Údarás Sábháilteachta Bia NA hÉIREANN
Food Safety AUTHORITY OF IRELAND

Report of the Scientific Committee
of the Food Safety Authority of Ireland

2024

Assessment of the safety of “probiotics” in food supplements

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Published by:

Food Safety Authority of Ireland
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ISBN: 978-1-910348-76-5

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Glossary

BIOHAZ Panel	The European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ) provides scientific advice on biological hazards in relation to food safety and food-borne diseases. This covers animal diseases transmissible to humans, transmissible spongiform encephalopathies, food microbiology, food hygiene and associated waste management issues.
cfu	colony-forming unit
decoction	A concentrated liquor resulting from heating or boiling a substance, especially a medicinal preparation made from a plant
Directive	A piece of legislation that requires European Union (EU) countries to achieve a certain result but leaves them free to choose how to do so. EU countries must adopt measures to incorporate a Directive into national law (transpose) in order to achieve the objectives set by the Directive. National authorities must communicate these measures to the European Commission. Transposition into national law must take place by the deadline set when the Directive is adopted (generally within 2 years).
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FBO	food business operator
FDA	Food and Drug Administration
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
food supplements	Food supplements are defined in Directive 2002/46/EC as "foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles

	and other similar forms of liquids and powders designed to be taken in measured small unit quantities"
G+C content	The percentage of heterocyclic nitrogen-containing bases in deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) that are either guanine (G) or cytosine (C) out of four total bases, including adenine and thymine in DNA or adenine and uracil in RNA
GRAS	Generally Recognized as Safe. GRAS is a term the United States Food and Drug Administration (FDA) applies to a substance that is intentionally added to food as a food additive and that is generally recognised, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use.
in silico	Performed on a computer or via computer simulation
LAB	lactic acid bacteria
MB	megabase
MIC	minimum inhibitory concentration
MS	Member State(s)
novel food	Is defined by Regulation (EU) 2015/2283 as any food that was not used for human consumption to a significant degree within the Union before 15 May 1997, irrespective of the dates of accession of Member States to the Union, and that falls under at least one of the 10 categories listed in the Regulation. 'Novel food' can be newly developed, innovative food; food produced using new technologies and production processes; as well as food that is or has been traditionally eaten outside of the EU.
probiotic	live microorganisms which when administered in adequate amounts confer a health benefit on the host (Food and Agriculture Organization (FAO)/World Health Organization (WHO) 2001)
QPS	Qualified Presumption of Safety. QPS is a safety assessment procedure used by EFSA for microbes used in the food chain. QPS uses existing knowledge about the safety of specific microbes to differentiate those that are not of concern (and can be given QPS status) from those that may represent a risk and should be subject to a full safety assessment.
Regulation	A piece of legalisation that applies automatically and uniformly to all EU countries as soon as it enters into force, without needing to be

	transposed into national law. Regulations are binding in their entirety on all EU countries.
taxonomic unit	The relative level of a group of organisms (a taxon) in an ancestral or hereditary hierarchy. A common system of biological classification (taxonomy) consists of species, genus, family, order, class, phylum, kingdom, domain.
USA	United States of America
VRE	vancomycin-resistant enterococci
WGS	whole genome sequencing
WHO	World Health Organization

Executive summary

Food supplements are defined in European Union (EU) food law (Directive 2002/46/EC,[1] S.I. No. 506 of 2007)[2] as "foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities". Directive 2002/46/EC provides for the setting of maximum safe levels of vitamins and minerals in food supplements. Other substances, including live microbes ("probiotics")[3] and their products, can be included in food supplements provided they are safe (Regulation (EC) No 178/2002,[4] S.I. No. 747 of 2007).[5] In the absence of EU guidance, the Food Safety Authority of Ireland (FSAI) through this document will provide guidance to food supplement producers and food business operators (FBOs) on assessing the safety of "probiotics" used in food supplements in Ireland.

To facilitate efficient monitoring of food supplements, Directive 2002/46/EC allows EU Member States (MS) to require the food supplement producer, or the person placing a food supplement product on the market, to notify the competent authority for that country. Ireland requires this notification. In Ireland, notification is not an approval or authorisation procedure. A notification is made by completing an online notification form,[6] which includes submitting a copy of the product label to the FSAI.

The most widely used "probiotics" in food supplements are strains of lactic acid bacteria (LAB), bifidobacteria, *Bacillus sporogenes* and the yeast *Saccharomyces boulardii*. The potential risks from consuming "probiotics" in food include infection, ill effects from microbial toxins produced by the microbial strains or contaminants, transmission of antimicrobial resistance and immunological

[1] Directive 2002/46/EC (as amended) of the European Parliament of the Council of 10 June 2002 on the approximation of the laws of Member States relating to food supplements. Available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32002L0046&qid=1684830323415
[2] S.I. No. 506 of 2007 – European Communities (Food Supplements) Regulations 2007. Available at https://www.irishstatutebook.ie/eli/2007/si/506/made/en/print
[3] "Probiotic" is defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Food and Agriculture Organization (FAO)/World Health Organization (WHO), 2001). The term "probiotic" is considered a non-authorised health claim under Regulation (EC) No 1924/2006 and, as such, the word "probiotic" is not allowed on labels of foods produced in Ireland.
[4] Regulation (EC) No 178/2002 (as amended) of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32002R0178&qid=1684830670157
[5] S.I. No. 747/2007 – European Communities (General Food Law) Regulations 2007. Available at https://www.irishstatutebook.ie/eli/2007/si/747/made/en/print?q=747/2007
[6] FSAI Online Food Notification System. Available at https://notifications.fsai.ie/login

effects. Reported adverse events resulting from consuming "probiotics" are few. Where opportunistic infections associated with "probiotics" in foods or food supplements are reported, they are usually in people at increased risk of infection where various underlying factors such as damage to the skin or mucous membranes, indwelling medical devices, alterations to the gut microbiome or impaired immune response may enable infection by organisms that are rarely or never associated with infection in otherwise healthy people.

The European Food Safety Authority (EFSA) introduced the concept of Qualified Presumption of Safety (QPS) to standardise its own safety evaluation of microorganisms used in "regulated products" (i.e. feed additive, food additive, food flavourings, food enzymes, novel foods and plant protection products). The QPS assessment is conducted separately from and independently of the safety assessment of a regulated product submitted for market authorisation. Therefore, having QPS status does not guarantee market authorisation. The first list of biological agents with QPS status was established in 2007. The QPS list is described at the species level for bacteria and yeasts, and at family level for viruses. EFSA performs an extensive literature search and then updates the QPS list every 6 months. In addition, the EFSA Panel on Biological Hazards (BIOHAZ panel) publishes a Scientific Opinion on the updated QPS list every 3 years. The FSAI uses the QPS list as a point of reference when assessing the safety of microbes or their by-products in foods.

In 2018, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) published "Guidance on the characterisation of microorganisms used as feed additives or as production organisms". There is currently no EU guidance on the criteria to be used for the safety assessment of "probiotics" in food supplements, or the evidence a food supplement producer should use to assess the safety of "probiotics" in food supplements.

This Scientific Committee report addresses two questions and makes the following recommendations:

Question 1. What is the committee's view on the most appropriate safety criteria to use when assessing the safety of "probiotics" in food supplements?

The Committee considers the most appropriate safety criteria to be that:

- There is long experience of use of the organism(s) in food or food supplements without substantiated report of harm in otherwise healthy people.
- Credible reports of infection or intoxication associated with the organism are isolated or rare and limited to those people at highest risk of infection.
- The organism(s) is readily identifiable to at least species level.

- There is an antimicrobial agent available for treatment of infection with the organism(s).
- The organism(s) has no known association with environmental harm.
- If the organism(s) was not used in food in the EU prior to 15 May 1997, its use has been authorised under the novel food Regulation (EU) 2015/2283.[7]
- If the organism(s) was genetically modified, it has been authorised under Regulation (EC) No 1829/2003[8] on genetically modified food and feed, and under Directive 2001/18/EC[9] on the deliberate release into the environment of genetically modified organisms.
- The organism(s) has been rigorously characterised as follows:
  - The definition of species and strain level is adequate to facilitate comparison in the event of suspected link to human infection.
  - There is evidence of the absence of properties associated with an increased potential to cause infection.
  - There is evidence of the absence of acquired (transferable) antimicrobial resistance genes.
  - There is evidence of the absence of a capacity for biogenic amine production.

Question 2. What evidence should a food supplement producer use to demonstrate the safety of "probiotics" when producing a food supplement?

The Committee considers that a food supplement producer should be able to demonstrate that:

- The organism(s) used meets the criteria set out above.
- The culture has been deposited in a recognised and accessible culture collection if not obtained from a culture collection.
- If cultures are stored, they are stored at -80 °C to ensure that they remain stable in storage.
- If cultures are propagated, the characterisation of the organism should be repeated at defined intervals to ensure that the organism has not significantly altered or been inadvertently displaced by, or contaminated with, another organism.

[7] Regulation (EU) 2015/2283 (as amended) of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council, and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. Available at https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32015R2283

[8] Regulation (EC) No 1829/2003 (as amended) of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32003R1829&qid=1685630140648

[9] Directive 2001/18/EC (as amended) of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EC – Commission Declaration. Available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32001L0018&qid=1685630232735

- The production process does not result in substantial change to the properties of the microorganism(s) between start of production and the end product.
- The production systems have adequate quality management systems to ensure consistent properties of the organism(s) in the final product.

In addition, the Scientific Committee made the following recommendations:

- Food supplement producers should document the food safety management system adopted to ensure consistent safe manufacture of the food supplement.
- The individual microbial strains used should be stored and accessible (either from the food supplement producer or from a culture collection) in the event of a suspected link to human infection.
- In line with the requirements of the Regulation on the provision of food information to consumers (Regulation (EU) No. 1169/2011),[10] accurate information should be provided to the consumer on the label. It is recommended that this information includes the type and number of organisms present as well as appropriate storage advice. In addition, the viability of the organism(s) throughout the shelf life of the product should be determined to ensure that the information on the product label is accurate throughout the shelf life. Furthermore, where it is known that there are groups of people for whom the strain or strains used may not be suitable, this should be indicated on the label.
- If an *Enterococcus faecium* strain is included as a "probiotic" in a food supplement, the label should clearly indicate the presence of *Enterococcus faecium* in the food supplement.

[10] Regulation (EU) No 1169/2011 (as amended) of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. Available at https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32011R1169

Purpose of the report

The FSAI has requested its Scientific Committee to establish guidance on assessing the safety of “probiotics” in food supplements. The Scientific Committee requested that the Biological Safety Subcommittee develop a draft report as a basis for consideration by the Scientific Committee.

The FSAI has specifically asked the Scientific Committee the following questions:

Q1. What is the committee’s view on the most appropriate safety criteria to use when assessing the safety of “probiotics” in food supplements?

Q2. What evidence should a food supplement producer use to demonstrate the safety of “probiotics” when producing a food supplement?

The purpose of this report is to provide scientific advice to the FSAI and guidance for food supplement producers and FBOs on the assessment of the safety of “probiotics” in food supplements.

The scope of this report does not include nutrients or other substances in food supplements, other than “probiotics”, and it does not address the efficacy of the microorganisms used.

1. Background

1.1 Regulation of food supplements containing "probiotics" in the EU

In the European Union (EU), Directive 2002/46/EC (S.I. No. 506 of 2007) sets out the requirements of the Member States (MS) relating to food supplements, and defines food supplements as "foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities". There is a body of opinion that considers that food supplements containing "probiotics" may be beneficial, and hence there are many examples of supplements on the market that contain between 6 to 12 $\log_{10}$ colony-forming units (cfu) g^{-1} of particular microorganisms.

The Food and Agriculture Organization (FAO)/World Health Organization (WHO) defined "probiotics" as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). In the EU, applications for health claims on "probiotics" have been submitted to the European Food Safety Authority (EFSA) for evaluation. To date, no application has been assessed by EFSA to have sufficient scientific evidence to support the health claim. The term "probiotic" is therefore considered a non-authorised health claim under Regulation (EC) No 1924/2006[11] and, as such, the word "probiotic" is not allowed on labels of foods produced in Ireland.[12] Despite the term "probiotic" lacking an authorised health claim in the EU, it is still widely used by the food and food supplements industry and the scientific community, and is therefore the term used in this document.

Food supplement regulations in Ireland require that a manufacturer, or person placing a food supplement on the Irish market, must notify the Food Safety Authority of Ireland (FSAI). This notification is not an approval or authorisation procedure, but merely a way to facilitate efficient monitoring of the supplements market.

[11] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Available at https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32006R1924
[12] FSAI frequently asked questions (FAQs) on Probiotic Health Claims. Available at https://www.fsai.ie/business-advice/nutrition/probiotic-health-claims

Food business operators (FBOs) that place food supplements containing "probiotics" on the market are required by food law (Regulation (EC) No 178/2002) to ensure that their products are safe. They are required to identify and control food safety hazards (Regulation (EC) No 852/2004).[13] This is especially important for products that are marketed to groups who tend to be more susceptible to infections and generally suffer more severe illness when they develop infection. These groups include those whose immune systems are either not fully developed, because they are very young, or are impaired due to age, illness or medical therapy, and those with indwelling medical devices. Organisms that are rarely or never associated with infection in otherwise healthy people (often called non-pathogenic or commensal) can be associated with serious infection in groups with specific risk factors for infection. This poses a greater challenge now than in the past because socioeconomic change and advances in medical treatment mean that a higher proportion of the population are living to very advanced years and for very long periods with conditions that, in the past, would have progressed rapidly without treatment.

Currently, there are no safety criteria in the regulations and no guidance available for assessing the safety of "probiotics" in food supplements.

1.2 EFSA: Qualified Presumption of Safety (QPS)

EFSA is an independent agency funded by the EU whose role is to provide scientific advice and perform risk assessments, as well as to communicate issues relating to food or feed safety and their possible impact on the environment. EFSA assesses the safety of microorganisms used in "regulated products" (i.e. feed additives, food additives, food flavourings, food enzymes, novel foods and plant protection products) before they are authorised for use in the European market. The concept of qualified presumption of safety (QPS)[14] was introduced by EFSA to facilitate a harmonised generic pre-assessment to support safety risk assessments. The QPS assessment is conducted separately from and independently of the safety assessment of a regulated product submitted for market authorisation. Therefore, having QPS status does not guarantee market authorisation. Nonetheless, if a product that requires a pre-market authorisation contains a microbial species that has QPS status, a simplified safety assessment may be done, that involves less data on potential risks as compared to a product containing a microbial species without QPS

[13] Regulation (EC) No 852/2004 (as amended) of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32004R0852&qid=1684830922214

[14] EFSA: Qualified Presumption of Safety (QPS). Available at https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps#efsa's-role

status (Herman *et al*., 2019). The FSAI uses the QPS list as a point of reference when assessing the safety of "probiotics" in food supplement notifications.

QPS status is granted at the species level for bacteria and yeasts, and at the family level for viruses. The QPS list was first introduced in 2007 as an opinion of the EFSA Scientific Committee, and included microbial species presented to EFSA and proposals made by stakeholders during a public consultation in 2005. The first QPS list comprised 72 microbial species that were notified to EFSA for market authorisation. Each microbial species was examined for the absence of virulence factors, toxic metabolites and transmissible antimicrobial resistance genes (Leuschner *et al*., 2010). Some microbial species have QPS status based on specific qualifications, such as that a particular microbial species may be used only for production processes, or that a microbial species must not express toxigenic activity (QPS list).[15] Currently, the EFSA Panel on Biological Hazards (BIOHAZ) updates the QPS list every 6 months based on a review of relevant scientific literature on the safety of microorganisms on the list and an assessment of new microorganisms notified to EFSA for possible QPS status.[16] EFSA performs a thorough, evidence-based systematic review of previously assessed microorganisms every 3 years and the updated list is published as a Scientific Opinion by the EFSA BIOHAZ panel.

The EFSA BIOHAZ panel only considers taxonomic units defined according to internationally accepted classification for inclusion in the QPS list. For prokaryotic microorganisms, the system of classification is the List of Prokaryotic names with Standing in Nomenclature (LPSN).[17] The International Code of Nomenclature for algae, fungi and plants (Turland *et al*., 2018) details the taxonomy and nomenclature for fungi, including yeasts, while the International Committee on Taxonomy of Viruses publishes regular updates on the accurate taxonomy of viruses.

In assessing the body of knowledge, the EFSA BIOHAZ panel performs a thorough review of the scientific literature related to the taxonomic unit. Particular attention is placed on the history of use of the microorganism, the ecology in the natural environment, clinical aspects, industrial applications and special properties of the taxonomic unit (Figure 1) (Herman *et al*., 2019). The possible presence of virulence determinants that could contribute to the pathogenicity of a microorganism for humans or animals or the production of antimicrobial agents or toxin(s) would raise safety concerns. Assessments also take into consideration whether adverse events were

[15] EFSA BIOHAZ Panel: Updated list of QPS-recommended biological agents for safety risk assessments carried out by EFSA. Available at https://zenodo.org/record/6902983#.Y85kHXbMKUk
[16] EFSA (24 January 2018) Qualified Presumption of Safety (QPS), *EFSA Journal*. Available at https://efsa.onlinelibrary.wiley.com/doi/toc/10.1002/(ISSN)1831-4732.QPS
[17] LSPN – List of Prokaryotic names with Standing in Nomenclature (founded by Jean P. Euzéby, 1997). Available at https://www.bacterio.net/

reported and if transmission was through food or other routes. The presence of acquired antimicrobial resistance-encoding genes conferring resistance to clinically relevant antimicrobial compounds is assessed, and the absence of these acquired genes is a requirement for all bacteria on the QPS list. Intrinsic antimicrobial resistance is not considered in this process. Environmental safety is also assessed, whereby the ability of the microorganism to survive, compete and proliferate in the environment, or to cause adverse health effects to animals or plants, is considered. The QPS evaluation process is primarily used for the evaluation of live microorganisms intentionally added to food or feed, although evaluations can also be for products derived from microbial metabolism that include enzymes, vitamins or amino acids. For the latter, QPS may apply only for a specific end use, rather than for the live microorganism. In such cases, a qualification is applied for the use of that microbial species and there is an expectation that there are no live microorganisms in the final product (Herman *et al*., 2019).

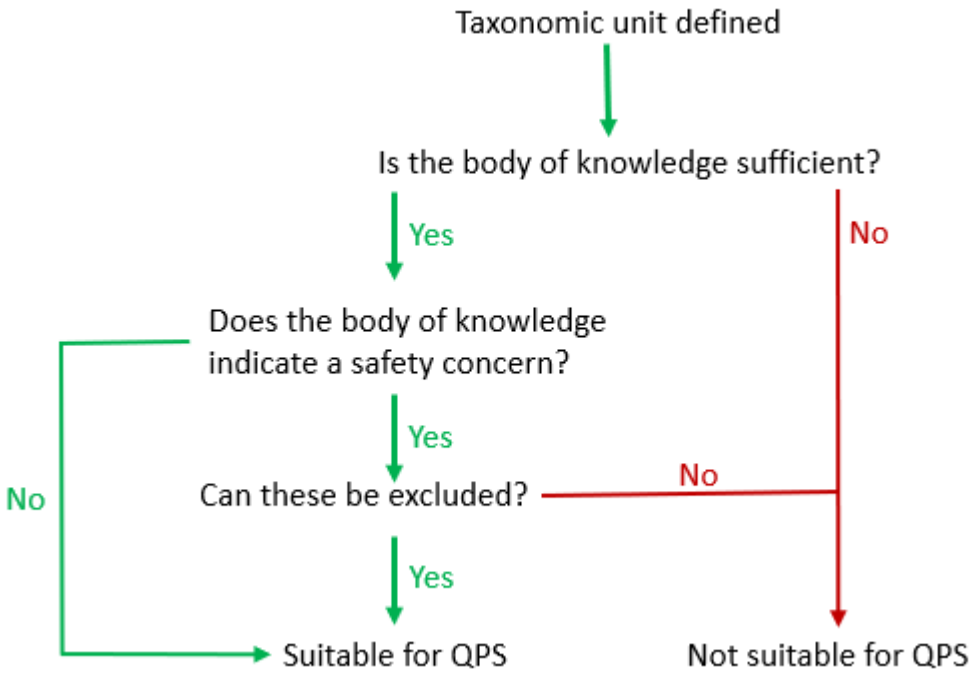

Figure 1 Scheme for assessing the suitability of a microorganism for QPS (adapted from Herman et al., 2019)

1.3 Comparison between QPS and Generally Recognized as Safe (GRAS)

There is potential for EFSA's QPS system to be misinterpreted as the European counterpart to the Generally Recognized as Safe (GRAS) guidelines, established by the United States Food and Drug Administration (FDA). However, there are important differences between the two systems. GRAS guidelines apply to food additives in general, while QPS is dedicated to microorganisms. GRAS applies to substances or organisms for a specific application. QPS is not applicable to single products containing a microbial strain but for a taxonomic unit, and usually at species level for bacteria and yeasts (Table 1). It is important to note that the GRAS status of ingredients, including microbes, has no regulatory status in the EU.

In both the EU and the United States of America (USA), the regulatory requirements do not consider the complex nature of food supplements that contain several microbial species and/or strains. "Probiotics" are dynamic, and characteristics vary significantly between species and strains. There are also additional complexities that arise in multi-species or multi-strain products where individual strains may interact with each other.

Table 1 A table showing the main differences between the QPS system and GRAS guidelines

QPS	GRAS
Applies to microorganisms only	Applies to food additives, including microorganisms
Assessment performed for microorganisms used as source of or contained in products that require EU market authorisation	Assessment performed after specific GRAS notification to the FDA
QPS status determined by EFSA	GRAS status determined by FDA and/or external experts
QPS applicable for whole taxonomic unit, usually species level for bacteria and yeasts, and families level for viruses	GRAS concerns a specific substance or microorganism (at the strain level)

1.4 Approaches to assessing the safety of "probiotics"

Sanders *et al.* (2010) have proposed that, when added to foods or food supplements, "probiotics" must be: sufficiently characterised, safe for intended use, supported by at least one positive human clinical trial conducted according to generally accepted scientific standards, and be viable and in sufficient numbers throughout the shelf life of the product.

One of the first steps in microbial strain characterisation is identification. Whole genome sequencing (WGS) is now regarded as the preferred technical method for bacterial characterisation for most purposes. In 2018, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) published "Guidance on the characterisation of microorganisms used as feed additives or as production organisms" (EFSA, 2018). It requires WGS for characterisation of bacterial strains. For yeasts, the genome is much larger and more complex, so sequencing the entire genome is not practical. Rather, the EFSA guidance recommends characterisation of yeasts by phylogenomic analysis such as using a concatenation of several conserved genes to produce a phylogeny against available related genomes. It also recommends that strains are deposited in an internationally recognised culture collection.

EFSA has published details of standard phenotypic tests with well-defined breakpoints for antimicrobial resistances (EFSA, 2012a). If a strain exhibits antimicrobial resistance, then further analysis, including WGS, is required. WGS and bioinformatics analysis allows for detection of described antimicrobial resistance-encoding gene(s) and mutations associated with antimicrobial resistance. Depending on the sequencing technique and analysis, it may establish whether these genes form part of mobile genetic elements. In addition, the genome sequence informs on the presence or absence of other putative genes of concern. For food supplements that contain multiple bacterial strains, it is important that each individual strain is properly identified and characterised, and safety assessments performed as appropriate.

While the EFSA FEEDAP Panel published "Guidance on the characterisation of microorganisms used as feed additives or as production organisms" (EFSA, 2018), there is currently no EU guidance on the criteria to be used for the safety assessment of microorganisms used in food supplements. Huys *et al*. (2013) proposed that characterisation of microbial strains could include testing for survival at relevant human anatomical and/or food production sites; the production of lactic acid or other short-chain fatty acids; adhesion to mucus or intestinal epithelial cells; interaction with human immune cells; resistance to digestive enzymes, bile or acid; antibacterial activity via competitive exclusion; or production of bacteriocins or hydrogen peroxide. Most "probiotics" in commercially available foods or food supplements belong to QPS species with a demonstrated history of safe use in foods. Therefore, the value of *in vivo* safety testing in healthy

animal models, such as mice or rats, is unclear since little or no adverse effect of QPS species is expected.

Human intervention studies allow documentation of the safety and tolerance of "probiotics" in foods or food supplements through rigorous monitoring of biological and clinical parameters for collection of safety data, and the reporting of adverse events. Unexpected adverse events might indicate a safety concern. However, it is important to establish whether the adverse events are actual differences between the placebo and microbial intervention groups and/or are considered to be intervention related (Snydman, 2008; Suez *et al*., 2019). To date in the literature, only rare, mild and transient microbial-related adverse events have been reported in "probiotic" food supplement human intervention studies with healthy individuals. In general, there are no significant safety concerns for the use of strains of lactic acid bacteria (LAB), bifidobacteria or yeasts in food or food supplements for healthy individuals, since they have a long history of safe use and have undergone rigorous QPS assessment and, as such, safety evaluations tend to focus on the intended use, the delivery format and dose.

Notably, the scope of the QPS list is for microorganisms being consumed by the general healthy population. QPS does not specifically take into consideration the potential risks for people at increased risk of infection where various underlying factors – such as damage to the skin or mucous membranes, indwelling medical devices, alterations to the gut microbiome or impaired immune response – may enable infection by organisms that are not associated with infection in otherwise healthy people. In addition, QPS does not consider the safety of microorganisms when they are consumed in a highly concentrated dose form in food supplements. A number of food supplement producers recommend medical supervision of the use of "probiotic" food supplements for people at increased risk of infection such as the young, old, pregnant or immunocompromised, as these groups are most likely to be more susceptible to adverse events.

If a strain does not belong to a species on the QPS list, it does not mean that it is unsafe. It means that it has not been assessed as a QPS species, and therefore a full safety assessment at the strain level should be performed. In addition, if a microbial strain has not been consumed in food prior to 15 May 1997, it must be assessed as a novel food according to Regulation (EU) 2015/2283 (S.I. No. 253 of 2022).[18]

In food supplement development, it is essential that the individual microbial strains can be cultured on an industrial scale that consistently yields cultures that are not materially different from the

[18] S.I. No. 253/2022 – European Union (Novel Foods) Regulations 2022. Available at https://www.irishstatutebook.ie/eli/2022/si/253/made/en/print

parent strain, as well as produce a sufficient yield to have adequate stability throughout the product shelf life. Notably, there is no EU guidance for a specific dose of "probiotics" when included as "other substances" in food supplements, and dose ranging studies for "probiotics" in food supplements are rare. For most safety studies of "probiotics", a dose between 10^8 and 10^{11} cfu is adopted, and the viability of the strains for the test products is recorded during clinical safety investigations.

1.5 "Probiotics" in commercially available food supplements

The most common microbial strains incorporated in commercially available food supplements belong to the genera *Lactobacillus* and *Bifidobacterium*. Other bacteria, including members of the genera *Streptococcus*, *Pediococcus*, *Enterococcus* and *Bacillus*, as well as members of the yeast genus *Saccharomyces*, are frequently incorporated in food supplements. The most common species included in commercially available food supplements are human-derived strains of *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus gasseri*, *Lactobacillus johnsonii*, *Lactobacillus casei*, *Bifidobacterium longum* subsp. *infantis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*. Although not naturally present in the gastrointestinal tract, bacterial species such as *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Leuconostoc* and *Lactococcus* genera are frequently incorporated in food supplements. These bacteria have QPS status in the EU due to their long history of use as starter cultures and in fermented foods.

1.5.1 Lactobacilli

Lactobacilli, which taxonomically belong to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales* and family *Lactobacilli* (*Lactobacillaceae* until 2020), include more than 250 species that are used in fermented food preservation and in biotechnology applications, or explored for their purported beneficial effects on health (Zheng *et al*., 2015). Lactobacilli are Gram-positive, mostly non-motile, catalase-negative, non-spore-forming rod-shaped bacteria that are found in several habitats, including food, feed, soil, plants, animals and humans. The genome size of lactobacilli is highly variable and ranges between one and more than four megabases (Mbs) Genome size also varies within a single species due to genome decay as strains become adapted to specific niches and where genes encoding for utilisation of various substrates are lost. The genus *Lactobacillus* was recently subdivided into 25 genera (Zheng *et al*., 2020), and the 37 species that have QPS status have been assigned to 13 of the genera. The species that are best characterised physiologically and technologically, and which are included on the QPS list, are

those relevant to food fermentation, food ingredients and biotechnological applications (Table 2). For this document, the pre-2020 nomenclature will be used, as it is still used on the QPS list and continues to be used on food and food supplement product labels.

Table 2 A table describing those lactobacilli used in food or feed fermentation, or as "probiotics"

Former name	Reclassification	Technological application
Lactobacillus acidophilus	Remaining as *Lactobacillus* genus	Food or food supplement products, fermented milk, cheese production, sausage fermentation
Lactobacillus delbrueckii subsp. *bulgaricus* and *lactis*		
Lactobacillus helveticus		
Lactobacillus crispatus		
Lactobacillus gasseri		
Lactobacillus iners		
Lactobacillus jensenii		
Lactobacillus johnsonii		
Lactobacillus brevis	*Levilactobacillus brevis*	Sourdough fermentation
Lactobacillus rhamnosus	*Lacticaseibacillus rhamnosus*	Cheese production, food or food supplement products
Lactobacillus casei	*Lacticaseibacillus casei*	
Lactobacillus paracasei	*Lacticaseibacillus paracasei*	
Lactobacillus plantarum	*Lactiplantibacillus plantarum*	Fermentation of vegetables, silage production, food or food supplement products
Lactobacillus pentosus	*Lactiplantibacillus pentosus*	
Lactobacillus fermentum	*Limosilactobacillus fermentum*	Food or food supplement products
Lactobacillus reuteri	*Limosilactobacillus reuteri*	
Lactobacillus salivarius	*Ligilactobacillus salivarius*	Food or food supplement products

1.5.2 Bifidobacteria

Bifidobacteria are gut inhabitants and represent non-motile, non-sporulating, non-gas-producing saccharolytic Gram-positive bacteria that belong to the family *Bifidobacteriaceae* and the phylum *Actinobacteria*. Actinobacteria exhibit a wide range of morphologies and properties and characteristically possess genomes of a high G+C content, ranging from 46% to over 70%. The

Bifidobacterium genus currently comprises 94 recognised species, with several of these species only very recently isolated, and from a wide range of mammalian, avian or insect sources (Alessandri *et al*., 2021). In the last three decades, bifidobacteria have become the subject of intensifying scientific scrutiny. They represent an abundant bacterial component of the human gastrointestinal microbiome. They are believed to be the dominant bacterial group in the microbiome of vaginally delivered, breastfed infants (Bottacini *et al*., 2014). Currently, five bifidobacterial species – namely *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium animalis*, *Bifidobacterium adolescentis* and *Bifidobacterium bifidum* – are included in the QPS list, and strains of these bifidobacterial species are included as "probiotics" in food and food supplements.

1.5.3 Bacillus

Bacillus species are members of the family *Bacillaceae*, a group of aerobic, sporulating organisms that are widespread in nature and are found in soil, air, fermented foods and the human gut. In the spore form, bacilli can survive in extreme environmental conditions, thereby enabling long-term survival in conditions that kill vegetative cells. Currently, EFSA has included 17 species from the genus *Bacillus* in the QPS list, all with the qualification of the absence of toxigenic activity. Among these, *Bacillus coagulans* is frequently found as an ingredient in food supplements, where it is referred to as *B. coagulans*, Lactospore or *Lactobacillus sporogenes*. *B. coagulans* was transferred to the genus *Weizmannia* in 2020 (Gupta *et al*., 2020). For this document, the pre-2020 nomenclature will be used, as it is still used on the QPS list and continues to be used on food and food supplement product labels*. B. coagulans*, originally named *Lactobacillus sporogenes*, was isolated from spoiled milk in 1933. *B. coagulans* is a Gram-positive, facultatively anaerobic non-pathogenic, spore-forming lactic acid-producing bacteria. It is heat resistant, with an optimum growth temperature of 35–50 °C and an optimum growth pH of 5.5–6.5. The spores of *B. coagulans* are terminal, differentiating it from other *Bacillus* species whose spores are central or sub-terminal. *B. coagulans* also differs from other *Bacillus* species by the absence of the enzyme cytochrome-C oxidase, and it does not reduce nitrate to nitrite. Strains of *B. coagulans* produce the bacteriocin coagulin, which has an antimicrobial effect against a broad spectrum of enteric microbes.

1.5.4 Enterococci

Enterococci are Gram-positive catalase-negative, non-spore-forming, facultative anaerobic LAB of the phylum *Bacillota*. Enterococci are predominantly gastrointestinal commensal bacteria in

humans and animals, but some members of the genus are opportunistic pathogens. Two species in the genus, namely *Enterococcus faecium* and *Enterococcus faecalis*, are recognised as commonly isolated Gram-positive pathogens in healthcare settings worldwide (Fiore *et al*., 2019). *E. faecium* and *E. faecalis* are associated with infection in humans, even in people who are not immunocompromised. Infections caused by *E. faecium* and *E. faecalis* include urinary tract infection (UTI), bacteraemia, intra-abdominal infections and endocarditis. Both *E. faecium* and *E. faecalis* have intrinsic antimicrobial resistance to a number of important classes of antimicrobial agents. For example, *E. faecium* generally has intrinsic resistance to ampicillin, and both *E. faecium* and *E. faecalis* are generally resistant to cephalosporin antimicrobial agents. Some *Enterococcus* species have intrinsic resistance to glycopeptide antimicrobial agents, including vancomycin. The emergence and spread of acquired resistance to vancomycin in *E. faecium* is a significant clinical concern. There are limited therapeutic options for healthcare-associated *E. faecium* bloodstream infections (septicaemia), particularly with those strains that have acquired resistance to vancomycin (Fiore *et al*., 2019). Furthermore, horizontal gene transfer from these species and acquisition of hemolysin-encoding genes by members of the gut microbiome is also a risk (Krawczyk *et al*., 2021; Ben Braïek and Smaoui, 2019; Fiore *et al*., 2019; Hanchi *et al*., 2018; Freitas *et al*., 2018).

In 2012, the EFSA FEEDAP panel published guidance on the safety assessment of *E. faecium* in animal nutrition (EFSA, 2012b). This guidance detailed that safety of *E. faecium* for inclusion in feed should be established by testing strains for susceptibility to ampicillin and the absence of the virulence factors and markers IS*16* (an insertion sequence conferring genomic plasticity), *hyl* (encoding a glycosyl hydrolase that has β-N-acetylglucosaminidase activity), and *esp* (encoding enterococcal surface protein located on a transferable pathogenicity island and enhancing initial cell adhesion, biofilm formation and pathogenesis in endocarditis). If the minimum inhibitory concentration (MIC) for ampicillin is greater than or equal to 2 mg/L, the *E. faecium* isolate is not considered safe. If the MIC for ampicillin is less than 2 mg/L, the absence of each of the genetic elements – IS*16*, *hyl,* and *esp* – must be confirmed for an *E. faecium* strain to be considered safe. If one or more of the three genetic elements are detected, then the strain is considered unsafe. This guidance for characterisation of *E. faecium* strains was included in the FEEDAP document (EFSA, 2018). While this guidance was developed for feed applications, the recommendations were, at that time, equally applicable to assessing the safety of *E. faecium* used in food supplements for human consumption. It is now recognised that conforming with these criteria does not necessarily make strains of *E. faecium* safe. In 2020, EFSA stated that *E. faecium* would be excluded from future QPS evaluations (BIOHAZ, 2020) as the precise genetic factors that make a strain of *E. faecium* pathogenic or non-pathogenic is not currently fully understood (Freitas *et al*., 2018).

1.5.5 Saccharomyces

Saccharomyces boulardii was discovered by the French microbiologist Henri Boulard in 1923 during a cholera epidemic in Indochina. Based on his observations, Boulard considered that people who consumed a drink prepared from the outer skin of the lychee and mangosteen fruits did not develop diarrhoea. Boulard isolated an agent to which he attributed this effect and named it *Saccharomyces boulardii*. *S. boulardii* is frequently incorporated in "probiotic" food supplements, and while it is closely related to baker's yeast, *Saccharomyces cerevisiae*, it differs from strains of *S. cerevisiae* by several metabolic and genetic characteristics. *S. boulardii* has an optimum growth temperature of 37 °C, is resistant to low pH and is tolerant to bile acids, whereas *S. cerevisiae* grows better at cooler temperatures (30–33 °C) and does not survive well in acid pH ranges (McFarland, 2010).

1.6 Reported adverse events associated with the consumption of "probiotics"

EFSA QPS designation considers selected lactic acid bacteria and *Bifidobacterium* species that are commensals of the human microbiome to be safe. Likewise, strains of lactobacilli and bifidobacteria incorporated in food supplements are also considered safe. However, it appears that adverse events are poorly reported in randomised clinical trials (Bafeta *et al*., 2018). The number of infections due to LAB or *Bifidobacterium* is reported to be very low, ranging between 0.05 and 0.4% for infective endocarditis and bacteraemia respectively (Kothari *et al*., 2019). Where infections occur, underlying factors such as damage to the mucous membranes, alterations to the gut microbiome or impaired host immune function may contribute to the occurrence of infection (Liong, 2008). *L. plantarum*, *L. paracasei*, *L. salivarius*, *L. acidophilus*, *Lactococcus lactis*, *Leuocnostoc, Pediococcus* and *Bifidobacterium* have been associated with cases of bacteraemia and endocarditis. However, the consumption of food or food supplements containing microorganisms is rarely demonstrated to be the source of infection (Campagne *et al*., 2020).

Several sporadic cases of infection have been linked to the consumption of *L. rhamnosus*. Kunz *et al*. (2004) reported infection in three infants with short bowel syndrome that developed bacteraemia following consumption of *L. rhamnosus* GG. Similarly, two paediatric patients were reported to have developed bacteraemia and sepsis as a result of ingesting *L. rhamnosus* GG (Land *et al*., 2005). Chiang *et al*. (2021) described lactobacillus bacteraemia in an extremely premature infant who had a central catheter and was administered *L. rhamnosus* GG for prevention of necrotising enterocolitis prevention. Isolates almost indistinguishable by WGS from *L. rhamnosus* GG were recovered from the patient, thereby implicating the *L. rhamnosus* GG

administered as probiotic as the infecting microorganism. Ingestion of *L. rhamnosus* GG was implicated as the cause of bacteraemia in a 17-year-old boy with ulcerative colitis. In this case, 99.78% similarity was observed in the 16S ribosomal ribonucleic acid (rRNA) gene sequences between the *L. rhamnosus* strain isolated from the patient's blood and the consumed *L. rhamnosus* GG (Vahabnezhad *et al*., 2013). The authors of this report suggested that individuals with ulcerative colitis may be susceptible to bacteraemia linked to consumption of lactobacillus strains. While Sendil *et al*. (2020) reported *L. rhamnosus* bacteraemia in a 75-year-old man who had received a renal transplant several years previously. Based on this case and a handful of cases reported in the literature, the authors concluded that *Lactobacillus* spp. might be an opportunistic pathogen in immunocompromised, transplant patients (Sendil *et al*., 2020).

The *Escherichia coli* strain Nissle 1917 has been used for over a century as a treatment for diarrhoea (Sonnenborn, 2016). While this organism is not licensed for this purpose in Ireland, experience with its use is relevant. Sepsis associated with *Escherichia coli* Nissle 1917 has been reported in a low-birth-weight infant (Guenther *et al*., 2010). In this case, a premature infant developed gastroenteritis due to rotavirus and adenovirus co-infection at postnatal day 13. The infant was administered *E. coli* Nissle 1917 and was assessed as having a positive response. However, at postnatal day 25 the infant developed sepsis. Blood cultures isolated an *E. coli* strain that had an indistinguishable pulsed-field gel electrophoresis (PFGE) profile to *E. coli* Nissle 1917. The infant received antimicrobial chemotherapy, was stabilised and was then discharged from hospital at 10 weeks of age (Guenther *et al*., 2010).

Fungemia and sepsis have been associated with *Saccharomyces boulardii*-based supplementary therapy that was taken with the intention of preventing antibiotic-associated diarrhoea or of treating recurrent *Clostridioides difficile*-associated diarrhoea (Doron and Snydman, 2015). These reports highlight that dietary supplementation with "probiotics" is associated with some risk for immunocompromised consumers.

Lactobacilli have also been reported to cause infective endocarditis. Strains isolated from patients with endocarditis were capable of platelet aggregation and adherence to fibronectin, fibrinogen and collagen (Antoun *et al*., 2020). *Lactobacillus*-mediated endocarditis has been associated with individuals who have impaired immunity, structural heart disease, recent surgery, prolonged antimicrobial therapy, dental infections and severe comorbidities (Antoun *et al*., 2020; Snydman, 2008). Reports of localised infections due to strains of lactobacilli or bifidobacteria are rare. Although lactobacilli have been associated with intra-abdominal abscesses, the implicated bacterial strains were not fully characterised, and it was not demonstrated that they were strains used in food supplements.

A case of mould contamination of a microbially enriched infant formula that led to mucormycosis and death of a preterm infant (Vallabhaneni *et al*., 2015) highlights the importance of ensuring that food containing “probiotics” meets stringent safety and quality standards to ensure that only the intended microorganisms are present in the product and that it is free from contamination.

In summary, reported adverse events associated with “probiotics” are few and tend to be ad hoc reports of infections – including bacteraemia, sepsis or endocarditis – in physiologically at-risk people. For most reports, evidence confirming that the isolate is a “probiotic” strain is lacking.

2. Questions to the Scientific Committee

Question 1: What is the committee's view on the most appropriate safety criteria to use when assessing the safety of "probiotics" in food supplements?

Recommended criteria for the safety assessment of "probiotic" strains in a food supplement should include detailed characterisation of the microorganisms (see Section 2.1) and information on the safety of production (see Section 2.2).

2.1 Microorganism characteristics

2.1.1 Characterisation of microorganisms to strain level

Each microorganism should be named according to currently validated nomenclature based on the International Code of Nomenclature for algae, fungi, and plants (Parker *et al*., 2019). An updated list of prokaryotic names is available at http://www.bacterio.net. The name should include the official genus, species and subspecies names according to the nomenclature rules, as well as a strain designation. The strain designation should be the assigned catalogue number of an internationally recognised culture collection where the strain is deposited. An additional commercial strain name or other widely used designation may also be relevant. It is recommended that the genome sequence of each microbial ingredient should be determined in accordance with guidance provided in the EFSA statement on the requirements for WGS analysis of microorganisms intentionally used in the food chain (EFSA, 2021).

2.1.2 Documentation of the strain origin, history of safe use in food or approval as novel or genetically modified

The origin of all microbial strains in a food supplement product should be detailed, i.e. where purchased from a commercial supplier the information should be available. Each microbial species should have a history of safe use in food, i.e. be included in the International Dairy Federation

(IDF)[19] and the European Food & Fermentation Cultures Association (EFFCA)[20] inventory of food cultures used in fermented foods, or be included on EFSA's QPS list. In addition, if a microorganism was not used in food in the EU prior to 15 May 1997, the microbial ingredient should be authorised as a novel food under Regulation (EU) 2015/2283 (S.I. No 253 of 2022). If a microorganism that was genetically modified were considered for use in a food supplement, it would have to be authorised under Regulation (EC) 1829/2003 on genetically modified food and feed, and under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

2.1.3 Absence of antimicrobial resistance determinants

In line with EFSA's approach for assessing the safety of microbial strains for inclusion in the QPS list, it is recommended that microbial strains used in food supplements should not add to the pool of antimicrobial resistance genes already present in the human gut bacterial population or otherwise increase the spread of antimicrobial resistance. An antimicrobial susceptibility profile should be determined for each microbial ingredient of a "probiotic" food supplement. EFSA's "Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance" details the phenotypic testing that should be performed to determine the susceptibility of a microorganism to a set of clinically relevant antimicrobials, together with guidelines for how to evaluate the test results according to cut-off values (EFSA, 2012a). The cut-off values are set via guidelines from relevant published research papers, the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and national and European monitoring programmes. When a strain of a typically susceptible species is resistant to a given antimicrobial drug, it is considered to have an acquired resistance for that compound. By contrast, intrinsic resistance to an antimicrobial is understood as being inherent to a bacterial species and typical of strains of that species.

It is recommended that culture-based methods for the determination of the antimicrobial susceptibility profile be complemented with *in silico* and molecular-based methods. WGS data should be examined for elements encoding resistance to antimicrobial agents of importance to human and animal health (i.e. critically important antimicrobials or highly important antimicrobials, as defined by the WHO (WHO, 2019)).

[19] IDF (2018) Inventory of microbial food cultures with safety demonstration in fermented food products. Bulletin of the International Dairy Federation (IDF No. 455-2012). Available at https://www.fil-idf.org/wp-content/uploads/woocommerce_uploads/2018/12/Bulletin-of-the-IDF-N%C2%B0-495_2018_Inventory-of-microbial-food-cultures-with-safety_Cat.pdf
[20] EFFCA (n.d.) Probiotics. Available at https://effca.org/microbial-cultures/probiotics/

In *Critically important antimicrobials for human medicine: 6th Revision 2018,* [21] the WHO has ranked 35 classes of antimicrobials into 3 categories according to their importance to human health (WHO, 2019):

- Critically important antimicrobials
  a) sole therapy or one of limited therapies to treat serious bacterial infections in humans and
  b) used to treat bacterial infections transmitted from non-human sources or with resistance genes from non-human sources
- Highly important antimicrobials: a or b
- Important antimicrobials: neither a nor b

It is recommended to conduct the search for elements encoding resistance against at least two up-to-date databases. These may include ResFinder,[22] the National Center for Biotechnology Information (NCBI) Bacterial Antimicrobial Resistance Reference Gene Database[23] and the Comprehensive Antibiotic Resistance Database (CARD)[24] resistance gene identifier for prokaryotes. The Mycology Antifungal Resistance Database (MARDy)[25] can be used to identify antifungal resistance.

If antimicrobial resistance determinants are identified, they should be assessed for the potential for horizontal gene transfer. If antimicrobial resistance is likely to be transferable (encoded on plasmids, bacteriophages or mobile genetic elements such as conjugative transposons or integrative and conjugative elements), the strain should not be considered for inclusion in a food supplement product. If antimicrobial resistance is considered intrinsic and unlikely to be transferable, the food supplement producers should consider if the resistance of the microbial strain is likely to make treatment difficult if the microorganism is associated with human infection.

[21] WHO (2019) Critically important antimicrobials for human medicine: 6th revision. Available at https://www.who.int/publications/i/item/9789241515528
[22] Center for Genomic Epidemiology; ResFinder. Available at https://cge.food.dtu.dk/services/ResFinder/
[23] Bacterial Antimicrobial Resistance Reference Gene Database. Available at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047
[24] Comprehensive Antibiotic Resistance Database (CARD). Available at https://card.mcmaster.ca/
[25] Mycology Antifungal Resistance Database (MARDy). Available at http://mardy.dide.ic.ac.uk/

2.1.4 Absence of virulence properties

Virulence factors are proteins or molecules produced by a microorganism that allow it to evade the immune system, colonise the host or produce toxins. The pathogenicity of a microorganism is generally related to its virulence factors. Virulence factors may be neutral, offensive (e.g. flagella or toxins) or defensive (e.g. acid resistance, antimicrobial resistance), and it may be acquired through vertical or horizontal transmission. It is recommended that databases such as VirulenceFinder,[26] Virulence Factor Database (VFDB)[27] or Bacterial and Viral Bioinformatics Resource Centre (BV-BRC, formerly PATRIC)[28] be used for *in silico* interrogation of an annotated genome sequence for the presence of virulence factors. *In silico* identification of virulence factors should be complemented with further phenotypic testing where appropriate. Strains of the *Bacillus* genus are known toxin producers. Therefore, a combination of *in silico* and *in vitro* analyses should be performed to confirm the absence of production of known toxins by *Bacillus* strains. For yeast, knowledge of virulence factors is not as extensive as it is for prokaryotes, so for yeast an approach that combines genetic, *in vitro* and *in vivo* analyses should be performed.

2.1.5 Assessment of biogenic amine production

Low molecular weight organic molecules – including histamine, tyramine, putrescine, phenylethylamine and cadaverine – that harbour additional amine groups are referred to as biogenic amines. These are widely detected in foods and beverages. The origin of biogenic amines may be exogenous, being formed by enzymes in plants or animals, or by microorganisms that can generate significant concentrations of biogenic amines through the action of amino acid decarboxylase enzymes. Strains of both Gram-positive and Gram-negative bacteria are capable of producing biogenic amines. In particular, LAB are significant producers. High intake of biogenic amines can lead to digestive, circulatory and respiratory symptoms. Symptom severity depends on the nature of the ingested biogenic amine, the amount ingested and the susceptibility of the human host, as well as the level of monoamine oxidase and diamine oxidase enzyme activity in the gut. Individuals with certain genetic deficiencies or who are taking a course of treatment with drugs such as monoamine oxidase inhibitors may experience more severe toxic or allergy-like effects when they are exposed to much lower concentrations of biogenic amine than the levels found in some foods. *In silico* identification of genes for microbially encoded amino acid decarboxylases

[26] Center for Genomic Epidemiology: VirulenceFinder. Available at https://cge.food.dtu.dk/services/VirulenceFinder/
[27] Virulence Factor Database (VFDB). Available at http://www.mgc.ac.cn/VFs/main.htm
[28] Bacterial and Viral Bioinformatics Resource Center (BV-BRC, formerly PATRIC). Available at https://www.bv-brc.org/

should be complemented with phenotypic testing to quantify the concentration of biogenic amine(s) produced by a microbial strain.

2.1.6 Absence of reported adverse events during human studies or reported among consumers

Serious adverse events associated with “probiotic” consumption have been identified in immunocompromised individuals, in particular those with an impaired intestinal barrier and those with a central venous catheter. Individuals who are particularly susceptible to infection should generally be advised to avoid “probiotics”. If a product is intended for a specific consumer group – e.g. pregnant women, children or the elderly – this should be stated on the package labelling. If a product is not considered safe or is not recommended for a specific group of consumers, this should be clearly stated on the label.

2.2 Safe production and incorporation of “probiotics” in food supplements

It is crucial that each individual strain in the final product is consistently indistinguishable from the strain that is used to initiate production. Genetic drift of the culture during production has the potential to result in an organism that is materially different from the organism(s) that the product is intended to contain. Where a food supplement producer does not purchase the strain(s) used to initiate production fresh from a reputable commercial supplier, a centralised in-house strain bank should be maintained. Reference stocks of the microorganisms should be stored at, or below, - 80 °C to minimise changes to the strain that can occur during long-term storage. If the strain used to initiate production is obtained from a third party, the food supplement producer is responsible for ensuring that cultures provided are appropriately produced and stored.

Question 2: What evidence should a food supplement producer use to demonstrate the safety of "probiotics" when producing a food supplement?

2.3 Evidence-based risk assessment of "probiotics" in food supplements

It is recommended that an evidence-based risk assessment of the microbes be performed for each "probiotic" food supplement product and that the forms below, or forms similar in content to those below, be completed as part of the risk assessment providing details of:

- The food supplement product
- The identification of the microbial strains
- The characterisation of each microbial strain
- Evidence of strain manufacturing safety.

2.3.1 Food supplement product details			
Food supplement product name:			
Date:			
Manufacturer/Distributor:			
2.3.2 Identification of the microbial strains			
	Microorganism*	**Phenotypic identification method**	**Genotypic identification method**
Identification details for each microbial strain, including genus, species and subspecies, if appropriate.	1		
	2		
	3		
	4		
	5		
	6		
	7		
	8		
	9		
	10		

**Add additional rows as appropriate if food supplement contains >10 microorganisms*

2.3.3 Characterisation of the microbial strains		
(Genus, species and strain designation) **Strain 1*.** _____		
Origin of the strain		
Strain number as given by the internationally recognised culture collection where it is deposited		
	Yes/No	**Details**
Adverse events associated with consumption of this microbial species or strain		
History of safe use of the microbial strain in food and/or food supplements		
Inclusion of the microbial species on EFSA's QPS list		
If the strain was not used in food in the EU prior to 15 May 1997, its use has been authorised under the novel food Regulation (EU) 2015/2283		
If the strain was genetically modified, it has been authorised under Regulation (EC) No 1829/2003 on genetically modified food and feed, and under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms		
Presence of plasmids, bacteriophage or mobile genetic elements such as insertion sequences, integrons or integrative and conjugative elements		
Antimicrobial susceptibility test results are available for the strain		
Resistance to antimicrobials (see Section 2.1.3) • Is there a known association between the resistance profile and the presence of resistance genes?		
• List identified acquired (transferable) antimicrobial resistance genes		
• List identified intrinsic antimicrobial resistance genes		
Encoded virulence factors (see Section 2.1.4)		
Phenotypic evidence of virulence (as relevant) • Haemolytic activity		
• Toxin production		
• Other virulence factors		
Biogenic amine production		

Relevant scientific publications related to the microbial strain		

* *For additional strains, please copy "Characterisation of the microbial strains" table*

2.3.4 Strain manufacturing safety

Manufacturer(s) of the microbial strains:

	Yes/No	Details
Microbial cultures are pure.		
Microbial cultures are maintained to minimise genetic drift.		
Microbial cultures are fully re-characterised at a minimum frequency of annual intervals to monitor for genetic drift.		

3. Conclusions

The most widely used "probiotics" in food supplements are strains of LAB, bifidobacteria, *Bacillus sporogenes* and the yeast *Saccharomyces boulardii*. The potential risks from consuming "probiotics" in food include infection, ill effects from toxins produced by the microbial strains or contaminants, transmission of antimicrobial resistance and immunological effects.

Reported adverse events associated with "probiotics" are few and tend to be ad hoc reports of infections, including bacteraemia, sepsis or endocarditis in physiologically at-risk people. For most reports, the evidence confirming that the isolate is a "probiotic" strain is lacking.

At present in the EU, the safety of food supplements containing "probiotics" is governed by food businesses' obligation to only place safe food on the market (Regulation (EC) No 178/2002), the novel food Regulation whereby a microorganism used to produce a food that has not been consumed in the EU to a significant degree must be authorised before being placed on the market (Regulation (EU) 2015/2283, S.I. No. 253 of 2022) and the obligation to produce food hygienically and to identify and control hazards (Regulation (EC) No 852/2004).

4. Recommendations

This Scientific Committee report addresses two questions and makes the following recommendations:

Question 1. What is the committee's view on the most appropriate safety criteria to use when assessing the safety of "probiotics" in food supplements?

The Committee considers the most appropriate safety criteria to be that:

- There is long experience of use of the organism(s) in food or food supplements without substantiated report of harm in otherwise healthy people.
- Credible reports of infection or intoxication associated with the organism are isolated or rare and limited to those people at highest risk of infection.
- The organism(s) is readily identifiable to at least species level.
- There is an antimicrobial agent available for treatment of infection with the organism(s).
- The organism(s) has no known association with environmental harm.
- If the organism(s) was not used in food in the EU prior to 15 May 1997, its use has been authorised under the novel food Regulation (EU) 2015/2283.
- If the organism(s) was genetically modified, it has been authorised under Regulation (EC) No 1829/2003 on genetically modified food and feed, and under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.
- The organism(s) has been rigorously characterised as follows:
    - The definition of species and strain level is adequate to facilitate comparison in the event of suspected link to human infection.
    - There is evidence of the absence of properties associated with increased potential to cause infection.
    - There is evidence of the absence of acquired (transferable) antimicrobial resistance genes.
    - There is evidence of the absence of capacity for biogenic amine production.

Question 2. What evidence should a food supplement producer use to demonstrate the safety of "probiotics" when producing a food supplement?

The Committee considers that a food supplement producer should be able to demonstrate that:

- The organism(s) used meets the criteria set out above.

- The culture has been deposited in a recognised and accessible culture collection if not obtained from a culture collection.
- If cultures are stored, they are stored at -80 °C to ensure that they remain stable in storage.
- If cultures are propagated, the characterisation of the organism should be repeated at defined intervals to ensure that the organism has not significantly altered or been inadvertently displaced by, or contaminated with, another organism.
- The production process does not result in substantial change to the properties of the microorganism(s) between start of production and the end product.
- The production systems have adequate quality management systems to ensure consistent properties of the organism(s) in the final product.

In addition, the Scientific Committee made the following recommendations:

- Food supplement producers should document the food safety management system adopted to ensure consistent safe manufacture of the food supplement.
- The individual microbial strains used should be stored and accessible (either from the food supplement producer or from a culture collection) in the event of a suspected link to human infection.
- In line with the requirements of the Regulation on the provision of food information to consumers (Regulation (EU) No. 1169/2011), accurate information should be provided to the consumer on the label. It is recommended that this information includes the type and number of organisms present as well as appropriate storage advice. In addition, the viability of the organism(s) throughout the shelf life of the product should be determined to ensure that the information on the product label is accurate throughout the shelf life. Furthermore, where it is known that there are groups of people for whom the strain or strains used may not be suitable, this should be indicated on the label.
- If an *Enterococcus faecium* strain is included as a "probiotic" in a food supplement, the label should clearly indicate the presence of *Enterococcus faecium* in the food supplement.

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Appendix 1 Request for Advice

Topic Title: Assessment of the safety of "probiotics" in food supplements

Date Requested: 11 October 2021

Date Accepted: 18 October 2021

Date revised: 15 September 2022

Target Deadline for Advice: End of August 2022

Form of Advice required: Report

Background/Context

Directive 2002/46/EC sets out requirements relating to food supplements and defines food supplements as "foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities". There is a body of opinion that food supplements containing "probiotics" may be beneficial and hence there are many examples on the market that contain between 6 to 12 log cfu g^{-1} of particular microorganisms. FAO/WHO defined "probiotics" as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). In the EU, applications for health claims on "probiotics" have been submitted for evaluation to the European Food Safety Authority (EFSA). To date, no application has received a positive opinion. The term "probiotic" is therefore considered a non-authorised health claim under Regulation (EC) No 1924/2006 and, as such, is not allowed on labels of foods produced in Ireland (FSAI advice on Probiotic Health Claims). Despite the lack of an authorised health claim associated with the term "probiotic" in the EU, it is widely used by the food industry and the scientific community, and is the term used in this document.

Food regulations in Ireland require that a manufacturer, or person placing a food supplement on the market, must notify Food Safety Authority of Ireland (FSAI). This notification is not an approval or authorisation procedure.

Food business operators that place food supplements containing "probiotics" on the market are required by food law (Regulation (EC) No 178/2002) to ensure their products are safe. This is especially important for products that are marketed to so-called vulnerable groups (i.e. groups who tend to be more susceptible to infections and generally suffer more severe illness when they

develop infection, because their immune systems are either underdeveloped, as they are very young, or impaired due to age or illness).

Currently, in Ireland there is no guidance for assessing the safety of "probiotics" in food supplements or guidance on hygienic aspects of microbial growth and incorporation into this food category.

EFSA introduced the concept of qualified presumption of safety (QPS) to harmonise its own safety evaluation of microorganisms used as food or feed additives, food enzymes, novel foods or pesticides. The first list of biological agents with QPS status was established in 2007 and is updated in the form of a scientific opinion from the BIOHAZ Panel every three years. EFSA also carries out an extensive literature search every six months to ensure that the list is up to date. FSAI uses the QPS list as a point of reference when assessing the safety of probiotics in food supplement notifications.

Questions to be addressed by the Scientific Committee

1. What is the committee's view on the most appropriate safety criteria to use when assessing the safety of "probiotics" in food supplements?
2. What evidence should a food producer[29] use to demonstrate the safety of "probiotics" ingredients when producing a food supplement?

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[29] For clarity, the term 'food producer' in Question 2 was modified in the report to 'food supplement producer' and the word 'ingredient' was deleted.

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Acknowledgements

The Scientific Committee would like to acknowledge Professor Lieve Herman, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium and the EFSA BIOHAZ panel for helpful discussions. Dr Mary O'Connell Motherway was the recipient of an SFI Public Sector Fellowship (19/PSF/7637).

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