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Novel Probiotics and Prebiotics: How Can They Help in Human Gut Microbiota Dysbiosis?

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Abstract

Background and Objectives: Novel probiotics and prebiotics designed to modulate the gut microbiota for improving health outcomes are in demand as the importance of the gut microbiota in human health is revealed. A review of the scientific literature regarding the current knowledge and novel species and novel oligosaccharides for the treatment of dysbiosis-associated diseases has been carried out due to their growing interest.

Results and Conclusions: 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. In European Union (EU), safety is assessed by novel food approval process and by the European Food Safety Authority (EFSA) established Quantitative Presumption of Safety (QPS) system for bacteria and other biologicals. Any messages on health benefits are covered by the European Regulation on Health Claims (ERHC), also assessed by EFSA. Examples of recent novel probiotics in EU include *Clostridium butyricum*, and *Bacteroides xylanisolvens* and examples of novel prebiotics include human milk oligosaccharides such as Lacto-*N*-neotetraose. Yacon root is an example on a previously novel prebiotic food which is allowed due to the reported existing cultivation and use in EU prior to the novel food regulation. Potential future candidates include further human milk oligosaccharides and bacteria such *Faecalibacterium prausnitzii* and *Akkermasia muciniphila*. Increasing knowledge on human intestinal microbiota and microbiota development enables the design of new more specific and hitherto unknown probiotics and prebiotics. Also understanding the microbe and microbe host interactions facilitates the search for novel probiotics and prebiotics.

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1. Introduction

Research interest in novel probiotics and prebiotics has increased rapidly due to the fast-paced discoveries in both composition and activity of human microbiome and their impact on health. Tools to manipulate gut microbiota and thereby improve both short-term and long-term health outcomes are also developing fast. Some of the current probiotics and prebiotics have been used for decades, but novel strains and components are identified for unique outcomes and are therefore expected to emerge rapidly [1]. The challenges for novel probiotic bacteria and prebiotics remain in the varying regulatory systems in different parts of the world. In Europe, two important legislative controls regulate the entry of novel probiotics and prebiotics in the European market. These include the safety assessment along with the recently revised Novel Food Regulation [2] and the health benefit assessment according to the Regulation on Health Claims [3]. An assessment of the current safety work in Europe and Health Claims in European Union will attempt to uncover the road to the market of novel probiotics and prebiotics with examples of their regulatory assessment and resulting decisions available until now.

2. Human intestinal microbiota

The human microbiota is a dynamic ecosystem established after birth and composed for all the

microorganisms living in human surface or inside our body in naturally symbiotic relationship with him [4].

The intestinal microbiota has the highest microbial diversity of the human body, with more than 1000 different bacterial species belonging in their majority to relatively few bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria [5, 6]. Although there is an increasing knowledge about the kinds of organisms, their abundance and taxonomical distribution in various parts of the human body; we still have to understand much better how they interact with each other or which of them play key functional roles for human health [7].

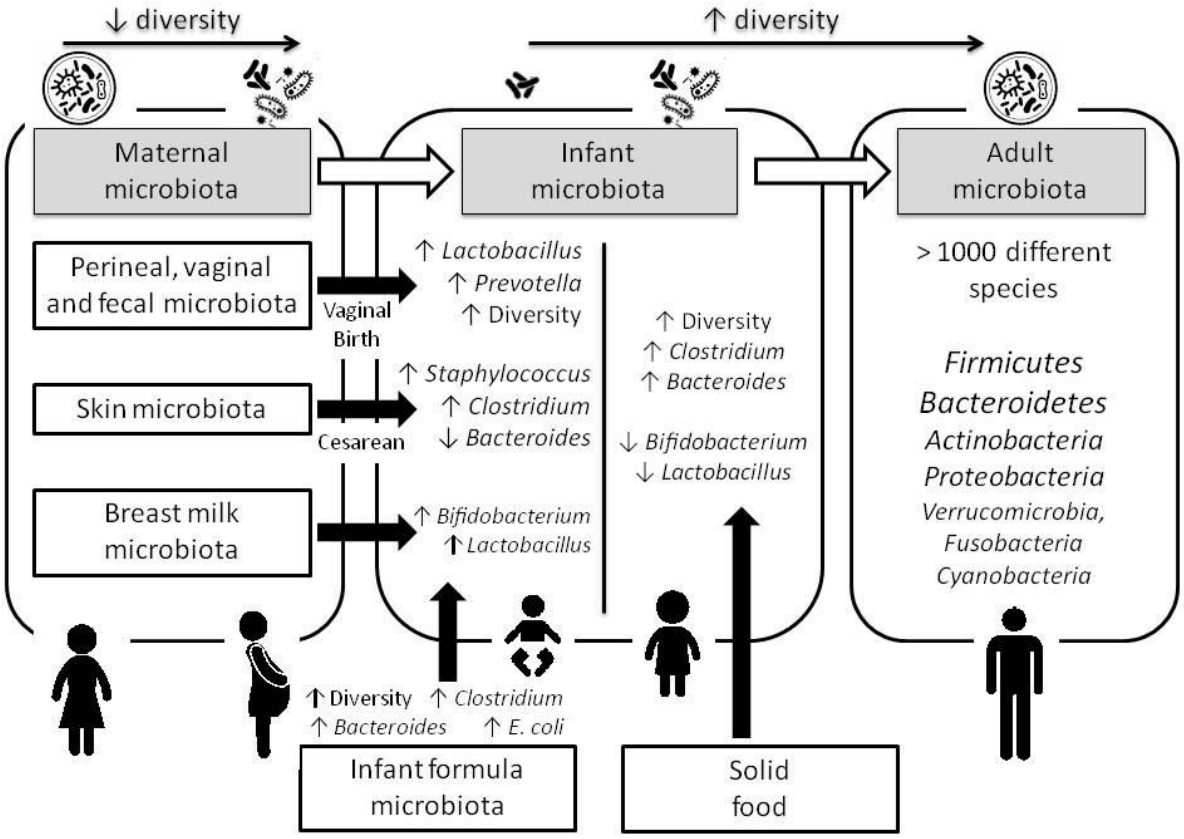

Figure 1. Changes in intestinal microbiota during life (adapted from Isolauri et al., [61]). Maternal microbiota changes between the first and third trimester during pregnancy and the first months postpartum, may be to promote transfer of specific strains to the infant [3]. During the first months of life the main bacterial groups are conditioned by the way of delivery and the food consumption pattern. Gradually, with the introduction of solid food, the bacterial diversity is increasing to rise the adult pattern after several years.

The establishment of our microbiota (Figure 1) begins already before birth by microbial contact through placenta and amniotic fluid [8] and seems to be greatly influenced by the mode of delivery through perineal, vaginal, and faecal microbiota inoculum by normal delivery or skin inoculum by caesarean section [9].

Differences in the initial inoculum are maintained along the next years, being detected even at the age of seven years [10], which might have an impact on infant health. After birth, the neonatal intestine becomes rapidly colonized by maternal and environmental bacteria and colonization continues during lactation increasing complexity and microbial diversity [11].

Another great influencing factor for the initial intestinal microbiota is the infant feeding. Breast milk contains living bacteria in a concentration of 10^2 to 10^4 viable bacterial per mL, prebiotic nutrients and bioactive components, playing an important role in the establishment of the neonatal microbiota [12, 13]. Infants that are exclusively breast-fed in harbour a microbiota dominated by Bifidobacteria and Lactobacillus, while exclusively formula-fed infants host a more diverse microbiota with increased abundance of *Escherichia coli*, Clostridia, and Bacteroides [4]. This differences might be caused not only for bacterial composition of human milk, but also for the presence of human milk oligosaccharides, a diverse family of unconjugated glycans with a prebiotic role that are

highly abundant in human milk and absent in infant formulas [4].

Solid food introduction and weaning increase the diversity of the microbiome and microbiota functionally maturates by a decrease in the relative abundance of genes involved in the degradation lactate utilization and towards enrichment of genes involved in the degradation of carbohydrates [6,11].

The intestinal microbiota closely resembles the diverse adult-like composition at the age of three years with high levels of Bacteroides and Clostridium, changes in Lactobacillus population and reducing Bifidobacterium levels [4]

Each human individual reaches a homeostatic composition, remaining relatively stable during most of a healthy adult's life [14,15]. Although each individual has a specific microbial composition at the species level [16], the overall phylogenetic profile might be categorized into different host-microbial ecosystems dominated by several clades with broad prevalence and relatively abundant carriage patterns that could have functional differences [16,17], doing that their host might respond differently to diet or drug intake. Defining normal healthy microbiota is impossible due to the great inter-individual variation among the species of microbes present at different body locations, together with variations in microbiota related with age, geographic area, genetic background, mode of delivery, breast-feeding, age, diet, hormonal cycles, travel, health status, and medical treatments of the host [5,14,18].

The microbiota has a profound impact on its host by providing a competitive barrier against invading pathogens, utilizing undigested food components and producing essential metabolites, modulating immune responses and immune system development, and stimulating intestinal maturation [12,14].

Microbial richness, intended as high bacterial diversity, is usually considered an indicator of a healthy status and makes the host less prone to a number of diseases [7]. Low richness is associated with several life-style related non-communicable diseases such as obesity, metabolic syndrome, immune-related, and inflammatory diseases [5]. The number and diversity of bacterial species within an individual's gastrointestinal tract remain relatively constant throughout life, as mentioned previously, but it is possible to stimulate the proliferation of specific microorganisms with beneficial health effects by manipulating the host diet [19].

3. Dysbiosis and diseases

Dysbiosis or dysbacteriosis is defined as a perturbation in the microbiota composition, with a decrease in the relative numbers of beneficial microbes and a thrive of harmful microbes in the intestinal tract [20]. Any defined imbalance between protective and harmful bacteria may have the ability to promote disease susceptibility and/or progression of a disease. Therefore, it is important to identify healthy microbiota development and the factors that cause deviations (Figure 2).

However, the distinction between beneficial and harmful bacteria is often not clear. It is important to consider that the effect of an intestinal microorganisms on the host and its pathogenic potential is also dependent on the specific circumstance (host state, genotype, diet, and lifestyle), meaning that microorganisms that are normally beneficial or commensal can become a potential threat to the host when conditions change [20,21].

Dysbiosis has been associated with several diseases in humans, and dysbiosis may increase the risk of diseases. Dysbiosis associated diseases include gastrointestinal and systemic problems, obesity, allergy and even cardiovascular diseases [1]. However is not clear whether dysbiosis is the cause of the disease, or whether both are concomitant phenomena [20]. Moreover there are multiple reasons for dysbiosis such as caesarean delivery, premature birth, short breast-feeding, diet, life style, hygiene or antibiotic use.

The successive development of intestinal microbiota from perinatal time to childhood and adolescence has been reviewed by Rautava et al. [22]. It is evident that the healthy individual microbial colonization pattern develops already during fetal life, and is influenced by the mode of delivery and feeding patterns during infancy modulating immune and metabolic development. Normal succession of microbes may improve infant health and reduce the risk of disease in later life.

After initial succession and development of richness and diversity typical to each person, the gut microbiome seems to be relatively stable during healthy adulthood. But qualitative and quantitative alterations, which lead to functional modifications, have been reported and associated with a number of human diseases [1].

The increased risk of obesity and childhood asthma in children born by caesarean section has been attributed to the different intestinal colonization pattern in these children [4].

Moreover, breastfeeding or formula feeding could impact in microbiota development (Figure 1). It has been shown that allergic infants display an abnormal "adult-type" Bifidobacterium flora, with high levels of *Bifidobacterium adolescentis* strain instead of the typical infant flora dominated by *Bifidobacterium bifidum* and lower total amount of Bifidobacteria [23].

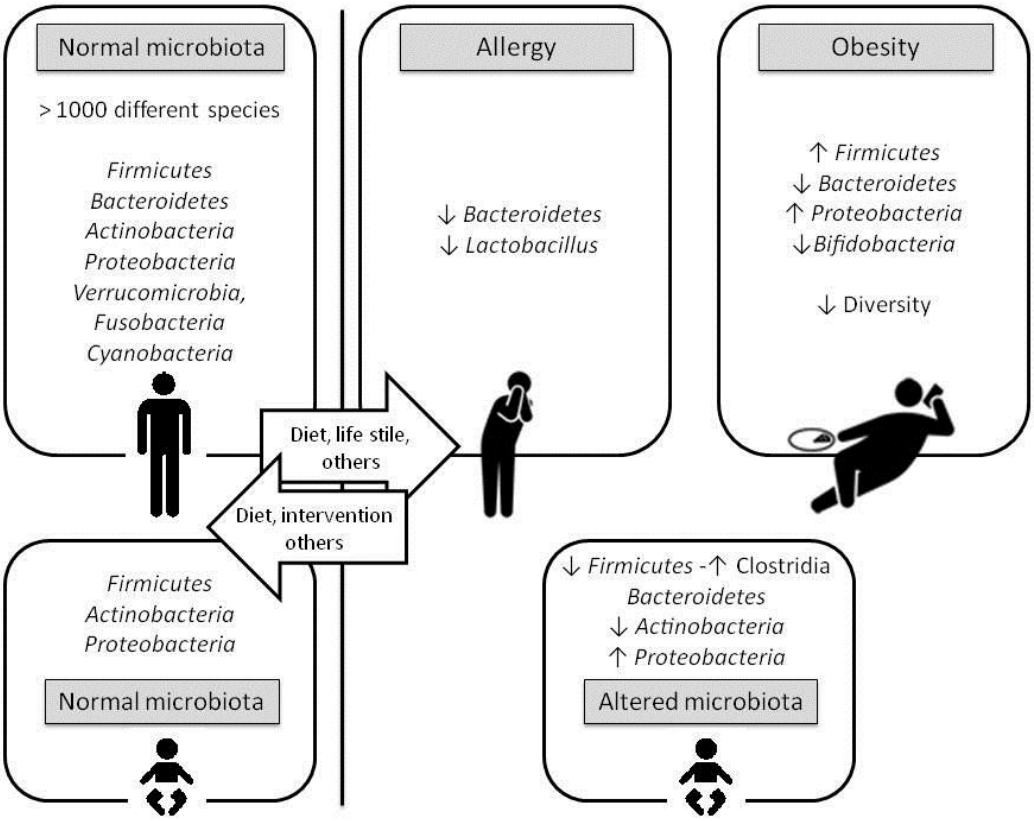

Figure 2. Changes in microbiota. Diet could have a great impact changing key populations in microbiota, which may increase the susceptibility to some diseases. Moreover, other factors related with life style or diseases like stress or antibiotic consumption may have an impact on microbiota. Specific intervention could balance this changes restoring microbiota and their function.

Breastfeeding is reported to be on factor influencing infant gut colonization and *Bifidobacterium longum*, appears to be the most common species found in breast milk. In a Finnish study, also *Bifidobacterium lactis*, one of the most commonly used probiotics, was found second most common in the milk of the study mothers [24], therefore breast milk microbiota and breast milk oligosaccharides are factors which direct infant gut microbiota development.

Emerging evidence suggests that variation in the microbiome may have a greater role than human genome variation in the pathogenesis of obesity given its direct interaction with environmental factors [25].

It has been suggested that an "obese microbiota" has high potential to extract energy from the diet [26]. It has been shown that the composition of bacteria in the gut differed between lean and obese individuals with a high rate of Firmicutes relative to Bacteroidetes, but some recent publications have contradicted these findings [27].

Allergic diseases has increased worldwide in recent decades and has been associated with the hygiene hypothesis [1] and changes in the life style. Alterations in gut microbiota have been reported in patients with allergic diseases and also, low bacterial diversity in intestinal microbiota during early life is associated with an increased risk of allergic disease [28,29]. Moreover, some bacterial phylotypes were associated with the development of allergy in infants as Clostridium, Enterococcus, Escherichia/Shigella, Staphylococcus, Faecalibacterium, or Prevotella [30-32]. The use of probiotics and prebiotic, or the combination of both as a synbiotics, may allow an adequate modulation of intestinal microbiota and could be a basis for nutritional tools against dysbiosis-associated diseases.

4. Probiotics

A recent review defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" supporting the use of this wording in the future. This definition includes a wide range of microbes and applications, defining probiotics (microbes, viable, and health benefits), and makes a difference between microbes used for technological purposes such as fermentation and those that are used for their health benefits [33].

The most frequently used bacterial genera are lactic bacteria, mainly from the Lactobacillus genus, but also Bifidobacterium genus, and other genera are also used such as the Enterococcus, Streptococcus, bacteria are commonly found in fermented dairy

products, human milk, the intestinal tract of healthy children or adults, and also from the digestive tract of animals and non-fermented food [1]. Moreover, probiotics may belong to other bacterial genera and even other domains such as Saccharomyces.

In Europe, European Food Safety Authority (EFSA) has not accepted probiotic health claims submitted due to stringent human study requirements. The mechanism of action of probiotics is strain dependent and not always well known, which is one of the problems considered by the authorities. The lack of sufficient evidence in healthy individuals is another reason [18]. In addition, live bacteria may not be powerful enough for induce measurable changes in healthy individuals which are necessary to obtain health claims [35]. Therefore, as proposed by Kumar et al. (2015), suitable scientific evidence is needed in different areas including genome information, antibiotic resistance profile, and selection criteria. Clearly defined target population is also [36].

Probiotic bacteria that are originally isolated from human milk are particularly interesting because they fulfil the main requirements recommended for human probiotics, such as human origin, a history of safe prolonged intake by a particularly sensitive population (infants), and they are adapted to reside in the human digestive tract and to interact with us in symbiosis [12].

Probiotics benefits are related with their ability to modulate intestinal microbiota of the host preventing or limiting pathogen colonization by bacteriocins and/or other metabolite production [21,37], and their improvement of barrier function of intestinal mucosa and immune and inflammatory responses of the host [38]. Moreover, specific probiotics are able to improve digestion by enzyme production as beta-galactosidase [1,39].

Several new probiotics have also been investigated and for examples *Clostridum butyricum*, a species previously used in animal feeds, has a long history of use in Japan and was recently accepted as a novel food in Europe [40]. Similarly, the safety evaluation and novel food approval was given by EFSA (2015) for heat inactivated *Bacteroides xylanisolvens* [40], but as the cells are not viable the product does not confirm to the probiotic definition [33]. It remains to be seen how the work on this species will progress in the future. Among probiotic candidates, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* have raised a lot of interest in the area of weight management [41, 42] and the potential safety assessment and human studies need to be completed.

Another direction is the use of probiotics to select defined mixed populations of faecal or intestinal bacteria from healthy subjects. Preparations such as 'RePOOPulate' have been studied already in human trials and they have investigated the use of a stool substitute preparation. The preparation, which may fulfill the requirements of a probiotic, was made from purified intestinal bacterial cultures derived from a single healthy donor, to treat recurrent *Clostridium difficile* infection [43,44].

Instead of fecal transplants, the defined microbial populations used to re-populate the intestinal tract may offer future solutions to serious intestinal dysbiosis problems such as recurrent or chronic *Clostridium difficile* [44]. Taken together, a large number of microbial preparations are considered for future use in both nutrition and medicine.

5. Prebiotics

The prebiotic concept refers to non-digestible food components which are selectively metabolized by intestinal health-promoting bacteria stimulating their growth and their activity [21]. But the definition of prebiotics changes between different organizations depending on parameters related to selectivity, site of action, grade of causality or association proved, and the requirement of fermentation or metabolism [45,46]. Recently, with the aim to clarify the prebiotic concept, Bindels et al. (2015), proposed to define prebiotic as "a non-digestible compound that, through its metabolism by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host" [21].

The mechanisms through prebiotics exert their benefits are: 1) selective stimulation of the growth and/or activity of intestinal bacteria associated with health, mainly Lactobacilli and Bifidobacteria [47]; and 2) production of short chain fatty acids (SCFAs), particularly butyrate, which have antimicrobial activity by reduction of intestinal pH and other immunological and physiological activities [21].

Most of the studies on prebiotics have been focused on fructans, such as inulin-type fructans, fructooligosaccharides (FOS) galactooligosaccharides (GOS) [47], and lactulose [48]. The location where they seems to exert their activity depend of their degree of polymerization (DP), acting in proximal areas of the colon those with a low DP, and in distal areas those with a high DP [49].

Several new sources of prebiotics and plants with high fructooligosaccharide content are under investigation. For example *Agave*-derived fructans [49, 50], dextran oligosaccharides [27], gluco-oligosaccharides, xylo-oligosaccharides, gluco-omannan, and galactoglucomannan oligosaccharides and Yacon root from South America are under investigation [51,52]. As has been suggested by Jakobsdottir and associates (2014), the development of future prebiotics should take into account their capacity to alter the gut microbiota and SCFA profiles; together with their ability to decrease systemic inflammation; increase satiety; and reduce oxidative stress and gastric emptying [26].

6. How probiotics and prebiotics may help?

6.1. Allergy and immune related diseases

Several studies correlate commensal microorganisms to balanced response of the immune system and oral tolerance acquisition. In infants, the establishment of normal microbiota is fundamental for the normal development of the immune system. Thus the modulation of infant gut microbiota by prebiotics and probiotics may have a broad influence on the immune response of the host [53]. In particular, dysregulation of T helper cell response is associated with allergy and autoimmune diseases [54].

Probiotics and prebiotics and allergy prevention is likely the most studied area in terms of health benefits. Specific probiotics or probiotic combinations, given either as a foods or supplements or in foods, have been evaluated in randomized double-blind controlled trials for primary prevention of allergic disease in infants. Such trials have been conducted using prenatal, post natal or perinatal administration of defined probiotic strains and not all strains or settings have been successful. The most convincing studies have applied perinatal administeration probiotics [55,56]. Recent World Allergy Organization (WAO) guidelines suggest that currently available evidence does not have a strong support for prevention of allergies by probiotic administration. However, WAO guideline panel determined that there is a likely net benefit from using probiotics resulting primarily from prevention of eczema. The WAO guideline panel suggests: a) using probiotics in pregnant women at high risk for having an allergic child; b) using probiotics in women who breastfeed infants at high risk of developing allergy; and c) using probiotics in infants at high risk of developing allergy [55].

Previous studies with FOS and GOS revealed low evidence in the prevention of eczema, as potential specific FOS/GOS combination benefit for infants at high risk of allergy [57]. Therefore, further research is required to document this effect in different populations. Inside this new kind of prebiotics, *Agave*-derived fructans increase expression of *FOXP3* transcription factor, which may influence reported imbalances of T helper cell response [50].

6.2. Obesity and metabolic syndrome

The number of cases of obesity and metabolic syndrome (characterized by obesity, hyperlipidemia, hypertension, insulin resistance, and type 2-diabetes) are doubled worldwide since 1980 [26,58]. Although the major cause of obesity is excessive energy intake and reduced energy expenditure, other factors contribute to the onset of obesity and its associated disorders. Among this factors which are able to impact the host response to nutrients, the gut microbiota represents an important one [58]. Development of overweight and obesity has been associated with early variations in microbiota development including both richness and diversity of microbiota [59]. Differences in microbiota have been reported in both pregnant women and their infants later gaining weight and becoming obese, being suggested that in breast-fed infants low levels of Bifidobacteria may predict later overweight and weight gain [60,61]. Therefore, the use of probiotics containing Bifidobacterium and Lactobacillus to ameliorate obesity and associated metabolic disorders has been shown to exert beneficial effects [62].

Obesity and high body weight can be altered by the consumption of dietary fibres not only through their satiating abilities and fat-fibre complex formation, but also by a SCFA-mediated physiological effect which are thought to influence satiety and energy intake [26,27]. Moreover this effect is coupled with microbiota modulation and commonly associated with a reduction in body weight, body fat and adipocyte size [62].

Recent studies demonstrate prebiotic potential of dextran oligosaccharides and xylooligosaccharides in increasing *Bifidobacterium spp*. and SCFA concentrations in obese subjects [27,51], who has been reported to have low number of Bifidobacterium [63]. Moreover, a recent review suggest that specific prebiotics may help modulating subjective satiety, reducing total energy consumption, reducing ghrelin concentration and reducing body weight in long duration trials, but future studies are necessary [19].

In obese patients, a recent study report changes in microbiota after caloric restriction and intervention with a mix of lactic acid bacteria, showing an increase in Bifidobacteria, Akkermansia and *Faecalibacterium prausnitzii* [41]. Further research with the aim to obtain more clinical evidence will be of great interest to guide modification of the microbiota by probiotics and prebiotics.

7. Regulatory aspects

Novel foods and ingredients are regulated in a different ways in different countries. The majority of evaluations systems are based on a risk or safety assessment reviews and most regulations require both notification and approval by a regulatory authority. Lists of approved novel food decisions are maintained by regulators and are made publicly available for instance in EU and Canada.

In general, safety assessment of new or novel probiotics and prebiotics is required worldwide. Regulations in European Union consider new probiotics from two different standpoints, safety and health benefits for health claims. Safety is very much directed according to the EFSA following regulation on novel foods (2015) and regulation on health claims [2]. Novel foods, i.e. foods and food components of processes not used in Europe prior to 1997 must undergo safety assessment prior to entering the market. A new probiotic may fall into two safety assessment categories as some microbial

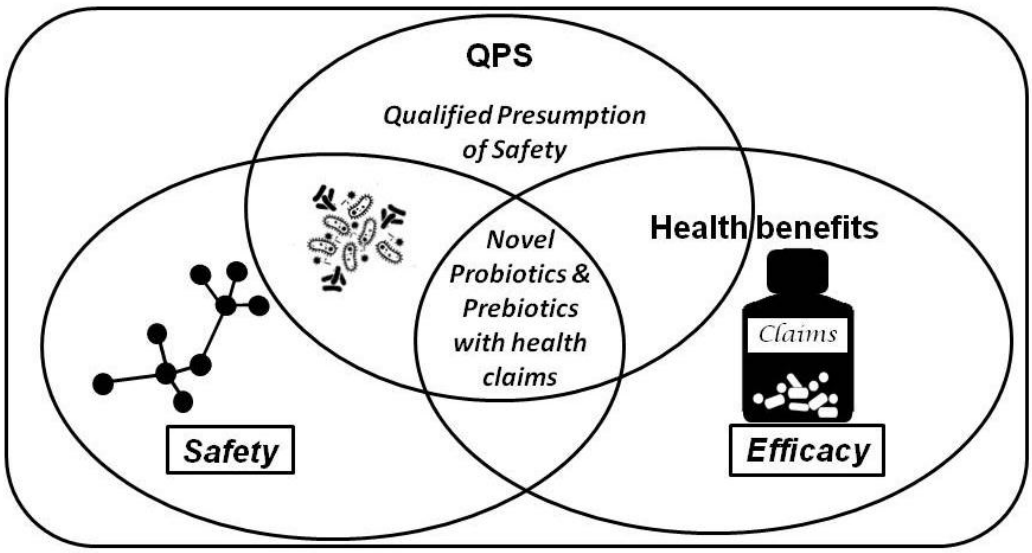

Figure 3. Differences between novel probiotic and prebiotic safety assessment in European Union (foods not previously consumed to a significant degree, and evaluation for safety either as live microbes or other novel components) and probiotics and prebiotic with health claims (evaluated for efficacy; adapted from Kumar et al., [36]).

species have been assessed by the EFSA as Qualified Presumption of Safety (QPS) and do not require an

extended safety assessment [40]. For example, bacteria isolated from human milk, species like *Lactobacillus gasseri*, *Lactobacillus salivarius*, *Lactobacillus reuteri*, *Lactobacillus fermentum* or *Biffidobacterum breve* are considered to have probiotic potential and enjoy the QPS status. Others, not belonging to the QPS status species need to be evaluated according to the new novel food regulation.

All probiotics, if a probiotic status is desired, need to be assessed for health effects in addition to safety, and this requires a number of human studies. The overlap of the three assessment systems is described in Figure 3. Apart from the QPS system, similar requirements of safety assessment are placed also on novel prebiotics. When considering other countries, Health Canada assesses the safety of all genetically-modified and other novel foods proposed for sale in Canada and also publishes a list of decision concerning novel foods and ingredients.

It is important also to consider the previous use of a novel probiotic or prebiotic worldwide and the newly revised European novel food regulation takes into consideration the "history of use" of new foods or food components outside Europe.

8. Conclusions

Taken together, the area of new probiotics and prebiotics is developing rapidly and benefits from intestinal microbiota research and new ways of dietary modulating microbiota development and activity. The expanding database on both mechanisms of action and clinical demonstrations in the area uncovers new possibilities of reducing the risk of both human and animal diseases by microbiota modulation. Human milk is an example of a bioactive food which contains both microbial and oligo-saccharide components which have potential in probiotic and prebiotic use. Understanding the mechanisms of action of these components provides new means of nutritional treatment and prevention modalities which will be able to improve human health.

In the future, more specific microbes and microbial combinations are likely to be introduced and the same applies also to new prebiotic components and compositions.

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6. Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. The funders had no role in the design, analysis, or writing of this article.

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