

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

Carlos Gómez Gallego, Seppo Salminen

Functional Foods Forum, Faculty of Medicine, University of Turku, Itäinen Pitkätatu 4 A, 20014, Turku, Finland.

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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Correspondence to

Carlos Gómez Gallego
Postdoctoral researcher
Functional Foods Forum
Faculty of Medicine
University of Turku,
Turku, Finland.
Tel: +358-23336820
Fax: +358-23336862
E-mail: cargom@utu.fi

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 E-

uropean 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].

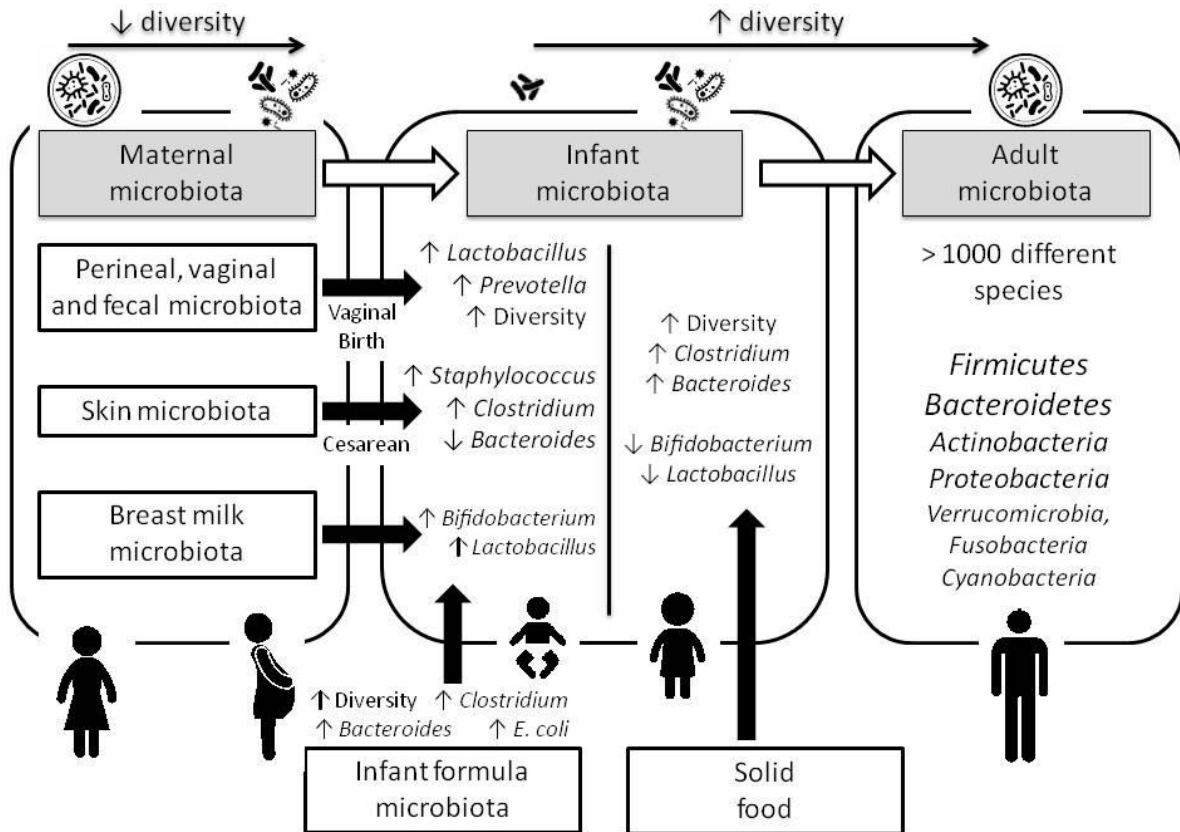


Figure 1. Changes in intestinal microbiota during life (adapted from Isolauri et al., [61]). Maternal microbiota changes between the first and third trimester during pregnancy and the first months postpartum, may be to promote transfer of specific strains to the infant [3]. During the first months of 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 matures 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].

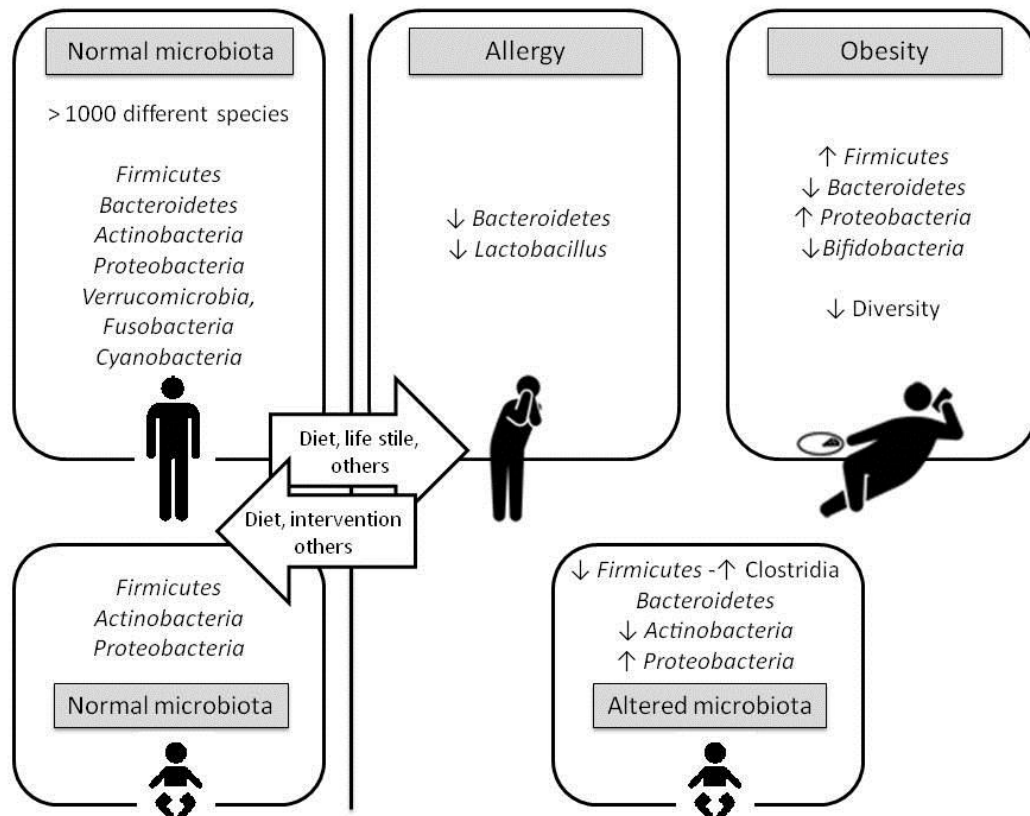


Figure 2. Changes in microbiota. Diet could have a great impact changing key populations in microbiota, which may increase the susceptibility to some diseases. Moreover, other factors related with life style or diseases like stress or antibiotic consumption may have an impact on microbiota. Specific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

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 administration 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

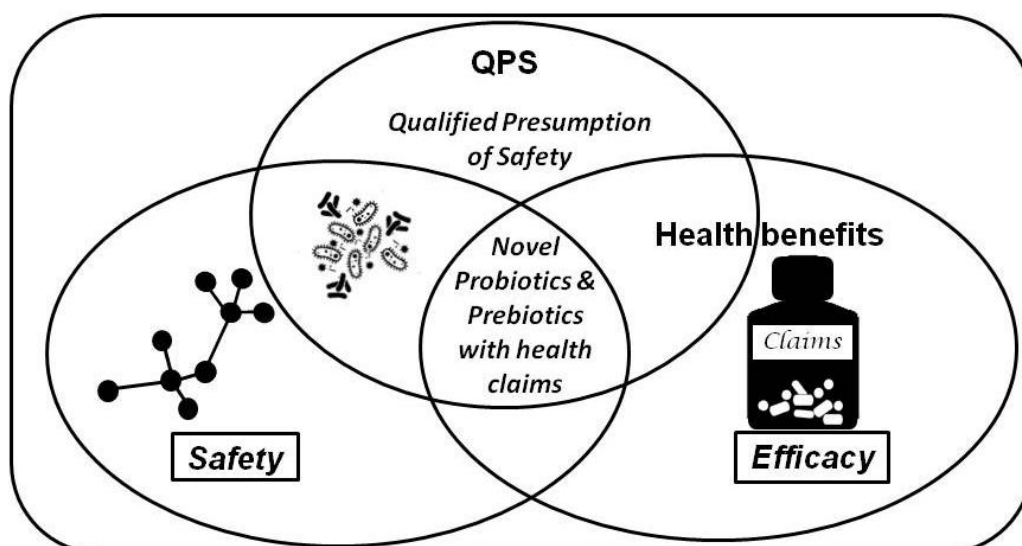


Figure 3. Differences between novel probiotic and prebiotic safety assessment in European Union (foods not previously consumed to a significant degree, and evaluation for safety either as live microbes or other novel components) and probiotics and prebiotic with health claims (evaluated for efficacy;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

References

1. Butel MJ. Probiotics, gut microbiota and health. *Med Mal Infect.* 2014; 44: 1-8.
2. Regulation (EU) 2015/2283 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.
3. Commission Regulation (EC) No 353/2008 (OJ L109, p11, 19/04/2008) of 18 April 2008 establishing implementing rules for applications for authorisation of health claims as provided for in Article 15 of Regulation (EC) No 1924/2006 of the European Parliament and of the Council. 2008.
 4. van Best N, Hornef MW, Savelkoul PHM, Penders J. On the Origin of Species: Factors Shaping the Establishment of Infant's Gut Microbiota. *Birth Defects Res C Embryo Today*. 2015; 105(4): 240-251.
 5. D'Argenio V, Salvatore F. The role of the gut microbiome in the healthy adult status. *Clin Chim Acta*. 2015; 451: 97-102.
 6. Subramanian S, Blanton LV, Frese SA, Charbonneau M, Mills DA, Gordon JI. Cultivating Healthy Growth and Nutrition through the Gut Microbiota. *Cell*. 2015; 161: 36-48.
 7. Jordan F, Lauria M, Scotti M, Nguyen TP, Praveen P, Morine M, Priami C. Diversity of key players in the microbial ecosystems of the human body. *Sci Rep*. 2015; 5: 10.
 8. Rodriguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, Avershina E, Rudi K, Narbad A, Jenmalm MC, Marchesi JR, Collado MC. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis*. 2015; 26: 26050-26050.
 9. Cabrera-Rubio R, Carmen Collado M, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr*. 2012; 96: 544-551.
 10. Salminen S, Gibson GR, McCartney AL, Isolauri E. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut*. 2004; 53: 1388-1389.
 11. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U S A*. 2011; 108: 4578-4585.
 12. Fernandez L, Langa S, Martin V, Maldonado A, Jiménez E, Martín R, Rodríguez JM. The human milk microbiota: Origin and potential roles in health and disease. *Pharmacol Res*. 2013; 69: 1-10.
 13. Ballard O, Morrow AL. Human Milk Composition Nutrients and Bioactive Factors. *Pediatr Clin North Am*. 2013; 60: 49-74.
 14. Ottman N, Smidt H, de Vos WM, Belzer C. The function of our microbiota: who is out there and what do they do? *Front Cell Infect Microbiol*. 2012; 2 (104): 1-11.
 15. Faith JJ, McNulty NP, Rey FE, Gordon JI. Predicting a Human Gut Microbiota's Response to Diet in Gnotobiotic Mice. *Science*. 2011; 333: 101-104.
 16. Huttenhower C, Gevers D, Knight R, *et al*. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012; 486: 207-214.
 17. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J; MetaHIT Consortium, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariac G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Mérieux A, Melo Minardi R, M'rimini C, Muller J, Oozeer R, Parkhill J, Renault P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P. Enterotypes of the human gut microbiome. *Nature*. 2011; 473(7346): 174-180.
 18. McFarland LV. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. *BMJ Open*. 2014; 4 (8): 1-18.
 19. Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br J Nutr*. 2014; 111: 1147-1161.
 20. Ducatelle R, Eeckhaut V, Haesebrouck F, Van Immerseel F. A review on prebiotics and probiotics for the control of dysbiosis: present status and future perspectives. *Animal*. 2015; 9: 43-48.
 21. Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol*. 2015; 12: 303-310.
 22. Rautava S, Luoto R, Salminen S, Isolauri E. Microbial contact during pregnancy, intestinal colonization and human disease. *Nat Rev Gastroenterol Hepatol*. 2012; 9: 565-576.
 23. He F, Ouwehand AC, Isolauri E, Hashimoto H, Benno Y, Salminen S. Comparison of mucosal adhesion and species identification of bifidobacteria isolated from healthy and allergic infants. *FEMS Immunol Med Microbiol*. 2001; 30: 43-47.
 24. Gueimonde M, Laitinen K, Seppo S, Isolauri E. Breast milk: a source of bifidobacteria for infant gut development and maturation? *Neonatology*. 2007; 92 (1): 64-66.
 25. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clément K, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T; MetaHIT consortium, Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. *Nature*. 2013; 500: 541-546.

26. Jakobsdottir G, Nyman M, Fak F. Designing future prebiotic fiber to target metabolic syndrome. *Nutrition*. 2014; 30: 497-502.
27. Sarbini SR, Kolida S, Deaville ER, Gibson GR, Rastall RA. Potential of novel dextran oligosaccharides as prebiotics for obesity management through in vitro experimentation. *Br J Nutr*. 2014; 112: 1303-1314.
28. Bisgaard H, Li N, Bonnelykke K, Chawes BL, Skov T, Paludan-Müller G, Stokholm J, Smith B, Krogfelt KA. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J Allergy Clin Immunol*. 2011; 128: 646-652.
29. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy*. 2014; 44: 842-850.
30. Ling Z, Li Z, Liu X, Cheng Y, Luo Y, Tong X, Yuan L, Wang Y, Sun J, Li L, Xiang C. Altered Fecal Microbiota Composition Associated with Food Allergy in Infants. *Appl Environ Microbiol*. 2014; 80: 2546-2554.
31. Penders J, Stobberingh EE, Van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders. *Allergy*. 2007; 62: 1223-1236.
32. Compare D, Nardone G. The role of gut microbiota in the pathogenesis and management of allergic diseases. *Eur Rev Med Pharmacol Sci*. 2013; 17: 11-17.
33. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014; 11: 506-514.
34. Belizario JE, Napolitano M. Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. *Front Microbiol*. 2015; 6: 1-16.
35. Gosalbez L, Ramon D. Probiotics in transition: novel strategies. *Trends Biotechnol*. 2015; 33: 195-196.
36. Kumar H, Salminen S, Verhagen H, Rowland I, Heimbach J, Bañares S, Young T, Nomoto K, Lalonde M. Novel probiotics and prebiotics: road to the market. *Curr Opin Biotechnol*. 2015; 32: 99-103.
37. Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin Production: a Probiotic Trait? *Appl Environ Microbiol*. 2012; 78: 1-6.
38. Andrade MER, Araujo RS, de Barros PAV, Soares AD, Abrantes FA, Generoso Sde V, Fernandes SO, Cardoso VN. The role of immunomodulators on intestinal barrier homeostasis in experimental models. *Clin Nutr*. 2015; 34: 1080-1087.
39. Khurshid M, Aslam B, Nisar MA, Akbar R, Rahman H, Khan AA, Rasool MH. Bacterial munch for infants: potential pediatric therapeutic interventions of probiotics. *Future Microbiol*. 2015; 10: 1881-1895.
40. EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. 2: Suitability of taxonomic units notified to EFSA until March 2015. *EFSA J*. 2015; 13(6): 4138.
41. Remely M, Hippe B, Geretschlaeger I, Stegmayer S, Hoefinger I, Haslberger A. Increased gut microbiota diversity and abundance of *Faecalibacterium prausnitzii* and *Akkermansia* after fasting: a pilot study. *Wien Klin Wochenschr*. 2015; 127(9-10): 394-398.
42. Schneeberger M, Everard A, Gomez-Valades AG, Matamoros S, Ramirez S, Delzenne NM, Gomis R, Claret M, Cani PD. *Akkermansia muciniphila* inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Sci Rep*. 2015; 5: 1-14.
43. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K, Allen-Vercoe E. Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: 'RePOOPulating' the gut. *Microbiome*. 2013; 1: 1-12.
44. Allen-Vercoe E, Petrof EO. Artificial stool transplantation: progress towards a safer, more effective and acceptable alternative. *Expert Rev Gastroenterol Hepatol*. 2013; 7: 291-293.
45. Pineiro M, Asp NG, Reid G, Macfarlane S, Morelli L, Brunser O, Tuohy K. FAO Technical Meeting on Prebiotics. *J Clin Gastroenterol*. 2008; 42(2): 156-159.
46. Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, Dubert-Ferrandon A, Gareau M, Murphy EF, Saulnier D, Loh G, Macfarlane G, Delzenne N, Ringel Y, Kozianowski G, Dickmann G, Lenoir-Wijnkoop I, Walker C, Buddington R. Dietary prebiotics: current status and new definition. *Food Sci Tech Bull Funct Foods*. 2010; 7 (1): 1-19.
47. Gibson GR, Probert HM, Van Loo J, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nut Res Rev*. 2004; 17: 259-275.
48. Ait-Aissa A, Aider M. Lactulose: production and use in functional food, medical and pharmaceutical applications. Practical and critical review. *Int J Food Sci Technol*. 2014; 49: 1245-1253.
49. Allsopp P, Possemiers S, Campbell D, Saldana Oyarzabal I, Gill C, Rowland I. An exploratory study into the putative prebiotic activity of fructans isolated from *Agave angustifolia* and the associated anticancer activity. *Anaerobe*. 2013; 22: 38-44.
50. Moreno-Vilet L, Garcia-Hernandez MH, Delgado-Portales RE, Corral-Fernandez NE, Cortez-Espinosa N, Ruiz-Cabrera MA, Portales-Perez DP. In vitro assessment of agave fructans (*Agave salmiana*) as prebiotics and immune system activators. *Int J Biol Macromol*. 2014; 63: 181-187.
51. Rastall RA, Gibson GR. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Curr Opin Biotechnol*. 2015; 32: 42-46.
52. Polari L, Ojansivu P, Makela S, Eckerman C, Holmbom B, Salminen S. Galactoglucomannan Extracted from Spruce (*Picea abies*) as a Carbohydrate Source for Probiotic Bacteria. *J Agric Food Chem*. 2012; 60: 11037-11043.
53. Choque Delgado GT, Cunha Tamashiro WMdS, Marostica Junior MR, Moreno YMF, Pastore GM. The

- putative effects of prebiotics as immunomodulatory agents. *Food Res Int.* 2011; 44: 3167-3173.
54. Gustafsson K, Willebrand E, Welsh M. Absence of the adaptor protein Shb potentiates the T helper type 2 response in a mouse model of atopic dermatitis. *Immunology.* 2014; 143: 33-41.
 55. Rautava S, Kainonen E, Salminen S, Isolauri E. Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant. *J Allergy Clin Immunol.* 2012; 130: 1355-1360.
 56. Fiocchi A, Pawankar R, Cuello-Garcia C, Ahn K, Al-Hammadi S, Agarwal A, Beyer K, Burks W, Canonica GW, Ebisawa M, Gandhi S, Kamenwa R, Lee BW, Li H, Prescott S, Riva JJ, Rosenwasser L, Sampson H, Spigler M, Terracciano L, Vereda-Ortiz A, Wasserman S, Yepes-Nuñez JJ, Brożek JL, Schünemann HJ. World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics. *World Allergy Organ J.* 2015; 8 (4): 1-13.
 57. Osborn DA, Sinn JKH. Probiotics in infants for prevention of allergy. *The Cochrane database of systematic reviews.* 2013; issue 3: 1-60.
 58. Cani PD. Gut microbiota and obesity: lessons from the microbiome. *Brief Funct Genomics.* 2013; 12: 381-387.
 59. Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. *Genes Nutr.* 2011; 6: 209-240.
 60. Kalliomaki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr.* 2008; 87: 534-538.
 61. Isolauri E, Rautava S, Collado MC, Salminen S. Early microbe contact in defining child metabolic health and obesity risk. In: Hester LGaR, ed. *Obesity: Intergenerational Programming and Consequences*: Springer, In press.
 62. Gerard P. Gut microbiota and obesity. *Cell Mol Life Sci.* 2016; 73: 147-162.
 63. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr.* 2008; 88: 894-899.