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Current Opinion in Biotechnology

Novel probiotics and prebiotics: road to the market☆

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Novel probiotics and prebiotics designed to manipulate the gut microbiota for improving health outcomes are in demand as the importance of the gut microbiota in human health is revealed. The regulations governing introduction of novel probiotics and prebiotics vary by geographical region. Novel foods and foods with health claims fall under specific regulations in several countries. The paper reviews the main requirements of the regulations in the EU, USA, Canada and Japan. We propose a number of areas that need to be addressed in any safety assessment of novel probiotics and prebiotics. These include publication of the genomic sequence, antibiotic resistance profiling, selection of appropriate *in vivo* model, toxicological studies (including toxin production) and definition of target population.

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Current Opinion in Biotechnology 2015, **32**:99–103

This review comes from a themed issue on **Food biotechnology**

Edited by **Michiel Kleerebezem** and **Christophe Lacroix**

http://dx.doi.org/10.1016/j.copbio.2014.11.021

Introduction

Issues pertaining to novel probiotics and prebiotics has increased in importance due to the fast-paced research in human microbiome science. Tools to manipulate the gut microbiota for improving health outcomes are in demand as the importance of the gut microbiota on health is revealed. Some probiotics and prebiotics have been used for decades, but probiotics and prebiotics targeted toward unique outcomes and functionalities can be expected to emerge.

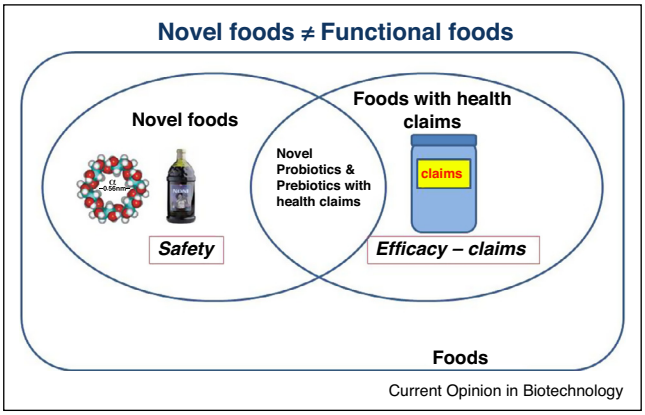
The regulations governing introduction of novel probiotics and prebiotics vary by geographical region. In some cases, confusion can result in differentiating novel foods from functional foods. The fundamental difference between these two categories of foods is that novel foods must be evaluated based on their safety, whereas functional foods need to be evaluated for any desired health claims. Figure 1 demonstrates that the terms are distinct but sometimes foods and food ingredients fall in both categories, which then necessitates evaluation for both safety and efficacy. This review summarises the discussion group views on current legislative framework in the USA, Europe, Canada and Japan regarding the assessment of probiotic and prebiotic novelty from the scientific, regulatory and consumer viewpoint. Additionally, it highlighted some of the major hindrances observed for 'novel' probiotics.

What are 'novel' probiotics and prebiotics?

In a recently published consensus report, the term 'probiotic' as originally defined by FAO/WHO was endorsed with minor corrections as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [1••]. The Food and Agriculture Organisation of the United Nations (FAO) defines 'prebiotic' as 'a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota' [2]. Previously, prebiotic studies were focussed on inulin, fructo-oligosaccharides and galacto-oligosaccharides and these prebiotics are now in the USA generally regarded as safe because of their long history of safe use.

☆ The International Scientific Association for Probiotics and Prebiotics (ISAPP) organised a discussion group comprising experts from probiotic research, industry and regulatory authorities from the United States, United Kingdom, Spain, the Netherlands, Finland, Canada and Japan during the 12th annual meeting in Aberdeen (Scotland), to characterise the requirements to be fulfilled and to establish differences in the path to the market for novel probiotic and prebiotic products.

Figure 1



Differences between novel foods (foods not previously consumed to a significant degree, and evaluation for safety) and foods with health claims (evaluated for efficacy).

Probiotics and prebiotics may also be novel foods, leading to challenges on whether or not a food or food ingredient is 'novel' especially in the EU [3•]. In the regulatory field, 'novel' is a legal construct determined by law, typically in relation to developments that occur after the regulation was enacted, thus leading to scientifically recognized grey area of novel foods [3•,4]. For the purpose of this paper, 'novel' will be used in the regulatory sense.

New probiotics and prebiotic components with varying functions have emerged. In the EU, these may be recognized as 'novel foods', thereby triggering a risk assessment procedure. In each case, the novel status will be assessed on a case-to-case basis. For example, a fructo-oligosaccharide or galacto-oligosaccharide with a significantly different degree of polymerization or with a different source or production method might be regarded as novel.

Regional differences in regulation of probiotics and prebiotics with respect to novelty and safety

European Union

Worldwide, the regulations governing novel foods, functional foods and traditional foods vary. In the EU, the introduction of novel foods that have not been used in the EU prior to 15 May 1997 is governed by the Novel Food Regulation 285/97/EC [5]. This Regulation clearly defines the risk assessment steps required for any authorisation of the novel food prior to introduction into the EU market. The regulation also defines 'substantial equivalence' to commonly used foods and in which case a simplified notification procedure applies. The Novel Food Regulation from 1997 is currently under revision and a proposed new regulation was published in December 2013 [6]. Potential changes in the update of the Regulation may cover traditional foods from 3rd countries, nanotechnology, as well as the submission and evaluation route (i.e. directly to EFSA rather than being conducted by competent authorities in member states) [6].

Based on the Regulation, a member state competent authority or European Food Safety Authority (EFSA) and the European Commission (EC) can make assessments of any food or food ingredient that has no history of safe use prior to 1997 in Europe and hence can be identified as 'novel'. The Regulation then requires an extensive safety assessment of the food or ingredient prior to acceptance to the EU market [4]. A list of novel foods and ingredients is available in the public registry by the EC and approvals are also explained in an inventory specifying the uses and restrictions for each component (http://ec.europa.eu/food/food/biotechnology/novelfood/nfnetweb/mod_search/index.cfm). For bacteria added into foods, which could also be considered novel [3•,4], there is an annually updated list of microbes intentionally added to foods (QPS, Qualified Presumption of Safety of Micro-organisms in Food and Feed, list) and this list forms the basis of organisms at the species level which are considered safe for foods and feeds in European Union (EFSA 2013 update) [7].

A novel probiotic or prebiotic can potentially be a component of conventional foods, food supplements or foods for particular nutritional uses ('Parnuts'). Parnuts foods incorporating probiotics or prebiotics comprise those designed for specific dietary requirements and may include infant formulas and follow-on formulas, processed cereal-based food and baby food, food for special medical purposes and total diet replacement for weight control.

When designated as a novel food, a safety assessment follows the European Novel Foods Regulation [3•], and an evaluation is needed for the EC to make a decision on the safety of the novel component.

The EC regulation on nutrition and health claims 1924/2006 requires that such claims are based on scientific evidence and acceptability [8]. EFSA has provided scientific and technical guidance for presenting applications for health claims on food. Neither Parnuts Directive 2009/39/EC nor EU Regulation 609/2013 are an escape route to circumvent the Health Claims Regulation 1924/2006; Hendriksen and Verhagen have developed a decision tree to discern Parnuts foods from ordinary foods (with health claims) [9•]. Following on from the publication of the health claims Regulation 1924/2006 [8], the EFSA has now evaluated about 3000 health claims for being scientifically substantiated (or not substantiated) [10].

United States

In the United States, all foods and food ingredients are regulated under the Food Drug and Cosmetic Act (FDCA). Safety of new and novel foods in the United

States is primarily the responsibility of the food manufacturer. The regulation states that 'any substance that is intentionally added to food is a food additive that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use'.

In the USA, probiotics and prebiotics, whether novel or not, intended for use in foods other than dietary supplements are regulated under the same regimen as all other food ingredients—that is, they may be introduced as food additives or as Generally-Recognized-As-Safe (GRAS) substances, at the discretion of the manufacturer [11]. There are two other conditions that pertain to GRAS substances but not to food additives. First, the information demonstrating safety must be generally available to the scientific community, usually regarded as requiring publication in the peer-reviewed scientific literature. Second, there must be general acceptance of the safety of the substances throughout the scientific community—there cannot be significant dispute regarding safety.

FDA has no fundamental role in GRAS determinations except in an advisory capacity. The law that established GRAS (the 1958 Food Additive Amendment to the FDCA [12]) specifically excluded GRAS substances from requiring FDA review and approval prior to entry into the food supply. The GRAS status of the intended use of a probiotic, prebiotic, or other substance is determined by a panel of qualified scientists who render the opinion that there is a 'reasonable certainty of no harm' from the intended use, and further that they believe that other equally qualified scientists would reach the same conclusion. This process may be internal to the company and maintained as confidential to the company and disclosed only to customers under confidentiality.

In 1997, the law was amended to provide for a GRAS notification whereby companies could submit it to the Food and Drug Administration (FDA). The submission usually consists of an assessment of existing data by a group of recognized experts. Such safety assessment through scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of the substance as a food additive and ordinarily is based on published studies, which may be corroborated by unpublished studies and other data and information. This is a voluntary submission whereby the FDA reviews the information and the favourable FDA response is 'FDA has no questions at this time.'

Probiotics have a long history of use in food as dietary supplements. A microorganism is a dietary ingredient if it is a dietary substance (an intentional constituent of food) [13]. An application of specific probiotics as New Dietary Ingredient (NDI) has to be made to FDA. For a probiotic that has been an ingredient in food, a notification is not needed when a dietary supplement product contains the probiotic which has been present in the food supply without having been chemically altered. A probiotic as dietary supplement may not claim to prevent, diagnose, mitigate, treat or cure a specific disease or classes of disease. It is also widely accepted that not all bacteria are dietary ingredients and bacteria that were never consumed as food are unlikely to qualify as dietary ingredient. For example, *Lactobacillus* strains used to produce cheese or yoghurt could be a dietary supplement used by humans. For new and novel probiotics, the route to market as a dietary supplement is to have the probiotic GRAS self-affirmed for use in food and then to use it in a dietary supplement in the same form.

Japan

In Japan, the assessment of novelty is based on both the source and the traditional use of foods or food ingredients in Japan. Details on novel microbes or prebiotics are not available currently.

The criteria for any potential claim related to human health would be the same as for traditional food. Probiotics and prebiotics can be components of the following categories of food that can make health claims:

1. Special dietary uses: Food broadly based on this category could be of medical purposes, pregnant women, infants and dysphagia patients.
2. Specific health application: FOSHU (Foods for Specified Health Uses), Product based on this category can make disease reduction claims.
3. Food with nutrient function claim.

FOSHU are based on following guidelines:

1. Foods with active constituents that affect the physiological function and biological activities of the body.
2. Foods that claim that if used in the daily diet could give the desired benefit.
3. Foods products are evaluated individually, according to their substantiation, validity, safety and quality; and approved by government.

A new law will come into force from April 2015 and will operate along with FOSHU health claim system. Interestingly, the new law will also permit medical professionals to recommend food supplements including in combination with drugs [14].

Canada

In Canada, the definition of 'novel food' and 'major change' are set out in B.28.001 of the *Food and Drug Regulations*. Manufacturers or importers (petitioner) are

required to submit a Safety Assessment Data Package to Health Canada prior to sale. Novel foods derived from microorganisms require history of safe use, detail of the novel process and genetic information and intended for use in or as a food. For new probiotics of eligible species safety is accepted in a manner similar to European QPS bacteria. The claims which are therapeutic in nature are regarded as 'drug Food and Drug Regulations' [15].

Novel probiotics are now being evaluated in the domain of natural health products based on a probiotic monograph. The monograph suggests the particulars of probiotic products including label information, general claim and specific claim.

Due to advent of 'omics' based technological advancements; better understanding can be achieved leading to clear scientific and regulatory guidelines worldwide. Some examples based on the recent literature include (Box 1).

Box 1 Examples of novel probiotics and prebiotics based on recent evaluation in European Union

Clostridium butyricum

Clostridium butyricum has been approved by the EU since 2009 for use in animal feed for fattening of chickens and pigs. Recently, it has been reclaimed for its application in humans. It has been marketed in Japan and other Asian countries for this purpose for more than 10 years. It is now classified as a complex novel food as its origin is considered as being from a source with no history of food use in EU. It is also not included in the QPS list and therefore requires for a full novel food application.

Faecalibacterium prausnitzii

As per current knowledge, *Faecalibacterium prausnitzii* has no regulatory approval as a probiotic. But recent evidence, supports its beneficial properties on human health, particularly in inflammatory intestinal diseases such as Crohn's disease [16]. Therefore, future probiotic uses are likely to occur when the safety of the species has been demonstrated to satisfy regulatory authorities in Europe and elsewhere.

Yacon

Yacon (*Smallanthus sonchifolius*) is a perennial plant that forms sweet tasting underground tuberous roots that vary greatly in shape and size and are commonly eaten raw (Herbal Guides 2010). Yacon belongs to the sunflower family of plants and is also related to Jerusalem artichoke (*Helianthus tuberosus*). In spite of its long history of use in South America and other continents, yacon was considered a novel food in Europe and yacon products were withdrawn from the market pending safety evaluation according to the novel food legislation [17]. However, due to more extensive documentation of the culturing and use of yacon in Europe the novel food status was removed.

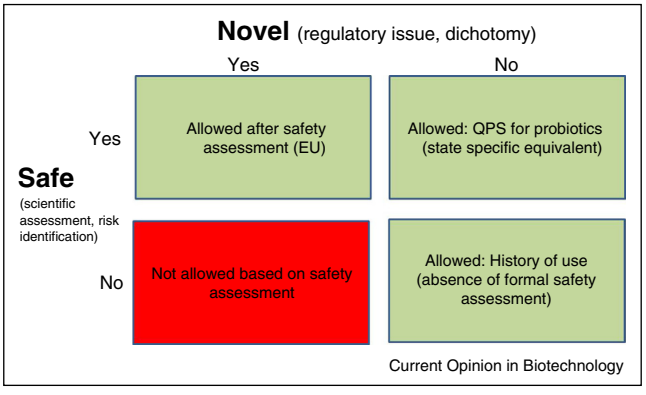
Lacto-N-neotetraose

Lacto-N-neotetraose (LNnT) is a tetrasaccharide naturally occurring in human breast milk, but not in cow's milk. An application for the authorisation of LNnT was submitted to the Food Safety Authority of Ireland (FSAI) and the authority assessed the safety properties. The novel ingredient is a synthetic oligosaccharide produced using D-lactose as a starting raw material. LNnT is commonly found in human milk and the concentrations proposed to be used are similar to those found in human milk [18]. No safety concerns were found and the process in the European Union is currently ongoing [19].

Novelty and safety

'Novelty' is a regulatory construct whereas 'safety' is a scientific and regulatory construct (Figure 2). The approach to establishing safety for a novel substance—including novel probiotics or prebiotics—does not differ in any way from establishing safety for a non-novel substance. Safety assessment is always a matter of an accumulation of evidence, including that gained by experience as well as that gained by planned research, and the weight given to the different types of evidence is a continuum. At one end of the continuum are those food substances that have been a component of the human diet for millennia with no evidence of harm and which, in many cases, were not subjected to any form of scientific review. Examples are those substances listed in the United States Code of Federal Regulations Title 21, Part 182—'Substances Generally Recognized as Safe' based on common use in food. Further along the spectrum are substances for which determination of safety is based on history of common use, but is confirmed by scientific review of the evidence; good examples are QPS microorganisms or microorganisms in the US GRAS notification registry. Novel substances differ only in that the entire weight of the safety assessment is borne by the scientific evidence, with little or no history of use to support it.

Figure 2



The interrelationship between safety assessment requirements and assessment of novelty of probiotics and prebiotics in European Union. First, Novel-yes, Safe-yes: the novel probiotic could be considered for human consumption after it has been found to be safe under the proposed conditions of use. Second, Novel-no, Safe-yes: these probiotics could be from list of probiotic microbes which are included in the QPS list of EFSA, Eligible list of species of Canada, GRAS of US. Third, Novel-no, Safe-no: these could be probiotic microbes which are considered to have a historical usage but it lack formal safety assessment. These probiotic microbes could be made available after safety evaluation. Fourth, Novel-yes, Safe-no: These kind of microbes which are claimed for probiotic attributes but there are identified risks or reports of adverse effects cannot be released as probiotics.

Box 2 Suggested information requirements for novel probiotics

1. Genome announcement: We recommend complete genome announcement and annotation. Functional annotation would help in predicting function.

2. Antibiotic resistance profile: All strains should be characterised for their antibiotic resistance potential and also the type of resistance. Conjugation studies could also be used to study transferability of antibiotic resistance.

3. Selection of proper *in vivo* model: There are growing numbers of studies which are based on mouse and rat models. It is important to realise that these models do not provide the 'actual' gastrointestinal conditions of humans. However, preliminary testing could be essential for newly characterised strains or species.

4. Toxicological studies: Some newly defined probiotic species are known to produce toxins. It should be scientifically assessed that the species or strain claimed for its probiotic properties does not produce any toxins.

5. Target population: Target population should also be clearly defined as a probiotic found to be effective in one population may have some adverse effect in another due to varied susceptibility to particular microbes. For example, application of probiotic for D-lactic acid production may lead to acidosis if used in infants.

Conclusion: panel view on assessing novelty and safety

Due to the increasing number of probiotic microorganisms and potential health claims based on these products or formulations, assessment of safety of these microorganisms for human consumption becomes very important. We consider that the scientific information summarised in Box 2 would be helpful in addition to that specified in the WHO/FAO guidelines 2002, for assessing probiotic strains for safety. Another dimension is the establishment of health messages, which are not mandatory prior to entering the market. However, they are often important for new strains and therefore reviews of the health claim assessment might be useful [15,20•].

Acknowledgements

The authors acknowledge the working group members for the discussion and expertise and the assistance of ISAPP International Scientific Association of Probiotics and Prebiotics in organising the discussion group in the Annual Meeting in Aberdeen in 2014. We also thank Mary Ellen Sanders for the suggestions and improving the article.

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