

Human gut microbiota - A lifetime history: from birth to adulthood (Riviera Maya, Mexico - June 26th, 2015)

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Summary

The objective of the Meeting was to raise recognition and expand knowledge of the gut microbiota among gastroenterologists, pediatricians and general practitioners in Latin American countries. Recognized international experts shared new findings on a number of topics including microbiota in health and disease, and probiotics in obtaining physiological effects and clinical benefits. This meeting report aims to provide a general overview of the topics discussed and the reader is referred to the cited references to gain further insight into the meeting's content.

Key words. Gut microbiota, gut-brain axis, probiotics, *saccharomyces boulardii* CNCM I-745.

Microbiota intestinal humana - Una historia de vida: del nacimiento a la edad adulta (Riviera Maya, México - Junio 26, 2015)

Resumen

El objetivo de la reunión fue ampliar el conocimiento de la microbiota intestinal entre los gastroenterólogos, pediatras y médicos generales en los países de América Latina. Reconocidos expertos internacionales compartieron los nuevos conocimientos sobre una serie de temas, incluyendo la microbiota en la salud y la enfermedad, así como efectos fisiológicos y beneficios clínicos de los probióticos. Este informe de la reunión tiene como objetivo proporcionar una visión general de los temas tratados y remitir al lector las referencias citadas para obtener una mayor comprensión del contenido de la misma.

Palabras claves. Microbiota intestinal, eje cerebro-intestinal, probióticos, *saccharomyces boulardii* CNCM I-745.

Role of the gut microbiota in human health

Of the 100 trillion cells inside each one of us, 90% are not ours but aliens: bacteria, fungi, and other microbes.^{1, 2} The human gastrointestinal tract harbors one of the most complex and abundant ecosystems colonized by more than 100 trillion microorganisms. The gut microbiota of young adults have higher proportions of firmicutes, whereas the elderly have a higher proportion of

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bacteroidetes.^{3, 5, 6} Two plenary sessions were respectively dedicated to review: (i) structural and functional aspects of the human gut microbiota, and (ii) changes in the microbial composition along the life cycle.

The key note address was given by Professor Francisco Guarner. Professor Guarner discussed the structure and functions of the human gut microbiota.³ Bacterial or fungal symbionts have evolutionarily adapted to provide the required organic compounds (essential amino acids and vitamins) and the ability to obtain energy from different sources.^{7, 8} The gut microbiota influences host metabolism,⁸ physiology⁹⁻¹² and immune system development.¹³ The overall structure of predominant genera in the human gut can be assigned into three robust clusters, which are known as 'enterotypes'.^{5, 14-16} Each of the three enterotypes is identifiable by the levels of one of three genera: bacteroides (enterotype 1), prevotella (enterotype 2) and ruminococcus (enterotype 3). Diet and antibiotics have an important impact on the structure and function of the intestinal microbiota.¹⁷ The bacteroides enterotype is associated with diets enriched in protein and fat. In contrast, the prevotella enterotype is linked to diets with predominance of fibres, carbohydrates and sugars. Use of antibiotics induces a decrease in microbial diversity (loss of richness in the ecosystem) and overgrowth of resistant species. Immense populations of viruses live in the human gut.¹⁸ They are mostly unique to each individual, and define the structure of gut bacterial communities by predation: every day, 7.5% of gut bacteria are killed by predators. Perturbations of the gut microbial ecosystem during infancy combined with genetic susceptibility may have a long-lasting impact on the immune system leading to disease or predisposition to disease later in life.¹⁹

Professor Luis Bustos Fernández followed on with a report on human gut microbiota onset and shaping through life stages. Up to date, the majority of studies have focused on the adult population, where the gut microbiota shows great stability and resistance to change. However, it appears unstable throughout childhood and in later stages of life.²⁰ At birth, the child is colonized by bacteria originating mostly from the mother and from the outside environment.²¹ The neonatal gut microbiome, beginning in utero, is affected by the nutritional status (breastfed versus formula fed) and gestational age (term versus preterm).²¹ Similarly, during the latter stages of life, the gut microbiota undergoes other pathological changes, which contribute to its further destabilization (high proportion of bacteroidetes, reduction in species diversity, in resistance to environmental fluctuations and in beneficial microorganisms, greater

susceptibility to infections, e.g. *C. difficile*). Therefore, it is during these extreme stages of life where different strategies for modulating the gut microbiota may have a strong impact on health.

The gut-brain axis

Gut and brain originated from the same tissue, the neural crest, and are in constant, bi-directional communication through the vagus nerve, the hypothalamic-pituitary-adrenal axis and the immune system.²²⁻²⁶ The microbiota was recently found to play a role in such "gut-brain axis", thus coining the term "microbiota-gut-brain axis".²²⁻²⁶

Doctors Luis Bustos Fernández and Jaime Ramírez-Mayans reviewed recent evidence in favor of a microbiota-gut-brain axis.²²⁻²⁶ Thus, differences in behavior and stress responses were reported between germ-free and conventional mice.^{10, 27, 28} The behavioral phenotype can be transferred between mice by microbiota transplantation.²⁵ Selected probiotics can modify brain activity and behavior in animals and humans.^{25, 29} Antibiotic or diet perturbations of the gut's flora (dysbiosis) can modify brain chemistry and behavior.^{30, 31} Doctors Bustos Fernández and Ramírez-Mayans concluded that, acute and chronic stress may alter gut microenvironment and give rise to a dysbiotic microbiota leading to anxious/depressive states. The exact pathways and mediators have not been completely elucidated (bacteria likely modulate behavior through an action on the enteric nervous system neurotransmitters). In the future, microbiota modulation using probiotics and/or symbiotics might play an increasingly important role in treating diseases such as IBS with anxiety and depression comorbidity.

Microbiota and related disorders

In Latin America, the prevalence of type 2 diabetes mellitus in urban areas ranges from 7% to 8%, versus only 1% to 2% in rural areas.^{32, 33} Recent studies have pointed out that, loss of biodiversity in the human gut microbiota is associated with far reaching consequences on host health.³⁴ Several disease states have been associated with changes in the composition of fecal and intestinal mucosal communities.³⁴

Professor Francisco Guarner presented data showing that a perturbed gut microbial colonization might be involved in some chronic non-communicable diseases of increasing incidence in modern society. Individuals with a low bacterial richness (23% of the population) are characterized by more marked overall adiposity, insulin resistance and dyslipidaemia and a more pronounced inflammatory phenotype when compared with high bacte-

rial richness individuals.³⁴ Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome.³⁶ Reduced microbial gene diversity was found in IBD.¹⁶ Low microbial gene diversity and depletion of *Akkermansia mucini-phila* was found associated with a relapsing course of ulcerative colitis.³⁷ Low gene richness and the enterotype bacteroides were found in Crohn's disease.³⁸ Recent studies suggest a role for the microbiota in autism spectrum disorders.³⁹ Diet, probiotics and gut microbiota transplantation are the principal tools in clinical practice for improving host-microbial symbiosis, and warrant further investigation for their ability to restore microbial richness in various disease states.

Professor Aldo Maruy followed on with a report on microbiota, obesity and type 2 diabetes mellitus (T2DM). Mice bred in a "germ-free" environment show 40% less body fat than those with a normal microbiota, despite eating less than 30% of what germ-free mice do.⁴⁰ A 60% increase in body fat and insulin resistance is observed when transplanting caecal content from a normal to a germ-free mouse, despite a decrease in food intake.⁴⁰ Obese mice show a 50% reduction in bacteroidetes with a proportional increase in firmicutes.⁴¹ This ratio changes in lean rats.⁴¹ Similar changes were found in humans⁴² and T2DM patients.⁴³ Gut microbiota in obese individuals and T2DM patients is altered and seems to be more efficient in extracting energy from food. It appears that dietary fat is an important factor which affects gut microbiota composition as well as the gut barrier function and the plasma levels of LPS.^{44, 45} This metabolic endotoxemia would contribute to the development of systemic low grade inflammation, insulin resistance and T2DM.⁴⁴⁻⁴⁶ Modulating gut microbiota through the use of prebiotics, probiotics, antibiotics and fecal transplant might be beneficial by improving glucose metabolism and insulin resistance in the host.^{36, 47-49}

Probiotic strains and products

In 1907, Élie Metchnikoff (Nobel Prize for Physiology in Medicine) suggested that: "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes"⁵⁰ and recommended that people should consume fermented milk containing lactobacilli to prolong their lives, as accelerated aging is due to autointoxication caused by the toxins produced by the gut microflora.⁵¹ There are thousands of different probiotics on the market, with very important differences between bacterial and yeast probiotics.^{52, 53} A plenary session was dedicated to discuss such differences.

Professor Yvan Vandenplas presented data regarding differences between probiotic strains and products. Some bacterial probiotic strains are part of healthy eating.⁵⁴ Probiotic strains have even been isolated from breast milk.^{55, 56} However, most of the strains in (fermented) food are only poorly resistant to gastric acid and other digestive secretions. Survival of strains in commercialized products can be very different. Strains of the same gender may also behave different in commercialized products because of differences in industrial preparation. Some are natural preparations (fermented milk products like yogurt), others are industrial preparations of fermented milk, others are food supplements commercialized in "health care shops" (encapsulated "medication-like" preparations), but all of these differ from approved medications.

Medicinal products can only be advertised if they possess scientific proof of benefit.⁵² Guidelines for the evaluation of probiotics in food leading to the substantiation of health claims have been edited by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).⁵⁷ Although most probiotics are bacteria (bifidobacteria, lactobacilli), one strain of yeast, *Saccharomyces boulardii* (*S. boulardii*), has been found to be an effective probiotic in double-blind clinical studies.⁵⁸ Conversely, Conway et al⁵⁹ failed to demonstrate that yoghurt has any effect on antibiotic-associated diarrhea.⁶⁰⁻⁶⁴ While many bacterial probiotic strains are poorly resistant to antibiotics, yeast probiotics are resistant to all antibiotics, but then very sensitive to antifungals. These differences may be relevant in the rare cases of sepsis or fungemia. The intrinsic resistance to antibiotics of some bacterial probiotic strains has been shown to be transferable to the gastro-intestinal flora of the host and to pathogens.

S. boulardii was discovered by Henry Boulard in the early 1920s and was first registered as a drug in 1961.⁶⁵ *S. cerevisiae* and *S. boulardii* CNCM I-745 are members of the same species with different metabolic and genetic properties.⁶⁶ One important difference in favor of *S. boulardii* CNCM I-745 is an increased expression of important genes for increased growth rate and better survival in acid pH.⁶⁷

A meta-analysis of data from five randomized-controlled trials showed efficacy of *S. boulardii* in preventing antibiotic-associated diarrhea in children and adults (mainly respiratory tract infections).⁶⁸ Finally, *S. boulardii* has a well investigated mode of action,⁶⁹ the quality of 15 probiotic products containing *S. boulardii* was verified⁷⁰ and the ESPGHAN Working Group for Probiotics and Prebiotics recommended *S. boulardii* for acute gastroenteritis.⁷¹

Professor Yvan Vandenplas concluded that probiotics do differ, and only products that have been clinically tested should be used in medical indications. The (theoretical but existing) possibility of transfer of intrinsic resistance is a valid reason to not use strains that have not been tested for this potential risk.

Saccharomyces boulardii CNCM I-745

This session was a series of 3 interactive workshops dedicated to *S boulardii* CNCM I-745.

Clinical evidence in children

Probiotics have been extensively studied over the past several years in treating sporadic infectious diarrhea in pediatric populations. The vast majority of the published trials show a statistically significant benefit of a few, well-identified probiotic strains, including *S boulardii*.⁷² Probiotics were also found to reduce the risk of antibiotic associated diarrhea (AAD) in children and for every 7-10 patients one less would develop AAD.⁷³

Professor Vandenplas reviewed clinical evidence of *S boulardii* CNCM I-745 in pediatrics. Recently, research has focused on newborn and preterm infants, despite of the fact that *S boulardii* is not registered or indicated in this age group.

A review and meta-analysis showed that *S boulardii* is safe and has clear beneficial effects in children who have acute diarrhea.⁷⁴ Pooling data from 22 trials showed that *S boulardii* significantly reduced the duration of diarrhea, stool frequency on day 2 and day 3, the risk for diarrhea on day 3 and day 4 after intervention compared with control.⁷⁴ A randomized trial in children with acute infectious diarrhea confirmed such results and showed that the mean length of hospital stay was shorter with more than 36 h of difference in the *S boulardii* group (4.60 ± 1.72 vs 6.12 ± 1.71 days, $p < 0.001$).⁷⁵ Moreover, the pooled evidence a total of 82 randomized clinical trials (RCTs) suggested that probiotics are associated with a reduction in AAD.⁷⁶ A randomized trial in 333 hospitalized children with acute lower respiratory tract infection, showed efficacy and safety of *S boulardii* to treat diarrhea and AAD.⁷⁷ A meta-analysis of 11 randomized clinical trials (RCTs; 2200 participants, among them 330 children) showed that the addition of *S boulardii* to the standard triple therapy significantly increased the eradication rate of *Hp* infection.⁷⁸

There are new promising results in newborns.⁷⁹ *S boulardii* has been shown to decrease and shorten neonatal jaundice. In preterm infants, born at 30-37 weeks

of gestation, an enhanced weight gain and feeding tolerance was shown, resulting in a decreased hospital stay. However, Prof Vandenplas does still not recommend the routine use of *S boulardii* in newborns.

Clinical evidence in adults

In the last twenty years we have witnessed a real revolution in the use of probiotics. However, choosing the right probiotic for each disease is a matter of discussion. To advance in this controversy, we need to know the available evidence.

Professor Henry Cohen reviewed clinical evidence of *S boulardii* CNCM I-745 in adults. *S boulardii* CNCM I-745 is used in adults to treat AAD, *C difficile* associated diarrhea (CDAD), traveler's diarrhea (TD) as well as in combination with therapy for *Helicobacter pylori* (*Hp*) infection.

Diarrhea is a relatively frequent adverse event, accounting for about 7% of all drug adverse effects.⁸⁰ More than 700 drugs have been implicated in causing diarrhea. Antimicrobials are responsible for 25% of drug-induced diarrhea, via the alteration of gut microbiota.^{80, 81} Several studies and meta-analyses provided a good body of evidence supporting the efficacy and safety of *S boulardii* CNCM I-745 to prevent AAD in adults.^{68, 69, 82, 83} Compared with placebo, treatment with *S boulardii* reduced the risk of AAD by 6.7%-17.2% (RR 0.43; 95% CI).⁶⁸

C difficile is the main cause of nosocomial infectious diarrhea and the causative agent of antibiotic-associated colitis, but is not the main agent of mild AAD in outpatients.⁸⁴ The combination of standard antibiotics and *S boulardii* CNCM I-745 was also an effective and safe therapy to prevent recurrence of CDAD.^{85, 86}

TD is a common health complaint among travelers, with rates ranging from 5% to 50%, depending on the destination.⁸⁷ A meta-analysis showed that several probiotics (*S boulardii* and a mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) had a significant efficacy for prevention of TD.⁸⁷

A recent meta-analysis showed that, the addition of *S boulardii* to the standard triple therapy significantly increased the eradication rate of *Hp* infection, although to levels still below target values.⁷⁸

Safety

Unique to probiotics is that they are alive when administered, and unlike other food or drug ingredients, possess the potential for infectivity or in situ toxin production.⁸⁸ Manufacturers of probiotics registered as drugs

such as *S boulardii* CNCM I-745, are subject to rigorous safety procedures by Health Evaluation Authorities. One of the main issues concerns the quality control and safety of probiotics that are being prescribed and sold over the counter. In other words, there are more and more probiotics with scarce scientific evidence on the safety of their composition and no knowledge of side effects or drug interactions.

Professor Keira Leon reviewed safety data of *S boulardii* CNCM I-745. Although very rare, side effects with *S boulardii* CNCM I-745 have been previously reported.⁸⁹ Fungemia was sometimes observed in patients with a central venous catheter, hospitalized in beds adjacent to patients treated with the yeast.⁹⁰ It is estimated that an average of 1 per 5.6 million patients, will develop fungemia from *S boulardii*.⁹¹ In a review of 92 cases of *Saccharomyces* invasive infection, *S boulardii* accounted for 51.3% of fungemias and was exclusively isolated from blood.⁹² Predisposing factors were similar to those of invasive candidiasis, with intravascular catheter and antibiotic therapy being the most frequent. These cases highlight that *S boulardii* should be used with caution in patients with central catheters and those with known or potentially compromised intestinal mucosal integrity or those with underdeveloped immune systems.⁸⁸

There is evidence supporting the safe use of probiotics in premature infants (<37 weeks and/or <1500 g).⁹³ Treatment with *S boulardii* decreases microbial translocation (LBP) and inflammation parameters (IL-6) in HIV-1-infected patients with long-term virologic suppression.⁹⁴ Further studies are required to assess the risk-benefit ratio in newborn patients with a risk of necrotizing enterocolitis and in immunosuppressed patients.^{93,95}

Conclusions

Main concluding points of the meeting can be summarized as follows:

The human gut is the natural habitat for a large, diverse and dynamic population of microorganisms, which over millennia have adapted to live on the mucosal surfaces or in the lumen.¹⁻⁵ The interaction between gut bacteria (gut microbiota) and their host is a symbiotic relationship mutually beneficial for both partners. The host provides a nutrient-rich habitat and the bacteria confer important benefits to the host. The gut microbiota helps to shape the immune system, the metabolic function, as well as behavior.⁸⁻¹³

The central nervous system and the gastrointestinal tract are in constant, bi-directional communication through various neural routes such as the vagus nerve cell

and the humoral mediators, including the immune system and the hypothalamic-pituitary-adrenal axis.²²⁻²⁶ Antibiotic or diet perturbations of the gut's flora (dysbiosis) can modify brain chemistry and behavior.^{30,31}

The gut microbiota is essential to the health and well-being of the host, a role which is becoming a booming area of research and presenting a new paradigm of opportunities for medical and food applications.² By mapping the normal microbial make-up of healthy humans using genome sequencing techniques, the researchers of the HMP (Human Microbiome Project) have created a reference database and the boundaries of normal microbial variation in humans.⁹⁶

The field progresses rapidly, owing to the availability of high-throughput molecular sequencing techniques combined with powerful bioinformatics for taxonomic identification and comparative analysis of datasets. Such studies have pointed out that, loss of biodiversity in the human gut microbiota is associated with far reaching consequences on host health. Several disease states have been associated with changes in the composition of fecal and intestinal mucosal communities including inflammatory bowel diseases, obesity and the metabolic syndrome.³⁴ Further, understanding of the importance of developing and maintaining gut microbiota diversity may lead to targeted interventions for health promotion, disease prevention and management.²

Currently, the majority of studies have focused on the adult population, and therefore we are far from understanding how the microbiota affects health during the different stages of life. It is, in fact, interesting to see how the microbiota shows great stability and resistance to change during adulthood, but appears unstable throughout childhood and in later stages of life.^{20,21}

Diet, functional foods and gut microbiota transplantation are areas that have yielded some therapeutic success in modulating the gut microbiota, and warrant further investigation of their effects on various disease states.² Targeted pharmacotherapy that acts synergistically with dietary manipulations or the provision of defined cocktails of intestinal microbes may well be the way of the future.

Are probiotic strains equal? Does it matter which probiotic you prescribe to your patient? The answer to these questions is of course negative. Probiotics do differ, as there are bacterial and yeast probiotic. Therefore, only these products should be used in medical indications that have been clinically tested.

S boulardii is one of the best studied probiotic strains.⁵⁸ In France, *S boulardii* CNCM I-745 is registered as medication since more than 50 years ago.⁶⁵

Its mode of action is well established at different levels: trophic, luminal and immunologic. Several meta-analyses published recently, show a good body of evidence supporting the efficacy and safety of this probiotic in adults in several situations, including antibiotic-associated diarrhea,^{68, 69, 82, 83} *C difficile* diarrhea⁸⁵ and travelers' diarrhea⁸⁷ as well as in combination with therapy for *H pylori* infection, to increase the eradication rate and reduce overall side effects.⁷⁸ *S boulardii* is effective in reducing not only the severity, but also the duration (with about 24 hours) of acute gastroenteritis.⁹⁷ *S boulardii* is one of the recommended probiotic strains in the treatment of acute gastroenteritis by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). Several studies also showed the addition of *S boulardii* to proton pump inhibitors and antibiotics resulted in a 10% better eradication rate of *Hp*.

S boulardii is safe and has clear beneficial effects in children who have acute diarrhea.^{74, 75} *S boulardii* showed efficacy and safety of *S boulardii* to treat AAD.⁷⁷ A meta-analysis of 11 RCTs showed that, the addition of *S boulardii* to the standard triple therapy significantly increased the eradication rate of *Hp* infection.⁷⁸

Probiotics are 'generally recognized as safe' and well tolerated in humans. The most common adverse effects include bloating and flatulence. Although very rare, *S boulardii* CNCM I-745 side effects have been reported. *S boulardii* CNCM I-745 is well tolerated within the usual indications as reported in the product SmPC (Summary of Product Characteristics).

Organizers. Dr Claudia Ferreira MD PhD and Mr Nicholas Coudurier (Biocodex international). President of the Meeting: Prof Jaime Ramírez-Mayans (México).

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