidt MA. Never say never again: protein glycosylation in pathogenic bacteria. *Mol Microbiol* 2002; **45**: 267-276 [PMID: 12123443]
40 **Schlee M**, Wehkamp J, Altenhoefer A, Oelschlaeger TA, Stange EF, Fellermann K. Induction of human beta-defensin 2 by the probiotic Escherichia coli Nissle 1917 is mediated through flagellin. *Infect Immun* 2007; **75**: 2399-2407 [PMID: 17283097 DOI: 10.1128/iai.01563-06]
41 **Grangette C**, Müller-Alouf H, Geoffroy M, Goudercourt D, Turneer M, Mercenier A. Protection against tetanus toxin after intragastric administration of two recombinant lactic acid bacteria: impact of strain viability and in vivo persistence. *Vaccine* 2002; **20**: 3304-3309 [PMID: 12213400]
42 **Steidler L**, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, Fiers W, Remaut E. Treatment of murine colitis by

Lactococcus lactis secreting interleukin-10. *Science* 2000; **289**: 1352-1355 [PMID: 10958782]
43 **Steidler L**, Neirynck S, Huyghebaert N, Snoeck V, Vermeire A, Goddeeris B, Cox E, Remon JP, Remaut E. Biological containment of genetically modified Lactococcus lactis for intestinal delivery of human interleukin 10. *Nat Biotechnol* 2003; **21**: 785-789 [PMID: 12808464 DOI: 10.1038/nbt840]
44 **Braat H**, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, van Deventer SJ, Neirynck S, Peppelenbosch MP, Steidler L. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin Gastroenterol Hepatol* 2006; **4**: 754-759 [PMID: 16716759 DOI: 10.1016/j.cgh.2006.03.028]
45 **Vandenbroucke K**, Hans W, Van Huysse J, Neirynck S, Demetter P, Remaut E, Rottiers P, Steidler L. Active delivery of trefoil factors by genetically modified Lactococcus lactis prevents and heals acute colitis in mice. *Gastroenterology* 2004; **127**: 502-513 [PMID: 15300583]
46 **Beninati C**, Oggioni MR, Boccanera M, Spinosa MR, Maggi T, Conti S, Magliani W, De Bernardis F, Teti G, Cassone A, Pozzi G, Polonelli L. Therapy of mucosal candidiasis by expression of an anti-idiotype in human commensal bacteria. *Nat Biotechnol* 2000; **18**: 1060-1064 [PMID: 11017043 DOI: 10.1038/80250]
47 **Krüger C**, Hu Y, Pan Q, Marcotte H, Hultberg A, Delwar D, van Dalen PJ, Pouwels PH, Leer RJ, Kelly CG, van Dollenweerd C, Ma JK, Hammarström L. In situ delivery of passive immunity by lactobacilli producing single-chain antibodies. *Nat Biotechnol* 2002; **20**: 702-706 [PMID: 12089555 DOI: 10.1038/nbt0702-702]
48 **Kruisselbrink A**, Heijne Den Bak-Glashouwer MJ, Havenith CE, Thole JE, Janssen R. Recombinant Lactobacillus plantarum inhibits house dust mite-specific T-cell responses. *Clin Exp Immunol* 2001; **126**: 2-8 [PMID: 11678893]
49 **Chatel JM**, Langella P, Adel-Patient K, Commissaire J, Wal JM, Corthier G. Induction of mucosal immune response after intranasal or oral inoculation of mice with Lactococcus lactis producing bovine beta-lactoglobulin. *Clin Diagn Lab Immunol* 2001; **8**: 545-551 [PMID: 11329455 DOI: 10.1128/cdli.8.3.545-551.2001]
50 **Repa A**, Grangette C, Daniel C, Hochreiter R, Hoffmann-Sommergruber K, Thalhamer J, Kraft D, Breiteneder H, Mercenier A, Wiedermann U. Mucosal co-application of lactic acid bacteria and allergen induces counter-regulatory immune responses in a murine model of birch pollen allergy. *Vaccine* 2003; **22**: 87-95 [PMID: 14604575]
51 **Daniel C**, Repa A, Wild C, Pollak A, Pot B, Breiteneder H, Wiedermann U, Mercenier A. Modulation of allergic immune responses by mucosal application of recombinant lactic acid bacteria producing the major birch pollen allergen Bet v 1. *Allergy* 2006; **61**: 812-819 [PMID: 16792578 DOI: 10.1111/j.1398-9995.2006.01071.x]
52 **Maassen CB**, Laman JD, den Bak-Glashouwer MJ, Tielen FJ, van Holten-Neelen JC, Hoogteijling L, Antonissen C, Leer RJ, Pouwels PH, Boersma WJ, Shaw DM. Instruments for oral disease-intervention strategies: recombinant Lactobacillus casei expressing tetanus toxin fragment C for vaccination or myelin proteins for oral tolerance induction in multiple sclerosis. *Vaccine* 1999; **17**: 2117-2128 [PMID: 10367944]
53 **Bermúdez-Humarán LG**, Cortes-Perez NG, Lefèvre F, Guimarães V, Rabot S, Alcocer-Gonzalez JM, Gratadoux JJ, Rodriguez-Padilla C, Tamez-Guerra RS, Corthier G, Gruss A, Langella P. A novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type 16-induced tumors. *J Immunol* 2005; **175**: 7297-7302 [PMID: 16301635]
54 **Drouault S**, Juste C, Marteau P, Renault P, Corthier G. Oral treatment with Lactococcus lactis expressing Staphylococcus hyicus lipase enhances lipid digestion in pigs with induced pancreatic insufficiency. *Appl Environ Microbiol* 2002; **68**: 3166-3168 [PMID: 12039786]
55 **Rosberg-Cody E**, Stanton C, O'Mahony L, Wall R, Shanahan F, Quigley EM, Fitzgerald GF, Ross RP. Recombinant lactobacilli expressing linoleic acid isomerase can modulate the fatty acid composition of host adipose tissue in mice. *Microbiology* 2011; **157**: 609-615 [PMID: 21178166 DOI: 10.1099/mic.0.043406-0]
56 **Park Y**, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. *Lipids* 1997; **32**: 853-858 [PMID: 9270977]
57 **Cherian G**, Ai W, Goeger MP. Maternal dietary conjugated linoleic acid alters hepatic triacylglycerol and tissue fatty acids in hatched chicks. *Lipids* 2005; **40**: 131-136 [PMID: 15884760]
58 **Yamasaki M**, Ikeda A, Oji M, Tanaka Y, Hirao A, Kasai M, Iwata T, Tachibana H, Yamada K. Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague-Dawley rats fed various fat-level diets. *Nutrition* 2003; **19**: 30-35 [PMID: 12507636]
59 **Navarro V**, Miranda J, Churruca I, Fernández-Quintela A, Rodríguez VM, Portillo MP. Effects of trans-10,cis-12 conjugated linoleic acid on body fat and serum lipids in young and adult hamsters. *J Physiol Biochem* 2006; **62**: 81-87 [PMID: 17217162]
60 **Ostrowska E**, Muralitharan M, Cross RF, Bauman DE, Dunshea FR. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. *J Nutr* 1999; **129**: 2037-2042 [PMID: 10539781]
61 **Blankson H**, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J Nutr* 2000; **130**: 2943-2948 [PMID: 11110851]
62 **Mougios V**, Matsakas A, Petridou A, Ring S, Sagredos A, Melissopoulou A, Tsigilis N, Nikolaidis M. Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat. *J Nutr Biochem* 2001; **12**: 585-594 [PMID: 12031264]
63 **Risérus U**, Berglund L, Vessby B. Conjugated linoleic acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trial. *Int J Obes Relat Metab Disord* 2001; **25**: 1129-1135 [PMID: 11477497 DOI: 10.1038/sj.ijo.0801659]
64 **Smedman A**, Vessby B. Conjugated linoleic acid supplementation in humans--metabolic effects. *Lipids* 2001; **36**: 773-781 [PMID: 11592727]
65 **Thom E**, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat in healthy exercising humans. *J Int Med Res* 2001; **29**: 392-396 [PMID: 11725826]
66 **Cho HJ**, Kim WK, Jung JI, Kim EJ, Lim SS, Kwon DY, Park JH. Trans-10,cis-12, not cis-9,trans-11, conjugated linoleic acid decreases ErbB3 expression in HT-29 human colon cancer cells. *World J Gastroenterol* 2005; **11**: 5142-5150 [PMID: 16127743]
67 **Kim EJ**, Holthuizen PE, Park HS, Ha YL, Jung KC, Park JH. Trans-10,cis-12-conjugated linoleic acid inhibits Caco-2 colon cancer cell growth. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G357-G367 [PMID: 12121883 DOI: 10.1152/ajpgi.00495.2001]
68 **Lee SH**, Yamaguchi K, Kim JS, Eling TE, Safe S, Park Y, Baek SJ. Conjugated linoleic acid stimulates an anti-tumorigenic protein NAG-1 in an isomer specific manner. *Carcinogenesis* 2006; **27**: 972-981 [PMID: 16286461 DOI: 10.1093/carcin/bgi268]
69 **Ochoa JJ**, Farquharson AJ, Grant I, Moffat LE, Heys SD, Wahle KW. Conjugated linoleic acids (CLAs) decrease prostate cancer cell proliferation: different molecular mechanisms for cis-9, trans-11 and trans-10, cis-12 isomers. *Carcinogenesis* 2004; **25**: 1185-1191 [PMID: 14976130 DOI: 10.1093/carcin/bgh116]

70 **Rosberg-Cody E**, Johnson MC, Fitzgerald GF, Ross PR, Stanton C. Heterologous expression of linoleic acid isomerase from Propionibacterium acnes and anti-proliferative activity of recombinant trans-10, cis-12 conjugated linoleic acid. *Microbiology* 2007; **153**: 2483-2490 [PMID: 17660413 DOI: 10.1099/mic.0.2006/001966-0]

71 **Barrett E**, Ross RP, Fitzgerald GF, Stanton C. Rapid screening method for analyzing the conjugated linoleic acid production capabilities of bacterial cultures. *Appl Environ Microbiol* 2007; **73**: 2333-2337 [PMID: 17277221 DOI: 10.1128/aem.01855-06]

72 **Coakley M**, Ross RP, Nordgren M, Fitzgerald G, Devery R, Stanton C. Conjugated linoleic acid biosynthesis by human-derived Bifidobacterium species. *J Appl Microbiol* 2003; **94**: 138-145 [PMID: 12492934]

73 **Rosberg-Cody E**, Ross RP, Hussey S, Ryan CA, Murphy BP, Fitzgerald GF, Devery R, Stanton C. Mining the microbiota of the neonatal gastrointestinal tract for conjugated linoleic acid-producing bifidobacteria. *Appl Environ Microbiol* 2004; **70**: 4635-4641 [PMID: 15294796 DOI: 10.1128/aem.70.8.4635-4641.2004]

74 **Verhulsta A,** Janssen G, Parmentier G, Eyssen H. Isomerization of polyunsaturated long chain fatty acids by propionibacteria. *Syst Appl Microbiol* 1987; **9:** 12-15 [DOI: 10.1016/S0723-2020(87)80049-8]

75 **Kim YJ**, Liu RH, Rychlik JL, Russell JB. The enrichment of a ruminal bacterium (Megasphaera elsdenii YJ-4) that produces the trans-10, cis-12 isomer of conjugated linoleic acid. *J Appl Microbiol* 2002; **92**: 976-982 [PMID: 11972704]

76 **Lee HY**, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y, Park JH. Human originated bacteria, Lactobacillus rhamnosus PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochim Biophys Acta* 2006; **1761**: 736-744 [PMID: 16807088 DOI: 10.1016/j.bbalip.2006.05.007]

77 **Lee K**, Paek K, Lee HY, Park JH, Lee Y. Antiobesity effect of trans-10,cis-12-conjugated linoleic acid-producing Lactobacillus plantarum PL62 on diet-induced obese mice. *J Appl Microbiol* 2007; **103**: 1140-1146 [PMID: 17897219 DOI: 10.1111/j.1365-2672.2007.03336.x]

78 **Mohamadzadeh M**, Pfeiler EA, Brown JB, Zadeh M, Gramarossa M, Managlia E, Bere P, Sarraj B, Khan MW, Pakanati KC, Ansari MJ, O'Flaherty S, Barrett T, Klaenhammer TR. Regulation of induced colonic inflammation by Lactobacillus acidophilus deficient in lipoteichoic acid. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4623-4630 [PMID: 21282652 DOI: 10.1073/pnas.1005066107]

79 **Palumbo E**, Favier CF, Deghorain M, Cocconcelli PS, Grangette C, Mercenier A, Vaughan EE, Hols P. Knockout of the alanine racemase gene in Lactobacillus plantarum results in septation defects and cell wall perforation. *FEMS Microbiol Lett* 2004; **233**: 131-138 [PMID: 15043879 DOI: 10.1016/j.femsle.2004.02.001]

80 **Steen A**, Palumbo E, Deghorain M, Cocconcelli PS, Delcour J, Kuipers OP, Kok J, Buist G, Hols P. Autolysis of Lactococcus lactis is increased upon D-alanine depletion of peptidoglycan and lipoteichoic acids. *J Bacteriol* 2005; **187**: 114-124 [PMID: 15601695 DOI: 10.1128/jb.187.1.114-124.2005]

81 **Grangette C**, Müller-Alouf H, Hols P, Goudercourt D, Delcour J, Turneer M, Mercenier A. Enhanced mucosal delivery of antigen with cell wall mutants of lactic acid bacteria. *Infect Immun* 2004; **72**: 2731-2737 [PMID: 15102782]

82 **Corthésy B**, Boris S, Isler P, Grangette C, Mercenier A. Oral immunization of mice with lactic acid bacteria producing Helicobacter pylori urease B subunit partially protects against challenge with Helicobacter felis. *J Infect Dis* 2005; **192**: 1441-1449 [PMID: 16170763 DOI: 10.1086/444425]

83 **Matsumoto S**, Hara T, Nagaoka M, Mike A, Mitsuyama K, Sako T, Yamamoto M, Kado S, Takada T. A component of polysaccharide peptidoglycan complex on Lactobacillus induced an improvement of murine model of inflammatory bowel disease and colitis-associated cancer. *Immunology* 2009; **128**: e170-e180 [PMID: 19740306 DOI: 10.1111/j.1365-2567.2008.02942.x]

84 **Rijkers GT**, Bengmark S, Enck P, Haller D, Herz U, Kalliomaki M, Kudo S, Lenoir-Wijnkoop I, Mercenier A, Myllyluoma E, Rabot S, Rafter J, Szajewska H, Watzl B, Wells J, Wolvers D, Antoine JM. Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. *J Nutr* 2010; **140**: 671S-676S [PMID: 20130080 DOI: 10.3945/jn.109.113779]

85 **Kalliomäki M**, Antoine JM, Herz U, Rijkers GT, Wells JM, Mercenier A. Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of allergic diseases by probiotics. *J Nutr* 2010; **140**: 713S-721S [PMID: 20130079 DOI: 10.3945/jn.109.113761]

86 **Rabot S**, Rafter J, Rijkers GT, Watzl B, Antoine JM. Guidance for substantiating the evidence for beneficial effects of probiotics: impact of probiotics on digestive system metabolism. *J Nutr* 2010; **140**: 677S-689S [PMID: 20107147 DOI: 10.3945/jn.109.113738]

87 **Wolvers D**, Antoine JM, Myllyluoma E, Schrezenmeir J, Szajewska H, Rijkers GT. Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of infections by probiotics. *J Nutr* 2010; **140**: 698S-712S [PMID: 20107143 DOI: 10.3945/jn.109.113753]

88 **Maupas JL,** Champemont P, Delforge M. Treatment of irritable bowel syndrome double blind trial of Saccharomyces boulardii. *Med Chir Dig* 1983; **12:** 77-79

89 **Gade J**, Thorn P. Paraghurt for patients with irritable bowel syndrome. A controlled clinical investigation from general practice. *Scand J Prim Health Care* 1989; **7**: 23-26 [PMID: 2657953]

90 **Halpern GM**, Prindiville T, Blankenburg M, Hsia T, Gershwin ME. Treatment of irritable bowel syndrome with Lacteol Fort: a randomized, double-blind, cross-over trial. *Am J Gastroenterol* 1996; **91**: 1579-1585 [PMID: 8759665]

91 **O'Sullivan MA**, O'Morain CA. Bacterial supplementation in the irritable bowel syndrome. A randomised double-blind placebo-controlled crossover study. *Dig Liver Dis* 2000; **32**: 294-301 [PMID: 11515626]

92 **Nobaek S**, Johansson ML, Molin G, Ahrné S, Jeppsson B. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 1231-1238 [PMID: 10811333 DOI: 10.1111/j.1572-0241.2000.02015.x]

93 **Niedzielin K**, Kordecki H, Birkenfeld B. A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome. *Eur J Gastroenterol Hepatol* 2001; **13**: 1143-1147 [PMID: 11711768]

94 **Kim HJ**, Camilleri M, McKinzie S, Lempke MB, Burton DD, Thomforde GM, Zinsmeister AR. A randomized controlled trial of a probiotic, VSL#3, on gut transit and symptoms in diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther* 2003; **17**: 895-904 [PMID: 12656692]

95 **Tsuchiya J**, Barreto R, Okura R, Kawakita S, Fesce E, Marotta F. Single-blind follow-up study on the effectiveness of a symbiotic preparation in irritable bowel syndrome. *Chin J Dig Dis* 2004; **5**: 169-174 [PMID: 15612887 DOI: 10.1111/j.1443-9573.2004.00176.x]

96 **O'Mahony L**, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005; **128**: 541-551 [PMID: 15765388]

97 **Kajander K**, Hatakka K, Poussa T, Färkkilä M, Korpela R. A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. *Aliment Pharmacol Ther* 2005; **22**: 387-394 [PMID: 16128676 DOI:

10.1111/j.1365-2036.2005.02579.x]
98 **Bittner AC**, Croffut RM, Stranahan MC. Prescript-Assist probiotic-prebiotic treatment for irritable bowel syndrome: a methodologically oriented, 2-week, randomized, placebo-controlled, double-blind clinical study. *Clin Ther* 2005; **27**: 755-761 [PMID: 16117982 DOI: 10.1016/j.clinthera.2005.06.005]
99 **Sen S**, Mullan MM, Parker TJ, Woolner JT, Tarry SA, Hunter JO. Effect of Lactobacillus plantarum 299v on colonic fermentation and symptoms of irritable bowel syndrome. *Dig Dis Sci* 2002; **47**: 2615-2620 [PMID: 12452404]
100 **Bausserman M**, Michail S. The use of Lactobacillus GG in irritable bowel syndrome in children: a double-blind randomized control trial. *J Pediatr* 2005; **147**: 197-201 [PMID: 16126049 DOI: 10.1016/j.jpeds.2005.05.015]
101 **Niv E**, Naftali T, Hallak R, Vaisman N. The efficacy of Lactobacillus reuteri ATCC 55730 in the treatment of patients with irritable bowel syndrome--a double blind, placebo-controlled, randomized study. *Clin Nutr* 2005; **24**: 925-931 [PMID: 16051399 DOI: 10.1016/j.clnu.2005.06.001]
102 **Kim HJ**, Vazquez Roque MI, Camilleri M, Stephens D, Burton DD, Baxter K, Thomforde G, Zinsmeister AR. A randomized controlled trial of a probiotic combination VSL# 3 and placebo in irritable bowel syndrome with bloating. *Neurogastroenterol Motil* 2005; **17**: 687-696 [PMID: 16185307 DOI: 10.1111/j.1365-2982.2005.00695.x]
103 **Whorwell PJ**, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, Kiely B, Shanahan F, Quigley EM. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol* 2006; **101**: 1581-1590 [PMID: 16863564 DOI: 10.1111/j.1572-0241.2006.00734.x]
104 **Long ZR**, Yu CH, Yang Y, Wang HN, Chi XX. [Clinical observation on acupuncture combined with microorganism pharmaceutical preparations for treatment of irritable bowel syndrome of constipation type]. *Zhongguo Zhen Jiu* 2006; **26**: 403-405 [PMID: 16813181]
105 **Gawrońska A**, Dziechciarz P, Horvath A, Szajewska H. A randomized double-blind placebo-controlled trial of Lactobacillus GG for abdominal pain disorders in children. *Aliment Pharmacol Ther* 2007; **25**: 177-184 [PMID: 17229242 DOI: 10.1111/j.1365-2036.2006.03175.x]
106 **Moon JT,** Kim HS, Park HJ. Effects of probiotics on the intestinal gas volume score and symptoms in patients with irritable bowel syndrome. A randomized double-blind placebo-controlled study. *Gastroenterology* 2007; **132** Suppl 2: A688
107 **Marteau P,** Holowacz S, Bieuvelet S. A randomized controlled trial of the probiotic combination Lactibiane® in irritable bowel syndrome, The Lactibiane® Study Group (ID# M1146). *Gastroenterology* 2007; **132:** A-371
108 **Simrén M,** Syrous A, Lindh A, Abrahamsson H. Effects of *Lactobacillus plantarum 299v* on symptoms and rectal sensitivity in patients with irritable bowel syndrome (IBS) – a randomized, double-blind controlled trial (#T2043). *Gastroenterology* 2006; **30:** A-600
109 **Simrén M,** Lindh A, Samuelsson L, Olsson J, Posserud I, Strid H, Abrahamsson H. Effect of yoghurt containing three probiotic bacteria in patients with irritable bowel syndrome (IBS) – a randomized, double-blind, controlled trial (ID# S1269). *Gastroenterology* 2007; **132:** A-210
110 **Guyonnet D**, Chassany O, Ducrotte P, Picard C, Mouret M, Mercier CH, Matuchansky C. Effect of a fermented milk containing Bifidobacterium animalis DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. *Aliment Pharmacol Ther* 2007; **26**: 475-486 [PMID: 17635382 DOI: 10.1111/j.1365-2036.2007.03362.x]
111 **Drouault-Holowacz S**, Bieuvelet S, Burckel A, Cazaubiel M, Dray X, Marteau P. A double blind randomized controlled trial of a probiotic combination in 100 patients with irritable bowel syndrome. *Gastroenterol Clin Biol* 2008; **32**: 147-152 [PMID: 18387426 DOI: 10.1016/j.gcb.2007.06.001]
112 **Sinn DH**, Song JH, Kim HJ, Lee JH, Son HJ, Chang DK, Kim YH, Kim JJ, Rhee JC, Rhee PL. Therapeutic effect of Lactobacillus acidophilus-SDC 2012, 2013 in patients with irritable bowel syndrome. *Dig Dis Sci* 2008; **53**: 2714-2718 [PMID: 18274900 DOI: 10.1007/s10620-007-0196-4]
113 **Enck P**, Zimmermann K, Menke G, Müller-Lissner S, Martens U, Klosterhalfen S. A mixture of Escherichia coli (DSM 17252) and Enterococcus faecalis (DSM 16440) for treatment of the irritable bowel syndrome--a randomized controlled trial with primary care physicians. *Neurogastroenterol Motil* 2008; **20**: 1103-1109 [PMID: 18565142 DOI: 10.1111/j.1365-2982.2008.01156.x]
114 **Hun L**. Bacillus coagulans significantly improved abdominal pain and bloating in patients with IBS. *Postgrad Med* 2009; **121**: 119-124 [PMID: 19332970 DOI: 10.3810/pgm.2009.03.1984]
115 **Dolin BJ**. Effects of a proprietary Bacillus coagulans preparation on symptoms of diarrhea-predominant irritable bowel syndrome. *Methods Find Exp Clin Pharmacol* 2009; **31**: 655-659 [PMID: 20140275 DOI: 10.1358/mf.2009.31.10.1441078]
116 **Ligaarden SC**, Axelsson L, Naterstad K, Lydersen S, Farup PG. A candidate probiotic with unfavourable effects in subjects with irritable bowel syndrome: a randomised controlled trial. *BMC Gastroenterol* 2010; **10**: 16 [PMID: 20144246 DOI: 10.1186/1471-230x-10-16]
117 **Moayyedi P**, Ford AC, Talley NJ, Cremonini F, Foxx-Orenstein AE, Brandt LJ, Quigley EM. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut* 2010; **59**: 325-332 [PMID: 19091823 DOI: 10.1136/gut.2008.167270]
118 **Kruis W**, Schütz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997; **11**: 853-858 [PMID: 9354192]
119 **Rembacken BJ**, Snelling AM, Hawkey PM, Chalmers DM, Axon AT. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 1999; **354**: 635-639 [PMID: 10466665]
120 **Venturi A**, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, Matteuzzi D, Campieri M. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther* 1999; **13**: 1103-1108 [PMID: 10468688]
121 **Ishikawa H**, Akedo I, Umesaki Y, Tanaka R, Imaoka A, Otani T. Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. *J Am Coll Nutr* 2003; **22**: 56-63 [PMID: 12569115]
122 **Guslandi M**, Giollo P, Testoni PA. A pilot trial of Saccharomyces boulardii in ulcerative colitis. *Eur J Gastroenterol Hepatol* 2003; **15**: 697-698 [PMID: 12840682 DOI: 10.1097/01.meg.0000059138.68845.06]
123 **Kruis W**, Fric P, Pokrotnieks J, Lukás M, Fixa B, Kascák M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C, Schulze J. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004; **53**: 1617-1623 [PMID: 15479682 DOI: 10.1136/gut.2003.037747]
124 **Tursi A**, Brandimarte G, Giorgetti GM, Forti G, Modeo ME, Gigliobianco A. Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. *Med Sci Monit* 2004; **10**: PI126-PI131 [PMID: 15507864]
125 **Cui HH**, Chen CL, Wang JD, Yang YJ, Cun Y, Wu JB, Liu YH, Dan HL, Jian YT, Chen XQ. Effects of probiotic on intestinal mucosa of patients with ulcerative colitis. *World J*

Gastroenterol 2004; **10**: 1521-1525 [PMID: 15133865]
126 **Kato K**, Mizuno S, Umesaki Y, Ishii Y, Sugitani M, Imaoka A, Otsuka M, Hasunuma O, Kurihara R, Iwasaki A, Arakawa Y. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther* 2004; **20**: 1133-1141 [PMID: 15569116 DOI: 10.1111/j.1365-2036.2004.02268.x]
127 **Furrie E**, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005; **54**: 242-249 [PMID: 15647189 DOI: 10.1136/gut.2004.044834]
128 **Bibiloni R**, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, De Simone C, Sartor RB. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. *Am J Gastroenterol* 2005; **100**: 1539-1546 [PMID: 15984978 DOI: 10.1111/j.1572-0241.2005.41794.x]
129 **Zocco MA**, dal Verme LZ, Cremonini F, Piscaglia AC, Nista EC, Candelli M, Novi M, Rigante D, Cazzato IA, Ojetti V, Armuzzi A, Gasbarrini G, Gasbarrini A. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 2006; **23**: 1567-1574 [PMID: 16696804 DOI: 10.1111/j.1365-2036.2006.02927.x]
130 **Henker J**, Müller S, Laass MW, Schreiner A, Schulze J. Probiotic Escherichia coli Nissle 1917 (EcN) for successful remission maintenance of ulcerative colitis in children and adolescents: an open-label pilot study. *Z Gastroenterol* 2008; **46**: 874-875 [PMID: 18810672 DOI: 10.1055/s-2008-1027463]
131 **Miele E**, Pascarella F, Giannetti E, Quaglietta L, Baldassano RN, Staiano A. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. *Am J Gastroenterol* 2009; **104**: 437-443 [PMID: 19174792 DOI: 10.1038/ajg.2008.118]
132 **Sood A**, Midha V, Makharia GK, Ahuja V, Singal D, Goswami P, Tandon RK. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol* 2009; **7**: 1202-1209, 1209.e1 [PMID: 19631292 DOI: 10.1016/j.cgh.2009.07.016]
133 **Matthes H**, Krummenerl T, Giensch M, Wolff C, Schulze J. Treatment of mild to moderate acute attacks of distal ulcerative colitis with rectally-administered E-coli Nissle 1917: Dose-dependent efficacy. *Gastroenterology* 2006; **130** (Suppl 2): A119-A119
134 **Sang LX**, Chang B, Zhang WL, Wu XM, Li XH, Jiang M. Remission induction and maintenance effect of probiotics on ulcerative colitis: a meta-analysis. *World J Gastroenterol* 2010; **16**: 1908-1915 [PMID: 20397271]
135 **Malchow HA**. Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease? *J Clin Gastroenterol* 1997; **25**: 653-658 [PMID: 9451682]
136 **Guslandi M**, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Dig Dis Sci* 2000; **45**: 1462-1464 [PMID: 10961730]
137 **Prantera C**, Scribano ML, Falasco G, Andreoli A, Luzi C. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. *Gut* 2002; **51**: 405-409 [PMID: 12171964]
138 **Schultz M**, Timmer A, Herfarth HH, Sartor RB, Vanderhoof JA, Rath HC. Lactobacillus GG in inducing and maintaining remission of Crohn's disease. *BMC Gastroenterol* 2004; **4**: 5 [PMID: 15113451 DOI: 10.1186/1471-230x-4-5]
139 **Bousvaros A**, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, Goldin B, Hartigan L, Kugathasan S, Levy J, Murray KF, Oliva-Hemker M, Rosh JR, Tolia V, Zholudev A, Vanderhoof JA, Hibberd PL. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. *Inflamm Bowel Dis* 2005; **11**: 833-839 [PMID: 16116318]
140 **Marteau P**, Lémann M, Seksik P, Laharie D, Colombel JF, Bouhnik Y, Cadiot G, Soulé JC, Bourreille A, Metman E, Lerebours E, Carbonnel F, Dupas JL, Veyrac M, Coffin B, Moreau J, Abitbol V, Blum-Sperisen S, Mary JY. Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial. *Gut* 2006; **55**: 842-847 [PMID: 16377775 DOI: 10.1136/gut.2005.076604]
141 **Chermesh I**, Tamir A, Reshef R, Chowers Y, Suissa A, Katz D, Gelber M, Halpern Z, Bengmark S, Eliakim R. Failure of Synbiotic 2000 to prevent postoperative recurrence of Crohn's disease. *Dig Dis Sci* 2007; **52**: 385-389 [PMID: 17211699 DOI: 10.1007/s10620-006-9549-7]
142 **Van Gossum A**, Dewit O, Louis E, de Hertogh G, Baert F, Fontaine F, DeVos M, Enslen M, Paintin M, Franchimont D. Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after lleo-caecal resection. *Inflamm Bowel Dis* 2007; **13**: 135-142 [PMID: 17206696 DOI: 10.1002/ibd.20063]
143 **Rolfe VE**, Fortun PJ, Hawkey CJ, Bath-Hextall F. Probiotics for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2006; (4): CD004826 [PMID: 17054217 DOI: 10.1002/14651858.CD004826.pub2]
144 **Rahimi R**, Nikfar S, Rahimi F, Elahi B, Derakhshani S, Vafaie M, Abdollahi M. A meta-analysis on the efficacy of probiotics for maintenance of remission and prevention of clinical and endoscopic relapse in Crohn's disease. *Dig Dis Sci* 2008; **53**: 2524-2531 [PMID: 18270836 DOI: 10.1007/s10620-007-0171-0]

P- Reviewer Franceschi F **S- Editor** Gou SX
L- Editor O'Neill M **E- Editor** Xiong L