Danone: The gut microbiome and probiotics – 100 years of shared history

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Danone was founded 100 years ago on a breakthrough concept of the time: that fermented foods and the bacteria they contained (not yet referred to as probiotics) could target the gut and its microbiome to bring health to all. One century later, Danone continues to place the gut and its microbiome at the core of its health strategy to deliver the company's mission "bringing health through food to as many people as possible". Today, the portfolio of the Danone Essential Dairy & Plant-Based division encompasses the traditional range of fermented dairy products and probiotics. The portfolio was recently broadened to include plant-based products, expanding the possibility to develop dairy and non-dairy ranges of healthy products that will contribute to nourish, enrich and protect the gut and its microbiome. Here we present the history of microbiology and Danone and reveal our research priorities for the coming years.

Figure 1. Danone yogurt was packaged and sold in ceramic pots in the early twentieth century. Source: *Danone.*

DANONE WAS BORN DURING THE MICROBIOLOGY REVOLUTION

"The secret to longevity may lie in the microbiome and the gut"[1]. "Why the gut microbiome is crucial for your health"[2]. These are two examples of headlines that were written over the past couple of years, but they could have been written one hundred years ago. By the end of the nineteenth century, the field of microbiology started to fulfil its first promises. Louis Pasteur discovered fermentation by lactic acid bacteria, which he described as beneficial bacteria to protect foods from spoilage. Despite the traditional use of fermentation in foods and beverages to improve storage time, safety, functionality, organoleptic quality and nutritional properties, it was Pasteur who demonstrated that the lactate produced during fermentation inhibited the growth of pathogens and avoided food spoilage[3]. The idea that not all bacteria were prejudicial was a breakthrough at the time. The scientific world was shaken by the promise of modern microbiology, which saw the rise of vaccines, the isolation of beneficial bacteria and, later in the century, with the discovery in 1928 of penicillin by Alexander Fleming, the development of antibiotics. Microbiology was seen by many as uncharted scientific territory holding promises to improve people's lives.

Building on Louis Pasteur's legacy, in 1899 Henri Tessier, a paediatrician from the Institut Pasteur, discovered *Bifidobacterium bifidum* in faecal samples of breast-fed infants and reported that the bacteria could be used to help prevent babies from developing diarrhoea. The Russian-born biologist Élie Metchnikoff, recipient of the Nobel prize in physiology or medicine in 1908 for his work on phagocytosis, hypothesized that "there is a link between the gut flora and the senile degeneration"[4]. The gut microbiome and its metabolites were linked to intestinal processes

beyond food digestion and the absorption of nutrients. At the time, it was a breakthrough concept: the bacteria in our guts could dictate our fate. Metchnikoff also observed that food could be used to beneficially modulate the gut flora, in particular the bacteria contained in fermented foods[4]. His hypothesis was that if the lactate produced by bacteria during fermentation can inhibit the growth of harmful bacteria, then the same process could occur in the gut. Consequently, lactic acid bacteria could inhibit the 'putrefaction' and production of toxins in the gut. He worked to isolate pure cultures from a sample of Bulgarian fermented milk, among them *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, the two species used today to produce yogurt.

The history of Danone begins in 1916, when Greek businessman Isaac Carasso took his wife and three children to Spain, his ancestral home. Much of Europe was experiencing the turmoil of the first world war and although Spain remained neutral, famine, malnutrition and poor living standards were present in many layers of society. Carasso was deeply affected by the levels of malnutrition and disease that he saw in Spanish children.

Carasso was open and curious about scientific developments and he was inspired by Metchnikoff's research into dairy ferments. Through experimentation and by trial and error, Carasso mixed fresh milk with ferments isolated at the Institut Pasteur to create in 1919, exactly 100 years ago, what became the first Danone yogurt. He named the yogurt after his son, Daniel, and in reference to his nickname 'Little Daniel'. The yogurt was packaged in porcelain pots (**Fig. 1**), which Carasso hand-delivered as a health food to pharmacies across the city. In 1924, the production site was visited by the College of Doctors of Barcelona who complimented the product[5].

From pharmacies, yogurt made it through to other channels accessible to a range of consumers and became a mainstream product. One decade later, Daniel joined the family business and successfully expanded Danone across France. In the late 1930s, with Europe once again on the brink of war, he decided to move to the United States. In the years following the first world war and, later, the second world war, yogurt was consumed as a way of providing healthy nutrients. The fermentation process means that yogurt contains higher quantities of vitamins B2, B6 and B12, and the nutrients calcium, potassium, zinc and magnesium than other dairy products such as milk[6]. Yogurt promotes adherence to dietary guidelines for many nutrients, particularly those of concern, such as calcium. During fermentation, the breakdown of lactose by enzymes present in *L. bulgaricus* and *S. thermophilus* reduces lactose levels and facilitates the consumption of dairy products by individuals who might be sensitive to lactose levels found in milk[7]. Recent population studies have suggested that yogurt might not only be a significant contributor in the provision of micronutrients but is also associated with a reduced risk of weight gain and increased satiety and that yogurt consumption might be linked to an overall healthier dietary and lifestyle pattern[8].

Building on Pasteur 's heritage, scientists in the twentieth century isolated other health-promoting strains from the human intestine among which were *Escherichia coli* Nissle (1917), *Lactobacillus acidophilus LB* (1907) and *Lactobacillus casei* Shirota (1935). In 1974, Richard B. Parker proposed a definition of probiotics as "organisms and substances which contribute to intestinal microbial balance"[9]. An official definition was issued in 2001 by the Food and Agricultural Organization of the United Nations and the World Health Organization as constituting "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Different types of bacteria (for example *Lactobacillus, Bifidobacterium, Streptococcus, Bacillus*) and yeast or mould (for example *Saccharomyces, Aspergillus, Candida*) are qualified as probiotics.

The first foodstuff to contain probiotics was probably fermented milk, but today many other matrices can contain probiotics. Danone started the production of two probiotic-containing fermented milks with *Bifidobacterium lactis* CNCM I-2494 (BFMP) and *L. casei* CNCM I-1518 (LFMP) in 1987 and 1994, respectively named Activia® and Actimel®. With the ambition to build and substantiate the health benefits of probiotics, a vast programme of studies was launched in the 1990s, resulting in more than 40 peer-reviewed scientific publications. The studied benefits of probiotic products range from reducing the incidence or duration of digestive discomfort symptoms, intestinal transit, common infection and illness, respiratory infection, gastrointestinal infection and prevention of diarrhoea following the use of antibiotics. Specifically, the results accumulated for BFMP showed a consistent and significant improvement of outcomes related to gastrointestinal discomfort in healthy adults[10,11] and a decrease in the severity of symptoms in people with IBS-C[12].

THE SECOND MICROBIOLOGY REVOLUTION FED DANONE'S GUT HEALTH RESEARCH

Scientific discoveries often rely on the development of new technologies. The birth of modern microbiology at the end of the nineteenth and beginning of the twentieth century makes no exception. The first microbiology revolution was possible because of the development of new technological tools, such as the microscope. The advancements in genomics, proteomics and epigenomics have propelled the field into a second revolution that will allow researchers to understand the precise mechanisms of action of the bacteria in the gut. The metagenomics sequencing tools available today represent similar technological advancements because they allow scientists to rapidly capture the complexity of microbiomes and study invisible microorganisms without the need to isolate them.

At the forefront of this second revolution were the European and United States consortia of laboratories, MetaHIT (Metagenomics of the Human intestinal Tract, a project financed by the European Commission, 2008–2012) and Human Microbiome Project (2007–2013), respectively, which have led the way and developed bioinformatics tools and concepts that have unlocked microbiome research. Inspired by its microbiology heritage, Danone Nutricia Research contributed to this frantic endeavour by joining the MetaHIT consortium, which was seen by many as a scientific gamble at the time.

Building on the emerging gut-centred science, Danone Nutricia Research rapidly focused its research efforts on understanding the mode of actions of its major probiotic-containing product targeting gut health: BFMP. After a decade of a broad range of collaborations with leading academics including those from Washington University at St. Louis, U.S., Harvard T.H. Chan School of Public Health in Boston, U.S., the University of California, Los Angeles, U.S. (UCLA), Institut National de la Recherche Agronomique, headquartered in Paris, and the University of Gothenburg, Sweden, we are able to provide a working model capturing the different potential mode of actions of BFMP[11] (**Fig. 2**). The evidence supports the hypothesis that BFMP confers health benefits by modulating the activity of the gut microbiome and the components of the gut–brain axis. On the gut microbiome, we have evidence showing that BFMP stimulates the pathways leading to the production of beneficial short-chain fatty acids and decreases the production of intestinal gas[13,14]. On the gut–brain axis, results obtained in different studies support the idea that this product decreases the visceral hypersensitivity and modulates activity of the brain[10,15].

During our research we found that not all individuals respond to BFMP to the same extent. Recently, we have shown that the decrease in intestinal gas production by BFMP was observed only in individuals exhaling high quantities of hydrogen[14]. We also observed evidence for two types of microbiomes: permissive and resistant[16]. The permissive microbiome was more susceptible to be modulated by BFMP than the resistant microbiome. Together,

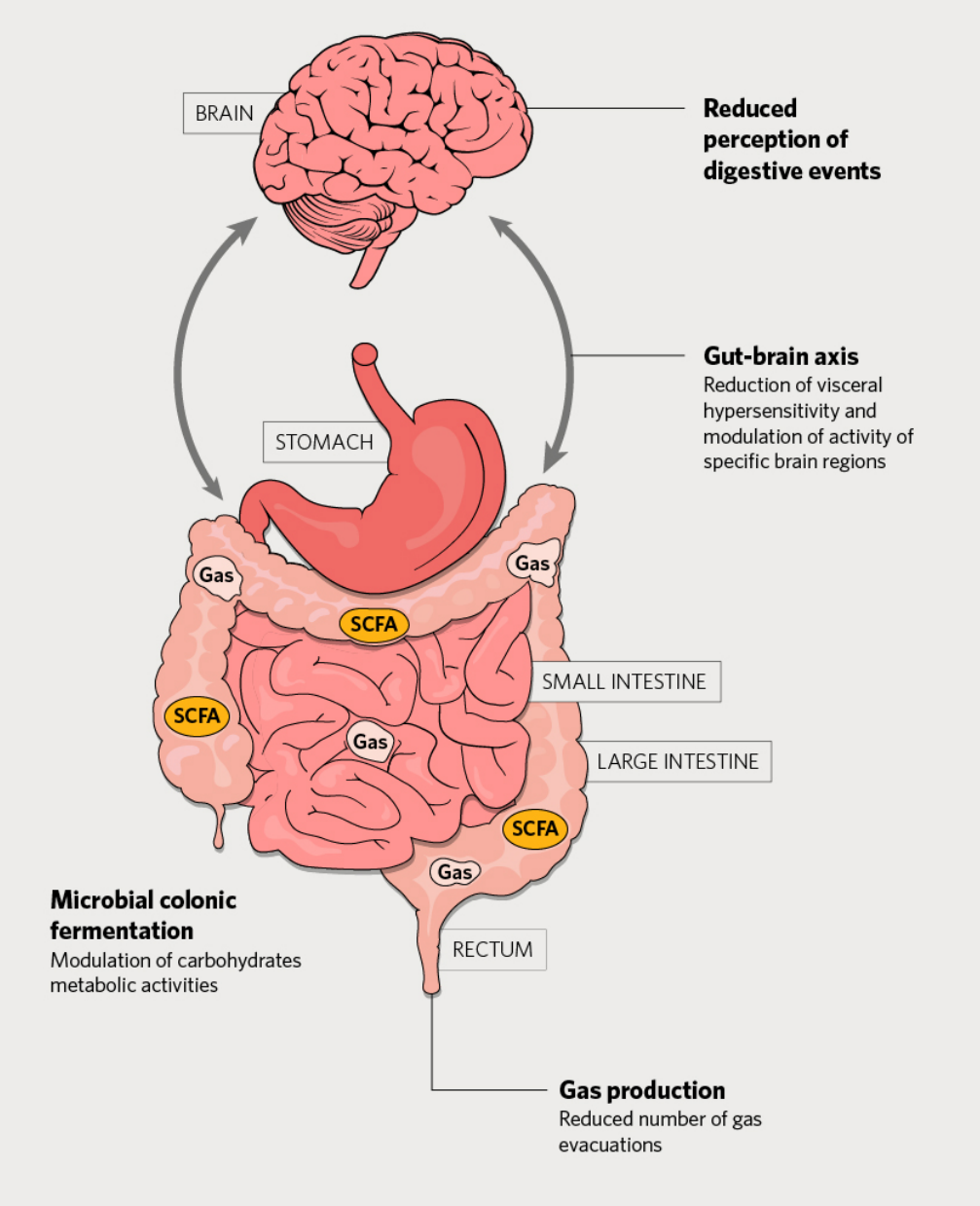

Figure 2. The potential mode of action of BFMP (Activia®). Source: *Danone / Boris Le Nevé.*

the results open the avenue for the 'precision probiotics' era. In other words, probiotics that will be selected and tailored to an individual's phenotype, genetics and microbiome.

Future investigations should focus on the mechanistic understanding of the benefits of probiotics. Such understanding will not just impact gut health but many aspects of health, from the neurological to the immune system, from paediatrics to geriatrics.

100 YEARS FROM NOW: CHALLENGES AND HOPES

In today's world, just as Isaac Carasso found 100 years ago, we face many challenges. In a world in constant and accelerated change and continuous interconnections, human health is interconnected through effects of the environment, especially diet, the microbiome and the intrinsic and specific background of each individual.

HEALTH THROUGH THE GUT

For decades, the scientific approach to health was

compartmentalized and focused on studying specific organs or cell types. Today, we are witnessing the emergence of a new era with the rise of integrative science, which defines health as the result of the adequate functioning of interconnected organs, organ systems or cell types. The intestine has been highlighted as one of the most interconnected organs because it is involved in mental, cardiovascular, metabolic, immune and musculoskeletal health and is essential for health and plays an important part in disease[17].

Scientists are (re)discovering the gut as a cornerstone of human health and well-being because the function of the gut goes way beyond its primary function of digesting and absorbing nutrients. The gut is mediated by four different mechanisms: i) signalling molecules produced by the gut make it a virtual endocrine organ, arguably the largest that can contribute to circulating signalling molecules within the host; ii) bacteria-derived compounds (for example, short-chain fatty acids, lipopolysaccharides, vitamins, and polyphenol metabolites) able to cross the epithelial barrier contribute or interfere with the functioning of extra-intestinal organs or tissues; iii) immune cells primed in the intestine and homing in extra-cellular tissues and; iv) a nervous system able to transmit signals from the gut into the brain, interfering with its activity.

Because the gut is important to human health, it is not surprising that more studies suggest the potential benefits of probiotics beyond the gut to include the brain, cardiovascular, metabolic, bone, muscular and immune systems. In 2013, Danone Nutricia Research and collaborators from UCLA[15] published the first gut-brain probiotic clinical trial in collaboration with scientists showing that a four-week intake of BFMP by healthy women affected the activity of brain regions that control central processing of emotion and sensation. More recently, we published a meta-analysis of 105 publications and demonstrated that the consumption of probiotics is associated with improvements in several metabolic risk factors in subjects with metabolic diseases[18]. Probiotics seem to be relevant candidates to bring health through the gut.

In line with our findings, we firmly believe in the concept of 'Health through the gut', which places the gut and its microbiome as an important target for health. More than one hundred years after Metchnikoff's insights, the idea that small molecules produced by the gut microbiome can modulate health is highly relevant and has catalysed a new gold rush in the gut microbiome field. The focus on health through the gut will allow the development of new solutions, including probiotics, to improve personal and hopefully contribute towards solving public health challenges.

PRECISION PROBIOTICS

Scientific and technological advances in the gut microbiome field hold promises for a new way to select the next generation of probiotics, named hereafter 'precision probiotics'. Precision probiotics will bring to the gut activities or functions that are not provided by our gut microbiome or our own genes. Precision probiotics will be tailored to a recipient's diet, phenotype, lifestyle, age, gender, genetics and microbiome.

The first precision probiotics were *L. bulgaricus* and *S. thermophilus*, the bacteria used for yogurt fermentation, because they allow people who are lactose intolerant to consume yogurt by providing the lactase (an enzyme missing in people who are lactose intolerant) to convert lactose into well tolerated glucose and galactose.

In the future, key species or functions of the gut microbiome will be identified by the scientific community, and probiotics will be a natural way to restore or support some of these functions. Probiotics could originate from our traditional lactic acid bacteria or *Bifidobacterium* but they will also come from bacteria isolated from the gut. *Akkermansia municiphila* is a gut-isolated bacterium that holds promises in the area of metabolic health because research suggests it improves several metabolic parameters in obese and overweight individuals[19].

OPEN SCIENCE

There is huge potential for probiotics and the gut microbiome to contribute to public health, therefore one company and one research team will not suffice to explore and exploit all possibilties. A new way of thinking is required, and that involves multiple partners working together. The Danone vision is collaboration and openness that allows complementary factors to work together towards common goals.

To celebrate 100 years since the creation of its first yogurt, Danone has opened its collection of 1,800 strains for research purposes[20]. The 193 lactic and *Bifidobacterium* ferment strains, as well as Danone's collection of 1,600 other strains, have been made available to researchers around the world, with the sole aim of sharing our legacy to benefit all. The bacteria strains in Danone's collection may have a range of additional uses, many of which have not yet been explored to their fullest potential. The strains could help to address a series of health, societal and environmental challenges[20].

Additionally, and due to the complexity and diversity of the microbiome, Danone Nutricia Research decided to collaborate with world citizens and open the possibility for citizens to help understand the complexity and diversity of the world's gut microbiomes. Traditionally, research on the gut microbiome focused on populations from the Western world because we know the diet and how a variety of foods can modulate the gut microbiome. Danone Nutricia Research partnered with the University of California, San Diego, U.S. to understand the best ways of nourishing the gut microbiome. By launching The Human Diets & Microbiome Initiative (THDMI) we aim to discover the best diets and foods consumed by humans in different parts of the world that can benefit our guts. Because of the high variability in gut microbiome composition between individuals, it is necessary to achieve the recruitment of several cohorts across all geographical regions to enable a full mapping of the gut microbiome combined with dietary data. Most importantly, unlike other similar initiatives, this programme is using full metagenome sequencing, which is the latest sequencing technology, to assess the gut microbiome composition and function.

The THDMI programme has a public health educational vocation and will be conducted to contribute to promote healthy dietary habits. The THDMI initiative will give participants the opportunity to become citizen scientists and to contribute to the 'health through the gut' revolution.

BRINGING IT ALL TOGETHER: THE GUT MICROBIOME AND DANONE SHARED HISTORY

The entrepreneurial spirit that inspired Isaac Carasso to search for solutions for malnourished children is as essential today as it was 100 years ago. And it is this spirit that propels us into the future at Danone. Entrepreneurs, in the purest sense, are those who identify a need – any need – and fill it. Every entrepreneur is a problem solver, someone who sees a problem affecting people and is driven to find a solution. Inspired by Carasso's entrepreneurship, openness and curiosity, research and innovation teams at Danone Nutricia Research share the same passion for innovation. Danone researchers use their scientific and technological expertise to create products that best fit the Danone mission to bring health through food to as many people as possible. The science and technology for developing probiotics has moved at the same pace as the science in the field of the microbiome. The gut microbiome history reflects the Danone history for food and health.

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