Science Publishers

CRC Press
Taylor & Francis Group

Probiotic Foods in Health and Disease

Editors
G. Balakrish Nair
Yoshifumi Takeda

PROBIOTIC FOODS IN HEALTH AND DISEASE

Editors

G. Balakrish Nair
Director, National Institute of Cholera and Enteric Diseases, Kolkata, India

Yoshifumi Takeda
Director, Collaborating Research Center of Okayama University for Infectious Diseases,
National Institute of Cholera and Enteric Diseases, Kolkata, India

National Institute of Cholera and Enteric Diseases, Kolkata, India
Indian Council of Medical Research, New Delhi

Yakult
DANONE

Yakult Danone India (P) Ltd., New Delhi

CRC Press
Taylor & Francis Group
an informa business
www.crcpress.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
270 Madison Avenue
New York, NY 10016
2 Park Square, Milton Park
Abingdon, Oxon Ox14 4RN, UK

Science Publishers
Enfield, New Hampshire

Published by Science Publishers, P.O. Box 699, Enfield, NH 03748, USA
An imprint of Edenbridge Ltd., British Channel Islands

E-mail: *info@scipub.net* Website: *www.scipub.net*

Marketed and distributed by:

CRC Press
Taylor & Francis Group
an informa business
www.crcpress.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
270 Madison Avenue
New York, NY 10016
2 Park Square, Milton Park
Abingdon, Oxon Ox14 4RN, UK

ISBN 978-1-57808-698-6

Library of Congress Cataloging-in-Publication Data

Probiotic foods in health and disease / editors, G. Balakrish Nair, Yoshifumi Takeda ; National Institute of Cholera and Enteric Diseases, Indian Council of Medical Research.

p. ; cm.

Includes bibliographical references and index.

ISBN 978-1-57808-698-6 (pbk.)

1. Probiotics. 2. Functional foods. I. Nair, G. Balakrish. II. Takeda, Yoshifumi, 1935- III. National Institute of Cholera & Enteric Diseases (India) IV. Indian Council of Medical Research.

DNLM: 1. Probiotics--pharmacology. 2. Food, Fortified--microbiology. 3. Lactobacillus--metabolism. 4. Nutritive Value. 5. Probiotics--therapeutic use. QU 145.5]

RM666.P835P68 2011

615'.329--dc22

2010040417

Printed in India

Published by Science Publishers, Enfield, USA

Foreword

It is sometimes easy to forget that scientific knowledge about probiotics and its role in health has its own history. Probiotics have been around since time immemorial with the realization of the impact that they were exercising but without understanding the science behind the impact. However, the situation changed markedly during the past decade or so with the advent of high throughput molecular tools and sequencing technologies providing greater insights into the complex assemblage of the gut microbial ecology unfolding its significant role in maintaining optimum health. With better understanding of the host-gut microbe interactions also came the recognition that interventions such as Probiotics that help in favorable modification of the gut flora may offer remarkable potential for improved health. This led to a rapid expansion of the probiotic arena through an escalation in probiotic research heralded by the large number of publications in the area highlighting the importance of this unique science. Scientific evidence soon pointed to the fact that besides playing a role in digestive health, probiotics could also modulate the immune system and have a significant effect on the alleviation of infectious diseases in children, adults and high risk groups. The past decade has validated its utility as an important therapeutic and preventive modality for gastrointestinal diseases, treatment and prevention of allergic disorders, chronic inflammatory diseases, prevention of cancers, immune stimulation and reduction of respiratory diseases.

This prompted the Indian Council of Medical Research (ICMR) to provide a thrust to the entire gamut of probiotic science in the country by initiating a series of annual probiotic symposia. The initiative aimed at providing a common scientific platform for basic scientists and clinicians to share and exchange knowledge and views and hence delve into newer areas of probiotic research. The 3rd India Probiotics Symposium in keeping with its theme "Probiotic Foods in Health and Disease" was an endeavor to bring together a panel of experts to review and present the more recent and relevant findings in the field that could finally contribute to integrating the advances for the development of scientifically substantiated products. This symposium organized for the third consecutive year by the National Institute of Cholera and Enteric Diseases, Kolkata also aimed at the dissemination of mechanisms involved in the translation of basic scientific findings into clinical studies and potentially new probiotic applications in the prevention and possible treatment of various diseases. Recognizing the need for establishing standards for the probiotic industry the symposium also helped address the Indian regulatory status of probiotics in food and reviewed the global regulatory milieu and its impact on scientific research. Policy recommendations that would advance the field of probiotics were made by experts present at the meeting.

The sponsors of the symposium, "Yakult Danone India" have more than 70 years of research to back the scientific efficacy of their strain, *Lactobacillus casei* strain Shirota in their product Yakult which has been accredited by the Ministry of Health, Labor and Welfare, Japan (FOSHU). Being pioneers in the area both Yakult and Danone engage in collaborative research through well conducted human trials across the globe to validate the health benefits of their probiotic products. Their endeavor remains to promote the science behind this niche category.

The 3rd India Probiotic Symposium, a continuation of a series, will be critical in establishing scientific credibility for this new evolving science in the country. I congratulate all those who have come along to create this difference and help develop a road map for the future……..

Prof. N.K. Ganguly
Former Director General Indian Council of Medical Research,
President, Jawaharlal Institute of Post Medical Education and Research, Pondicherry
Distinguished Biotechnology Fellow & Advisor, Translational Health Sciences &
Technology Institute, National Institute of Immunology, New Delhi

Preface

Microbiology is witnessing change of unprecedented dimension. Microbiologists have worked for eons on how microbial pathogens overwhelm the host to cause disease. Extensive work has been done on a whole range of microbial armaments that pathogens use to evade host defences and cause disease. The fact that microbes can be used to treat and prevent disease is an emerging concept permeating into the way of thinking of pathogen hunters. There is, therefore, a paradigm shift in the mindset of how microbes are conceptualized. Such a change has also been catalysed by the knowledge that only about 5% (if not lower) microorganisms have been cultured and the vast majority remain anonymous, uncultured and unrecognized. The other central citadel in microbiology, the concept of culturability, is crumbling. And the concept that is gaining a foothold is assessing a niche by its community rather than individual culture. The intestinal microbiota of humans and animals is an ecological niche of great importance and is a formidable organ within an organ and its function in immune stimulation, competitive inhibition and a host of other beneficial roles is beginning to be recognized in the past decade or so. Commensurate with these changes, microbiologists are now homing on how the intestinal microbiota protects through a range of mechanisms and how the microbiota can be manipulated with probiotics is which are just being revealed. These changes have also ushered the science of Therapeutic Microbiology.

While there have been rapid advances in the science and practice of probiotics in the west, the pace has been much slower in countries like India. The grand plan of the symposium and this book is to disseminate this new found enthusiasm in probiotics both among clinicians and basic scientists. The intention is to spread the message of the need for more research and the need for application based on solid science. This proceeding captures the essence of the talks delivered at the third Probiotic symposium conducted in Delhi.

We would like to thank the authors for their valuable contribution and for cooperating with us to meet deadlines. The invaluable help extended by Dr. Neerja Hajela, Head, Science, Yakult Danone India, Delhi in skilfully coordinating, editing the first draft and collating the chapters of this book is gratefully acknowledged. We are certain that this book would not have come to this stage without her assistance. We also wish to thank Mr. Sunil Bernard and Mrs. Saheli Samanta, both from National Institute of Cholera and Enteric Diseases, for help rendered in formatting and compiling the references.

G. Balakrish Nair, Ph.D.
Yoshifumi Takeda, M.D., Ph.D.

Contents

Foreword iii

Preface v

1. Probiotic Foods Today and the Future: Where Science and Commerce Need to Meet 1
Gregor Reid, Jean Maclaim, Ruben Hummelen, Joke Dols and *Wayne L. Miller*

2. Probiotics- Guidelines, Regulations and Health Claims 15
B. Sesikeran and *R. Hemalatha*

3. Legislation of Probiotic Fermented Milk Drinks—An Important Functional Food in Asia 21
Akiyoshi Hosono

4. "FOSHU" Japanese Regulations for Probiotic Foods 34
Osamu Chonan

5. Probiotics: A Pharmacologist's Perspective 42
S.D. Seth and *Mohua Maulik*

6. Host-microbe Interactions in the Gut: A Microbiologist's Perspective 49
R. Chaudhry, V.D. Bamola and *P. Panigrahi*

7. Probiotics and Intestinal Defensins: Augmenting the First Line of Defence in Gastrointestinal Immunity 61
Dipankar Ghosh

8. Toward the Identification of Gut Microbial Markers Associated to Irritable Bowel Syndrome 75
Raish Oozeer and *Patrick Veiga*

9. Probiotics and their Role in Reducing Allergic Disorders 81
S.K. Kabra and *Rakesh Lodha*

10. Oral Microflora, Oral Health and Probiotics 87
Mahesh Verma and *Ruchika Roongta Nawal*

11. Proiotics and Women's Health 111
Indrani Ganguli, Mala Srivastava and *Mamta Dagar*

Index 123

1 Probiotic Foods Today and the Future: Where Science and Commerce Need to Meet

Gregor Reid, Jean Maclaim, Ruben Hummelen, Joke Dols and Wayne L. Miller
Canadian Research and Development Centre for Probiotics
Lawson Health Research Institute
London
Canada
E-mail: gregor@uwo.ca

PROBIOTICS TODAY

The field of probiotics has never been stronger, as measured by numbers of publications on the topic and the growing number of products entering the marketplace. Still, nine years after the United Nations and World Health Organization landmark definition of probiotics "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (22), this is a good time to reflect on where the field stands and the challenges and opportunities that lies ahead.

The recognition of the importance of microbiota in human and animal health is long overdue, given the volume of organisms inhabiting the body. The exogenous application of selected organisms to restore and maintain health has biblical roots, yet has taken nearly a century since Metchnikoff's observations, to be delivered to the masses. Modern methods of bulk fermentation, drying and delivery of probiotic organisms in various food types have made it possible to widely distribute products. However, many challenges remain.

All too often, companies ignore the FAO/WHO guidelines (23) as to what it takes to be appropriately called a probiotic. The majority of products in the market claiming to be 'probiotic' are in fact foods with bacteria added. We need to reiterate that the companies that sell a product are responsible for proving that it confers a health benefit and the only way to do this is through placebo controlled clinical studies. Studies that report probiotic use when infact the product had never been shown to be probiotic can lead to adverse publicity and confusion amongst consumers (9). Examples abound for this point, with three main categories being the most problematic:

1. Using say two strains shown to be probiotic in capsules, and adding them to another two strains in milk and expecting the same outcomes.

2. Composing a formulation of multiple strains and claiming that more the merrier and there must somehow be highly functional in the host.
3. Making claims about one product that has not been appropriately tested in humans, and using the work of another product to support it being efficacious.

(1) In the first example, distributors often go to fermentation companies and ask, or are recommended to purchase, 'probiotic' strains so that they can be added to a product. But, what if the same strain in dried encapsulated form shown to increase natural killer cells in elderly people, is now to be added to milk, fruit juice, a capsule with other strains, a yogurt with two starter cultures, a dried yogurt, frozen produce, or to a capsule that will not be appropriately handled in terms of exposure to temperature and moisture? What if the end users are children or people with diarrhoea? Even though regulatory agencies will not normally permit disease claims for foods, the company is still claiming that it is selling a 'probiotic'. But, while the product is unlikely to cause harm, unless studies are performed there is no way of knowing if it will actually confer measurable health benefits: thus, it does not meet the criteria for 'probiotic'. This might seem pedantic or asking for standards more associated with pharmaceutical agents, but it speaks to the core of the field. If probiotics are to gain wide acceptance and the respect of scientists, healthcare professionals and others, there must be an expectation that they meet minimum standards and not just the normal absence of contaminants and suitable viable count at expiry.

If two strains administered in milk daily to elderly subjects increases tumouricidal effects of natural killer cells (27), could we expect the same effect with these strains in a different formulation and concentration or in younger healthy adult subjects? These issues are at the core of the current probiotic field and there are no clear answers. In the elderly subjects there can be an age-related decline in lymphoid cell activity (immunosenescence) and therefore a benefit to administering probiotics. But, if no such impact (benefit) occurs with a different formulation or younger target group, then the product would not be probiotic. Thus, if the new product is marketed to children and adults, there should be at least one randomized controlled study showing it to be beneficial—probiotic.

(2) The issue here is not to make foods adhere to pharmaceutical evidence-based standards, but rather to ensure that a standard for 'probiotic' is upheld. If companies want to add Lactobacilli or Bifidobacteria or other 'probiotic' strains to their foods, without doing human studies, they should be permitted to do so but be made to call the product something other than probiotic. For example, Theralac capsules contain "Five Probiotic Species": *Lactobacillus acidophilus* LA-1–10 billion CFU; *Lactobacillus paracasei* F-19–5 billion CFU; *Lactobacillus rhamnosus LR-44*–2 billion CFU; *Bifidobacterium lactis* BL-34–10 billion CFU; and *Bifidobacterium lactis Bi-07*–3 billion CFU with a guarantee of 30 billion CFUs altogether (http://www.theralac.com/). It also contains 2 prebiotics that stimulate probiotics (patent pending) and natural acid-proof gel formulation (patented). The company claims that Theralac promotes a healthy soft-lining (wall) in the intestinal tract which results in improved digestion, regularity and nutrient absorption. A search of PubMed will be futile if you want to find any studies on this formulation. Strain LA-1 has been used in combination with *B. lactis* Bb12 as a potential immunomodulator (53), F-19 has been used orally in a pilot study which used vaginally applied *L. acidophilus* to treat bacterial vaginosis (BV) (20), while *Bifidobacterium animalis* subsp *lactis* Bi-07 has been used with *L. acidophilus* NCFM daily for 6 months to reduce fever, rhinorrhea and cough incidence and duration and antibiotic prescription incidence and the number of missed school

days attributable to illness, for children 3 to 5 years of age (39). None of these studies examined the softness of the gut wall, nor would it be likely that such a thing could be assessed easily. None of the studies examined improved digestion, regularity or nutrient absorption. Furthermore, different strains induce different immune responses in the host (30, 32, 40), meaning that some strains could counter the activity of another.

Another example is iFlora which contains *Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus* with a total 15 billions cells per capsule, apparently designed to help maintain normal levels of yeast in the body and support healthy vaginal flora and urinary tract balance, as well as healthy digestion and normal bowel function (http://sedonalabs.com/shop/pc/viewPrd.asp?idcategory=2&idproduct=2). This is a lot of very different health effects for a product whose strains are not designated and therefore impossible to track through PubMed. Not all probiotic strains enhance vaginal or urinary health (6, 37) and there is no evidence of what a 'normal' level of yeast in the body represents.

(3) Three examples will be used to illustrate the third point. In 1973, Bruce and others showed that women who did not have recurrent urinary tract infections (UTI) had a vaginal microbiota dominated by lactobacilli (13). This led to an extensive research program that culminated in two strains, *L. rhamnosus* GR-1 and *L. reuteri* RC-14 being successfully shown to populate the vagina and out-compete a range of pathogens. The selection of these strains was a laborious process that included *in vitro* assays, some animal studies, extensive development of drying and encapsulation, and a number of clinical trials followed by acquisition of FDA approval in the USA and regulatory approvals elsewhere. Assessments were performed on the strains alone and in combination, in milk and dried formulations and studies were undertaken to provide insight into mechanisms of action. The latter includes displacement of pathogens (15, 52) and modulation of immunity (35, 36, 61). Thus, although the process could be shortened, it is far from simple.

It is gratifying when others cite your work and indeed model theirs after yours, however, this is different from implying that a completely different product in content, strain properties and formulation is somehow the same or could give the same responses *in vitro* or *in vivo*. A product sold by Natural Factors as Women's Multi Probiotic comprises the following:

Proprietary Synergistic Blend:	
Total Active Cell Count 12 Billion:	
Lactobacillus acidophilus	2.4 Billion
Lactobacillus casei	2.4 Billion
Lactobacillus rhamnosus	2.4 Billion
Bifidobacterium longum	1.2 Billion
Lactobacillus plantarum	1.2 Billion
Lactobacillus fermentum	0.6 Billion
Lactobacillus lactis	0.6 Billion
Streptococcus thermophilus	0.6 Billion
Bifidobacterium breve	0.4 Billion
Bifidobacterium bifidum	0.2 Billion
Cranrich Cranberry Concentrate	250mg
Other ingredients: Vegetable capsule (cellulose, purified water), FOS (Fructooligosacchardies) inulin, Magnesium stearate (vegetable grade), Ascorbic acid	

The product does not contain either *L. rhamnosus* GR-1 or *L. reuteri* RC-14, yet on its web site ostensibly set up to explain their product "The ideal probiotic blend for every woman", it is the successful studies on strains GR-1 and RC-14 that are prominently cited and no studies on the actual product being sold. The site also does not explain how the 250mg of cranberry affects bacterial viability, or why fructooligosaccharide and inulin are added and what they do (http://www.naturalfactors.com/ca/en/products/137/womens-multi-probiotic-with cranrich). A disclaimer is included presumably to appease the FDA by stating that the information is not intended to self-treat conditions that should be treated by a healthcare provider, when in fact the whole document covers infections, cancer and other diseases for which foods cannot be used to prevent, at least not without a major portfolio of studies.

In a somewhat related case, a press report on a Winclove Bio Industries six-strain probiotic supplement that received a vaginal infection-related health claim certification, included not only a description of the six strains: *Bifidobacterium bifidum, Lactobacillus plantarum, L. acidophilus, L. rhamnosus, L. salivarius and L. casei,* but also "a Winclove version of one (*L. rhamnosus*) of the two strains highlighted as being most efficacious by the leading researcher and developer in the area – Professor Gregor Reid." (http://www.nutraingredients.com/ Industry/Winclove-targets-female-niche-with-probiotic-health-claim). To the reader, it may appear that the Winclove product somehow contains a strain as good as or better than *L. rhamnosus* GR-1 in terms of functionality, or that the product has an extra five strains that could make it even better than GR-1 and RC-14. The report then stated that the claim certification was based on a pilot study, several *in vitro* studies and clinical trials conducted with similar products, and that a spokesperson for the company said "Further studies have been commissioned but it was not necessary to have any clinical trials for this claim."

Two issues need to be raised here. The first is to emphasize that no two strains or products are alike and the onus is on the manufacturers to perform studies proving that a product confers health benefits. The second is to show that even regulatory agencies do not enforce the use of the term 'probiotic' or the requirement for clinical efficacy before allowing an infection-related health claim, thus making it very confusing for consumers and healthcare professionals to know which products have been clinically proven.

The final example is so-called probiotics that survive heat treatment. This has become popular for production of chocolates, bread, cookies and potentially other 'probiotic' foods. With 523 new stock keeping units globally in the probiotic foods and beverages sector in 2007, the question is how many truly meet the minimum requirements with designation of strains, suitable end-of-shelf viability, and proven benefits? (http://www.nutraingredients-usa.com/Industry/Probiotics-grow-on-innovation-Datamonitor). In the case of chocolate, one technique combines three strains in microencapsulation, while another uses *Bacillus coagulans*, a spore forming organism that was long marketed as *Lactobacillus sporogenes*, and which has limited human data on its potential benefits (21). While there are merits to technical advances that allow different organisms to be added to food, the consumer needs to know how many pieces of chocolate or bread should be eaten per day and to what physiological benefit? In the case of chocolate, such studies need also to assess potential negative outcomes such as on cholesterol. If consumers are to decide between taking only one daily probiotic, how can they do this with limited information permitted on the label, few or no studies for certain products on Pub Med, and web pages that cite studies on other products?

In the developing world, the diversity of probiotic products is small at present, but as distribution channels open up and more people become financially able to purchase products, the same issues listed above will come to the forefront. Hopefully, by then the standards will have risen in the developed world.

PROBIOTICS IN THE DEVELOPING WORLD

Fermented foods with specific *Lactobacillus* strains play a major role in the diet of several regions in the developing world. Fermentation improves the digestibility of the fermented product and degrades anti-nutritive factors and toxins. Fermented products are consumed in the developing world because of an inherent belief that they promote health. An intriguing example is found in Kenya, where recent typing of *Lactobacillus plantarum* strains used by the Massai was found to have probiotic potential. The strains attached well to epithelial cell walls, were bile resistant and able to survive intestinal passage (43), albeit such characteristics are insufficient to be certain of what the organism will do in the host.

Besides the traditional use of *Lactobacillus* strains, the addition of probiotic *L. rhamnosus* GR-1 to a locally made yogurt has been proven to be successful in Mwanza, Tanzania. At this site, local women produce GR-1 supplemented yogurt for approximately 230 people each day of which at least 120 are living with HIV (59). Early results of this administration are presented below. It is important to note that probiotic supplementation is not merely dependent on a milk supply. Fermentation of cassava porridge with *L. plantarum* was shown to be feasible and improve the pace of recovery of malnourished children (Mbugua, M.K. personal communication). The use of probiotic strains in fermented products in a controlled way could have substantial benefits in the developing world in at least the following areas:

1. Gastrointestinal infections and diarrhoea.
2. HIV and immunity.
3. Women's health.
4. *Helicobacter pylori* infections in the stomach.

(1) Especially in developing countries, diarrhoea is a major killer. In 1998, diarrhoea was estimated to have killed 2.2 million people, most of them less than five years of age. In Tanzania, 17% of mortality in children under 5 years of age can be attributed to diarrhoeal diseases compared to 9.3% due to HIV. Besides efforts to increase sanitation and the availability of clean water, a simple food-based intervention could make a significant difference. In Peru, where 12% of mortality in children under 5 years of age are due to diarrhoeal diseases, a randomized controlled trial (RCT) of 200 children showed that *L. rhamnosus* GG reduced the number of episodes from 6.02 episodes/year to 5.21 episodes/year ($p = 0.03$) (45). This is still only making a small dent in the overall incidence, but other benefits are provided once diarrhoea occurs. A Randomized, Controlled Trial (RCT) of 287 children showed that the mean duration of a diarrhoeal episodes was reduced from 72 hours to 58 hours with the addition of *L. rhamnosus* GG to Oral Rehydration Solution (ORS) ($p = 0.003$) (29). Another RCT among 559 children showed that not only the duration of diarrhoea was reduced with this approach, but also the length of stay in hospital