# Health Claims in Europe: Probiotics and Prebiotics as Case Examples

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## Abstract

Health claims regarding foods imply a relationship between a specific food and maintenance of good health, or that food can reduce the risk of disease. Health claim legislation in the European Union sets out from the concept of consumer protection. Health claim assessment focuses on defining given foods, assessing their health relationship, and evaluating relevant studies with an emphasis on controlled human intervention research. Challenges include the focus of claims on healthy populations, although most intervention studies have been conducted among patients. A further problem attends the risk reduction claim, which requires changes in generally accepted biomarkers reflecting the risk of disease. Scientific assessment and guidance documents direct the development of health claims both in Europe and elsewhere. Experience from completed assessments should make it possible to provide consumers with reliable claims to help them make healthier choices and develop lifestyles supporting long-term well-being.

## INTRODUCTION

Health claims for foods generally imply that there is a relationship between a specific food and maintenance of a good health status, or that a given food can reduce the risk of a particular disease. Such claims include, for example, statements on dental caries and foods reducing the risk of it, or on calcium and foods strengthening the bones and reducing the risk of fractures. The details of health claim regulations vary from one country to another. Often the main differences are to be found in defining target consumers or populations and in the degree of evidence required to back a health claim. The thin line between definitions of food products and pharmaceutical products is often far from established.

For the past two decades, food manufacturers in Europe and elsewhere have adopted a strategy of selling "functional foods," which claim to make the consumer healthier. In fact, no official definition for functional foods exists. Currently, regulators in Europe are asking manufacturers to provide scientific evidence to support these claims, and a system for assessment has been developed. As no legal definition for functional foods has been adopted, one must consider functional foods to comprise foods with approved health claims.

Health claim legislation in the European Union (EU) is relatively new, and a variety of health claims have been introduced (Verhagen et al. 2010). The background to the regulation lies in the concept of consumer protection, its substance focusing on consumer rights and consumer protection and on innovative developments of food. The regulation requires more detailed nutrition labeling, including a declaration of nutrient content, e.g., energy, fat, saturated fat, and carbohydrate levels in the food. The regulation has been created within the framework of a settled EU background. In detail, it brings together several elements of legislation. In the broadest terms, it grants few directly effective entitlements to individuals, whereas by contrast various responsibilities are imposed on the food industry. The economic dimension covers intra-EU trade, which accounts for approximately 75 percent of all food trade within the European Union. Another feature is that it applies in an area where national food information laws are considered the exception and may therefore only be adopted if they meet the conditions of the regulation.

The Regulation on Health and Nutrition Claims aims to ensure that consumers are not misled by unsubstantiated, exaggerated, or untruthful claims about food products. With the current legislation, consumers should be able to rely on clear and accurate information on food labels, enabling them to be properly informed on the food they choose. This approach is associated with the EU campaign for healthier lifestyle choices as well as the Commission's consumer protection objectives. The regulations also aim to provide all food producers and all manufacturers with clear and harmonized guidelines, which will ensure fair competition and help protect innovation in the food industry. The objective is to ensure that manufacturers make genuine health and nutrition claims to prevent competition with false or inaccurate claims. There are thus important challenges in health claim assessment, which need careful consideration before any claim can be adopted in the European Union common register or permitted health claims, or placed in the register of claims not acceptable in the European Community.

## HEALTH CLAIMS IN EUROPE

On July 1, 2007, Regulation (EC) No. 1924/2006 of the European Parliament and the Council on Nutrition and Health Claims Made for Foods came into force. It harmonizes the provisions related to nutrition and health claims and lays the basis for rules governing the authorization of health claims made for foods.

As indicated, the regulation was planned on the one hand to protect the consumer against misleading information, economic losses, and health problems, and on the other hand to harmonize

legislation in member states. Meaningful health claims are conceived as helping the ordinary healthy European consumer to make healthy food choices and further develop healthy lifestyles.

The terms pertaining to health claims in the regulations are defined in three different categories. The so-called Article 13.1 health claims, also referred to as function claims, encompass (*a*) the role of a nutrient or other substance in growth, development, and the functions of the body, (*b*) psychological and/or behavioral functions, and (*c*) slimming or weight control or a reduction in the sense of hunger or an increase in the sense of satiety or the reduction of the energy available from the diet. These claims need to be based on generally accepted scientific precepts. Such claims could include products where the function is generally known and relates to phenomena that have an impact on health.

Another category formed by Article 13.5 claims involves claims based on newly established science—that is, the claims are novel and possibly also based on patented products, methods, or processes. In the future, these might also include probiotic and prebiotic products and newly developed data on health and health benefits.

A third category includes the following areas for Article 14 health claims: (*a*) reduction of disease risk claims and (*b*) claims pertaining to children's development and health.

One important aspect of this category of claims is that any scientific evidence presented must be demonstrated in the target population or in children. In risk reduction claims, a key factor is that the risk reduction should be demonstrated in a healthy population and based on documented changes in generally accepted biomarkers associated with disease risk. An example is the reduction of cholesterol, which is associated with a reduction in the risk of coronary heart disease. Another instance might be dental caries, a disease affecting virtually all otherwise healthy Europeans. Risk reduction could be demonstrated in reduced numbers of *Streptococcus mutans* or *Streptococcus sorbinus* in the saliva or on dental surfaces, decreased pH change following a meal, or reduced thickness of dental plaque.

A graphic outline of the claim categories is presented in **Figure 1**. The terminology of health claims and related matters has been described by Magalhaes and coworkers (2011).

The existing claims in different EU member states (Article 13.1 claims) were collected by national authorities and submitted to the European Commission, which subsequently passed them to the European Food Safety Authority (EFSA) for assessment. The claims submitted have been

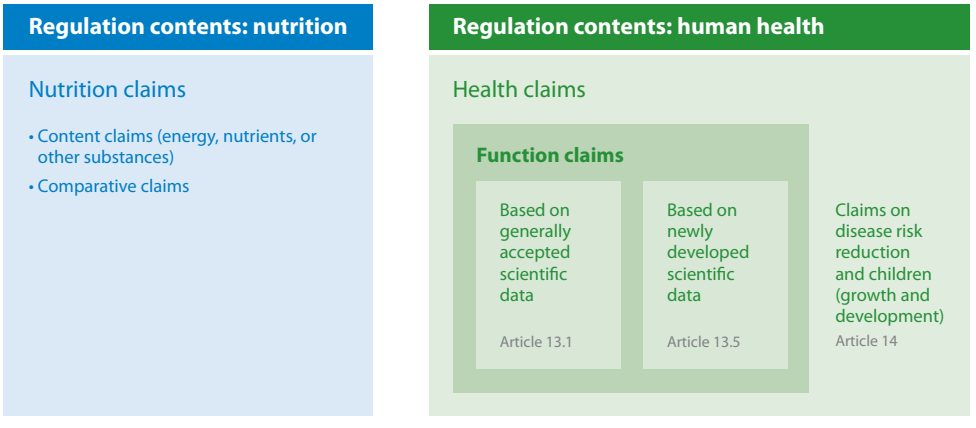


**Figure 1**

The types of health claims and their content in Europe according to Regulation 1924/2006. Adapted with permission from Verhagen et al. 2009.

assessed separately to verify whether a scientific basis for them exists, and in positive cases, they are added to the list of permitted European health claims. Member states had the opportunity to submit national lists with claim proposals and scientific references to the EC up to January 31, 2008. Subsequently, additional data and clarifications were requested, and in 2011 the Commission allowed further data to be submitted for the limited number of claims eligible for reassessment, as the microorganisms that were not considered to be sufficiently characterized. The Commission asked the EFSA, and specifically the Scientific Panel on Nutrition, Dietetic Products, and Allergies (NDA) for a scientific evaluation of individual claims. The claims and their conditions, as well as rejected claims with the reason for rejection, were to be listed in a Commission decision. The first decisions of authorized claims were published by the EU Commission in October 2009 [Commission Regulation (EC) No. 983/2009] and will be updated following the evaluation of new claims.

The regulation on health claims has been in force in different European countries for two years, but practical measures are still in process and the EU Commission is working on the finalized format of a common European list of approved health claims. The European Regulation has already had a great impact on all European food sectors. The process may have an impact on developments elsewhere, although the evidence used for health claims is similar. The need for approval of any health claim made for foods on the basis of scientific evidence promises to modify the marketing strategies used to communicate the beneficial effects attributed to products within and outside Europe. Although regulations on health claims in other parts of the world may vary greatly, there is a clear call for more scientific substantiation of these claims in the context of human intervention studies. On the basis of the European regulation, high-quality human intervention studies are needed to substantiate any specific health claim for a certain product. This requirement for randomized placebo-controlled studies will constitute the focus of research efforts in the future.

## HEALTH CLAIM DEVELOPMENTS OUTSIDE EUROPE

There has been a long process of development of health claims, with varying results, in different countries around the world. Health claims in the field of functional foods were first defined in Japan and came into force with a law on foods for specified health uses (FOSHU). The FOSHU regulation has been further developed from the starting point in 1991 to wider use of health claims in 2001, with a division in 2005 into three categories of FOSHU claims (standard FOSHU, reduction of disease FOSHU, and qualified FOSHU). Most claims in Japan are backed by human studies, animal studies, and supporting in vitro studies (He & Benno 2011). The number of FOSHU products has rapidly increased and now offers a multitude of claims and functions for different types of foods. Following the Japanese system, several countries have decided to regulate health messages or other information of health benefits on foods or food components.

In the United States, a health claim constitutes a particular item in the regulatory area—not all health messages are classified as health claims. The so-called structure-function claims, which focus on maintaining or supporting particular body structures or functions, do not require regulatory approval. However, health claims must be approved by the U.S. Food and Drug Administration or a scientific body of the U.S. government or the National Academy of Sciences (Sanders 2011).

## PREREQUISITES FOR A HEALTH CLAIM

According to Regulation (EC) No. 1924/2006, the use of health claims shall only be permitted if the food/constituent for which the claim is made has been shown to have a beneficial physiological effect. In assessing each claim, EFSA makes a scientific appraisal on whether the claimed effect is

to be considered a beneficial physiological effect for the intended population. For function claims, a beneficial effect may relate to maintenance or improvement of a function.

For reduction of disease risk claims, beneficial refers to whether the claimed effect relates to the reduction (or beneficial alteration) of a risk factor for the development of a human disease (not a reduction in the risk of disease). A risk factor is a factor associated with the risk of a disease that may serve as a predictor of the development of that disease. Whether or not the alteration of a factor is considered to be beneficial in the context of a reduction of disease risk claim depends on the extent to which it is established that (*a*) the risk factor is an independent predictor of disease risk (such a predictor may be established from intervention and/or observational studies) and that (*b*) the relationship of the risk factor to the development of the disease is biologically plausible.

Except for well-established risk factors, the extent to which a reduction in some factors is beneficial in the context of a reduction of disease risk claim needs to be considered on a case-by-case basis. A health benefit is a condition that has been demonstrated to improve the health of the subject. A major issue in this context is that health claims should be assessed for the healthy European population.



## GUIDANCE FOR HEALTH CLAIM APPLICATIONS

EFSA recently published a guidance document on scientific requirements for health claims related to gut and immune function with a view to facilitating study design for submissions. This manual addresses the beneficial effects and outcome measures that are acceptable for substantiation of claims in these areas (EFSA 2011). Other guidances are still being prepared and draft guidance is available (**http://www.efsa.europa.eu/en/ndaclaims/ndaguidelines.htm**). In relation to microorganisms proposed as probiotics and to prebiotics, the guidance document on gut health is discussed in greater detail.

## GUIDANCE FOR GUT AND IMMUNE HEALTH CLAIMS

### Beneficial Physiological Gastrointestinal Functions

In relation to the physiological functions of the gastrointestinal tract, EFSA considers the following to constitute beneficial effects: contribution to normal bowel function, reduction in gastro-intestinal discomfort, and improvement in the digestion/absorption of nutrients in specific situations.

Outcome measures acceptable for the substantiation of claims on normal bowel function include reduction in transit time, increased frequency of bowel movements, increased fecal bulk, and softer stools, provided they do not result in diarrhea. Outcome measures acceptable for the substantiation of claims on reduction in gastrointestinal discomfort include data on the severity and frequency of symptoms [e.g., abdominal pain, cramp, bloating, straining, borborygmi, and the sensation of incomplete evacuation (EFSA 2011)]. In relation to the digestion/absorption of nutrients, improvement in lactose digestion has been considered a beneficial physiological effect in that it may alleviate lactose maldigestion symptoms when the food/constituent that is the subject of the claim (e.g., lactose-hydrolyzing bacteria or enzymes) is consumed with lactose-containing foods. In this particular case, appropriate outcome measures include assessment of symptoms and/or measurement of breath hydrogen and methane. However, whether improved digestion of other nutrients is considered a beneficial physiological effect or not may depend on the health consequences of reduced digestion, e.g., if the undigested nutrient exerts an adverse effect on the

gastrointestinal tract or whether its absorption and retention is limited by digestion. Similarly, improved nutrient absorption (e.g., iron) has been considered beneficial when absorption is a limiting factor for the maintenance of an adequate status of the nutrient and when the absorbed nutrient can be utilized (EFSA 2011).

## Beneficial Effects Related to the Gut Microbiota

In relation to the gastrointestinal microbiota and pathogens, EFSA regards as a beneficial effect a contribution to the defense against pathogens because their presence in the gastrointestinal tract may cause infections. For these function claims, outcome measures considered appropriate include clinical outcomes related to gastrointestinal infections (e.g., number of episodes and severity or duration of infection) and/or reduction in the presence of specific pathogens, their toxins, and other virulence factors, provided that their pathogenicity is well established. Otherwise, evidence for the pathogenicity of the microorganisms targeted by the food (constituent) should be provided. Even if some groups of bacteria inhabiting the human intestinal tract, e.g., enterobacteria and clostridia, can include pathogenic species or strains, the group as a whole is not considered pathogenic but rather commensal (EFSA 2011).

Other outcome measures, such as decreased stool pH, increased short-chain fatty acid production, and a reduction in intestinal permeability, are not considered sufficient alone to substantiate this type of claim, although they may provide evidence for the mode of action of a food (constituent) and the biological plausibility of the claim. For reduction of disease risk claims related to gastrointestinal infections, appropriate outcome measures are relevant reductions in specific pathogenic microorganisms or their toxins in the gastrointestinal tract, as their presence constitutes a risk factor for infections. The relevance of such reductions should be justified in relation to the type of infection, by the magnitude of reduction, and/or by evidence of a reduction in clinical outcomes related to infections. This approach also applies to function and disease risk reduction claims related to pathogens and infections in other parts of the body. For claims related to the microbiota composition, changes in the number of nonpathogenic microorganisms, including lactobacilli and bifidobacteria, have not been considered beneficial unless accompanied by a beneficial physiological or clinical outcome. It is considered that the mere passage of probiotic microorganisms and their survival in the gastrointestinal tract does not imply any beneficial influence on the host physiology. Moreover, current scientific knowledge is still insufficient to define the bacterial groups and their relative abundance such as would constitute a healthy microbiota. Many efforts have been made to identify the specific bacteria that contribute to a normal microbiota by comparing the composition in healthy populations with a specific disease or populations at disease risk (Luoto et al. 2011, Sanz et al. 2011). Thus, associations between alterations in specific bacterial groups and certain conditions have been established, but all these alterations do not necessarily reflect the causality of the underlying disease (Bloom et al. 2011). The use of metagenomic approaches could potentially contribute to progress in the definition of what is a normal microbiota on a population basis and its possible association with certain host phenotypic features (e.g., age or body-mass index) (Arumugam et al. 2011, Qin et al. 2010). The latest studies of the fecal metagenomes of individuals from four countries have thus led to the identification of three common clusters or enterotypes in the microbiota of adults, mostly driven by species composition, that appeared not to be nation or continent specific. Nevertheless, other studies across Europe have reported that, for instance, the dominance of bifidobacteria in infants is relatively variable and affected by the country of birth with significant differences in bifidobacterial levels within European countries (Fallani et al. 2010).

## Beneficial Effects Related to the Immune System

In relation to claims on the function of the immune system, EFSA considers a contribution to maintaining a normal immune function to constitute a beneficial physiological effect, but the specific aspect of immune function, which is the subject of the claim, should be further defined. Specific beneficial effects for function claims that are acceptable include a contribution to immune defense against pathogens and to immune resistance to allergens. Appropriate outcome measures for the substantiation of a claim related to immune defense against pathogens include (*a*) changes in relevant immunological parameters together with clinical outcomes related to infections or with microbiological endpoints (e.g., reduction in pathogens and toxins), as described for claims on defense against pathogens, and (*b*) enhanced vaccination responses (increased numbers of individuals attaining protective levels and in titres in groups of individuals), which may indicate increased protection against infections.

For function claims on immune resistance to allergens, appropriate outcome measures are clinical outcomes related to a specific type of allergy (e.g., the incidence, severity, and frequency of allergic manifestations), applying appropriate diagnostic procedures together with evaluation of appropriate immunological parameters.

Changes in markers of the immune function (e.g., in numbers of various lymphoid subpopulations, proliferative responses of lymphocytes, phagocytic activity of phagocytes, lytic activity of natural killer cells, and production of cellular mediators) as proposed in many applications per se have not been considered beneficial physiological effects unless accompanied by physiological or clinical outcomes. In the particular case of population groups considered to be at risk of immunosuppression (e.g., older adults, individuals experiencing stress or engaging in heavy physical exercise, or after exposure to UV radiation), studies showing improvement in symptoms and/or restoration or maintenance of immune markers may be considered appropriate.

For claims on the reduction of a risk factor for allergy, appropriate outcome measures may comprise alterations in immunological markers accompanied by an improvement in clinical outcomes related to allergy (e.g., reduced incidence, severity or frequency of allergic manifestations).

Other possible claims, such as reduction of inflammation, have also been proposed. In these cases, EFSA considers appropriate outcome measures to be changes in markers of inflammation associated with the development of the disease, provided that they are accompanied by beneficial physiological or clinical outcomes. In the context of a reduction of disease risk claim, a reduction in the levels of markers of inflammation might also indicate a beneficial physiological effect if accompanied by a reduced incidence of a disease associated with chronic inflammation (EFSA 2011).

# HUMAN INTERVENTION STUDIES FOR HEALTH CLAIMS

Health claim documentation is based mainly on human intervention studies conducted in a target population using the relevant food and ingredient at the intended dose level. This is especially true in the case of claims intended for children's health or claims associated with reduction in risk of disease. Prior to human intervention studies, it is important to establish the rationale for the probiotic strain or strain combination and to obtain information on the preclinical properties of the strain or strain combination.

For human intervention studies, it is vital to select the appropriate target population that corresponds to the intended claim. It is as important to use the same dose of the food product or ingredient in studies intended to be featured in the claim.

Human intervention studies can be classified in a number of ways, but the following hierarchy of study design forms a common basis: (*a*) blind, randomized controlled intervention studies,

other randomized studies (noncontrolled), controlled (nonrandomized) studies, other intervention studies; (*b*) human observational studies, cohort studies, case-control studies, cross-sectional studies, other observational studies (such as case reports); and (*c*) other human studies dealing with the mechanisms of action of given substances.

Human studies should be conducted according to international guidelines, and they should provide information on markers or factors that are important or on intermediate markers associated with clear endpoints in the disease or health area in the claimed effect. Examples could be cholesterol levels in the case of heart disease risk or numbers of *S. mutans* on dental surfaces, oral pH, and dental plaque as risk factors for caries and tooth decay. Guidelines for human studies and other information required for health claim applications are available (EFSA 2009b).

## TOTALITY OF EVIDENCE

As specified in the European health claim regulation, all claims should be substantiated, taking into account the totality of the available scientific data and weighing the evidence. This should be undertaken with an eye to the specific conditions of use. In particular, the total evidence should demonstrate the following: (*a*) the extent or importance of the claimed effect of the food/constituent for human health; (*b*) whether a scientific cause and effect relationship can be established between the consumption of food or the food constituent and the claimed effect in humans (define, for example, the strength, consistency, specificity, dose-response, and biological plausibility of the relationship); (*c*) the quantity of the food/ingredient and pattern of consumption required to obtain the claimed effect and the quantity and daily portions should be such that they can be reasonably achieved as part of a recommended balanced diet in European countries; and (*d*) the target population for which the claim is intended is defined, and the specific study populations in which the evidence was obtained is representative of the target population.

## CASE EXAMPLES OF HEALTH CLAIMS: PROBIOTICS AND PREBIOTICS

In the case of probiotics, the main health benefits are associated with regulation of the intestinal tract microbiota by reducing the numbers or colonization of pathogenic bacteria or viruses and with maintenance of the intestinal gut integrity or barrier function. Specific probiotics may also be associated with other health benefits, including the following: desirable modulation of lactose intolerance, diarrhea prevention and symptom alleviation, immune and allergy response, mineral absorption, and inhibition of procarcinogen-activating enzymes.

A probiotic claim or a prebiotic claim is any claim that states, suggests, or implies that a probiotic containing food or a prebiotic food has particular characteristics relating to its origin, nutritional properties, and health (EC 2006, FAO 2007, WHO 2002).

### Criteria for Microorganisms Proposed as Probiotics and Health Claims

The three important areas the regulations pinpoint for assessment in health claims and probiotic and prebiotic claims for each potentially probiotic strain or prebiotic component are the following: (*a*) characterization of the strain or each of the strains in a probiotic mix or combination or the prebiotic components, (*b*) identification of the health relationship to benefit the general population or a defined part of it, and (*c*) demonstration of health effects in a normal healthy target population.

These three criteria form the essential basis for the establishment of potential human health claims for microorganisms and prebiotics, and these steps are discussed in detail in each of the following paragraphs.

## Definition of Probiotics and Prebiotics

The definition of a probiotic has evolved over the years, and its development has been at times difficult to approach. Resulting from this process, the definition most commonly applied is based on work of International Life Sciences Institute-Europe and the World Health Organization (WHO) (Salminen et al. 1998; WHO 2001, 2002). The WHO expert group definition of probiotics states that probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2002). This differs from the definition often used in Japan, where the term probiotic covers not only live microorganisms but also cells of nonviable microorganisms that confer health benefits (Salminen et al. 1999).

The definition probably requires further evaluation now that it is understood that the viability of microorganisms is not always directly associated with culturability. It is also now acknowledged that the majority of intestinal microorganisms are viable but not culturable, and also probiotics may thus be viable without necessarily being culturable (Lahtinen et al. 2006, 2007). Because of this development, the term viability needs to be redefined. One of the first areas addressed in viability assessment has been in the approval of animal feed containing viable but nonculturable probiotic bacteria. Noncultivable cells may contribute to the beneficial effects of the feed, as indicated by the absense of cultivable lactobacilli in the feed used in efficacy trials.

A prebiotic is a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota (FAO 2007). The qualifications for a prebiotic are defined in the FAO report as follows: A prebiotic is a component not an organism or drug. It is also a substance that can be chemically characterized, and in most cases the substance is a food grade component. The health benefit defined needs to be measurable and not due to absorption of the component into the bloodstream or due to the component acting alone. It should be shown that the mere presence of the prebiotic component and the formulation in which it is being delivered alters the composition or activity of the microbiota in the target host. Potential mechanisms might include fermentation, receptor blockage, or production of health-promoting components from the prebiotic by the microbiota. According to the definition, a prebiotic can be a fiber, but a fiber need not be a prebiotic (FAO 2007).

## Characterization of Probiotic Bacteria

An appropriate identification of and nomenclature for microorganisms constitutes the starting point in the assessment of microbial properties. A reliable identification by adequate methods confirms the identity of the strain in commercial use, this also being necessary for the proper labeling of products containing them (Felis & Dellaglio 2007). A correct identification allows linkage of the microorganism to what is already known regarding the corresponding microbial group and prediction of some of its properties.

During the recent years molecular techniques have been introduced as a replacement or complement to traditional phenotypic methods. DNA-DNA hybridization has been the gold standard for determination of bacterial identity, two strains being considered to belong to the same species if their DNA-DNA relatedness is $\geq$70%. However, because of the difficulties associated with this technique and the need for expertise not normally available in the food industry, phylogenetically based approaches, such as sequence analysis of the 16S rRNA gene, have been the most commonly used methods for bacterial species identification.

A recently completed EU-funded project (Prosafe) concluded that biochemical tests should not be used as stand-alone methods for the identification of probiotic cultures and recommended the use of 16S rRNA gene sequence analysis as the best tool for routine species identification

because of its high reproducibility and data exchangeability (Vankerckhoven et al. 2008). The FAO-WHO expert group recommends that phenotypic tests should be made first, followed by genetic identification, using methods such as DNA/DNA hybridization, 16S rRNA sequence analysis, or other well-established methods (FAO 2006). Nevertheless, it must be emphasized that in some cases 16S rRNA gene sequencing has a limited resolution, and it may not suffice for discrimination between closely related species (Felis & Dellaglio 2007, Vankerckhoven et al. 2008), necessitating the use of other methods. It is also important to point out that when comparing a gene sequence with those held in the databases, the quality of the sequences deposited in the database has a great impact on the accuracy of the identification. In this regard, the Prosafe project underlined the presence of unreliable or incomplete sequence entries in public sequence databases, pointing out the need for a list of validated complete 16S rRNA gene sequences for the purposes of identification.

Establishing the identity of a microorganism is thus the first step in the assessment of its safety and efficacy. In this respect, the qualified presumption of safety (QPS) approach established by EFSA considers the identification of microorganisms as the first pillar in safety assessment (EFSA 2007a,b; Leuschner et al. 2010). EFSA is requested to assess the safety of biological agents notified in the context of authorization requests. A wide range of microorganisms and viruses have been or are expected to be referred to EFSA for safety assessment. EFSA's Scientific Committee has recommended that EFSA panels use a common risk assessment approach in order to ensure a consistent approach: the QPS. The QPS approach can be utilized for pre-market safety assessment of notified biological agents by all EFSA's Scientific Panels. QPS aims at harmonizing risk assessment and allowing risk assessors to focus on the microorganisms with the greatest risks or uncertainties. EFSA also updates the QPS list annually, and the 2010 update is currently available (EFSA 2010). In a similar manner, some probiotic strains are also listed in the U.S. Food and Drug Administration's generally regarded as safe (GRAS) inventory (FDA 2011). However, in evaluating microorganisms proposed as probiotics, in addition to proper species identification, it is of importance to take into account that probiotic effects are strain specific, making it necessary to identify them at strain level. According to the FAO-WHO working group (FAO 2006), strain typing must be conducted using a reproducible genetic method or a unique phenotypic trait. DNA macrorestriction followed by pulsed field gel electrophoresis is considered the gold standard for strain identification, and it has been extensively used in differentiating between probiotic strains. Other molecular methods are also available to this end and recent advances have increased the applicability of genomics in general for identification and characterization of probiotic microorganisms (O'Flaherty & Klaenhammer 2010).

Within the framework of the EU Regulation on Nutritional and Health Claims Made for Foods [Regulation (EC) no 1924/2006], in assessing applications, EFSA has considered appropriate identification at species and strain level a restriction criterion for the further assessment of health claims related to probiotics. There is clear call for proper species identification and strain characterization (genetic typing), using internationally accepted molecular methods. In addition, strains should be named according to the International Code of Nomenclature. In the context of this regulation, the purposes of characterization are to confirm the identity of the food/constituent that is the subject of the health claim and to establish that studies undertaken for substantiation of the health claim were performed with the food/constituent for which the claim is made. Characterization should also be sufficient to allow control authorities to verify that the food/constituent that bears a health claim is the same as that which was the subject of community authorization (EFSA 2009a). According to the recommendations of WHO (WHO 2002), and although not mandatory but recommended by EFSA, strains should also be deposited in an internationally

recognized culture collection. These are important criteria that assure access of authorities to the strain, tracking of the strain, and related information whenever necessary.

Proper identification of any investigated strain may constitute the critical starting point for probiotic studies. A number of papers have reported that the identity of microorganisms isolated from probiotic products often does not correspond to the information on the product label (Hamilton-Miller et al. 1999, Gueimonde et al. 2004, Huys et al. 2006). Accurate and reliable identification of probiotic strains is necessary for evaluation of both the documented health benefits and the safety of probiotic products.

Currently, the increasing availability of genome sequences is allowing genome-wide and/or multilocus phylogenetic analysis more often. During recent years, the development of high-throughput sequencing technologies has enormously enhanced sequencing capability while significantly reducing costs. Although final genome assembly still remains a time-consuming task, the number of completed bacterial genomes and especially that of draft genomes is increasing very rapidly and these may change the way we assess bacterial strain identity (Chain et al. 2009, de Vos 2011). In fact, some probiotic strain genomes have already been sequenced and in some cases the sequences have been deposited in public databases. In the future, the deposit of genomes from probiotic strains in commercial use, in public or restricted-access databases, may overcome all the current limitations regarding identification at species and strain levels. The genome sequence constitutes the best possible genetic fingerprint of a given strain. In addition, the availability of genomes of commercial strains would allow researchers and authorities a ready access to information on potential traits of a strain in cases where new markers related to the efficacy or safety of probiotics are identified in the future.

It is clear that strains used by the food industry and scientists should be identified using molecular methods and up-to-date taxonomical nomenclature. In this respect, it is also important to make strains available in international culture collections. Even nowadays, many scientific articles are published without reporting data on the tested strains, thus hampering scientific development in this area as well as assessment of the efficacy and safety of probiotics.

## SPECIFIC CHALLENGES IN THE CONTEXT OF PROBIOTICS

### Viability

The directions for the future of probiotics involve a number of major challenges. The first particularly specific challenge for probiotics in the future is assessment of viability. The current WHO definition of probiotics characterizes them as viable food supplements, but viability is defined by most regulatory authorities as culturability. Culturability itself depends on specific media and culture conditions. As demonstrated in human intestinal microbiota assessment studies, only a small part of the intestinal microbiota can be cultured. Nonetheless, these microbes may be viable and even if nonviable cells are present may have an effect on human health (Ben-Amor et al 2005; Lahtinen et al. 2006, 2007; Salminen et al. 1999).

Reliable determination of the viability of bacteria in probiotic products is essential in both human studies and product quality control. In most regulatory analyses, the plate count method has traditionally been used for controlling the viability of bacteria, but there are several disadvantages. The plate count method requires specific culture media and relatively long incubation times and is hampered by technical difficulties in incubation conditions for specific species. For many species residing in the human intestinal tract, a suitable growth medium is not even known. Furthermore, for fastidious microorganisms, such as *Bifidobacterium* species, of intestinal origin, which are increasingly used as probiotics, it may be difficult to find an optimal growth medium

for reliable enumeration. Such bacteria have specific and unique nutritional and environmental requirements for optimal growth, and plate counts for certain strains may vary by several log units when grown on different nutrient-rich culture media (Lahtinen et al. 2006a,b; 2008). It is often not feasible to identify all potential growth media for a particular strain or purpose. Such difficulties may lead to underestimation of the real probiotic counts. Another recently reported major challenge for the plate count method is the presence of so-called dormant bacteria, which are unable to grow on conventional growth media but may nevertheless be measured as viable using cytological viability assays (Lahtinen et al. 2006a). Such a dormant population may exist in many probiotic products and food starters, and a similar population occurring in bile acid–stressed bifidobacteria has been demonstrated by Ben Amor and colleagues (2002). Recent reports suggest that probiotic bacteria in a fermented product may become dormant during prolonged storage (Lahtinen et al. 2005, 2006). The strain-specific properties and the objects of analysis should be taken into account when future enumeration methods for different probiotic strains are chosen, and this should also be considered in regulatory control.

## WHY HAVE HEALTH CLAIMS ON PROBIOTIC AND PREBIOTICS NOT GAINED ACCEPTANCE?

In contrast to Japan, where health claims may be based on evidence from animal experiments, in Europe, substantiation of health claims evidence from human studies is required. This is a prime reason why, in contrast to Japan, opinions on pre- and probiotics so far published by the EFSA have not been favorable. For many probiotic microorganisms, another main issue has been the lack of characterization. Although it is known that effects are strain dependent, the information on which the assessments are to be made has often failed to satisfactorily provide the strain characterization. This pertains to article 13.1 claims rather than article 13.5 and 13.14 claims. Nonetheless, also in the latter type of claims, combinations of bacteria have sometimes been used in studies, and the actual claims have related to specific strains and vice versa. Otherwise a combination of bacterial strains has been used, and only part of them have been sufficiently characterized.

For microorganisms as well as potential prebiotics, a problem often encountered in assessments has been that the health relationship claimed is not defined. Either the claimed health relationships have been too general, or they were considered by EFSA not to be beneficial. In this context, one of the main reasons for not granting a positive opinion has been that many claims have stated that merely increasing the proportion of lactobacilli or bifidobacteria in the gut should be considered as a beneficial health effect. EFSA has not accepted this view and has required evidence of beneficial clinical outcomes. Such information has often not been provided. Other examples of overgeneralized claims have been statements such as "improves gut health" or "boosts the immune system."

Claims have sometimes been judged as not eligible by EFSA in that they have pertained to treatment of pathological situations rather than maintaining normal physiological conditions or reducing disease risk factors. Effects in patients can, under certain circumstances, be accepted as evidence for effects in the general population, but claims oriented to subjects beyond the scope of the claims regulation were judged not eligible. Some claims have been oriented to reducing the risk of a disease but have failed to identify risk factors, and this is required in the claims regulation.

Other claims have failed because the studies provided to substantiate them have had flaws in their design. Intervention studies have not always been sufficiently randomized, measures for blinding subjects and/or observers have not always been adequate, and often statistical analysis has been inadequate. One flaw often encountered has been that many measures have been studied, but statistical analysis has failed to correct for multiple comparisons.

Flaws in the actual measurements have also been encountered. For example, the immunologic basis for a runny nose or a rash to corroborate the allergic nature of these measures has been absent, and the infectious nature of diarrhea has not been addressed.

Most probiotic studies in humans have been conducted among subjects who have been either ill or critically ill. A further challenge is thus built-in in the form of the EU regulation that health claims are designed for normal healthy populations or populations at risk of specific disorders.

Taken together, a number of challenges have appeared in the probiotic and prebiotic area, and health claims have not been established according to the regulatory requirements. In spite of extensive studies in the area, the focus has been beyond the scope of the regulations as approved by the European Parliament. The research tools must thus be redirected to areas that support future health claims. This can be achieved through focusing on the requirements of the regulation and by careful assessment of probiotic properties and related health outcomes. The improved and more detailed guidance documents provided by EFSA also benefit all future research and applications. Learning from the experience of previous assessments should make for health claims for probiotics and prebiotics, providing the consumers with reliable advice and new directions to help them make healthier choices and develop lifestyles that support their long-term well-being.



## SAFETY ISSUES

Microorganisms that are proposed as probiotics are generally regarded as safe, as their consumption has a long history of safe use in foods. Most of them are included in the list of microorganisms that have EFSA QPS status for their use in foods (EFSA 2010, Leuschner et al. 2010). For this reason, safety evaluation is usually not an issue in the assessment of health claims. It is true that incidents of adverse effects due to consumption of such microorganism are few. Some prudence in the use of microorganisms as probiotics is nevertheless warranted. There is concern over conveying antibiotic resistance. For this reason, it may be considered adequate to treat microorganisms as novel foods in those cases in which specific strains are introduced in food of which no prior consumption has taken place, or when the use of these microorganisms would lead to consumption patterns significantly different from those of the present. In these cases, safety evaluation along the novel food regulation may be recommended (Verhagen et al. 2009).

## CONCLUSIONS

Whereas a vast literature database on effects of probiotic microorganisms on health exists, studies have generally been designed as exploratory and were not sufficiently designed to fulfill the criteria for substantiation of a health claim under the current claims regulation. With the current guidelines in place, it should be possible for companies to design studies so that for those microorganisms that exert beneficial effects, the information provided by these studies suffices to gain a positive evaluation by EFSA.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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