Review

Safety Aspects and Implications of Regulation of Probiotic Bacteria in Food and Food Supplements

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ABSTRACT

The application of living bacteria as probiotics in food or food supplements requires a careful safety assessment. This review summarizes key issues concerning the safety aspects of bacteria added to particular products marketed for improvement of general health or treatment of (post)infectious symptoms. The bacteria used in such products should be completely safe; however, it can be challenging to provide evidence for absence of all virulence properties. In some cases, virulence factors have been detected in probiotic bacterial strains, and the implications of these traits for safety assessments are discussed. Horizontal gene transfer can result in acquisition of virulence genes or antimicrobial resistance in probiotic bacteria. Antimicrobial resistance in these bacteria can possibly aid the spread of undesired resistance in intestinal bacterial populations. The relative risk of such gene transfers is considered. The generation of complete bacterial genome sequences can both resolve and create safety issues. Current practices of safety assessment procedures in the United States and the European Union are briefly reviewed and a future outlook is provided.

Bacteria deliberately added to food products are used either as technological additives (starter or protection cultures) or as functional additives for human health benefits. An increasing number of commercial products (food and food supplements) containing viable bacteria are marketed for their beneficial effect on the immune system or on human health in general. Starter or protection cultures are added for technological purposes and are not considered in this study. Various terms (e.g., probiotics, synbiotics, and functional food) and definitions are in use for such products. Gibson and Roberfroid (30) defined probiotics as “microbial food supplements that beneficially affect the host by improving its intestinal microbial balance.” Six years later, experts consulted by the World Health Organization and the Food and Agriculture Organization (26) refined this definition as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” We suggest the following slightly modified definition of probiotics: “food or food supplements containing defined microorganisms in sufficient numbers to reach the gut in viable status resulting in positive health effects after consumption.” This definition does not contradict international and scientific definitions but adds both qualitative (defined microorganisms) and quantitative (sufficient numbers) requirements to the presumed positive health effect.

Probiotic agents are used for prevention of enteric diseases and intestinal microbial imbalance or as treatment for these conditions. Described health benefits of probiotics have been extensively reviewed elsewhere (18, 31, 35, 39, 40). These benefits include prevention and treatment of acute gastrointestinal infections (notably in children), treatment of antibiotic-associated and Clostridium difficile-associated diarrhea, and reduction of symptoms in cases of inflammatory bowel disease (18, 41, 48) among more far-reaching claims such as prevention or treatment of colon cancer (31). Reduction of diarrhea in lactose-intolerant individuals also has been described (46). Other suggested benefits are enhancement of the immune system (42), synthesis of nutrients in the gut, and decrease in the prevalence of allergies (39). Young children are specific target groups for probiotics (31, 44, 56). The effectiveness of probiotics is not the subject of this review. Instead, we address the safety aspects of products containing purposefully added viable bacteria.

Probiotics are commonly (though not exclusively) based on fermented milk products, e.g., yogurt and yogurt products, in which the fermenting microorganisms are viable at the time of consumption, although consumers are not always aware of this fact (34). Examples of products and the bacterial species they contain are given in Table 1. The marketing of fermented dairy products is rapidly expanding in the United States and Europe; in Europe, probiotics currently represent up to 20% of such products (15). In addition to their use in human foods, probiotics are used in animal foods to enhance and stabilize the growth of fattening animals (51). Probiotics are commonly used as replacements for antibiotic growth-promoting feed additives, especially because the use of such additives is now banned.

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in the European Union (EU) (36). Use of feed additives requires strict evaluation of efficacy and safety done by the European Food Safety Authority, whose scientists conduct experimental studies based among other things on Scientific Committee on Animal Nutrition (SCAN) opinions (47). This review is focused on uses of probiotics for humans.

Probiotic products frequently contain specific strains of *Bifidobacterium, Lactobacillus, or Streptococcus thermophilus* (Table 1). Products can contain combinations of species or strains. Less commonly used are specific strains of *Escherichia coli, Enterococcus faecalis*, or the yeast *Saccharomyces boulardii* (15, 18). *Bifidobacterium* and *Lactobacillus* species have a long history of safe use, although in rare cases infections with some species have been described. Because *E. coli* and *Enterococcus* have recognized pathogenic strains, the safety of strains used in probiotic applications must be carefully examined for these species.

The term prebiotics is reserved for food or food supplement ingredients that require predigestion by gut bacteria, are specifically targeted to enhance the growth of beneficial microorganisms in the gut, and have beneficial effects on the host (16, 30). Products combining pre- and probiotics and containing both bacteria and some of their nutrient requirements are known as synbiotics. All these products fall under the general description “functional foods,” which are foods or nutrients that promote health, beyond satisfying basic nutrition demand, because of their inclusion of functional ingredients (bacteria or other components).

This review deals exclusively with products that contain, or at least should contain, deliberately added viable probiotic bacteria. Such products are increasingly aimed at consumers with weakening or weakened immune systems, such as the elderly, children, and immunocompromised individuals. Such targeting prompts safety concerns. Probiotic bacteria should not cause disease in humans; they should be completely nonpathogenic and should not be able to evolve into pathogenic variants. Evidence of these characteristics is not simple to obtain. Here, we concentrate on the requirement that probiotic bacteria must be proven nonpathogenic. Complications associated with providing evidence of the absence of virulence characteristics are discussed. The ability to acquire DNA from residual microbiota also is considered because acquisition of either virulence genes or antibiotic resistance genes is undesirable. Safety aspects of shelf life, packaging, and preparation are not considered here.

**PROBIOTIC BACTERIA SHOULD BE NONPATHOGENIC AND SHOULD NOT POSE A RISK TO THE HOST**

The definition of probiotic bacteria includes their beneficial effects on the host. The organisms must not be pathogenic to any individual host, including immunocompromised individuals. Probiotic bacteria are mainly *Bifidobacterium* species or lactic acid bacteria (38). The lactic acid bacteria mainly belong to the order *Lactobacillales* containing, among others, the genera *Lactococcus, Lactobacillus, Enterococcus, and Streptococcus*. Lactobacilli and bifidobacteria are naturally present in the human gut in relatively high numbers (32). Commercially exploited strains can at best colonize the human gut for a short period (transient colonization) (9, 17). Lactic acid bacteria are considered mostly harmless and are members of the genera *Lactococcus* and *Lactobacillus* that have obtained the status “generally recognized as safe” (GRAS) in the United States (45, 50). Nevertheless, some species of *Lactobacillus* have been associated with opportunistic infections (13, 29). Other bacterial species frequently used as probiotics, such as *E. coli* or *Enterococcus* species, contain both nonpathogenic variants and pathovars (28). In such cases, the attribute “pathogen” is strain specific rather than species spe-

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**TABLE 1. Systematic summary of commercially available probiotic products**

<table>
<thead>
<tr>
<th>Type of product</th>
<th>Most common microbiological contents</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt and yogurt-derived products (milk or soy based)</td>
<td><em>Lactobacillus</em> spp., e.g., <em>L. johnsonii, L. paracasei, L. acidophilus, L. delbrueckii</em> subsp. <em>bulgaricus</em>, and <em>L. rhamnosus</em> GG</td>
<td>Products may contain one or more species; prebiotics (e.g., inulins) also may be included</td>
</tr>
<tr>
<td>Dairy-based drinks</td>
<td>Predominantly single strains, e.g., <em>Lactobacillus casei</em> Shirota, <em>L. lactis</em></td>
<td>Single strains frequently present in high concentrations (products such as Yakult and Actimel)</td>
</tr>
<tr>
<td>Other dairy products</td>
<td>Same as yogurts, frequently single strains</td>
<td>Cheese, buttermilk, etc.</td>
</tr>
<tr>
<td>Nondairy products</td>
<td>Same as yogurts, frequently single strains</td>
<td>Fermented cereals, fruit drinks, raw sausages</td>
</tr>
<tr>
<td>Dietary supplements (tablets, powders, drops, and capsules)</td>
<td>Single strains of various species</td>
<td>Often in combinations with vitamins, etc.</td>
</tr>
<tr>
<td>Products used in human medicine</td>
<td>Single strains, e.g., <em>E. coli</em> Nisslè, <em>Enterococcus faecalis</em>, <em>Saccharomyces boulardii</em>, and <em>Lactobacillus rhamnosus</em> GG</td>
<td>Used as therapeutic agents</td>
</tr>
<tr>
<td>Products used in animal feed</td>
<td>Single strains or combinations of strains of different genera</td>
<td>Frequently used in combination with prebiotics</td>
</tr>
</tbody>
</table>
cific (24). As a consequence, general conclusions about safety are not always applicable to all members of a given species or genus. Strain-specific characterization is required in such instances to prove absence of pathogenicity. GRAS status cannot be granted for lactic acid bacteria in general, and a safety evaluation must be at least species specific or better yet strain specific.

For characterizing probiotic bacteria, evidence should be provided that particular pathogenic features are absent, notably those involved in invasion and translocation, toxin production, and the ability to survive and multiply in the blood stream (4). Some bacteria can take up DNA from the environment and incorporate this DNA into their genome. Nonpathogenic bacteria could, at least in theory, gain virulence genes in this way, especially while inhabiting the human gut. Whether this would result in pathogenic properties that were previously absent is debatable.

As a consequence of DNA uptake, there is the additional risk that probiotic bacteria could acquire resistance against antimicrobial agents by horizontal gene transfer, which is generally considered undesirable. When residual nonpathogenic bacteria (probiotics or natural microbiota) have gained antimicrobial resistance genes, they could donate such genes to pathogenic bacteria, with possibly adverse health consequences. Therefore, the safety of bacterial products must be considered in terms of their virulence repertoire, antibiotic resistance, and ability to acquire foreign DNA.

**PROVING THE ABSENCE OF VIRULENCE GENES**

Providing evidence of presence of virulence properties in a given bacterial strain is, in most cases, relatively straightforward. Virulence properties such as hemolytic activity, production of enterotoxins or cytotoxins, or the capacity to invade host epithelial cells are easily demonstrated. By analogy, the absence of such properties should be equally simple to demonstrate. Because genes involved in these activities could be present but not expressed, negative phenotypic results should be confirmed by proving the absence of key genes with PCR techniques and/or DNA hybridization. However, such experimental approaches have their own difficulties. PCR primers or oligonucleotide probes used for detection of genes may be too specific, weak DNA hybridization may occur below detection levels, or chosen cutoffs may result in incorrect interpretations.

Setting aside experimental flaws and false-negative results, proving the absence of virulence genes is nevertheless complicated for a number of reasons. First, not all virulence properties are well characterized for all (entero)pathogens, and for some pathogens (e.g., *Campylobacter jejuni*) we are still uncertain about their pathogenic mechanisms (52). These bacteria may rely on so far unknown mechanisms and genetic properties for virulence, which cannot be detected.

Because some probiotic bacteria have the ability to colonize, we should allow a distinction between colonization properties and virulence properties, but unfortunately these activities are related. It is not always possible to define what properties help bacteria colonize and adapt to a niche, i.e., the gut epithelium (an ability that may be pertained in probiotic strains), and what properties are exclusively or largely responsible for disease. This difficulty is in part due to the way we identify virulence factors (53).

To prove a gene’s role in virulence, inactivation of the gene must result in attenuation, and complementation should reverse the phenotype (23); however, the same effect could be obtained if colonization genes or housekeeping genes were inactivated. Thus, such evidence is inconclusive for defining virulence genes, as has been discussed elsewhere (53). For example, many researchers consider fimbriae, which are required for adhesion and colonization, to be virulence factors (25) because they determine tissue tropism and their inactivation would result in loss of colonization and hence absence of disease. Commensal bacteria, however, also express fimbriae, and some fimbriae classes are conserved between pathogenic and nonpathogenic strains or species. If we follow the molecular Koch’s postulates stated by Falkow (23), virulence genes should not be present in nonpathogenic strains or species. Thus, only particular types of fimbriae should be considered virulence factors, e.g., those found only on uropathogenic *E. coli* strains with specific binding affinity for particular epithelium cells (19).

A third complication arises when true virulence genes are present in commensal organisms. Such an example is *hlyA*, which encodes hemolysin in *E. coli*. Pathogenic *E. coli* strains usually are strongly hemolytic, and this property is considered a virulence factor for strains causing extra-intestinal infections (54) and for enterohemorrhagic *E. coli* (2). *E. coli* strains isolated from healthy individuals may nevertheless carry the *hlyA* gene, which can be expressed in such strains, although activity is found at lower frequency than in strains isolated from patients (6, 10). Clearly, the mere presence of *hlyA* is not enough to categorize a strain as pathogenic.

Studies of complete genome sequences have revealed that virulence genes in commensals are quite common. It is now possible to determine the complete genome sequence of a bacterial isolate, and the price of such efforts is rapidly decreasing (7). Ideally, a genome sequence should reveal the true beneficial or pathogenic nature of a given isolate, in particular when genome sequences of both beneficial and pathogenic strains of a bacterial species are compared. In reality, the majority of genes of beneficial and pathogenic variants within a species are shared in what can be defined as the core genome. This sharing was demonstrated for *E. coli* when seven complete genome sequences of pathogenic and nonpathogenic strains were compared (55). Thus, the variable genetic content (not belonging to the core genome) would have to be responsible for virulence or lack thereof. Even when researchers concentrate on this subset of genes, virulence properties cannot easily be predicted from gene content. Virulence genes can be redundant, or they can function in synergy in complex processes. Genes also are frequently misannotated. All of these complications have been described for enteropathogens and
make it difficult to predict virulence (or absence thereof) from a genome sequence.

Thus, it is difficult to obtain evidence that a given bacterial strain is nonvirulent, even when complete genome sequences are available. A genome sequence even may reveal the presence of virulence genes that had not been expected. Evidence of the safety of bacterial species based on the proven absence of virulence genes cannot be considered conclusive. Yet, conclusive evidence is expected from the producers of probiotics within the animal nutrition field (47, 51).

**PROVING THE ABSENCE OF ANTIBIOTIC RESISTANCE**

Acquired resistance to antimicrobial compounds used in clinical applications must be avoided in probiotic bacteria. Although such resistance would not have direct effects on the health of the host, such resistance genes can serve as genetic reservoirs for other potentially pathogenic bacteria. The absence of antibiotic resistance in a given bacterial strain can be proven in a relatively straightforward manner. Determination of the MICs for common antibiotics is a routine and highly standardized procedure, and evidence for the absence of resistance genes can be obtained by molecular methods. The most suitable method for determining MICs must be determined; at the moment, the Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) method has the broadest application (43). Unfortunately, not all standards applied nationally or internationally are as detailed as the CLSI standards. Safety evaluation protocols for probiotics do not always include a list of which antibiotic compounds should be tested, each member of a particular antibiotic class or only compounds in use in human medicine (which is required by CLSI standards). The guidelines related to antibiotic testing of food bacteria need improvement.

Because many (although certainly not all) bacterial virulence genes and antibiotic resistance genes are encoded on plasmids, the carriage of plasmids could be considered a potential risk factor. Nevertheless, assessment of the presence of plasmids currently is not required for food supplements in Europe, and we do not think that this should become a requirement. When the complete genetic content (chromosomal plus extrachromosomal entities) is included in the analysis for presence of virulence and resistance genes, the mere presence of plasmids is not relevant because the genes they carry would be assessed in any case. An exception could be made for plasmids that enable conjugation, because these plasmids would make it easier for the cell to acquire DNA from the environment. The presence of prophages is of less concern, although at some stage prophages may become lytic and may influence the gut microbiota in undesired ways. A large proportion of intraspecies bacterial variation seems to be phage related (8) (personal observations, 2003, 2006).

**SHOULD GENE ACQUISITION BY PROBIOTIC BACTERIA BE CONSIDERED A RISK?**

Inactivation of a single virulence gene can result in a severe decrease, if not complete loss, of virulence. However, the opposite experiment, i.e., adding a single virulence gene to a bacterium that is not by itself pathogenic (complementation of a mutation in an otherwise virulent strain is not meant here) does not necessarily introduce or increase virulence. The life style of a pathogen is well adapted to survival in its host and its preferred ecological niche, and the disease resulting from an infection is often multifactorial. Thus, inactivation of one factor will (likely) decrease virulence, but addition of that factor to an avirulent background may remain without consequences.

Virulence genes frequently are present on plasmids, as for example in hemorrhagic \textit{E. coli} O157:H7, and plasmids can be transferred to a new genetic background. Hayashi et al. (33) concluded that the \textit{E. coli} O157:H7 chromosome encodes 1,632 proteins and 20 tRNAs that are not present in commensal \textit{E. coli} K-12 strains. Among these 1,632 proteins, at least 131 were assumed to have “virulence-related functions” (33). Thus, the virulence plasmid is not solely responsible although it is indispensable for the pathogenicity of \textit{E. coli} O157:H7. It is thus unlikely that the uptake of one or two virulence genes by a commensal \textit{E. coli} strain or even the uptake of a complete virulence plasmid (if it were compatible with the genetic background of the recipient strain) would have a major effect on the benevolent phenotype of that strain. Uptake of a complete virulence plasmid is highly unlikely, as was demonstrated in a study of the evolutionary relationship between \textit{Shigella} and \textit{E. coli} (20). The results of that work revealed that fragments of virulence plasmids are more likely to be transferred between virulent strains than are complete virulence plasmids, further decreasing the chance that nonpathogenic bacteria can acquire virulent properties in a single step.

Uptake of virulence genes by probiotic strains would be possible only if pathogen were present at the time of consumption, which applies for probiotics taken during bouts of (bacterial) diarrhea. Although under laboratory conditions it is quite possible to transfer virulence genes into probiotic bacteria, to our knowledge no evidence has been published of probiotic strains that have spontaneously acquired virulence genes after being consumed by humans, although it would be difficult to identify such a rare event. It is questionable what influence a transfer event would have on the health status of a patient. Most likely, the pathogen originally present would be the main culprit for any symptoms because it is already equipped to cause disease. Because probiotic bacteria usually colonize transiently (9), repeated intake of bacteria is required for persistent colonization. As a consequence, in the possible event of gene uptake, these naturally modified bacteria eventually would be washed away and unmodified bacteria would take their place. Because bacterial species used as probiotics are also naturally colonizing the gut, the addition of transiently colonizing probiotic strains does not add to the background risk of gene transfer. For these reasons, the risk to human health as a result of transfer of virulence genes to probiotics can be considered negligible.

Most of these arguments also apply to the acquisition of resistance genes. Genes conferring antimicrobial resistance frequently reside on mobile DNA fragments (e.g.,
plasmids, transposons, or integrons), which facilitates the spread and amplification of resistance (12). These genes can be transferred to other strains by conjugation or transformation, although genetic barriers exist that limit DNA exchange between bacteria. Such transfer of resistance genes is a natural process and poses a hazard only when a pathogen becomes resistant to a drug used in treatment situations (49).

For probiotic bacteria to take up resistance genes, such genes would have to be residual; thus, resistance is not created but merely transferred from one organism to the next (and possibly amplified). Because lactic acid bacteria and bifidobacteria usually reside in the gut naturally, these provide a potential reservoir for antibiotic genes even in the absence of probiotic bacteria (1). Gene transfer is more likely to occur within than between species, but interspecies transfer of resistance genes can occur with low frequency. Transfer rates also can vary among strains within a species. For instance, enterococcal strains from food seem to have a reduced rate of acquisition compared with that of clinical isolates (37). Variations in acquisition potential between strains used for sausage and cheese ripening also have been observed (14).

The relative risk of antibiotic gene transfer from gut bacteria to probiotic bacteria must be compared with the background risk of an intestinal microbiota without probiotic inhabitants. In view of the diversity and complexity of the gut microbiota and the transfer rates occurring in vivo, we consider the relative importance of probiotics as an intermediate for transfer of resistance genes to potential pathogens low.

To avoid selection for resistance, consumers should not use antibiotics while taking probiotic products. We strongly discourage the current practice (31) of application of probiotics during antibiotic treatment. Susceptible probiotic bacteria would not be able to survive during the course of medication, rendering the product useless. When probiotic products are used as postantibiotic treatment, there should be a time gap long enough to allow removal of the antibiotic from the gut. Under these conditions, there is no positive selection for resistance.

SAFETY ASPECTS RELATED TO PRODUCT REGISTRATION—STATUS QUO

There is no common worldwide safety assessment standard with legal status for dietary supplements containing probiotics. Approaches in the United States, the European Union (EU), and the member states of the EU differ significantly. Formal registration for bacterial supplements within the EU is necessary only for feed additives or therapeutics for human medicine. Food deliberately containing live bacteria is not regulated and is subject only to the general requirement that food must not impose a risk to human health.

In contrast, the EU regulations concerning feed additives include a strict procedure that must be followed before a microorganism or a mixture of bacteria or yeasts can be officially approved as a probiotic feed additive (3, 51). The assessment follows a premarket authorization, a positive list principle, and an in-depth risk assessment of strain-specific effects on human health. Figure 1 shows an example of a decision tree for the acceptance of probiotic enterococcal strains concentrating on antibiotic resistance, which is based on a SCAN opinion (47). Safety aspects are regarded with respect to the target animal species, the final consumer, the environment, and the safety of workers in contact with the microorganism during production or application (EU Council Directive 89/391/EEC).

The GRAS system applied in the United States by the Food and Drug Administration (FDA) covers substances that are intentionally added to food (by definition a food additive), which are then subject to premarket review and approval by the FDA “unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use” (50). These substances include live microorganisms added to food for uses other than as starter cultures. Thus, GRAS organisms are regarded as safe within their specific conditions of use and do not need formal approval by the FDA. Information about safety can be deduced from the scientific literature or can be empirically obtained through the long history of safe use, i.e., a substantial history of consumption in foods by a significant number of consumers. Obviously, the latter route is not available for products containing novel species.

Within the EU, diverse approaches are applied on a national level. For example, in France only microorganisms without prior history of use are considered for approval (22). In the United Kingdom, there is no specific program for probiotics; however, the British Food Standards Agency (FSA) (27) and similar national agencies in other member states of the EU must approve food additives following the Novel Food legislation (21). The German regulation does not foresee a special procedure for the application of live microorganisms in food. Instead, a classification originally developed for worker safety is used. A detailed list is available that divides bacterial species (including subspecies) into four risk groups (5). Bacteria used in the food industry are classified in risk group 1 (no human health hazard) or
TABLE 2. Bacterial properties to be excluded before the application of probiotic organisms, according to a German expert group (11)

<table>
<thead>
<tr>
<th>Bacterial properties to be excluded before application</th>
<th>Remarks and examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation of biogenic amines</td>
<td>Examples: tyramine, histamine, and phenylethylamine</td>
</tr>
<tr>
<td>Activation of procarcinogens</td>
<td>Examples: activation by azo reductase, nitro reductase, and β-glucuronidase</td>
</tr>
<tr>
<td>Induction and/or destruction of thrombocytes</td>
<td>Examples: specific hydrolases</td>
</tr>
<tr>
<td>Activation of platelet aggregation</td>
<td></td>
</tr>
<tr>
<td>Binding to fibrinogen and fibronectin</td>
<td></td>
</tr>
<tr>
<td>Mucin reduction</td>
<td>Detectable in certain bifidobacteria; can be regarded as a condition for invasive properties</td>
</tr>
<tr>
<td>Hemolytic activity</td>
<td>Example: glycopeptide (e.g., vancomycin) resistance in enterococci</td>
</tr>
<tr>
<td>Transferable resistance to antibiotics</td>
<td>Effect of these properties in vivo as yet unclear</td>
</tr>
<tr>
<td>Capacity for deconjugation and dehydroxylation of bile salts</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3. Comparison of assessment schemes for food supplements in the United States (FDA GRAS system) and proposed scheme in the EU (EFSA QPS system)

<table>
<thead>
<tr>
<th>GRAS guidelines</th>
<th>QPS guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applies to food additives in general</td>
<td>Applies to microorganisms only</td>
</tr>
<tr>
<td>Determination of GRAS status by FDA and/or external experts</td>
<td>Determination of QPS status by EFSA</td>
</tr>
<tr>
<td>Open list</td>
<td>Positive list</td>
</tr>
<tr>
<td>Based on common use</td>
<td>Based on history of use and adverse effects</td>
</tr>
<tr>
<td>Describes specific substance or microorganism</td>
<td>Describes taxonomic unit (e.g., genus, species, or strain)</td>
</tr>
<tr>
<td>Case-by-case assessment</td>
<td>General assessment</td>
</tr>
</tbody>
</table>

THE QUALIFIED PRESUMPTION OF SAFETY CONCEPT

Recently, the European Food Safety Authority (EFSA) has launched a system for a common European approach to safety of bacterial dietary supplements, the qualified presumption of safety (QPS) (21). This is a well-designed concept that could set an example for other authorities, and we present it here in detail as an example of how internationally accepted standards of safety aspects could be developed. More details can be found on the EFSA Web site (http://www.efsa.europa.eu/EFSA/efsa-locale-1178620753812_1178667590178.htm). A comparison of the assessment schemes of the FDA and EFSA is given in Table 3.

To establish the QPS status for a given microorganism, several requirements must be fulfilled, which are divided into four steps (Fig. 2). As a starting point (step I), a decision must be made about which taxonomic entity (i.e., the taxon or taxonomic unit such as genus, species, subspecies, or other grouping such as homofermentative lactobacilli) should be considered. The highest possible taxonomic level is preferred to avoid reassessment of microorganisms with the same characteristics. In step II, a body of knowledge is collected that is required for establishing the QPS status, including history of use, industrial applications, ecological data, clinical data (where available), and information from the scientific literature. Currently, there is no standardized notification system for clinical cases related to the use of bacterial food additives. Case reports are only occasionally published and do not cover all observations. Therefore, ongoing screening of the scientific literature for new observations is necessary, even for bacteria with a long history of use. The exclusion of pathogenicity (step III) as a further element of QPS relates again to the chosen taxonomic unit. The difficulties related to this requirement have been discussed in this review. When differentiation between virulent and avirulent strains cannot be achieved, those species (subspecies, etc.) with pathogenic potential would be ex-
cluded from QPS status. Not all strains of that species will be excluded from application in food or feed, but an assessment on a case-by-case basis would be required. The final criterion (step IV) is the application or end use of the microorganism and the food. In specific environments (foods), particular microorganisms can be desirable, whereas under different circumstances these same organisms are undesirable or dangerous. For example, lactobacilli in yogurts are desirable, but these same bacteria can lead to spoilage in sausages. Therefore, the QPS status relates to application in a specific product or group of products. Specifically for Lactobacillus, this approach has been recently supported with reference to the wide application of members of this genus in food and feed (4). The EFSA will start with the evaluation of the most common groups of microorganisms used in food and feed production, e.g., lactic acid bacteria, Bacillus spp., yeasts, and commonly encountered filamentous fungi (21).

CONCLUSIONS AND FUTURE PERSPECTIVES

The currently applied systems for safety assessments of bacterial supplements are not standardized. The European system for the approval of bacterial feed additives is very prescriptive and is based on criteria that have been criticized here, e.g., the focus on exclusion of virulence factors or specific antibiotic resistance determinants. The system in use for human therapeutics is similar and not completely appropriate or suitable for probiotics. No specific regulation exists for probiotics within the EU. Nevertheless, the QPS approach envisioned by the EU and EFSA is a promising alternative for safety assessment of bacterial dietary supplements and probiotic food products. Compared with the GRAS system in use in the United States, the QPS system appears more flexible and takes into account additional emerging safety risks, such as acquisition of antibiotic resistance or virulence determinants. Evaluation of these characteristics is essential, because evidence to prove the absence of virulence genes or traits is by definition never exhaustive. Nevertheless, the risk of adverse effects due to transfer of virulence genes can be considered low. The QPS system includes additional criteria to evaluate the safety of bacterial additives (such as a history of safe use in the food industry), clearly describes the relation to a taxonomic unit, and specifies the intended application. Such a system is preferred over a regulatory system that assesses only the presence of certain virulence or resistance factors.

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