The Gut-Brain Axis
Dietary, Probiotic, and Prebiotic Interventions on the Microbiota

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Preface

Given the ever-increasing body of evidence that the gut, and more particularly the enteric microbiota, can affect central nervous system (CNS) function, it is perhaps not unsurprising that alterations in the microbiome, in many instances also accompanied by gut dysfunction, have been associated with significant CNS disorders. Some of these have their focus intuitively in the gut, such as obesity (chapter: Gut Microbiota and Metabolism) and irritable bowel syndrome (chapter: Dietary Interventions and Irritable Bowel Syndrome), whereas others, until recently at least, may have been considered primarily disorders of the CNS. However, many common (pathophysiological) features are shared among these disorders, including, although not exclusively, alterations in gut permeability, microbiota diversity, and gut-brain signaling; the latter perhaps occurring consequent to alterations in the former. This of course represents a simplistic, albeit logical, explanation for the ensuing inflammation often associated with disorders of the microbiota-gut-brain axis, but nonetheless it establishes an attractive pathway for intervention. However, the temporal nature of the alterations in the microbiota, changes in gut barrier integrity and manifestation of pathology, and whether these represent predisposing factors or disease consequence remain unclear. Indeed both possibilities are plausible. To date, there has also been perhaps an underappreciation of the enteric nervous system (chapter: Influence of the Microbiota on the Development and Function of the "Second Brain"—The Enteric Nervous System), or "second brain," which is juxtaposed with the microbiota and represents an accessible window into the pathophysiology of CNS disorders. As our ability to study the complexity of the microbiota and microbiota–host interactions progresses, by harnessing the power of sequencing technologies and use of relevant animals models, such as germ-free or gnotobiotic species, our understanding of the interplay among the microbiota, gut, and brain continues to rapidly develop. This pace of discovery will undoubtedly help address the causality dilemma, but it requires coordinated efforts by multidisciplinary teams; the importance of such studies will only be truly demonstrated by translation to human populations.

In this book, we present evidence establishing a role for the microbiota in disorders of the gut-brain axis, and we have specifically invited commentary from our contributors on the potential for intervention by dietary, probiotic, or prebiotic means in their management. In this regard, advances in sequencing technology and metabolite analysis have provided insight into the identification of putative microbial-based interventions. However, this strategy is most likely to be further influenced by environmental factors in early life and by aging, diet, and exposure to antibiotics. These may well be viewed as confounding factors in experimental studies, but they are real, and variable, among populations and patients and are likely to influence and inform the success or failure of any given microbiota-targeted or dietary intervention. They may also be viewed as risk factors for gut-brain axis disorders. Here again, a common theme emerges throughout several chapters of this book, pointing toward critical periods in early life as key for establishing an appropriate microbiota profile for

future well-being. This in turn raises questions about the optimum time for intervention and reversibility of established microbiota-associated alterations in the host (eg, "Can adverse microbiota-associated programming of the host in early life be later reversed to overcome CNS dysfunction?").

We also explore the characterization and optimal delivery of microbiota-targeted interventions. Strategies to restore the gut microbiota using probiotics are discussed, with examples of food- and nonfood-based probiotic carriers (chapter: Probiotics as Curators of a Healthy Gut Microbiota: Delivering the Solution) and the scientific basis for their use in a microbial endocrinology context and consideration as "drug delivery vehicles" (chapter: Microbial Endocrinology: Context and Considerations for Probiotic Selection). However, we also acknowledge the importance of diet as a possible and logical intervention given the global evidence-based literature for its impact on mental well-being. There is no doubt that diet must be considered as an intimate partner in the microbiota-gut-brain axis. However, the delivery of such therapeutic promise is not without its (regulatory) challenges, not least of which is how the field should define a probiotic that influences brain function (ie, a psychobiotic) and the need to demonstrate efficacy for the general population, excluding studies in disease subjects, validation of risk factors of developing a disease, and elucidating their mode of action. This of course applies more broadly and well beyond dietary probiotic and prebiotic interventions affecting the microbiota-gut-brain-axis. There are also global challenges to overcome, including how to ensure that populations in the developing world will benefit from microbial interventions on human health.

This book brings together a group of contributors, all experts in their respective fields, from those involved in brain-gut axis research to cross-cutting areas of technology, epidemiology, and regulation. With this in mind, the book is organized into four main areas. The first two provide background into the technologies, tools, and strategies used to explore the microbiome in health and disease and provide insight into the regulatory framework in which investigators will have to work to deliver the promise of microbial-based interventions to human populations. The third area explores the microbiome at the extremes of life and the importance of critical developmental periods that may provide opportunities for microbial-based interventions. We also introduce the importance and evidence for the role of diet in maintaining good mental health with a global perspective. The final area then addresses specific disorders of the gut-brain axis that may prove amenable to dietary interventions.

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CHAPTER

1 Regulatory Considerations for the Use and Marketing of Probiotics and Functional Foods

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REGULATORY IMPACT OF DEFINITIONS

Scientific research is the driving force of innovation in nearly all fields of human activity, including nutrition. In the context of nutrition science the management of enteric microbiota to achieve a "health effect" in a human host has enjoyed a long history. During his stay in the early 1900s at the Institute Pasteur, Elie Metchnikoff noticed the "…different susceptibilities of people to the harmful action of microbes and their products. Some can swallow without any evil result a quantity of microbes which in the case of other individuals would produce a fatal attack of cholera. Everything depends upon the resistance offered to the microbes by the invaded organism" (Metchnikoff, 1907). He focused on the sensitivity to low pH of pathogens most commonly isolated from the human gut at that time (*Enterobacteriaceae*); lactic acid-producing bacteria able to colonize the human gut seemed to Metchnikoff to constitute an ideal tool for inhibiting the growth of pathogens.

The following 50 years witnessed more efforts to develop Metchnikoff's ideas; for example, in Europe with *Escherichia coli* strain Nissle 1917 (Möllenbrink and Bruckschen, 1994) and in Japan with *Lactobacillus casei* Shirota (Morotomi, 1996). In the United States Nicholas Kopeloff studied *Lactobacillus acidophilus* (1926) (by lucky coincidence with the focus of this book, Kopeloff was an associate professor in bacteriology at the Psychiatric Institute of Ward's Island, New York), as did Rettger et al. (1935). However, the impact of these investigations on the market was limited, and these studies were ignored by regulatory agencies.

A breakthrough occurred with the appearance in the scientific literature of the term *probiotic*, which seems to have been coined during the 1950s (Hamilton-Miller et al., 2003) to identify substances able to support the growth of microorganisms; this term appears to have been chosen to oppose the concept of an antibiotic. However, the first

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clear definition of the term *probiotic* in relation to beneficial bacteria emerged in the 1960s (Lilly and Stillwell, 1965). At that time research mainly focused on the selection and use of bacteria for use as feed additives. This peculiarity was made evident by Fuller (1989), who proposed to define probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance."

Probiotic use was extended to humans by Havenaar and Huis in't Veld (1992), who proposed the definition "a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora." The definition further evolved with the introduction of references to the quantity of viable cells necessary to exert probiotic action. For example, Guarner and Schaafsma (1998) suggested that probiotics be defined as "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host." A further step was taken by the Food and Agricultural Organization (FAO)/World Health Organization (WHO) Joint Expert Consultation that redefined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO Joint Working Group, 2001). The verb *administered* was introduced instead of the word *consumed* to include beneficial bacteria in the urogenital tract or bacteria applied topically, according to studies published at the end of the last century that were the basis for products appearing on the market at the beginning of the 2000s (Ocaña et al., 1999; Parent et al., 1996). Further specification of the term *probiotic* was provided by the same expert group in 2002 (FAO/WHO Joint Working Group, 2002). Thus it is clear that definitions of the term *probiotic* have followed the advancement of scientific research, from the quest for substances with actions opposite to those of antibiotics to the selection of bacteria beneficial for humans (not only in the gut).

The two FAO/WHO documents strongly impacted not only science but also regulation, which is relevant for this chapter. Since 2002 these documents have been used as references by health and food-safety agencies all over the world. The European Food Safety Authority, the US Food and Drug Administration, and Health Canada have used them as templates for their own guidelines for probiotics, as have agencies in China, India, Brazil, Argentina, and other nations (Table 1.1). Thus these documents have clarified and improved the regulatory profile of probiotics.

At the time of this writing, the term *probiotic* has reached a consensus definition with two components: (1) viable bacteria (2) with documented (at the strain level) potential to confer health benefits in the host when administered in the necessary amount; this action could be independent of any effect on the composition of the host's gut microbiota. It is also assumed that a clear taxonomy has been assigned to the strains and that their intended use is safe. These considerations should be taken together with more general considerations about "active substances" from the regulatory point of view: (1) the need for accurate bacterial identifications, which imply precise definitions of the active substances; (2) the need to assess safety on the basis of a long history of safe use if the product is food or on the basis of specific testing if the product is pharmaceutical; and (3) the need to evaluate efficacy, which should be assessed in healthy people for food and in patients for drugs.

Table 1.1 List of Health and Food Safety Agencies Referring to Food and Agricultural Organization/World Health Organization Guidelines for Probiotic Definition and Evaluation

Regulatory Authority or Author (Country)	Document
US Food and Drug Administration (United States)	*Complementary and Alternative Medicine Products and Their Regulation by the Food and Drug Administration* (Food and Drug Administration, 2006).
US Pharmacopoeia (United States)	*Appendix XV: Microbial Food Cultures Including Probiotics* (US Pharmacopoeia, 2012).
Health Canada	*Guidance Document : The Use of Probiotic Microorganisms in Food* (Health Canada, 2009). The document "clarifies the acceptable use of health claims about probiotics, and provides guidance on the safety, stability and labeling aspects of food products containing probiotic microorganisms."
Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT; Argentina)	*Codigo Alimentario Argentino. Capitulo XVII: Alimentos de Regimen o Dietéticos* (A.N.M.A.T.).
Ministry of Health, China Food and Drug Administration (People's Republic of China)	*Regulatory for Probiotic Health Food Application and Examination* (interim; China Food and Drug Administration (CFDA), 2005). Most of the Food and Agricultural Organization/World Health Organization guidelines have been adopted. A list of 10 allowed probiotic species is furnished.
Indian Council of Medical Research-Department of Biotechnology (ICMR-DBT; India)	*ICMR-DBT Guidelines for Evaluation of Probiotics in Food* (Indian Council of Medical Research Task Force et al., 2011).
International Life Sciences Institute (ILSI)-India	*Guidelines and Criteria for Evaluation of Efficacy, Safety and Health Claim of Probiotic in Food Products in India* (ILSI–India, 2012).
Bureau of Food and Drugs, Department of Health (Philippines)	*Bureau Circular No. 16S 2004* (Bureau of Food and Drugs, 2004). Guidelines for definition and regulation of probiotics as food supplements in the Philippines. Bacterial groups different from Lactobacilli, Bifidobacteria, nonpathogenic Streptococci, *Bacillus clausii*, and *Saccharomyces boulardii* "shall be subject to demonstration of evidence of safe use as food supplement."
European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) working group (Poland, Italy, Croatia, Israel, Belgium)	*Use of Probiotics for Management of Acute Gastroenteritis* (Szajewska et al., 2014). Systematic review giving recommendation on the use of probiotics in previously healthy children with acute gastroenteritis. *Lactobacillus rhamnosus* GG and *S. boulardii* are strongly recommended as an adjunct to rehydratation therapy.

Continued

Table 1.1 List of Health and Food Safety Agencies Referring to Food and Agricultural Organization/World Health Organization Guidelines for Probiotic Definition and Evaluation—cont'd

Regulatory Authority or Author (Country)	Document
Nutrition and Metabolism Group of the Spanish Neonatology Society (Spain)	*Recommendations and Evidence for Dietary Supplementation with Probiotics in Very Low Birth Weight Infants* (Narbona López et al., 2014). It is associated with lower risk of enterocolitis and death, but protocols (dosage, strains, duration) are still not established.
World Allergy Organization	*Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics* (Fiocchi et al., 2015). Recommendations for the use of probiotics in pregnant women or women breastfeeding otherwise healthy infants with risk of eczema, in which a likely net benefit is present, albeit with no clear evidence of a risk reduction of allergy. Clinical studies in this field present several methodological limitations.
World Gastroenterology Organisation	*World Gastroenterology Organisation Guideline: Probiotics and Prebiotics* (Guarner et al., 2012).
Institute of Food Technologists	*Health Benefits of Probiotics and Prebiotics* (Ohr, 2010). The global retail market for probiotic and prebiotic foods and drinks reached in 2008 approximately $15.4 billion (estimated by Packaged Facts); several probiotics approved by health claims are presented, such as oral probiotic gum prototype and herbal tea with probiotics.
Hoffmann DE, Fraser CM, Palumbo F, Ravel J, Rowthorn V, Schwartz J	*Federal Regulation of Probiotics: An Analysis of the Existing Regulatory Framework and Recommendations for Alternative Framework* (Hoffmann et al., 2012). Output resulting from a National Institutes of Health grant with the aim of examining the legal and regulatory issues raised by probiotics and to determine whether the current regulatory framework is a good fit for the range of probiotics that are on the market, under development, or that may be developed in the future.
Superior Health Council (Belgium)	*Publication of the Superior Health Council No. 8651, Probiotics and Their Implications for Belgian Public Health* (Publication of the superior health council no. 8651, 2012). The paper describes the importance of safety assessment of probiotics and bacterial identification by phenotypical and molecular approaches, with particular attention to the construction of a database.

Another fundamental regulatory issue must be addressed: the two FAO/WHO documents only deal with the use of probiotics in food, as clearly indicated with "the Consultation agreed that the scope of the meeting would include probiotics and prebiotics in food, and exclude reference to the term biotherapeutic agents, and beneficial microorganisms not used in food." The working group defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" and restricted the scope of the discussion to this definition (FAO/WHO Joint Working Group, 2001). Therefore the working group appeared to focus on members of the genera *Lactobacillus* and *Bifidobacterium* and paid much less attention to beneficial microorganisms not used in food. This restriction has a strong regulatory impact because food legislation all over the world deals with products aimed to be provided to healthy people; substances aimed to treat, cure, and/or prevent pathological conditions are addressed under different legislation that covers drugs, medical devices, etc.

To underscore the relevance of this restricted area of applications, we refer to the second FAO/WHO document (2002), in which the expert working group provided a scheme (Fig. 1.1), entitled "Guidelines for the Evaluation of Probiotics for Food Use," in which actions, depicted as boxes, to be performed to grant probiotic status to a food

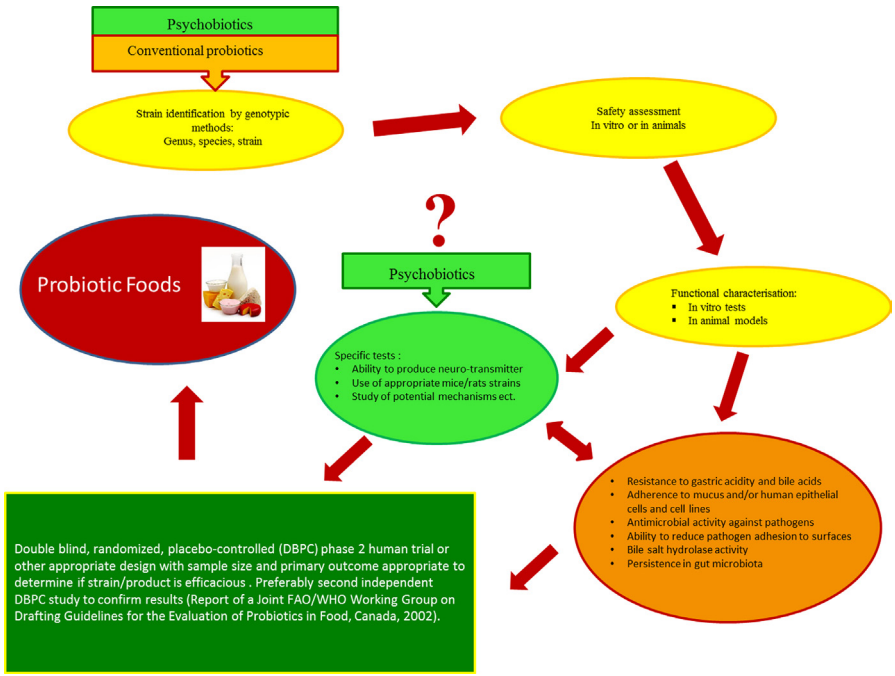

FIGURE 1.1

A possible evaluation scheme of psychobiotics compared to conventional probiotics for food use.

are listed in order and outlined by a row of arrows that connect each action to the next one. Not surprisingly the box containing the action "Phase 3, effectiveness trial is appropriate to compare probiotics with standard treatment of a specific condition" is not connected by an arrow to the final box granting probiotic food status. Therefore we infer that the word *probiotic* was proposed by the FAO/WHO to define bacteria with a beneficial action in pathological and healthy conditions, and that the word *probiotic* can be used for applications that are not related to food (e.g., vaginal or dermal administration). However, the scheme is to be used only for food applications—products targeted to healthy people. These observations are particularly relevant for the assessment of probiotic safety; the long history of safe use of *Lactobacillus* and *Bifidobacterium* provides a solid body of knowledge on their safety as food ingredients consumed by healthy people, but their use in pathological conditions remains unclear.

It is unfortunate that some members of the scientific and clinical worlds have paid little, if any, attention to this last point. Clinical trials have been conducted that did not pay enough attention to safety assessments of specific strains used in specific pathological settings. For example, there is little information about viable bacteria directly administered through a nasal tube into the intestine, which may result in a dose to the intestine that is higher than the dose that would be delivered via the usual oral route (Besselink et al., 2008). Obvious adverse effects were reported (Didari et al., 2014; Fijan, 2014; Kochan et al., 2011; Sanders et al., 2014; Shanahan, 2012; Urben et al., 2014) by some clinicians when probiotics were administered in clinical settings. It is less obvious whether the revision of safety guidelines for probiotics is being sought, although these guidelines are only applicable to healthy people. Use in pathological conditions is subject to safety-assessment procedures for drugs.

Because the probiotic definition is now very popular, not only in the scientific and clinical worlds (in the last 5 years three papers per day were uploaded to PubMed with the keyword *probiotics*) but also as a marketing tool, misuse of the term has boomed. For instance, it has been applied to cosmetic products such as shampoos and aftershave, for which no viability or efficacy of bacterial cells has ever been established. Moreover, in papers and meeting proceedings bacteria isolated from the gut are called *probiotics* even when characterization of their health effects is not provided.

These types of misuse of the term *probiotic* prompted the International Scientific Association for Probiotics and Prebiotics to publish a consensus statement on the appropriate use and scope of the term *probiotic* (Hill et al., 2014). This document categorizes the beneficial mechanisms of probiotics into three groups. The first group deals with mechanisms identified at the genus level, such as colonization resistance. The second group is related to species-specific effects, such as vitamin production in the gut. The third group addresses strain-specific effects; for the purposes of this book we include action on the gut–brain axis (neurological effects) in this group. The final recommendations of Hill et al. (2014) reinforce the concept that properly controlled studies supporting health effects are essential to properly define some microbes as probiotics. These studies may be conducted at the genus, species, or strain level according to the desired beneficial effect. This recommendation also implies that language any more specific than "contains probiotics" must be further substantiated.

Starter cultures may not be defined as probiotics when there is no evidence of health benefits, even if the cultures are traditionally associated with fermented foods. The same restriction applies to fecal microbiota transplants. It is interesting to note that an opportunity exists to define commensal microbes without a history of use in food as probiotics if they are well characterized and supported by adequate evidence of safety and efficacy. This strategy widens the potential for use of newly characterized gut-derived bacteria that exert beneficial actions. However, from the regulatory point of view it seems clear that this last group of probiotics will fall into the "pharma" category because they do not belong to the group of bacteria with a long history of safe use in food.

The scientific and regulatory histories of prebiotics are more recent than those of probiotics. The first definition of the term *prebiotic* appeared in 1995 when Gibson and Roberfroid introduced this neologism to identify a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson and Roberfroid, 1995). In 2004 the definition was slightly modified: "a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host wellbeing and health" (Gibson et al., 2004). The second version lacked the phrase *non-digestible* while retaining the concept of fermentation by certain groups of bacteria. The definition proposed by the FAO was contained in the final report of a FAO Technical Meeting in 2007: "a prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota" (FAO Technical Meeting on Prebiotics, 2007). This definition was recently challenged (Bindels et al., 2015) because it "does not require the prebiotic to be fermented or metabolized by the gut microbes, and therefore does not distinguish among substances that modulate gut microbiota composition solely through an inhibitory action. As a consequence, antibiotics would be prebiotics according to this definition." However, this remark does not account for the fact that antibiotics are pharmaceuticals and therefore cannot be defined as, or considered to be, food components.

A total of seven (Table 1.2) slightly different definitions of the term *prebiotic* were recently reviewed and discussed by Bindels et al. (2015), who also proposed a new definition. Five of the six available definitions refer to a specific/selective action of prebiotics on gut microbiota composition (Bindels et al., 2015); the only one that simply links the action of prebiotics to "modulation" is the FAO definition, as stated in the publication. It is important to note that it is not easy to establish a clear-cut differentiation between beneficial and detrimental members of communities of gut bacteria. Culture-independent, DNA-based approaches have determined that even the best-characterized prebiotics are not as specific as previously assumed.

From the regulatory point of view, the actions of probiotics and prebiotics are fundamentally different, as stated in their respective definitions; the former may directly exert their action whereas the latter may mediate changes in the composition of the gut microbiota. It may be simpler to assess the safety of probiotics than the safety of prebiotics. It is surprising to note that in contrast to the abundant literature on the

Table 1.2 Evolvement of Prebiotic Definition

References	Prebiotic Definition
Gibson and Roberfroid (1995)	"Nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health."
Reid et al. (2003)	"Nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria."
Gibson et al. (2004)	"Selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host wellbeing and health."
Roberfroid (2007)	"Selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health."
FAO Technical Meeting (2007)	"Nonviable food component that confers a health benefit on the host associated with modulation of the microbiota."
Gibson et al. (2010)	"Dietary prebiotic: a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health."
Bindels et al. (2015)	"A nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host."

safety of probiotics (AlFaleh and Anabrees, 2013; Didari et al., 2014; Fijan, 2014; Kochan et al., 2011; Sanders et al., 2014; Shanahan, 2012; Urben et al., 2014), very little information is available for the assessment of the safety of prebiotics; most data are confined to prebiotic use in infant nutrition (López-Velázquez et al., 2013; Van den Nieuwboer et al., 2014, 2015a,b). Because "modern community-wide molecular approaches have revealed that even the established prebiotics are not as specific as previously assumed" (Bindels et al., 2015), it seems prudent to suggest that more data on the impact of prebiotics on the overall composition of the gut microbiota be pursued.

GUT–BRAIN AXIS: WHAT COULD BE RELEVANT FOR ESTABLISHING REGULATIONS?

We have established that the term *probiotic* is historically associated with the intestinal environment and functions such as the homeostasis or balance of gut microbiota. However, in the last 5 years the hypothesis that probiotics can influence brain

functions and contribute to the amelioration or prevention of diseases such as depression, anxiety, and mood disorders has gained support from a growing body of evidence, which is reviewed in other chapters of this book. Because this research is opening new areas of applications that are currently not covered by existing regulations, we should expect new challenges from the regulatory point of view.

Should specific regulations be established for this class of probiotics? This question is pertinent because several studies have reported positive effects after probiotic administration in animal models, mostly germ-free or conventionally housed mice or rats. Fewer studies, which are often preclinical pilot trials, have been conducted in human subjects.

The first challenge is to refine or change the only available definition of probiotics that influence brain function. Dinan et al. (2013) proposed the term *psychobiotic* to mean "a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness." Note that this definition matches the definition of a drug; the reference to patients and illness clearly excludes the possibility of categorizing a psychobiotic as food. Moreover, Dinan et al. (2013) explained that the observed health benefit is related to strain-specific actions, such as the production and delivery of neuroactive substances such as gamma-aminobutyric acid (GABA) and serotonin. This example illustrates a "rare" probiotic effect (Hill et al., 2014), meaning that it is strain related and not widespread in all strains of a species. The claim of such an effect requires extensive trials in humans to be substantiated.

FAO/WHO guidelines (2001) and guidelines from the European Food Safety Authority (EFSA, 2009) recommend identification of bacteria at the levels of species and strain for several reasons, including safety, but mainly because it is important "to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies" (FAO/WHO Joint Working Group, 2001). This important aspect, which has been recognized for food-related probiotics, is a *conditio sine qua non* for psychobiotics (Fig. 1.1). For example, Barrett et al. (2012) reported that some strains of *Bifidobacterium* and *Lactobacillus* produced GABA when grown in the presence of monosodium glutamate. GABA is a neurotransmitter that regulates several psychological and physiological processes in the brain that contribute to depression and anxiety (Schousboe and Waagepetersen, 2007). To understand the prevalence of this microbial property in the bacteria of the same genus, Barrett et al. (2012) found that only one *Lactobacillus* strain and four strains of *Bifidobacterium* produced GABA out of 91 tested strains. This evaluation was performed using an in vitro test; when these strains were additionally evaluated in fecal fermentation medium, only *Lactobacillus brevis* DPC6108 produced GABA at high levels (Barrett et al., 2012). The authors concluded that this physiological property could be expressed in vivo and perhaps defined as "strain related and rarely present in lactobacilli and bifidobacteria" (Barrett et al., 2012). Therefore it seems clear that psychobiotics should be handled as pharmaceutical products and should be subject to pharmaceutical legislation.

Nonetheless, some studies indicate that probiotic bacteria play a role in the gut–brain axis in healthy people (Messaoudi et al., 2011a, b), which suggests that "food probiotics" may be exploited for management of the gut–brain axis. Here we only

consider human studies (Benton et al., 2007; Rao et al., 2009; Steenbergen et al., 2015), although several studies reported positive effects after probiotic administration in animal models (Desbonnet et al., 2008, 2010). These animal models are often used to obtain insight into neurochemical changes induced by the modulation of intestinal microbiota via the administration of psychobiotic strains. Germ-free animal models are particularly useful for neurogastroenterology research, but animal data often cannot be translated to humans because they do not accurately reflect the physiology and environments of human populations.

Regulatory bodies require evidence obtained in humans. For example, in 2008 the European Union approved Commission Regulation (EC) No. 353/2008, which established and implemented rules for applications for the authorization of health claims. Article 5a of this regulation states that the scientific evidence to be provided to support the application for a health claim "shall consist primarily of studies in humans and, in the case of claims referring to children's development and health, from studies in children" (Commission Regulation (EC) No 353/2008).

Regarding the gut–brain axis in healthy subjects, in a pioneering study Marcos et al. (2014) monitored anxiety in young subjects under academic examination stress. Although fermented probiotic milk reduced the effect of stress on the immune system, there was no significant effect on anxiety (outcomes were similar in control and treatment groups; Marcos et al., 2014). A more recent investigation (Mohammadi et al., 2015) reported more promising results from a randomized, double-blind, placebo-controlled trial of 70 petrochemical workers who were healthy but under stress due to working conditions. Subjects were randomly assigned to receive 100 g/day probiotic yogurt plus one placebo capsule ($n=25$), one probiotic capsule daily plus 100 g/day conventional yogurt ($n=25$), or 100 g/day conventional yogurt plus one placebo capsule ($n=20$) for 6 weeks. Both probiotic-consuming groups received significantly improved scores on a general health questionnaire; stress-scale scores also improved. In contrast, no significant improvements were detected in the conventional yogurt group.

Another very recent study (Steenbergen et al., 2015) sought to assess whether a multispecies probiotic containing bifidobacteria, lactobacilli, and lactococci reduced cognitive reactivity in nondepressed individuals. In this triple-blind, placebo-controlled, randomized study, 20 healthy participants received a 4-week probiotic food supplement and 20 control participants received an inert placebo. A validated index of depression was used to evaluate cognitive reactivity to sad moods before and after the intervention. The treated group reported significant reductions in rumination and aggressive thoughts, leading to an overall reduced cognitive reactivity to sad mood versus participants who received the placebo intervention.

From the regulatory point of view, encouraging observations must be confirmed to enable food use that is supported by an approved health claim. A nonexhaustive list of example questions to be answered includes:

1. What is the rationale for using a seven-strain mixture in a particular probiotic?
2. What is the role of each bacterial component in the observed effect?
3. If the ratio of bacterial members in a marketed blend is different from that used in a clinical trial, will the results remain consistent?

We encourage prudence in drawing conclusions and recommend accounting for differences in approaches and needs between peer reviewers of scientific journals and examiners of regulatory administration.

CONCLUSIONS

If probiotics are to be used to manage human functions influenced by the gut–brain axis, then a clear definition of *psychobiotics* must be crafted. Results from animal trials must be confirmed in human trials in which healthy or unhealthy subjects are enrolled to support the development of food or pharmaceutical products.

As with conventional probiotics, it is important to identify the final target of individual psychobiotics, which will inform trial design. For healthy subjects, evaluation of probiotic/psychobiotic effectiveness should differ from evaluation in the context of illness. Trials should consist of randomized, double-blind placebo studies with rigorous definitions for measuring the effectiveness of psychobiotics, particularly for healthy people.

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CHAPTER

2

Targeting the Microbiota: Considerations for Developing Probiotics as Functional Foods

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THE PROBIOTIC CONCEPT AND EVOLUTION

The probiotic concept is based on the notion that the commensal microbiota contributes to human physiology; consequently, favorable modifications in its composition may help to maintain health and reduce disease risk (Neef and Sanz, 2013). The rationale behind the probiotic concept dates back to the times of Elie Metchnikoff (1907), who established associations between the consumption of fermented milk with lactic acid bacteria and longevity in rural populations of Bulgaria (Bibel, 1988). At around the same time (1900), bifidobacteria were isolated from healthy breast-fed infant feces by Henry Tissier, who suggested that they could prevent infections by displacing bacteria causing colitis in breast-fed infants (reviewed by Bertazzoni et al., 2013). The term *probiotic*, which originates from the Greek term *pro bios* ("for life"), was first used as such in 1965 by Lilly and Stillwell to describe substances produced by bacteria that, unlike antibiotics, stimulate the growth of other bacteria. Then in 1989 Roy Fuller finally suggested a description of probiotics similar to the currently accepted definition, indicating that they are "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance." Since then, this term has been widely used on labels and in publicity to communicate a health benefit to consumers. The probiotic concept was finally defined by scientific consensus in 2001 in an attempt to categorize these functional food ingredients under harmonized criteria because of their increased commercialization. The currently accepted definition of a probiotic was developed in 2001 by an expert consultation group working under the umbrella of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) on the health benefits of probiotics in foods. Probiotics were then defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). In 2002 a joint FAO/WHO working group also published the first guidelines for the evaluation of probiotics in foods. This definition implies that

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