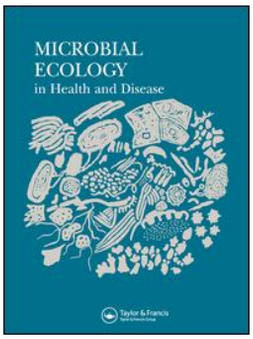

Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: informahealthcare.com/journals/zmeh20

Relationship between number of bacteria and their probiotic effects

Professor Elisa Bertazzoni Minelli & Anna Benini

To cite this article: Professor Elisa Bertazzoni Minelli & Anna Benini (2008) Relationship between number of bacteria and their probiotic effects, Microbial Ecology in Health and Disease, 20:4, 180-183, DOI: 10.1080/08910600802408095

To link to this article: https://doi.org/10.1080/08910600802408095

© 2008 The Author(s). Published by Taylor & Francis.

Published online: 11 Jul 2009.

Submit your article to this journal

Article views: 9673

View related articles

Citing articles: 17 View citing articles

Full Terms & Conditions of access and use can be found at
https://informahealthcare.com/action/journalInformation?journalCode=zmeh20

Microbial Ecology in Health and Disease. 2008; 20: 180–183

informa
healthcare

EXTENDED ABSTRACT

Relationship between number of bacteria and their probiotic effects

ELISA BERTAZZONI MINELLI & ANNA BENINI

Department of Medicine and Public Health, Pharmacology Section, University of Verona, Policlinico "G.B. Rossi" P. le L.A. Scuro, Verona, Italy

Abstract
The effects of probiotics on human health are positive and well defined in diarrhoea treatment. There are no clinical results regarding the relationship with dose or duration of treatment. The results from clinical studies have not been conclusive in that the effects of probiotic are dependent on strains, acute or chronic gastrointestinal infection and immunological or inflammatory disease and different dose and duration of treatment. The concentration of probiotics needed to obtain a clinical effect is often quoted as $\geq 10^6$ cfu/ml in the small bowel and $\geq 10^8$ cfu/g in the colon. The dose for treatment of an acute illness by a particular probiotic agent may be lower or higher, in the order of 10-fold or 100-fold or more in terms of colony forming units (cfu). In acute infectious diarrhoea it seems that higher doses of probiotics given for short courses are more effective than lower doses. In chronic or immunological diseases (allergic, inflammatory and/or immune diseases) the effects depend also on the interaction with gut immune system and duration of treatment. To evaluate the efficacy of probiotics it may be essential to identify specific target groups of individuals with more specific higher susceptibilities to the potential effects of probiotics.

Key words: *Probiotics, acute infectious diarrhoea, immunological disease, efficacy*

Introduction

As defined by FAO/WHO, probiotics are live micro-organisms which, when administered in adequate amounts, confer a health benefit to the host. Probiotics encompass live bacteria, belonging to the natural non-pathogenic bacterial flora, which are thought to exert healthy benefits beyond inherent basic nutrition. They consist of different strains of bacteria (lactic acid bacteria (LAB), bifidobacteria, bacilli, *Escherichia coli,* clostridia, propionibacteria) and yeasts (*Saccharomyces*) (1,2). Their utilization in antibiotic-associated diarrhoea (AAD), *Clostridium difficile* diarrhoea and diarrhoea caused by virus or bacteria demonstrated positive results at standard doses (10^7–10^8 cfu/day), e.g. reduction of stool frequency and mean duration of diarrhoea in adults and children (3).

Probiotics are effective in the treatment of diarrhoea but are variable in diarrhoea prevention (4).

The literature on the efficacy of probiotics in the prevention of travellers' diarrhoea and *Helicobacter pylori* infection gives conflicting findings.

Protective effects have been shown with probiotic preparations of *L. rhamnosus* GG and *Saccharomyces boulardii*, while preparations comprising a mixture of *L. acidophilus* and *L. bulgaricus*, *L. acidophilus* or *L. fermentum* have not been shown to be effective.

Preparations of non-viable *L. acidophilus* showed no efficacy in prevention of travellers' diarrhoea.

A number of clinical studies on the effects of probiotics on *H. pylori* infection indicate a suppressed growth of *H. pylori* without eradication, although there are differences in the effectiveness between strains.

Differences in the populations involved in the studies, the probiotic strains used (and their viability), and methodological and statistical problems – such as subgroup analysis or similar – could explain the discrepancies. Additional trials may still be worth considering with probiotics that have demonstrated a protective effect for the prevention and the treatment of acute infectious diarrhoea in children (3). Lactic acid bacteria seem to exert better effects when administered in combination.

Correspondence: Professor Elisa Bertazzoni Minelli, Department of Medicine and Public Health, Pharmacology Section, University of Verona, Policlinico "G.B. Rossi" P.le L.A. Scuro, 10, 37134-Verona, Italy. Tel: +39 0458027603. Fax: +39 0458027452. E-mail: elisa.bertazzoni@univr.it

ISSN 0891-060X print/ISSN 1651-2235 online © 2008 Informa UK Ltd.
DOI: 10.1080/08910600802408095

A body of data has stressed the differences among bacterial species and strains as regards resistance to intestinal conditions, their survival and colonization, as well as their different probiotic effects.

Stability

To be effective probiotic cultures must be able to withstand processing conditions, retain their probiotic properties after processing and survive in sufficient numbers in the product during shelf-life storage. The stability of a probiotic is linked to various factors, including genus, species, strain biotype and, above all, the formulation storage conditions.

Viability

The survival capacities of various strains of *L. acidophilus*, *L. plantarum*, *L. salivarius*, *L. casei* and *L. johnsonii* in acid conditions are higher than those of *L. bulgaricus*. Approximately 1–10% of *L. acidophilus* ingested in fermented product were found to survive until the ileum in several human studies using intestinal intubation techniques (2).

L. plantarum NCIB 8826, *L. salivarius* 433118 and some *Bifidobacterium* spp. (commercial milk product) showed a very high survival capacity. Their concentration in the ileum reached 10^8 and 10^7 cfu/ml, respectively, after a single dose; they passed through the ileum at a concentration above 10^5 cfu/ml for more than 5 h. No small bowel colonization was observed.

Similarly, some *Bifidobacterium* spp. from fermented dairy products and *L. plantarum* NCIB 8826 exhibited a high survival in the whole gastrointestinal tract; 25–30% of the ingested bacteria being recovered from faeces. Faecal concentrations reached 10^8 cfu/g, and these bacteria did not colonize the gut (5).

Other studies in healthy volunteers with different probiotic preparations showed that the faecal concentrations of ingested *L. acidophilus*, *L. reuteri*, *L. salivarius* UCC118 and *L. rhamnosus* strain GG reached around 10^6 cfu/g (2).

The faecal recovery of bifidobacteria and lactobacilli in healthy subjects exhibited a dose–response relationship. Despite the amount up to 10^{11} cfu/day of *Lactobacillus paracasei* subsp. *paracasei* (CRL-431) viable CRL-431 bacteria could not be isolated from the fresh faecal samples from 2 weeks of treatment and 2 weeks of wash-out. In contrast, recovery of *Bifidobacteriun animalis* ssp. *lactis* (BB-12) exhibited a dose–response relationship, with 10^{10} cfu/day being the lowest dose giving a statistically significant chance of recovering viable strain BB-12 from the faeces (6).

A 10-fold increase of ingested bacteria caused the average number of recovered viable strain BB12 to increase by a factor of 20 (10^{13}). It seems evident that the higher the ingested dose, the greater the number of subjects positive for viable bacteria (10^{11} cfu/day) in young healthy adults (1).

The concentration of probiotics needed to obtain clinical effects is often quoted as $\geq 10^6$ cfu/ml in the small bowel and $\geq 10^8$ cfu/g in the colon (7).

The pharmacokinetics of three strains of LAB were studied in the human gastrointestinal tract.

L. plantarum NCIMB 8826 in the ileum reached 10^8 cfu/ml after a single dose (10^8 cfu/ml) in fermented milk. *L. fermentum* KLD and *Lactococcus lactis* MG 1363 showed lower and shorter ileal survival (8).

L. plantarum NCIMB 8826 was present at high concentrations (10^8 cfu/g) in the faeces on day 7 of the 1 week ingestion period. It was undetectable in the faeces 2 weeks after the end of the ingestion period (2).

In a healthy human subject receiving 1 g/day (about 3×10^{10} viable cells) of lyophilized *S. boulardii* fecal levels were reported to be 1.4×10^7/g (9).

A dose–response effect was observed in the prevention of castor oil-induced acute diarrhoea, where *S. boulardii* at 120×10^{10} cfu/kg protected rats from acute manifestations (10). A linear relationship was obtained ranging from no protection with 3×10^8/ml viable *S. boulardii* to 85% survival when a preparation containing 3.3×10^{10}/ml was employed in a mouse experimental model of *C. difficile* colitis. The transient presence of high levels of living *S. boulardii* in the gastrointestinal tract of gnotobiotic mice seems to be necessary to protect from *C. difficile* mortality in an animal model for human pseudomembranous colitis (9).

In most cases, even if viability is not required, it is likely correlated with most effects, as it is a useful indicator of the number of cells present, regardless of what cell component may be active.

Situations where viability is not required for probiotic activity include improved digestion of lactose, anti-hypertensive effects, and some immune system modulation activities. Certain effects have been linked to non-viable cells, e.g. cell components, enzyme activities or fermentation products (11).

Probiotic dose

As yet not much is known about the minimal dose and/or frequency of probiotics required for the probiotic effect. The dose for treatment of an acute illness by a particular probiotic agent may be lower

or higher, in the order of 10-fold or 100-fold or more in terms of cfu. In acute infectious diarrhoea it seems that higher doses of probiotics given for short courses are more effective than lower doses (4). In chronic or immunological diseases (allergic, inflammatory and/or immune diseases) the effects also depend on the duration of treatment.

Probiotic effects seem to be dose-dependent. However, the dose effect is controversial and most of the reported studies were *in vitro* experiments. The usual effective dosage in humans is 10^7–10^9 cfu/mg per day.

Effects on the immune system

The effects on health or physiology may be either direct or indirect through modifications of the endogenous ecosystem or the immune response, suggesting that a single mechanism of action for all probiotics and all effects is unlikely.

In addition to a direct impact on epithelial cells and cytokine responses, probiotics may also influence the development and activity of regulatory T cells (2).

The study of immune-modulating effects in healthy adults is problematic, because it cannot be concluded that the tested bacteria exert no health-promoting effects. The relationship between the immune system and commensal flora is a precarious one, and perturbation in immune or epithelial homeostasis can led to gut inflammation. Therefore, to evaluate the efficacy of probiotics it may be essential to identify specific target groups of individuals with more specific higher susceptibilities to the potential effects of probiotics, e.g. low bifidobacteria or lactobacilli count, microflora imbalance or intestinal immunological alterations (inflammatory bowel disease, irritable bowel syndrome, atopic dermatitis, etc.).

Different preparations of LAB stimulate intestinal lymphoid foci and their accessory cells in different ways, lending further support to the notion that live forms of LAB can stimulate specific compartments of the immune system differently to killed forms.

L. rhamnosus HN001, delivered orally as a viable probiotic supplement in a milk-based substrate, is able to enhance phagocytic capacity in mice (6). In the case of immune enhancements a dose of 10^7 *L. rhamnosus* daily for 14 days was enough to enhance the phagocytic capacity of blood leucocytes in mice but a dose of 10^9 was found necessary to enhance the phagocytic capacity of peritoneal cells. Heat-killed *L. rhamnosus* HN001 was as effective as live cells in enhancing innate cellular immune function, while only live forms enhanced specific gut mucosal antibody responses to orally administered cholera toxin vaccine (6).

No significant statistical differences were observed for phagocytic activity in blood lymphocytes, IgA faecal concentrations or production of interferon (IFN)-γ and interleukin (IL)-10 in blood cells, following *L. johnsonii* administration (10^6 cfu/ml). The IFN-γ and IL-10 production in blood cells was significantly reduced when evaluated according to number of viable faecal bacteria (12). A minimum daily dose of 10^9 seems to be required to modulate certain forms of non-specific, anti-infective mechanisms of defence.

Cell debris of *L. delbrueckii* subsp. *bulgaricus* MB453 and *L. plantarum* MB 452 stimulates peripheral blood mononuclear cells (PBMNCs) when used at a concentration higher than 10^4 cfu/ml, while both *L. azidophilus* MB443 and *L. casei* MB451 strains only require concentrations higher than 10^6 and 10^5 cfu/ml. *L. casei* subsp. *rhamnosus* (*L. GG*) had a very low stimulation capacity compared with other strains (13). Bifidobacteria stimulate pro- and anti-inflammatory cytokines more significantly than lactobacilli, but the stimulation pattern is different. The highest concentration of bifidobacteria (10^7 cfu/ml) induces PBMNCs to produce less pro- and anti-inflammatory cytokines than the lower concentration of the strains (10^3 cfu/ml).

E. coli Nissle, which has been shown to be effective in maintaining remission of ulcerative colitis, has a high stimulating capacity for IL-10 and IL-1β, compared with other strains, but a low capacity for tumour necrosis factor (TNF)-α (13).

The dose, timing and selection of patients are critical in clinical results of atopic dermatitis. Early treatment, age and long periods of administration (2 years) induce better and long-lasting improvement in newborns than in children and/or short-course therapy with *Lactobacillus* species (*L. rhamnosus* 119070/2, *L. GG*, *L. reuterii*) (14). Lactobacilllus species are beneficial in decreasing severity and extent of moderate-to-severe atopic dermatitis among children <2 years old (15).

Inflammatory bowel diseases

The intestinal microflora has been suggested to be involved in the pathogenesis of inflammatory bowel diseases in genetically predisposed subjects with immunological alterations, triggering an overly aggressive cell-mediated immune response (16). In experimental dextran sodium sulfate-induced colitis *L. crispatus* reduced the severity of tissue damage in a dose-dependent manner, while *B. subtilis* was ineffective (17). In trinitrobenzene sulfonic acid-induced experimental colitis in mice similar results

were obtained following administration of high dose combination of different lactobacilli and bifidobacteria by immunomodulation and IL-10 production (18).

The intestinal microbiota plays a critical role in the pathophysiology of pouchitis, a major complication after ileal pouch and anastomosis in patients with ulcerative colitis (19).

Recent studies have shown that probiotic treatment with VSL#3, a mixture of eight different probiotic bacterial strains at high dose (300 billion viable lyophilized bacteria) is effective in maintaining remission in pouchitis. Patients received doses of VSL#3 twice daily (3+3 g) for 9 months or until relapse: 17 of 20 patients remained in remission while all those on placebo relapsed. The same preparation administered as prophylaxis once daily (VSL#3, 6 g) maintained antibiotic-induced remission for at least a year in patients with recurrent or refractory pouchitis, ameliorating their quality of life (20). The administration of lactobacilli to patients with acute pouchitis showed no effects while in patients with mild active pouchitis it induced partial effects.

Results in Crohn's disease and irritable bowel disease are variable, but several probiotics are promising. The timing of probiotic administration, the dose and the duration of treatment increased the positive effects in selected patients (8,20).

Conclusions

We need adequate clinical trials on microbial strains with defined characteristics, as well as a better definition of patients and a more appropriate use of probiotic in terms of number of bacteria, administered doses and expected effects.

At present, we cannot define the optimal amount of bacteria for probiotic effects. Thus, we need further investigations to define the effective dose for each strain, and their appropriate utilization for different clinical situations.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Christensen HR, Larsen CN, Kaestel P, Rosholm LB, Sternberg C, Michaelsen KF, et al. Immunomodulating potential of supplementation with probiotics: a dose-response study in healthy young adults. FEMS Immunol Med Microbiol. 2006;47:380–90.
2. Marteau P, Shanahan F. Basic aspects and pharmacology of probiotics: an overview of pharmacokinetics, mechanisms of action and side-effects. Best Pract Res Clin Gastroenterol. 2003;17:725–40.
3. Allen SJ, Okoko B, Martinez E, Gregorio G, Dans LF. Probiotics for treating infectious diarrhoea. Cochrane Database Syst Rev 2004;CD003048.
4. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. Lancet Infect Dis. 2006;6:374–82.
5. Vesa T, Pochart P, Marteau P. Pharmacokinetics of *Lactobacillus plantarum* NCIMB 8826, *Lactobacillus fermentum* KLD, and *Lactococcus lactis* MG 1363 in the human gastrointestinal tract. Aliment Pharmacol Ther. 2000;14:823–8.
6. Donnet-Hughes A, Rochat F, Serrant P, Aeschlimann JM, Schiffrin EJ. Modulation of nonspecific mechanisms of defense by lactic acid bacteria: effective dose. J Dairy Sci. 1999;82:863–9.
7. Sanders ME. Probiotics: considerations for human health. Nutr Rev. 2003;61:91–9.
8. Oozeer R, Leplingard A, Mater DD, Mogenet A, Michelin R, Seksek I, et al. Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk. Appl Environ Microbiol. 2006;72:5615–7.
9. Elmer GW, Corthier G. Modulation of *Clostridium difficile* induced mortality as a function of the dose and the viability of the *Saccharomyces boulardii* used as a preventative agent in gnotobiotic mice. Can J Microbiol. 1991;37:315–7.
10. Girard P, Pansart Y, Lorette I, Gillardin JM. Dose-response relationship and mechanism of action of *Saccharomyces boulardii* in castor oil-induced diarrhea in rats. Dig Dis Sci. 2003;48:770–4.
11. Sanders ME, Veld JH. Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labelling issues. Antonie van Leeuwenhoek. 1999;76:293–315.
12. Gill HS, Rutherfurd KJ. Viability and dose-response studies on the effects of the immunoenhancing lactic acid bacterium *Lactobacillus rhamnosus* in mice. Br J Nutr. 2001;86:285–9.
13. Helwig U, Lammers KM, Rizzello F, Brigidi P, Rohleder V, Caramelli E, et al. *Lactobacilli, bifidobacteria and E. coli* nissle induce pro- and anti-inflammatory cytokines in peripheral blood mononuclear cells. World J Gastroenterol. 2006;12:5978–86.
14. Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, et al. Effect of probiotic Lactobacillus strains in children with atopic dermatitis. J Allergy Clin Immunol. 2003;111:389–95.
15. Weston S, Halbert A, Richmond P, Prescott SL. Effects of probiotics on atopic dermatitis: a randomised controlled trial. Arch Dis Child. 2005;90:892–7.
16. Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology. 2004;126:1620–33.
17. Castagliuolo I, Galeazzi F, Ferrari S, Elli M, Brun P, Cavaggioni A, et al. Beneficial effect of auto-aggregating Lactobacillus crispatus on experimentally induced colitis in mice. FEMS Immunol Med Microbiol. 2005;43:197–204.
18. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M. Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-beta-bearing regulatory cells. J Immunol. 2005;174:3237–46.
19. Kühbacher T, Ott SJ, Helwig U, Mimura T, Rizzello F, Kleessen B, et al. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. Gut. 2006;55:833–41.
20. Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut. 2004;53:108–14.