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Guidelines for assessment of safety aspects of probiotic (food) products

Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety

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Persons working for VKM, either as appointed members of the Committee or as ad hoc experts, do this by virtue of their scientific expertise, not as representatives for their employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a project group consisting of both VKM members and external experts. The members of the ad hoc group are acknowledged for their valuable work on this opinion.

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Contents

Abbreviations

FAO, Food and Agriculture Organization of the United Nations

WHO, World Health Organization

CFU, Colony Forming Units

DBPC, Double-Blind Placebo-Controlled

ISAPP, International Scientific Association for Probiotics and Prebiotics

GRAS, Generally Recognized As Safe

QPS, Qualified Presumption of Safety

NFSA, Norwegian Food Safety Authority

VKM, Norwegian Scientific Committee for Food Safety

EFSA, European Food Safety Authority

NDA, EFSA Panel on Dietetic Products, Nutrition and Allergies

Background

VKM has conducted a number of risk assessments of probiotics on request from the Norwegian Food Safety Authority (NFSA). With the exception of one report, which was of general nature, reports were assessing benefits / risks of specific commercial products. NFSA's requests were partly due to notifications from the food business operator (FBO). The Panel for Biological Hazards and The Panel for Food Additives - VKM have been involved in all these cases and their experience with these assessments is valuable for compiling a document concerning which elements should be included in the evaluation of a probiotic product and how they should be judged.

Products containing probiotics are increasingly marketed and consumed, despite concerns pertaining to the use of such products for certain groups, i.e. critically ill hospitalized patients, pregnant women, infants and small children. NFSA can, on the ground of safety concern instigate a risk assessment of the probiotic strain/product.

Products containing probiotic strains that have not previously been used are considered as novel food and are subject to authorisation before marketing.

In order to standardize and simplify the assessments relating to safety of probiotic products as much as possible, it was proposed that a general guideline would be useful for the producers, food authorities (NFSA), and VKM.

The aims of the guidelines are:

- to make risk assessments of probiotics more transparent by showing FBOs what documentation is required (producers may also use Table 1Table 1in Annex 1 as an aid). The guideline should display which aspects VKM emphasizes in risk assessment of probiotics and also what the mandatory requirements are. Thus the number of requests to VKM from NFSA would be limited,

- to enable NFSA, on the basis of the guidelines, to advise which notifications need to be assessed by VKM and also which applications lack the necessary documentation that would preclude a risk assessment by VKM. In order to evaluate whether the provided documentation is sufficient, NFSA may use Table 1 in Annex 1,
- to enable VKM, on the basis of these guidelines, to conduct more streamlined risk assessment of probiotics, and thus save time and resources.

According to the mandate from VKM, the guidelines focus on safety aspects. Other aspects pertinent to probiotics, but outside the VKM mandate, may be found in FAO (2002). We expect the guidelines to be updated as new data become available and will be accessible at www.vkm.no and www.mattilsynet.no.

Terms of reference

The working group has defined following terms of reference:

The aim of the guidelines is to provide information about which documentation concerning probiotic products must be provided to allow a safety assessment of the products.

Consideration of any health claims, for example related to gut and immune function, are not part of the mandate. Novel foods must be assessed by EFSA and are not included in these guidelines.

Definitions

Probiotics[1]
In 2001, Food and agriculture organisation (FAO) of United Nations and World Health Organisation (WHO) defined probiotics as: Live microorganisms, which when administered in adequate amounts confer a health benefit on the host (FAO 2002).

Generally Recognized As Safe (GRAS)[2]
Generally recognized as safe (GRAS) is an American Food and Drug Administration (FDA) designation that a chemical or substance added to food is considered safe by experts, and so is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) food additive tolerance requirements.

Qualified Presumption of Safety (QPS)
The QPS approach is a system similar in concept and purpose to the GRAS definition used in the USA, but has been modified to take account of the different regulatory practices in Europe. With respect to probiotics, QPS represents a possible route to harmonisation of approaches for the safety assessment of microorganisms used in feed/food production.

Novel Foods and Food Ingredients
Novel foods are foods and food ingredients that have not been used for human consumption to a significant degree within the European Community before 15 May 1997.

With regards to probiotic products, the use of a new probiotic strain, that has not previously been consumed (to a significant degree) in the EU, would require a novel foods assessment. The use of a novel food matrix including an established probiotic strain may also require novel foods assessment.

The following terms are not used in the document but are included because they are frequently used in connection with probiotic products.

Prebiotics
A prebiotic is a selectively fermented food ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health[3].

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[1] The International Scientific Association for Probiotics and Prebiotics, ISAPP, proposed that when combined with the specifications outlined by the FAO/WHO Working Group for the Evaluation of Probiotics in Food (2002), the key aspects of this definition should be more precise and in addition include the following aspects:

- A probiotic must be alive when administered,

- A probiotic must have undergone controlled evaluation to document health benefits in the target host,

- A probiotic must be a taxonomically defined microbe or combination of microbes (genus, species and strain level),

- A probiotic must be safe for its intended use.

[2] http://en.wikipedia.org/wiki/Generally_recognized_as_safe

[3] (Gibson et al. 2010. Food Science and Technology Bulletin: Functional Foods 7 (1) 1–19.)

Currently three criteria are required for a prebiotic effect
- Resistance of the prebiotic to degradation by stomach acid, mammalian enzymes or hydrolysis
- Utilisation (breakdown, metabolism) of the prebiotic by intestinal microorganisms
- Selective stimulation of the growth and/or activity of beneficial microorganisms in the gut.

Synbiotics

The term synbiotic is used for a product that contains both probiotics and prebiotics. Since the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favours the probiotic component.

1 Characterisation of microorganism and product information

This section describes product information that the producer must submit in order to document the safety of the product, before placing a product containing probiotic microorganisms on the market.

1.1 Characterisation of microorganism

The correct identification (genus, species and strain number) of the added microorganism(s) must be clearly stated. The names used must be in accordance with The International Code for Nomenclature of Microorganisms.
The strain must be deposited in a recognized international bacteria culture collection and the strain number for the strain in the collection should be provided.
Trade names for a strain may be used, but only in addition to the correct strain designation.
The strain must be identified using both phenotypic and genotypic methods. The information of the type of classification methods must be provided.

Identification of the strain

The potential effects of probiotics are considered to be strain specific. It is therefore necessary to identify and specify not only the genus and species of the probiotic in question, but also the actual strain used. In addition it must be stated how, and how often, the strain-specificity is secured and maintained.

A *strain* is a sub-population of a species with intra-species properties and is usually identified by a name, number, or letter. Strain identity is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies.
Extrapolation of data from one strain to another, even from the same species is not permissible.

The origin of the strain has to be stated as it is generally accepted that a probiotic intended for human use should be of human origin.

The nomenclature used must conform to the current, scientifically recognized names as can be retrieved for example from the validation lists published in the International Journal of Systematic and Evolutionary Microbiology. This is to ensure understanding and transparency on an international basis.

Specification of the microorganism must be established using the most current, valid methodology, preferably by using a combination of phenotypic (morphological and biochemical) and genotypic (*e.g.* 16s rRNA gene sequencing) tests.

Strain typing has to be performed with internationally accepted molecular typing methods with high discriminatory power (*e.g.* pulsed field gel electrophoresis (PFGE) or other appropriate methods) and using unique phenotypic traits, including antibiotic resistance profile, since genetic alteration of microorganisms due to acquisition of a resistance gene may be undetectable even by using molecular epidemiological methods with high resolution.

Characterization of the strain

Data regarding extra-chromosomal DNA, like plasmids and other mobile genetic elements including Insertion Sequence (IS)-elements, integrons and transposons and absence of virulence factors should be provided. Any possible association between mobile genetic elements and antimicrobial resistance genes/virulence factors should be reported.

Antibacterial resistance profiles of the strain with respect to all major classes of antibacterial agents and the most commonly clinical relevant agents in each class should be provided. Possible association between antimicrobial resistance phenotypes and the presence of resistance genes in the strain should be determined by molecular methods. The accession number of the complete sequence data of the strain, if available, should be submitted.

Proposed antimicrobial activity of the probiotic strain against pathogenic microorganisms should be examined by *in vitro*-testing (e.g. agar-method) and may require substantiation of efficacy with human trials (*in vivo*-testing). Documentation must be in the form of published peer-reviewed articles.

Results from studies on possible interaction between the probiotic microorganism and medicinal products should be available when relevant, or described and explained if not considered relevant. Documentation must be in the form of published peer-reviewed articles.

1.2 Product information

Number of viable probiotic bacteria per gram of product/per serving

The number of viable probiotic bacterial cells in the product within the time frame of its shelf life should be clearly given including a proviso that recommended storage conditions have been upheld.
The numbers may be expressed as log Colony Forming Units (CFU) per gram of product or per serving of a specified size. If the product as sold is intended to be mixed with water or milk before consumption, the number of cells per gram of ready- to- eat product, or per serving should also be given.

Number of recommended daily serving

The producer should state the recommended number of times the product should be eaten per day and the size of serving.

Food matrix

Specification of the food matrix, ingredients and nutritional content must be given, as in accordance with ordinary regulations for foods.
If outsourced ingredients are used, information on the manufacturer of these ingredients should be given.

Storage conditions and shelf life

The necessary conditions of storage for the product, which are important for ensuring survival and full viability of the probiotic strains within the specified shelf life, should be clearly described. These conditions may be more stringent than the storage conditions applicable to the carrier food. Storage conditions will normally refer to time, temperature, exposure to air and relative humidity. Information regarding documentation of the survival during storage of added strains is required.

For the probiotic ingredient, the shelf life of the product is determined by the requirement for survival of the number of viable probiotic bacterial cells considered necessary for provision of a health benefit. If the bacterial survival (and hence shelf life) is affected by opening the packaging, thus allowing access of oxygen, moisture, contamination etc, then information regarding the shelf life after opening should also be given.

2 Safety

Products should only contain probiotic bacteria granted QPS[4] and/or GRAS status.

2.1 Maintenance of the strain by the food product producer

Strain integrity

Control systems for ensuring the integrity of the added probiotic strain(s) must be described by the producer.
If the producer uses a culture supplied by a recognised international culture collection company, then the procedures used by the culture producer to ensure the purity, stability and integrity of the cultures should be included in the notification.

Number of viable probiotic bacteria per gram of product/per serving

The methodology to determine the number of viable cells should be given, including type of agar used and incubation parameters.

Viability during storage

The producer is responsible for implementing control procedures for monitoring the number of viable probiotic organisms in the product during storage. Details of the quality control monitoring procedures should be provided.

2.2 Antagonistic or synergistic effects

If the product should or should not be consumed together with other foodstuffs or therapeutic agents, the details of this should be stated on the packaging and further detailed information must be given with the application.
If consumption of other foods or medicinal products can increase or decrease the health effect of the product, this should be documented and stated.

2.3 Intestinal survival

In vitro tests are insufficient to predict the functionality of probiotic microorganisms in the human body, but may nevertheless be useful in order to gain knowledge of strains and the mechanism of the probiotic effect.
Appropriate target-specific *in vitro* tests that correlate with *in vivo* results are recommended. The main currently used and recommended tests for probiotic strains include:

[4] Table 1 in "Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA." The EFSA Journal (2007) 587, 1-16, http://www.efsa.europa.eu/en/efsajournal/doc/587.pdf

- Tolerance to body temperature
- Resistance to gastric acidity and bile salts
- Survive passage to its target organ (most commonly the intestinal tract)
- Adherence to mucus and/or human epithelial cells and cell lines
- Survival in the competitive environment of the intestinal tract (*in vitro* gut)

The results obtained from all appropriate tests carried out, should be submitted.

2.4 *In-vitro* and *in-vivo* safety assessments

Theoretically, probiotics may be responsible for four types of side-effects:

- Systemic infections
- Deleterious metabolic activities
- Excessive immune stimulation in susceptible individuals
- Gene transfer (e.g. antimicrobial resistance gene) to other bacteria

FAO[5] has recommended that the safety of probiotic strains should be documented using:

***In vitro* studies:**

- Assessment of certain metabolic activities (e.g. D-lactate production, bile salt deconjugation)
- Determination of antibiotic resistance patterns and whether resistance genes are located on, or associated with, mobile genetic elements (*e.g.* plasmids).
- If the strain under evaluation belongs to a species with known haemolytic potential, determination of haemolytic activity is required.

***In vivo* studies**

Animal studies:

- If the strain under evaluation belongs to a species that is a known to produce toxins of relevance to mammals, it must be tested for toxin production.

Human studies

If available, the following should be documented:

- Assessment of short term side-effects during human studies
- Assessment of long term side effects
- Epidemiological surveillance of adverse incidents in consumers (post-market)
- If the species under evaluation is known ever to have caused systemic infections in humans, the actual documentation must be presented
- Population-based surveillance for the isolation of probiotic bacteria from patients with infections

[5] FAO/WHO Guidelines for the Evaluation of Probiotics in Food, 2002

- Studies regarding consumption in special subpopulations; pregnant, children, elderly people, critically ill patients

If it is inadvisable to consume the product more than (for example) once per day, this should be clearly stated and reasons given. Consumption of some probiotics products may in some cases constitute a major proportion of the diet. Food for small children is a particular example. Any documented safety aspects concerning long-term consumption of probiotic products in relation to the total diet, should be provided by the notifier.

3 Consumer group(s)

If the product is intended for a specific consumer group, for example pregnant, small children, the elderly or ill people, this should be stated on the packaging. Where relevant, approval from the Food Authority must be obtained. If the product is unsuitable for a specific consumer group, this should be stated.

The EFSA Scientific opinion on the scientific requirements for health claims related to gut and immune function (EFSA Journal 2010) [6] states which requirements must be met in order to accept a health claim for a particular food or food ingredient. Information on claims can be found in Annex 2.

[6] http://www.efsa.europa.eu/en/efsajournal/pub/1984.htm

Annex 1 – Documentation check list

Table 1 Documentation provided by producers/notifiers (More information has been provided in the body of the text)

	Yes/no	Comments/references
Characterization of microorganism		
Correct name (genus, species) as given by the recognized international bacteria culture collection where it is deposited		
Identification methods used		
• Phenotypic methods		
• Genotypic methods		
Characterization of the strain		
Strain number as given by the bacteria culture collection where it is deposited		
Methods used to secure the strain specificity		
How often is the strain specificity secured?		
Origin of the strain (of human origin?)		
Data regarding extra-chromosomal DNA (plasmids, mobile genetic elements like Insertion Sequence, Integron, etc)		
Antibacterial resistance profiles		
• Any known association between the resistance profile and presence of resistance genes?		
• Any known antimicrobial activity of the strain against human pathogenic microorganism (usually *in-vitro* study)		
Intestinal survival		
• Tolerance to body temperature		
• Resistance to gastric acidity and bile salt		
• Adherence to mucus and/or human epithelial cells and cell lines		
• Survival in the competitive environment of the intestinal tract		
Haemolytic activity (if belonging to a species with known haemolytic potential)		
Toxin production (if belonging to a species with known potential for toxin production)		
Product information		

Recommended storage condition (time, temperature, exposure to air and relative humidity)		
Number of viable probiotic bacteria per gram of product/per serving		
• If mixed with water or milk: Number of bacteria per gram of the ready-to-eat product or per serving		
Number of recommended doses daily		
Food matrix (according to ordinary regulations for food)		
Shelf life (If affected by opening the packaging, give the shelf life after opening)		
Safety		
Control systems for ensuring the integrity, purity and stability of the culture		
Method for determining the number of viable cells (agar used, incubation parameters, etc)		
Control procedures implemented to determine viability during storage –		
Antagonistic/synergistic effects (if any).		
• Should the product or should the product not be consumed with other foodstuffs or therapeutic agents?		
• Are there any known deleterious health effects if consumed with other foods or medicinal products?		
Safety assessments:		
In vitro studies: Assessment of certain metabolic activities (e.g. D-lactate-production, bile-salt deconjugation)		
In vivo studies (if available)		
• Epidemiological surveillance of adverse incidents in consumers (post-market)		
• If the bacterium is known ever to have caused systemic infections: actual documentation		
• Studies regarding consumption in special subpopulations, e.g. pregnant, children, elderly, critically ill patients		
• Short term and long term adverse effects		
If it is inadvisable to consume more than the		

recommended daily dose, the reason must be stated		
Any documented safety aspects concerning long term consumption of probiotic products in relation to the total diet (e.g. for small children) should be provided.		

Contact information (name, address, telephone and website of the Company) should be stated on the packaging.

NFSA's evaluation of the documentation received	
Accepted	
Rejection of application	
Need for further documentation	
Need for risk assessment – request to VKM	

Comments:

Annex 2 - Claims

Health Claims

A Health Claim is any statement conferred by labelling or advertising that states, suggests, or implies that a relationship exists between consumption of a food or food constituent (including an ingredient in the food) and a person's health. Only health claims approved by EFSA can be used in marketing.

The main beneficial effects of probiotics are claimed to be:
• Antimicrobial effects that can result in reduced growth of pathogenic bacteria,
• Detoxifying effect that may include inactivation or neutralizing substances that may be harmful to the host,
• Immunological effect regarding positive stimulation of the immune system.

The EFSA Scientific opinion on the scientific requirements for health claims related to gut and immune function (EFSA Journal 2010) states which requirements must be met in order to accept a health claim for a particular food or food ingredient. Studies appropriate for substantiation of claims must be carried out with the food/constituent for which the claim is made, be carried out in a study group representative of the population for which the claim is intended and the design and quality of the studies must allow scientific conclusions to be drawn. All health claims pertaining to food and food ingredients will be evaluated in the EFSA Panel on dietetic products, Nutrition and allergies.

Product specific claims - Claims on gastrointestinal microbiota

The FAO/WHO working group recommends that specific health claims on foods be allowed relating to the use of probiotics, where sufficient scientific evidence is available, as statements like "improves gut health" may be misleading and not informative.

For claims related to maintaining normal defence against pathogens in the gastrointestinal tract, maintaining normal immune function and stimulation of protective antibody titres related to defence against pathogens, this should be documented in clinical studies.