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Microbial diversity in the human gut: bifidobacterial prospective

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Microbial world inhabits many parts of Earth by colonizing different ecological niches (e.g. soil, marine communities, human and animal gut). These complex communities interact with human beings and they are narrowly linked to human life for their significance in medicine, engineering, food and agriculture (Sloan et al. 2006). In this context, the gastro-intestinal tract (GIT) of humans is the home of vast arrays of bacterial cells, named microbiota, which are estimated to be more than ten times of the total number of human body cells (Backhed et al. 2005). The microbial populations residing in the distal human intestine are predicted to consist of more than 500 bacterial species and around 1,000 phylotypes, belonging to a limited number of broad taxonomic units (Eckburg et al. 2005; Turroni et al. 2008). This complex microbiota is considered to be crucial for various gut functions, such as host nutrition, regulation of epithelial development, regulation of host fat storage, stimulation of intestinal angiogenesis, inflammatory immune responses and protection against pathogens (Rakoff-Nahoum et al. 2004; Backhed et al. 2004; Stappenbeck et al. 2002; Noverr and Huffnagle 2004; Savage 1977; Corr et al. 2007).

Despite the importance of gut microbiota for human health, there is a big gap of knowledge about the real composition of intestinal microflora and the precise mechanisms operating between microorganisms and microbe-host body.

The study of complex ecosystem has been carried out, until recently, by traditional culture techniques with, at most, biochemical methods to identify organisms (Furrie 2006). Around 1980 microbiologists understood the importance of applying non-traditional techniques to understand the compositions of the microbial world (Olsen et al. 1986). Nowadays, it is estimated that culture recovery is less than 1% of the total bacterial diversity in the most environmental niches (Schmeisser et al. 2007; Riesenfeld et al. 2004). The underestimation of true microbial diversity urged microbiologists to use and develop new methodologies based on DNA molecular tools to find explanations of many interrogatives. In addition, new methods that combine molecular biology and genetics has shown to provide important output respect to classical microbiology. First of all, genome sequencing gives the possibility to reveal the complete genetic make-up of an organism and the combination of genomics and functional genomics techniques allow exploring not only the potentiality but also what microorganisms do really in their environments. Metagenomic technology, among all -omics techniques, is an excellent tool to better analyze microbial communities and it represents a possible key to investigate genome heterogeneity and evolution in environmental contexts. Furthermore, it provides access to a more detailed overview of microbial diversity than it has been viewed by cultivation/isolation on Petri dish (Schmeisser et al. 2007; Handelsman 2004). Recently, many workers have focused their attention on metagenomic analyses of the human gut microbiota (Eckburg et al. 2005; Wang et al. 2005). Metagenomic studies of mucosal and faecal samples retrieved from healthy subjects demonstrated the presence of eight dominant phylogenetic phyla belonging to the *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Spirochaeates* and *Actinobacteria* (Eckburg et al. 2005). Between *Actinobacteria* phylum, *Bifidobacterium* genus is the most abundant bacterial group identified in the gut microbiota of infants (Turroni et al. 2008). Six are the species commonly found in human gut (*Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium catenulatum*) in different quantity according to age, diet and health status. Much attention has been focused on these species by food companies and

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consequently research because of their presumptive healthy promoting activities exerted on human beings. The probiotic concept dates back to 1908 when Metchnikoff observed that the consumption of certain fermented foods had positive effects on human health (Metchnikoff 1908). The first time that the term probiotic was used it was in 1965 by Lilly and Stillwell (Lilly and Stillwell 1965), but the universal accepted definition was coined by Fuller, i.e "Probiotics are live microorganisms which beneficially affect the host by improving the intestinal microbial balance" (Fuller 1989). There is accumulating evidence describing the capacity for probiotic strains to modulate intestinal microflora, immunomodulation, reduction of allergic disease symptoms, alleviation of acute gastro-enteritis, reduction of lactose intolerance, stimulation of immune response, reduction of intestinal inflammation, short chain fatty acid production and alleviation of constipation (Goldin 1998; Saxelin et al. 2005; Ouwehand et al. 2002).

Many interrogatives are nowadays open to discussion even if recently complete genome sequences of few *Bifidobacterium* strains have given a significant start point to explain some genetic and evolutionary aspects (Schell et al. 2002; Lee et al. 2008; Sela et al. 2008; Kim et al. 2009; Barrangou et al. 2009). For example, genomics has provided insights into the capabilities of these microorganisms to breakdown complex carbohydrates in human gut. In fact, human body is poor of catabolic enzymes required for polysaccharides degradation (Sonnenburg et al. 2005). Many of the sugars that escape digestion by the host's enzymes are used as carbon- and energy sources by the various components of the microbiota. Such host's indigested carbohydrates include fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), gluco-oligosaccharides, xylo-oligosaccharides, inulin, starch, arabinoxylan and arabinogalactan, lactulose and raffinose (Guarner and Malagelada 2003). Genomic data has provided important information to understand the genetic mechanisms of these carbohydrates utilization. The genome of *B. longum* subsp. *longum* NCC2705 was shown to contain many genes involved in sugar utilization, in particular a large variety of glycosyl hydrolases (> 40), required for utilization of diverse plant-derived dietary sugars or complex carbohydrates (Schell et al. 2002). Moreover, in several bifidobacterial genomes are present many genes encoding carbohydrate-modifying enzymes that modify, create or degrade glycosidic bonds (Ventura et al. 2007).

Genomic information also provided insights about the capabilities of bifidobacteria to adapt to the GIT environment as well as to interact with their host. In fact, little is known about the molecular basis of interactions between the intestinal host epithelium and bifidobacteria. Bifidobacteria are predicted to encode cell envelope-associated structures which may play a key role in determining microbe-host interaction. All sequenced bifidobacteria appear to encode an extracellular polysaccharide (EPS) or capsular polysaccharide, and such an extracellular structure may be important in bacterial adherence to host cells, while it could also contribute to resistance to stomach acids and bile salts (Perez et al. 1998; Ventura et al. 2007). Moreover, genes predicted to encode glycoprotein-binding fimbria-like structures, which have been identified in the genome sequences of both *B. longum* subsp. *longum* NCC2705 and *B. longum* subsp *longum* DJO10A, may mediate another type of interaction with the host (Klijn et al. 2005; Schell et al. 2002). In addition, *B. longum* subsp *longum* NCC2705 encodes a serpin-like protease inhibitor that has been demonstrated to contribute to host interaction in the GIT (Ivanov et al. 2006). The NCC2705 serpin is an efficient inhibitor of human neutrophil and pancreatic elastases, whose release by activated neutrophils at the sites of intestinal inflammation represents an interesting mechanism of innate immunity (Ivanov et al. 2006). Recently, it has been shown that the serpin gene of *B. breve* is highly induced when bacterial cultures are exposed to various host-derived proteases (Turroni et al. 2010). The protease-inducible serine protease inhibitor synthetized by *B. breve* may have an anti-inflammatory activity that can reduce the negative effects of serine proteases activity at the intestinal inflammation sites (Turroni et al. 2010).

Genome sequencing efforts have provided a vast reservoir of molecular information that promises to revolutionize our understanding of life. However, genome sequences alone provide only limited insights into the biochemical pathways that drive cell functions. All 'omics' approaches may be crucial to identify the precise mechanisms by which bifidobacteria affect human health. Thus, all these studies could help to answer questions about the real impact of many of the so far considered probiotic/commensal bifidobacteria on the composition and function of the human gut microbiota. The knowledge of the molecular mechanisms by which probiotic bacteria interact with their host and with the other microbial components of the human gut microbiota appears to be indispensable for the development of the next probiotic generations.

REFERENCES

Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 101:15718-15723.

Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI (2005) Host-bacterial mutualism in the human intestine. Science 307:1915-1920.

Barrangou R, Briczinski EP, Traeger LL, Loquasto JR, Richards M, Horvath P, Coute-Monvoisin AC, Leyer G, Rendulic S, Steele JL, Broadbent JR, Oberg T, Dudley EG, Schuster S, Romero DA, Roberts RF (2009) Comparison of the complete genome sequences of Bifidobacterium animalis subsp. lactis DSM 10140 and Bl-04. J Bacteriol 191:4144-4151.

Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG (2007) Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. Proc Natl Acad Sci U S A 104: 7617-7621.

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005) Diversity of the human intestinal microbial flora. Science 308:1635-1638.

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66: 365-378.

Furrie E (2006) A molecular revolution in the study of intestinal microflora. Gut 55:141-143.

Goldin BR (1998) Health benefits of probiotics. Br J Nutr 80:S203-207.

Guarner F, Malagelada JR (2003) Gut flora in health and disease. Lancet 361:512-519.

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669-685.

Ivanov D, Emonet C, Foata F, Affolter M, Delley M, Fisseha M, Blum-Sperisen S, Kochhar S, Arigoni F (2006) A serpin from the gut bacterium Bifidobacterium longum inhibits eukaryotic elastase-like serine proteases. J Biol Chem 281:17246-17252.

Kim JF, Jeong H, Yu DS, Choi SH, Hur CG, Park MS, Yoon SH, Kim DW, Ji GE, Park HS, Oh TK (2009) Genome sequence of the probiotic bacterium Bifidobacterium animalis subsp. lactis AD011. J Bacteriol 191:678-679.

Klijn A, Mercenier A, Arigoni F (2005) Lessons from the genomes of bifidobacteria. FEMS Microbiol Rev 29: 491-509.

Lee JH, Karamychev VN, Kozyavkin SA, Mills D, Pavlov AR, Pavlova NV, Polouchine NN, Richardson PM, Shakhova VV, Slesarev AI, Weimer B, O'Sullivan DJ (2008) Comparative genomic analysis of the gut bacterium Bifidobacterium longum reveals loci susceptible to deletion during pure culture growth. BMC Genomics 9:247.

Lilly DM, Stillwell RH (1965) Probiotics: Growth-Promoting Factors Produced by Microorganisms. Science 147:747-748.

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DOI 10.1007/s12263-010-0204-8

Metchnikoff E (1908) The prolungation of life. Optimistic studies.

Noverr MC, Huffnagle GB (2004) Does the microbiota regulate immune responses outside the gut? Trends Microbiol 12:562-568.

Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA (1986) Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol 40:337-365.

Ouwehand AC, Salminen S, Isolauri E (2002) Probiotics: an overview of beneficial effects. Antonie Van Leeuwenhoek 82:279-289.

Perez PF, Minnaard Y, Disalvo EA, De Antoni GL (1998) Surface properties of bifidobacterial strains of human origin. Appl Environ Microbiol 64:21-26.

Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118:229-241.

Riesenfeld CS, Schloss PD, Handelsman J (2004) Metagenomics: genomic analysis of microbial communities. Annu Rev Genet 38:525-552.

Savage DC (1977) Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol 31:107-133.

Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM (2005) Probiotic and other functional microbes: from markets to mechanisms. Curr Opin Biotechnol 16:204-211.

Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, Pessi G, Zwahlen MC, Desiere F, Bork P, Delley M, Pridmore RD, Arigoni F (2002) The genome sequence of Bifidobacterium longum reflects its adaptation to the human gastrointestinal tract. Proc Natl Acad Sci U S A 99:14422-14427.

Schmeisser C, Steele H, Streit WR (2007) Metagenomics, biotechnology with non-culturable microbes. Appl Microbiol Biotechnol 75:955-962.

Sela DA, Chapman J, Adeuya A, Kim JH, Chen F, Whitehead TR, Lapidus A, Rokhsar DS, Lebrilla CB, German JB, Price NP, Richardson PM, Mills DA (2008) The genome sequence of Bifidobacterium longum subsp. infantis reveals adaptations for milk utilization within the infant microbiome. Proc Natl Acad Sci U S A 105:18964-18969.

Sloan WT, Lunn M, Woodcock S, Head IM, Nee S, Curtis TP (2006) Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ Microbiol 8:732-740.

Sonnenburg JL, Xu J, Leip DD, Chen CH, Westover BP, Weatherford J, Buhler JD, Gordon JI (2005) Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 307:1955-1959.

Stappenbeck TS, Hooper LV, Gordon JI (2002) Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc Natl Acad Sci U S A 99:15451-15455.

Turroni F, Foroni E, O'Connell Motherway M, Bottacini F, Giubellini V, Zomer A, Ferrarini A, Delledonne M, Zhang Z, van Sinderen D, Ventura M (2010) Characterization of the serpin-encoding gene of Bifidobacterium breve 210B. Appl Environ Microbiol 76:3206-3219.

Turroni F, Ribbera A, Foroni E, van Sinderen D, Ventura M (2008) Human gut microbiota and bifidobacteria: from composition to functionality. Antonie Van Leeuwenhoek 94: 35-50.

Ventura M, Canchaya C, Fitzgerald GF, Gupta RS, van Sinderen D (2007) Genomics as a means to understand bacterial phylogeny and ecological adaptation: the case of bifidobacteria. Antonie Van Leeuwenhoek 91:351-372.

Wang M, Ahrne S, Jeppsson B, Molin G (2005) Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. FEMS Microbiol Ecol 54:219-231.