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Diversity, vitality and activities of intestinal lactic acid bacteria and bifidobacteria assessed by molecular approaches

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Abstract

While lactic acid bacteria and bifidobacteria have been scientifically important for over a century, many of these are marketed today as probiotics and have become a valuable and rapidly expanding sector of the food market that is leading functional foods in many countries. The human gastro-intestinal tract with its various compartments and complex microbiota is the primary target of most of these functional foods containing lactic acid bacteria and bifidobacteria (LAB&B). In addition, their use as vectors for delivery of molecules with therapeutic value to the host via the intestinal tract is being studied. This review focuses on molecular approaches for the investigation of the diversity of lactic acid bacteria and bifidobacteria in the human intestine, as well as tracking of probiotic bacteria within this complex ecosystem. Moreover, methodologies to determine the viability of the lactic acid bacteria and bifidobacteria and molecular approaches to study the mechanisms by which they adapt, establish and interact with the human host via the digestive tract, are described.

Keywords: Lactic acid bacteria; Molecular techniques; Diversity; 16S rRNA; Viability; Activity; Functionality

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Contents

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1. Introduction

Scientific interest in the lactic acid bacteria and bifidobacteria can be traced back over a century to the pioneering activities of Louis Pasteur, Ilya Mechnikov and Henri Tissier. Pasteur's work at the end of the 19th century illustrated that lactic acid fermentation was due to microorganisms while solving a failed wine production in which lactic acid bacteria replaced the alcohol fermentation of yeast. Mechnikov in fact was most famous for describing phagocytosis, but he proposed the ingestion of lactic acid bacteria in order to promote human health, and essentially founded the probiotic concept in the early days of 1900. At the same time, Tissier discovered the bifidobacteria and speculated about their use as infant probiotics. Probiotics have now been defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [1]. Today, fermented dairy foods supplemented with probiotics have grown into a multi-million Euro business [2]. In most cases, these functional foods include lactic acid bacteria and bifidobacteria (collectively termed LAB&B for this review) that are marketed as probiotics. This explains the large interest in the survival and activity of LAB&B in the human intestine. The food industry and academia continue research in order to understand the physiology, interactions and mechanisms by which probiotic LAB&B provide benefits to the host via the human digestive tract [1,3,4].

The human digestive tract harbours a wealth of niches with many microbial ecosystems that vary according to location of the intestinal tract. Many members of the lactic acid bacteria as well as bifidobacteria naturally form part of this dynamic ecosystem. These include the so-called autochthonous or indigenous ones, as well as the allochthonous LAB&B that are acquired from the environment of which ingested food is presumably the main source. The main lactic acid bacteria found in the human intestine comprise *Lactobacillus* and *Leuconostoc* spp., while *Bifidobacterium* spp. are dominant among the first colonizers of newborns and continue to persist at a low level in adults (see below).

Apart from being indigenous members of the human gut, lactic acid bacteria are found in a plethora of niches, including plant material, fermented dairy, vegetable and meat products and sour dough breads. Foods fermented by lactic acid bacteria are rendered safe by preservation and have improved textures, flavours and tastes. Hence, a variety of lactic acid bacteria, notably *Lactococcus* and *Lactobacillus* spp., are used as starter cultures for the production of fermented foods and persist in these in high numbers. However, many of the lactic acid bacteria that are ingested via consumption of these fermented foods do not survive passage of the human intestinal tract. *Lactococcus lactis* cells used as a starter for industrial cheese production, provide the largest load of living consumed lactic acid bacteria. Studies with a genetically marked *Lactococcus lactis* strain revealed approximately 1% survival in human volunteers followed by a rapid decline after consumption cessation that resembled a first-order kinetics as expected for a stressed population [5]. Hence, specific strains of lactic acid bacteria that are used to enrich probiotic foods are chosen for their resistance to passage through the human gastro-intestinal tract. This also holds for bifidobacteria that are used as probiotics but as their primary niche is the digestive tracts of humans and other animals, survival is less of an issue than culturing them in industrial environments.

All lactic acid bacteria that include among others the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Weisella* belong to the phylum of the *Firmicutes* [6], and include a great number of species that is most numerous for the lactobacilli, for which over 80 species are known [7]. The genus *Bifidobacterium* belongs to the phylum of the *Actinobacteria* and, as such, is not closely related to the lactic acid bacteria [8]. However, all LAB&B are gram-positive bacteria that differ in the G + C content of their genomes: low G + C for the lactic acid bacteria and over 50% G + C content for bifidobacteria. Similar to the lactic acid bacteria, bifidobacteria are fermentative and produce several acids including lactate, and are predominantly catalase negative. Due to their phylogenetic relations, shared metabolic properties, and common food-grade status leading to their incorporation in functional foods, lactic acid bacteria are aligned with the bifidobacteria for the purpose of this review and the term LAB&B is used to collectively indicate both groups of bacteria.

During the last few years novel benefits are being discovered of adding specific LAB&B probiotic strains to our food [1,9–11]. This is not so surprising considering we have co-evolved together with our intestinal microbes over millions of years, and these have been programmed to manipulate networks of genes [12–14]. The intestinal microorganisms, collectively called microbiota, consist of at least 10^{13} microbes and are dominated by anaerobic bacteria, comprising over a 1000 species (see [14–16] for reviews and [17] for a recent inventory). The digestive tract offers a relatively non-hostile environment and supply of nutrients that is produced and consumed by the host. Studies with germ-free and conventional animals that are colonized by intestinal bacteria have shown that the microbiota contributes to diverse processes including roles in host nutrition, intestinal epithelial development and activity, and fat storage, educates the immune system, and maintains the integrity of the mucosal barrier amongst other functions [18–20]. Lactic acid bacteria and bifidobacteria, both the commensal or ingested members, play a role in the intestinal niche. The rising number of genome