The Journal of Nutrition
Supplement: Guidance for Assessing Probiotics Beneficial Effects—How to Fill the GAP

Guidance for Substantiating the Evidence for Beneficial Effects of Probiotics: Current Status and Recommendations for Future Research[1–3]

Ger T. Rijkers,[4] Stig Bengmark,[5] Paul Enck,[6] Dirk Haller,[7] Udo Herz,[8] Marko Kalliomaki,[9] Satoshi Kudo,[10] Irene Lenoir-Wijnkoop,[11] Annick Mercenier,[12] Eveliina Myllyluoma,[13] Sylvie Rabot,[14] Joseph Rafter,[15] Hania Szajewska,[16] Bernhard Watzl,[17] Jerry Wells,[18] Danielle Wolvers,[19] and Jean-Michel Antoine[20]

[4]Department of Surgery, University Medical Center Utrecht, and Department of Medical Microbiology and Immunology, St. Antonius Hospital, Nieuwegein, The Netherlands; [5]University College London, London, United Kingdom; [6]Department of Internal Medicine VI, University Hospital, Tübingen, Germany; [7]Chair for Biofunctionality, ZIEL-Research Center for Nutrition and Food Science, Technische Universität München, Freising-Weihenstephan, Germany; [8]Research and Development, Mead Johnson Nutrionals a Bristol-Myers Squibb Company, Evansville, IN; [9]Department of Paediatrics, University of Turku, Turku, Finland; [10]Yakult Europe, Almere, The Netherlands; [11]International Dairy Federation FIL-IDF, Paris, France; [12]Allergy Group, Nutrition and Health Department, Nestlé Research Center, Lausanne, Switzerland; [13]Valio Ltd., Research Centre, Helsinki, Finland and Institute of Biomedicine, Pharmacology, University of Helsinki, Helsinki, Finland; [14]INRA, UR 910 Ecology and Physiology of the Digestive Tract, F-78350 Jouy-en-Josas, France; [15]Department of Medical Nutrition, Karolinska Institute, Huddinge, Sweden; [16]The Medical University of Warsaw, Warsaw, Poland; [17]Department of Physiology and Biochemistry of Nutrition, Max Rubner-Institute, Karlsruhe, Germany; [18]Department of Host-Microbe Interactomics, University of Wageningen, Wageningen, The Netherlands; [19]Unilever Food & Health Research Institute, Vlaardingen, The Netherlands; and [20]Danone Research Center, Palaiseau, France

Abstract

Probiotic bacteria are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. There is a growing interest in probiotics within the scientific community, with consumers, and in the food industry. The interactions between the gut and intestinal microbiota and between resident and transient microbiota define a new arena in physiology, an understanding of which would shed light on the "cross-talk" between humans and microbes. The different beneficial effects of specific probiotic strains may be translated into different health claims. However, there is a need for comprehensive and harmonized guidelines on the assessment of the characteristics and efficacy of probiotics and of foods containing them. An international expert group of ILSI has evaluated the published evidence of the functionality of different probiotics in 4 areas of (human) application: *1*) metabolism, *2*) chronic intestinal inflammatory and functional disorders, *3*) infections, and *4*) allergy. Based on the existing evidence, concrete examples of demonstration of benefits and gaps are listed, and guidelines and recommendations are defined that should help design the next generation of probiotic studies. J. Nutr. 140: 671S–676S, 2010.

Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (1). The first written documentation of the health-promoting effect of probiotics can be found in the Persian bible, in which Genesis 18:8 states that Abraham owned his longevity to the daily consumption of fermented milk products. The scientific literature on probiotics starts with Metchnikoff (2), which incidentally also dealt with longevity. His hypothesis was that ingestion of lactic-acid-producing bacteria as yogurt in

[1] Published in a supplement to *The Journal of Nutrition*. Presented at the workshop "Guidance for Assessing Probiotics Beneficial Effects: How to Fill the GAP," held in Montreux, Switzerland, May 22–24, 2008 and organized by ILSI Europe in association with the International Dairy Federation. The supplement coordinator for this supplement is Agnès Méheust, ILSI Europe. Publication costs for this supplement were defrayed in part by the payment of page charges. This publication must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact. Supplement Coordinator disclosure: Agnès Méheust is employed as a Scientific Project Manager for ILSI Europe, which is largely funded by the food and related industries. Supplement Guest Editor disclosure: Josef Neu declares no conflict of interest. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, editor, or editorial board of *The Journal of Nutrition*.

[2] Author disclosures: This work was commissioned by the Probiotics Task Force of ILSI Europe composed of the following industry members: Barilla G. & R. Fratelli, Danisco, Danone, Friesland Campina, Kraft Foods, Mead Johnson Nutrition, Nestlé, Seven Seas, Unilever, Valio and Yakult Europe. G.T.R., S.B., D. H.,M.K., S.R., J.R., H.S., B.W., and J.W. have no conflicts of interest to declare. J.-M. A. and I.L.-W. are employees of Danone. P.E. is consultant for SymbioPharm. U.H. is an employee of Mead Johnson Nutrition. S.K. is an employee of Yakult. A.M. is an employee of Nestlé. E.M. is an employee of Valio. D.W. is an employee of Unilever. The opinions expressed herein are those of the authors and do not necessarily represent the views of ILSI Europe.

[3] Supplemental Tables 1–13 are available as Online Supporting Material with the online posting of this paper at jn.nutrition.org.

Correspondence should be addressed to ILSI Europe. E-mail: publications@ilsieurope.be.

0022-3166/08 $8.00
First published online February 3, 2010; doi:10.3945/jn.109.113779.

Ukrainia was the protective factor enhancing longevity. Coincidentally, in Paris, a physician reported that healthy breast fed-babies carried a Y-shaped gut microbe absent in formula-fed babies suffering from diarrhea. He hypothesized that this "*Bifidus*" was a "good" (protective) microbe. At that time, life expectancy in Europe was 51 y for men and 53 y for women. Today, life expectancy is 74 for men and 80 for women. The 23- to 27-y gain in life expectancy in the past century is the combined result of better hygiene, better housing, feeding, and clothing, and less physically demanding labor. Also, a better control of infectious diseases through many of these previous factors, through vaccination, and through antibiotics and the general improvement in preventive and curative medicine has contributed to this gain. With all these variables, direct proof for the first part of Metchnikoff's hypothesis still is lacking. As far as the second part of his hypothesis is concerned, probiotic bacteria do have the ability to interfere with growth of members of the intestinal microbiota and thus may have the ability to contribute to a better control of infectious diseases and as such promote a "healthy life."

The basic definition of probiotics, although adequate, is not sufficiently detailed to allow for an unbiased use of probiotics in the various fields of application. This especially holds true when effects of different probiotics are compared on diverse physiological and pathological processes including metabolism, infection, chronic intestinal disorders, and allergy, among other potential effects. It is impossible to extrapolate results obtained with a given probiotic strain to other strains, or to extrapolate beneficial effects of 1 strain in a particular health area to other benefits. Probiotic microorganisms are, by definition, alive at the moment of administration. Little data exist on viability of probiotic bacteria in the various parts of the human intestine, but many strains currently used as probiotics have a documented ability to survive transiently in the human gut and are recovered alive in feces (3–10). Some effects of probiotics may be bound to their metabolic activity (11). However, other effects of probiotics may not necessarily require active replication of the strain in situ, as they may be mediated, for example, by bacterial DNA or cell wall components.

Although the mechanisms of action of probiotics are largely unknown at the molecular level, a probiotic can act (**Fig. 1**) in a number of ways, including the following: *1*) Within the gut lumen by direct interaction with the complex ecosystem of the gut microbiota. Probiotics can also have a direct metabolic effect in the gut by providing enzymatic activities; *2*) by interaction with the gut mucus and the epithelium, including barrier effects, digestive processes, mucosal immune system, and enteric nervous system; and *3*) through signaling to the host beyond the gut to the liver, systemic immune system, and other potential organs such as the brain.

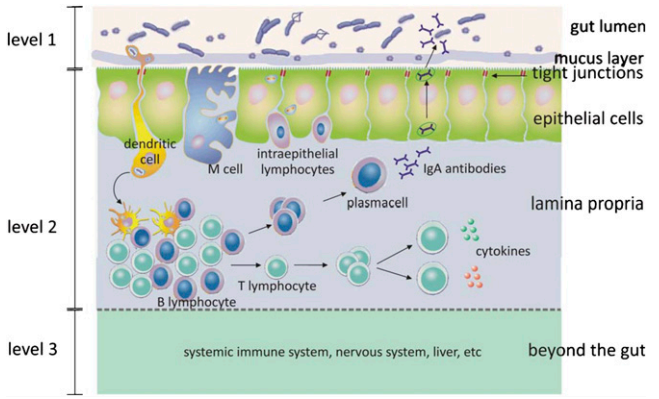

FIGURE 1 The 3 levels of action of a probiotic. Probiotic bacteria can interfere with growth or survival of pathogenic microorganisms in the gut lumen (*level 1*). Probiotic bacteria can improve the mucosal barrier function and mucosal immune system (*level 2*) and, beyond the gut, have an effect on the systemic immune system as well as other cell and organ systems such as liver and brain (*level 3*).

Most of these effects have been established in animal models or in vitro assays, but in humans direct demonstration of effects of probiotics on relevant biomarkers is limited.

Probiotic science is a rapidly expanding field and still relatively young. By the end of 2009 there were 5466 publications in the PubMed database, of which >28% (1571) were reviews. For comparison, the search term "antibiotics" yields 506,706 publications of which 8% (43,323) are reviews. Clearly, the field of probiotics does not suffer from lack of reviews but from insufficient original research.

An international expert group from ILSI set out to define recommendations for the next generation of studies and conducted an extensive literature analysis with the aims to *1*) establish the state of the art in selected benefit areas; *2*) identify gaps in the methodology to demonstrate the functionality of probiotics; and *3*) provide recommendations of best-practice approaches to substantiate the health benefits of probiotics.

The analysis was restricted to the use of probiotics in humans (**Table 1**), although probiotics for use in animal husbandry has been extensively researched and represents a considerable field of interest. Data from animal experiments were included in the analysis only when they shed a light on the mechanism of action of the probiotic strains or when they were used to conduct

TABLE 1 Applications of probiotics covered in this manuscript

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

experimental studies that are unethical in humans. Furthermore, we decided to focus their evaluation on 4 documented areas of application, i.e., metabolism, infections, inflammatory bowel diseases and irritable bowel syndrome, and allergy.

We collected clinical studies on probiotics using PubMed as the source of information, and key words with on 1 hand descriptors for probiotic(s) [probiotic(s), lactic bacteria, yog(h)urt, fermented milk(s)], and on the other hand, specific descriptors for each topic: metabolism (metabolism, carbohydrates, lactose, lipids, xenobiotics, phytoestrogens, oxalate), infections [(infectious) diarrhea, traveler's diarrhea, antibiotic-associated diarrhea, *Clostridium difficile*, respiratory, otitis, pharyngitis, *Helicobacter pylori*, necrotizing enterocolitis, critically ill, surgical patients], IBS, IBDs (inflammatory bowel diseases, Crohn's disease, ulcerative colitis, pouchitis), and allergy (allergic disease, allergic rhinitis, allergic rhinoconjunctivitis, allergy, asthma, atopic disease, atopic eczema, eczema, food allergy). The search was conducted from January 2000 to October 2008 except for lactose, where most studies have been reported before 2000.

The summary of each study is tabled on a separate document available as accompanying online material on the website of *The Journal of Nutrition*. Summary tables indicating the current level of evidence for effects of probiotics applying the criteria for evidence-based medicine (12) are included in the separate section papers (13–16).

This report focused on the scientific definition of products, and to avoid potential conflict of interest, every commercial name or brand name was deleted and replaced by the name of the strains included in the product.

The document was split into 5 parts, with 1 general introduction, including conclusions that were common to the 4 core papers, and 4 core papers dealing with 1 specific target for probiotic benefit.

General gaps and recommendations

Probiotics and their benefits are an area of intensive research in different domains. Even though well-conducted and well-focused clinical studies have started to appear [discussed in this supplement (13–16)], this area of probiotic research is still in its "infancy." Yet, clear-cut positive and negative results have now been obtained that may help to refine and focus ongoing and further research. Many human data are available on the 4 benefit areas selected by the expert groups, which made it possible to come up with conclusions and recommendations. Most of them are applicable to the 4 topics, although some of them, reported at the end of each chapter, are specific to 1 domain (13–16).

It should be kept in mind that functional foods, although their mode of action is complex, may provide an alternative to the pharmacological approach in patients who require lifetime treatment and/or suffer from serious side effects or drug resistance development.

We have identified several key points that need to be acknowledged to be able to appreciate the existing evidence and that need to be addressed to stimulate progress in substantiation of probiotics efficacy.

Because the effect size of nutritional intervention studies is recognized to be smaller than those of classical drug trials (17,18), confounding factors such as diet and lifestyle are expected to have a relatively bigger impact and thus potentially mask the exerted benefit (19). In addition, many of the conditions typically targeted in probiotic research, such as those evaluated by this Expert Group, cover multiple manifestations of closely related interdependent dysfunctions leading to a syndrome, rather than monocausal, single-symptom conditions. It is therefore of great importance to better define study populations, carefully design study protocols (including diet and lifestyle as potential confounders), and better characterize probiotic strains, such that future studies will increase our understanding of the field and will be able to help realize the great potential of probiotics.

Identification of target and study population. Assessment of the functionality of probiotics should ideally be performed directly in the target population, which may be either the general population or a subgroup of subjects with a given condition. In designing a probiotic study for, e.g., risk reduction for a certain infection, the investigator(s) must realize that within the healthy population variation will exist in immune status, ranging from fully immunocompetent to a certain degree of immunodeficiency (suboptimal health state) (20). This scale will be gradual, and thus it will be difficult to mark clearly the border between these states (**Fig. 2**). In such a model, the projected functionality of probiotics in risk reduction of infection would mainly be measurable in the segment of the population with a suboptimal immune function, whereas the effect may be undetectable in the fully immunocompetent subjects (Fig. 2). The impact of the gut microbiota on human health is increasingly recognized nowadays. Yet, it has not been established to date to what extent differences in microbiota composition and/or function of the studied subjects may have influenced the results of similar clinical trials. Therefore, a better characterization of the microbiota of the study population, relying on the high-throughput technologies under development, could become an important addition to future trials.

Probiotics may impact different pathways to end up in an integrated functional benefit or in modulating different risk factors. Therefore, the selection of the adequate study population should take into account the global physiology. The link between the recorded risk factors and their role in the disease

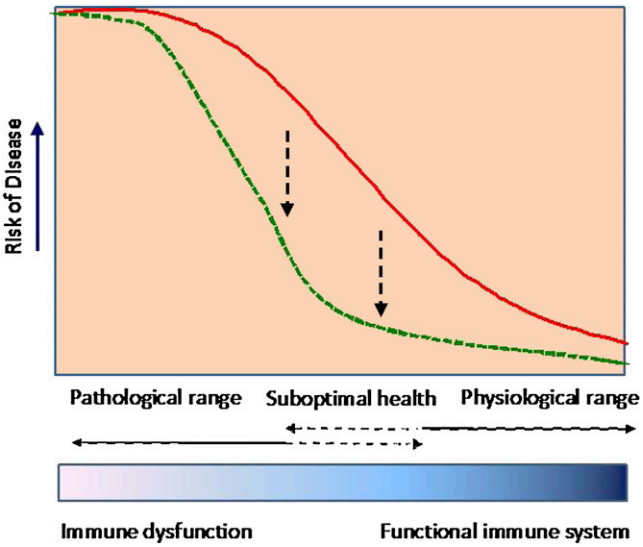

FIGURE 2 Model for functionality of probiotics. The drawn curve indicates the risk in the population on disease (such as infections, inflammatory diseases, allergy) as a function of immunocompetence. The dotted curve shows the (hypothetical) situation after intervention with probiotics; broken arrows indicate the reduction in risk for disease.

development/maintenance of a healthy state should be described. To achieve this we might benefit from new tools such as -omics technologies that will contribute to better define biomarkers or surrogate markers for healthy, at-risk, or (transiently) diseased subjects. It is generally recognized that the influence of nutrition on health is characterized by small effects over a long period of time. It is necessary to be able to extrapolate from a small study population to the overall target group. This extrapolation may be carried on the basis of relevant epidemiological background data as well as interaction and intervention modeling methods.

Considerations for protocol design. The objective of a clinical study on probiotics is to demonstrate either a significant improvement of a clinical condition or a reduction of a risk factor. This objective holds true for clinicians as well as for consumers. When changes are relatively small, this would require either a very large population or quite a long trial, as is the case, for example, for allergies. Therefore, surrogate markers of endpoints are commonly used in clinical trials. Most often the markers used in probiotics studies were not validated surrogate markers. For example, several studies have been reporting a stimulating effect of probiotics on components of the innate immune system including natural killer cell activity or phagocytosis (21). However, the correlation of these biomarkers with a clinical endpoint, e.g., resistance against infections, has not consistently been explored. To advance our knowledge on the working mechanisms of probiotics, and to increase our understanding of the role of various biomarkers in a number of health conditions, it is essential that more probiotic studies incorporate both clinical outcomes and measurement of biomarkers.

Background diet. Most studies excluded other potential active microorganisms from the diet of the tested subjects during run-in and/or wash-out periods. Potential dietary components that may bias the effect of the probiotics being explored must be reported or standardized in the diets of both the control and the probiotic group; e.g., the amount of saturated fat or cholesterol when exploring the efficacy of a potential probiotic on blood lipids, or the amount of indigestible carbohydrates when measuring the breath test response to a lactose load.

Product effects. Most recent studies clearly described the tested probiotics, but did not describe the food matrix composition or general processing, whose impact has not been extensively studied in human trials. This can interfere with the efficacy of the probiotic in terms of viability, stability, and the quantity of active biocompounds that are responsible for the studied health effect. The most documented example is the deleterious effect of heat-process treatment on the ability of yogurt cultures to break down lactose (22).

Probiotic strain selection and characterization. The ILSI Europe Task Force and Expert Group on Probiotics agreed with the conclusion of the International Organization for Standardization (ISO) joint action team on probiotics that the strain-specific benefits of probiotics emphasize the need for proper strain identification (23).

For strain identification, different molecular microbiological typing techniques may be applied such as pulsed-field gel electrophoresis, amplified fragment-length polymorphism, or mulltilocus sequence typing. At the International Dairy Federation, efforts have been undertaken to establish 1 of these techniques as a standardized ISO/IDF method for unambiguous strain identification. The techniques mentioned generate a "fingerprint" of a particular bacterial strain. Nevertheless, molecular fingerprinting does not provide detailed information on structure or sequence of functionality-encoding genes. Therefore, strains that appear different on molecular typing may carry the same or different functionality-encoding genes (**Fig. 3**). With a few exceptions (24–28), the genes that determine the health benefit delivered by specific probiotic strains have not been identified to date. Genome comparison of strains belonging to the same species but that differ in functionality or are nonfunctional may help in identifying key genes involved in the interaction with the host. With this information in hand, genetic approaches, i.e., knock-out and knock-in of candidate genes, will contribute to definitively attributing a role to purported functionality-encoding genes. In addition, efforts remain to be invested in the identification of bioactive compounds of strains with a proven health benefit.

Selection of strains should be based not solely on molecular typing and comparison of different strains but also on metabolic characterization as well as the metabolic consequences of interactions with the matrix, with the background diet, and with the host.

Growth conditions and biological/physiological state of the probiotics strains may modulate their metabolic capacity in the human gut or their impact on the interaction with the host as illustrated in the work of van Baarlen et al. (29). This is an important area for future research and should be better recorded (23).

In addition, there is a need to invest more efforts to delineate the predictive value of in vitro assays and animal models. This in turn will improve the selection of candidate probiotic strains to be included in clinical trials. It might be argued that a number of published clinical trials have failed because of poor prior selection of the probiotic strain and of a limited capacity to mimic in preclinical assays surrogate models for different diseases, risk factors, or syndromes. These pilot (small-scale) human trials would allow benchmarking of candidate probiotic strains and studying mechanisms of actions in humans.

Metaanalyses should be applied with caution

Metaanalyses are powerful tools to explore and strengthen the scientific evidence; however, this statistical tool must be used wisely. When a metaanalysis pools data on different probiotics (efficacious and nonefficacious), different conditions, and dif-

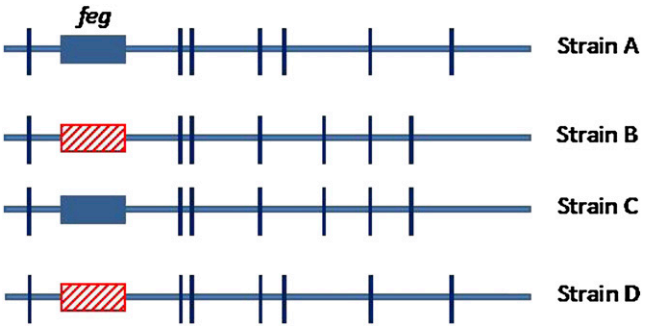

FIGURE 3 Molecular fingerprinting of probiotic strains. In this hypothetical example strain A would be a proven effective probiotic strain because of expression of the functionality encoding gene *feg* and a given molecular fingerprint. Strain B lacks this *feg* gene and has a different fingerprinting pattern than strain A. Strain C, however, does express the *feg* gene with a "nonfunctional" fingerprint, and strain D has an identical fingerprint as strain A but lacks the *feg* gene.

ferent patient characteristics, the result can only be an average noneffect because of the heterogeneity of benefits and probiotics included in the metaanalysis. Alternatively, metaanalyses can point toward generic activities of bacteria rather than the discrete function of 1 particular strain. Schematically, a metaanalysis can be designed to aim at a variety of targets for various purposes:

Compiling the results of trials exploring the effect of the same microorganism on a specific condition. This makes it possible to confirm the efficacy of that given probiotic on that condition and to explore the variability of the effects among different study groups.

Comparing the effect of different candidates on the same specific benefit ending in: *1*) Identification of 1 (or a cluster of) "active" probiotic(s) on that specific benefit, and 1 (or a cluster of) "nonactive" microorganism(s) on that condition illustrating the specificity of some probiotics; *2*) Identification of different strains within a species that are able to provide a similar benefit; *3*) Theoretically, when all tested strains and species have a similar beneficial effect, this could point toward a "generic" bacterial benefit. Thus far this has not been found with the possible exception of necrotizing enterocolitis (30).

Most of the time the aggregate results of all tested probiotics, active and nonactive together, are nonsignificant, highlighting the fact that there is no "generic" benefit common to all probiotics. Therefore, it is important, when using and designing a metaanalysis, to identify the objective and accordingly to select the right inclusion criteria to achieve useful conclusions.

General conclusions

In conclusion, to demonstrate that a strain is able to provide a given benefit, and therefore be recognized as a probiotic, the following are needed:

Identify the tested strain and give a fair description of the food matrix or probiotic carrier as well as an indication of the process used to make the product tested in clinical trials. This will help to assess the totality of evidence for a given probiotic.

Conduct human intervention studies according to good clinical practices, which include monitoring and reporting on confounding factors, e.g., ingestion of other potentially active microorganisms, dietary components, drugs, or lifestyle that may interfere with the explored benefit.

When available, use a protocol that has been able to discriminate between an active and an inactive strain for that specific benefit: similar design, number of subjects, and duration. In the near future, that will become more and more accessible.

Harmonize the way results are expressed. Different teams using similar markers have expressed the results of their trials in different ways. For example, the effect of probiotics on lactose absorption has been measured by the breath test technique by different teams. Results are expressed either by the area under the curve or the peak hydrogen concentration or the slope of the increase in hydrogen concentration and compared either to a standard nonabsorbable carbohydrate (lactulose) or to milk or to another control product. This does not ease the comparison of the different strains tested by different teams.

Use metaanalyses to distinguish between (clusters of) active and nonactive strains on a given benefit and in compiling data on a given probiotic for a specific benefit.

Carefully select and define the target population to be able to detect efficiently the benefit (e.g., lactose absorption in lactose malabsorbers, reduction of hypercholesterolemia, reduction of diarrhea) and to further extrapolate to the general population.

Acknowledgments

All authors wrote, read, and approved the final manuscript. G.R and J.M.A. had primary responsibility for final content. We thank the following past and present scientific project managers from ILSI Europe: Sandra Tuijtelaars (also from International Dairy Federation), Carina Madsen, Fiona Samuels, Margarita Corrales, and Agnès Méheust.

Literature Cited

1. Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. 2001 Ref Type: Report. Available from: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf.
2. Metchnikoff E. The prolongation of life. Optimistic studies. London: Butterworth-Heinemann, 1907.
3. Rochet V, Rigottier-Gois L, Levenez F, Cadiou J, Marteau P, Bresson JL, Goupil-Feillerat N, Dore J. Modulation of *Lactobacillus casei* in ileal and fecal samples from healthy volunteers after consumption of a fermented milk containing *Lactobacillus casei* DN-114 001Rif. Can J Microbiol. 2008;54:660–7.
4. Rochet V, Rigottier-Gois L, Ledaire A, Andrieux C, Sutren M, Rabot S, Mogenet A, Bresson JL, Cools S, et al. Survival of *Bifidobacterium animalis* DN-173 010 in the faecal microbiota after administration in lyophilised form or in fermented product—a randomised study in healthy adults. J Mol Microbiol Biotechnol. 2008;14:128–36.
5. Tuohy KM, Pinart-Gilberga M, Jones M, Hoyles L, McCartney AL, Gibson GR. Survivability of a probiotic *Lactobacillus casei* in the gastrointestinal tract of healthy human volunteers and its impact on the faecal microflora. J Appl Microbiol. 2007;102:1026–32.
6. Elli M, Callegari ML, Ferrari S, Bessi E, Cattivelli D, Soldi S, Morelli L, Goupil FN, Antoine JM. Survival of yogurt bacteria in the human gut. Appl Environ Microbiol. 2006;72:5113–7.
7. Bezkorovainy A. Probiotics: determinants of survival and growth in the gut. Am J Clin Nutr. 2001;73:399S–405S.
8. Matto J, Fonden R, Tolvanen T, Wright A, Vilpponen S, Satokari R, Saarela M. Intestinal survival and persistence of probiotic *Lactobacillus* and *Bifidobacterium* strains adminsitered in triple-strain yoghurt. Int Dairy J. 2006;16:1174–80.
9. Johansson ML, Nobaek S, Berggren A, Nyman M, Bjorck I, Ahrne S, Jeppsson B, Molin G. Survival of *Lactobacillus plantarum* DSM 9843 (299v), and effect on the short-chain fatty acid content of faeces after ingestion of a rose-hip drink with fermented oats. Int J Food Microbiol. 1998;42:29–38.
10. Goldin BR, Gorbach SL, Saxelin M, Barakat S, Gualtieri L, Salminen S. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. Dig Dis Sci. 1992;37:121–8.
11. Goossens D, Jonkers D, Russel M, Stobberingh E, van den Bogaard A, Stockbrugger R. The effect of *Lactobacillus plantarum* 299v on the bacterial composition and metabolic activity in faeces of healthy volunteers: a placebo-controlled study on the onset and duration of effects. Aliment Pharmacol Ther. 2003;18:495–505.
12. Michail S, Sylvester F, Fuchs G, Issenman R. Clinical efficacy of probiotics: review of the evidence with focus on children. J Pediatr Gastroenterol Nutr. 2006;43:550–7.
13. Rabot S, Rafter J, Rijkers G, Watzl B, Antoine JM. Guidance for substantiating the evidence for beneficial effects of probiotics: Impact of probiotics on digestive system metabolism. J Nutr. 2010;140:677–89.
14. Haller D, Antoine JM, Bengmark S, Enck P, Rijkers G, Lenoir-Wijnkoop I. Guidance for substantiating the evidence for beneficial effects of probiotics: Probiotics in chronic inflammatory bowel disease (IBD) and the functional disorder, irritable bowel syndrome (IBS). J Nutr. 2010;140:690–7.
15. Kalliomaki M, Antoine JM, Herz U, Rijkers G, 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;140:713–21.
16. Wolvers D, Antoine JM, Myllyluoma E, Schrezenmeir J, Szajewska H, Rijkers G. Guidance for substantiating the evidence for beneficial effects of probiotics: Prevention and management of infections by probiotics. J Nutr. 2010;140:698–712.
17. Franz MJ, VanWormer JJ, Crain AL, Boucher JL, Histon T, Caplan W, Bowman JD, Pronk NP. Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. J Am Diet Assoc. 2007;107:1755–67.
18. Khan NA, McAlister FA, Rabkin SW, Padwal R, Feldman RD, Campbell NR, Leiter LA, Lewanczuk RZ, Schiffrin EL, et al. The 2006 Canadian Hypertension Education Program recommendations for the management of hypertension: Part II—Therapy. Can J Cardiol. 2006;22:583–93.
19. Westrate JA, Van Poppel G, Verschuren PM. Functional foods, trends and future. Br J Nutr. 2002;88:S233–5.
20. Kudo SE. Intestinal flora and health. In: Van der Kamp JW, Asp NG, Miller Jones J, Schaafsma G, editors. Dietary fibre bio-active carbohydrates for food and feed. Wageningen (The Netherlands): Wageningen Academic Publishers; 2004, p. 197–202.
21. Albers R, Antoine JM, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartin S, Sanderson IR, Van LJ, et al. Markers to measure immunomodulation in human nutrition intervention studies. Br J Nutr. 2005;94:452–81.
22. Savaiano DA, AbouElAnouar A, Smith DE, Levitt MD. Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactase-deficient individuals. Am J Clin Nutr. 1984;40:1219–23.
23. Mercenier A, Lenoir-Wijnkoop I, Sanders ME. Physiological and functional properties of probiotics. Bull Int Dairy Fed. 2008;429:2–6.
24. Tabasco R, Garcia-Cayuela T, Pelaez C, Requena T. *Lactobacillus acidophilus* La-5 increases lactacin B production when it senses live target bacteria. Int J Food Microbiol. 2009;132:109–16.
25. Kim Y, Oh S, Kim SH. Released exopolysaccharide (r-EPS) produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic *Escherichia coli* O157:H7. Biochem Biophys Res Commun. 2009;379:324–9.
26. Lebeer S, Vanderleyden J, De Keersmaecker SC. Genes and molecules of lactobacilli supporting probiotic action. Microbiol Mol Biol Rev. 2008;72:728–64.
27. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453:620–5.
28. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. Proc Natl Acad Sci USA. 2007;104:7617–21.
29. van Baarlen P, Troost FJ, van Hemert S, van der Meer C, de Vos WM, de Groot PJ, Hooiveld GJ, Brummer RJ, Kleerebezem M. Differential NF-kappaB pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. Proc Natl Acad Sci USA. 2009;106:2371–6.
30. Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2008;CD005496.