Original Article

Intestinal colonisation, microbiota and future probiotics?

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The human intestine is colonized by a large number of microorganisms, collectively termed microbiota, which support a variety of physiological functions. As the major part of the microbiota has not yet been cultured, molecular methods are required to determine microbial composition and the impact of specific dietary components including probiotics. Probiotics are viable microbial food supplements, which have a beneficial impact on human health. Health-promoting properties have been demonstrated for specific probiotic products. The most significant demonstrations for probiotic efficacy include prevention and treatment of antibiotic associated diarrhea, rotavirus diarrhea and allergy prevention. *Lactobacillus rhamnosus* GG (=ATCC 53103) and *Bifidobacterium lactis* Bb12 are the among the best-characterized and most studied probiotic strains with demonstrated impact on human health. New complex targets for probiotics include irritable bowel syndrome and *Helicobacter pylori* infection. For future probiotics the most important target is a demonstrated clinical benefit supported by knowledge on the mechanistic actions in the microbiota of the target population. Molecular and genomics-based knowledge of the composition and functions of the microbiota, as well as deviations from the balanced microbiota, will advance the selection of new and specific probiotics. Potential combinations of specific probiotics may prove to be the next step to reduce the risk on intestinal diseases and reconstruct specific microbial deviations.

Key Words: Intestinal microbiota, diversity, genomics, probiotic, clinical studies, *Lactobacillus* GG

Introduction

The development of intestinal microbiota in the human gastrointestinal tract depends on the original inoculum at birth, living environment and the early feeding practises. Microbial colonization of the human infant begins at birth and continues during the early feeding and weaning periods, and results in relatively stable microbial communities in adults.[1] The mature intestinal microbiota forms a physical and immunological barrier between the host and the environment. The barrier function of microbiota appears to support the intestinal health and protect the host by a healthy microecology in the gut.[1]

The early and matured intestinal microbiota are unique to each human being. From birth on, during breast feeding and weaning the microbiota diversifies and becomes stable with complex metabolic functions and it facilitates a barrier protecting the host against microbial and other invasions from the environment.[1-4] There is a great need to further characterize the normal and aberrant microbiota in humans. New molecular methods have been developed for this work and more details are revealed at present. The importance of resident bacteria for the host's physiology include metabolic activities, trophic effects on the intestinal epithelium and protection of the host against the overgrowth of potential pathogens in the gastro-intestinal tract.[2,3] Above these effects, specific strains of the gut microbiota have been found to elicit anti-inflammatory responses in the intestinal epithelial cells thus strengthening the intestinal homeostasis.

Probiotics are defined as "viable microbial food supplements, which, when taken in the right doses, beneficially influence human health".[5,6] These definitions require that safety and efficacy have to be scientifically demonstrated for each strain. Probiotics often act upon modifying the process of intestinal microbiota development or the composition and activity of fully developed microbiota. Probiotics can also act by direct contact with the mucosal cells facilitating cross talk between the host and microbes. Current probiotics have several demonstrated beneficial effects on human health. These include maintenance of healthy intestinal microbiota development and counteracting deviations observed in gut inflammatory diseases or preceding them.

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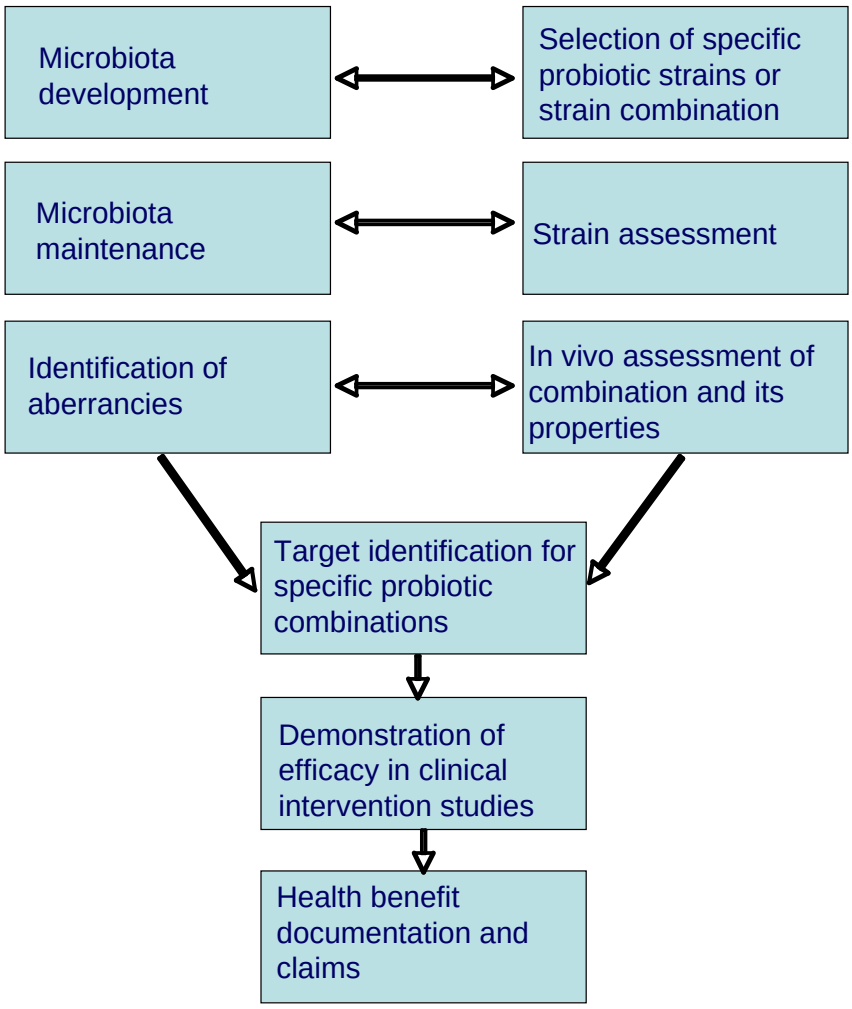

Figure 1. Steps for selecting new target-specific probiotics and probiotic combinations.

Aberrant intestinal microbiota – can we define and influence it?

Several deviations in the intestinal microbiota render us vulnerable to intestinal inflammatory diseases. Various deviations that predispose us to gastrointestinal problems have been characterised. As the methods to characterize the microbiota improve, more targets for intervention will be described to form a basis for probiotic action. Specific *Bifidobacterium* species in the healthy infant gut are most predominant and metabolically active and also specific *Clostridium* spp. are often present.[1] Changes in their quantitative and qualitative composition appear to serve as useful indicators of deviations from the balanced microbiota, denoted here as abberrancies. However, other specific bacterial genera and species remain to be defined among the developing intestinal microbiota that impact on both early and later infant health. It has been reported that specific deviations in intestinal microbiota (such as, for example, decreased numbers and an atypical composition of bifidobacteria and aberrancies in the *Clostridium* content and composition) may predispose infants to allergic disease.[7] In a similar manner, deviations from the normal microbiota are associated with frequent antibiotic side-effects and microbiota changes. Aberrant microbiota during childhood may comprise a factor predisposing to both inflammatory gut diseases and diarrhoea.[7,8] The differences in faecal bacterial population between irritable bowel syndrome (IBS), one of the most common gastrointestinal disorders, and control subjects have been reported in several studies. These observations suggest that *Clostridium* spp., some *Bifidobacterium* spp. and the instability of the active predominant faecal bacterial population, may be involved in IBS.[8-10] Such deviations again, may form the target for probiotics in the future. In conclusion, advanced understanding of the quantitative and qualitative aspects of microbiota composition is expected to enhance our knowledge basis for gut-associated diseases and their clinical impact.

Developing future probiotic combinations

It has been suggested that the intestinal microbiota consists of mainly symbiotic microorganisms.[11] These dedicate part of their genomes to processes that are beneficial to the host. Identification of such processes along with aberrancies in intestinal microbiota will help in the identification and development of new probiotics. The combination of specific probiotics to target new microbiota aberrancies offers the next challenge to the research and development, as single strains have not always been effective for particular target populations. Negative outcomes for probiotic interventions have been reported for irritable bowel syndrome, *Helicobacter pylori* eradi-

cation, travellers diarrhoea and Crohn's disease patients. Probiotic combinations may have additive and synergistic effects, but at times different strains and species may also counteract each other's beneficial effects. These effects have been described *in vitro* and also in this issue.[12] Thus, new *in vitro* tests and innovative approaches are required to facilitate the design of probiotic combinations, as is illustrated here following the outline of Fig. 1.

The properties of probiotic combinations have been studied in detail with two different mixtures of strains and reported also in this issue.[12] Two of the strains in the combinations have been assessed as effective in different types of gastroenteritis.[13,14] These have combined the well-characterized strains *Lactobacillus rhamnosus* GG (=ATCC 53103) and *Bifidobacterium lactis* Bb12, as well as *Bifidobacterium breve* BB99. Additional strains in the combinations assessed include *Lactobacillus rhamnosus* LC 705 and *Propionibacterium freudenreichii* subsp. *shermanii*, which have provided health benefits in human studies on toxin binding and metabolic activity enhancement.[15] Several meta-analysis studies attest to the efficacy of *L. rhamnosuss* GG in clinical trials and efficacy data is available on *B. lactis* either alone or in combination with other probiotics. These are some times additive in their in vitro properties and for instance adhesion can be increased in probiotic combinations. The presence of *L. rhamnosus* s GG significantly enhances the adhesion of *B. lactis* Bb-12 and *P. freudenreichii* strain.[16] The combinations have demonstrated efficacy in the treatment of irritable bowel syndrome[17] (Kajander *et al.*, 2006) and *Helicobacter pylori* infection.[18] In meta-analysis studies also *Lactobacillus plantarum* has been reported to have a trend in decreasing irritable bowel syndrome symptoms. [19]

Although clinical intervention trials provide important proof of efficacy for different probiotics and combinations in various disease states, advancing molecular and genomics-based research will provide data on identification of key processes related to microbiota development and maintenance. These include nutrient-microbe interactions and more detailed chatacterization of the transfer of microbial communities from parent to the infant. Incorporating microbial meta- and post-genomics approaches together with host gene expression data from the exposed mucosal sites or aberrant microbiota activity will advance the understanding the roles of probiotics, microbiota and microbe-microbe and host-microbe interactions. The availability of probiotic genomes will be important for the prediction of the intestinal functions and potential to be used as single strains or in strain combinations. It will also provide information about the mechanisms of action of probiotics, facilitating the development or selection of a new generation of specific probiotic combinations with enhanced and more site-specific and target disorder-specific properties. Such data will reveal new rational improvement of probiotic strains for long-term use. Comparative genomics in combination with expression analysis will allow the assessment of interaction between probiotic, symbiotic, pathogenic microorganisms, providing valuable insight in the features of these different lifestyles. Ultimately, this would increase our understanding of the functional properties of probiotics and their safety as well as the evolutionary relation among them.

Conclusion

The intestinal microbiota in healthy humans is a metabolic organ which provides a defence system against harmful environmental exposures. Deviations in composition can be related to multiple disease states within the intestine but also beyond it. Similarly, components of the human intestinal microbiota or organisms entering the intestine may have both harmful and beneficial effects on human health. This is illustrated with a specific combination of well-established probiotic strains which in this issue of the journal have been demonstrated to have additive and synergistic in vitro properties, have safe history of use and have verified clinical benefits for humans. The future target is to increase the genomic information on both probiotic combinations and microbiota activities to improve the understanding of specific intestinal diseases. Then the goal is to apply the knowledge of intestinal microbiota composition and aberrancies on selecting the right probiotic combinations for defined target populations to maintain healthy intestinal microbiota and to improve human health and well-being.

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腸道移生性、微生物菌叢及益生菌的未來?

人體腸道有許多的微生物體移生，整體稱之為菌群，提供各種的生理功能。當菌群中主要的部分尚未被培養出來時，需要採用分子方法去測量微生物的組成及特殊飲食成分的影響，其中包含益生菌。益生菌可當作微生物的食物補充品，對人體健康有益。特定的益生菌產品已被指出具有健康促進的特性。益生菌最顯著的功效包括：預防及治療抗生素相關的腹瀉、輪狀病毒腹瀉及預防過敏。*Lactobacillus rhamnosus* GG (=ATCC 53103) and *Bifidobacterium lactis* Bb12是兩種對人體的健康研究得最透徹的菌株。益生菌新的標的，包括大腸急躁症及幽門螺旋桿菌感染。對未來的益生菌而言，最重要的標的是對目標族群的菌群，在機械式作用知識支持下，證實其臨床優勢。菌群組成及功能及平衡菌群偏差之分子及基因體相關知識，將促進新的特定益生菌的選擇。潛在的特定益生菌結合體可能是下一步被證實可能降低腸道疾病危險性及重整特定微生物偏差。

關鍵字:益生菌、益生菌結合體、腸道菌群、臨床研究、*Lactobacillus GG*。