Probiotics: 100 years (1907-2007) after Elie Metchnikoff's Observation

Kingsley C. Anukam [1,2*] PhD, MHPM and Gregor Reid [1,2,3] PhD, MBA, ARM, CCM

[1]Canadian Research and Development Centre for Probiotics, Lawson Health Research Institute, 268 Grosvenor Street, London, Ontario, N6A 4V2, Canada.
[2]Department of Microbiology & Immunology, University of Western Ontario, Canada.
[3]Department of Surgery, University of Western Ontario, Canada.

The scientific rationale for the use of live microbes in the prevention and treatment of infections came to lime-light most transparently at the beginning of the 20th century when Elie Metchnikoff in 1907 hypothesized that replacing or diminishing the number of 'putrefactive' bacteria in the gut with lactic acid bacteria could normalize bowel health and prolong life. After more than half a century the term probiotics was coined to reflect Metchnikoff's idea. It is now defined as 'live microorganism, which when administered in adequate amounts confer a health benefit on the host'. In the last 10 years, scientific research in probiotic microbiology has progressed considerably and significant advances have been made in the selection and characterization of specific probiotic cultures and substantiation of health claims relating to their use. Molecular and genetic studies have helped to uncover the mechanistic basis for the beneficial activities of probiotics. It has taken one hundred years, but there are signs that Metchnikoff's hypothesis is now truly being brought to life and many health attributes will indeed be conferred by use of probiotics.

Key words: probiotics; prevention; treatment; infections; health maintenance; fermented foods.

Brief history of Elie Metchnikoff

Born on May 16, 1845 in Ukraine, Elie Metchnikoff studied natural sciences at the University of Kharkoff and pioneered research in immunology leading to the discovery of intercellular digestion in a flatworm. In 1904, he became the deputy director at the Pasteur Institute laboratory in Paris from where he discovered the process of phagocytosis which demonstrated how specific white blood cells can break down harmful bacteria in the body. For this work, he was awarded the Nobel Prize for medicine in 1908.

Although there is reference to sour milk or fermented cultures as far back as the Bible, Elie Metchnikoff is regarded as the grand father of modern probiotics. He made a landmark observation that the regular consumption of lactic acid bacteria in fermented dairy products, such as yogurt, was associated with enhanced health and longevity in Bulgarian peasant populations. He linked this to the 'Bulgarian bacillus' which was discovered by a 27-year old Bulgarian physician Stamen Grigorov, and he later demonstrated how healthy bacteria in yogurt helped digestion and improved the immune system.

The scientific rationale for the health benefit of lactic acid bacteria was provided in his book "The prolongation of life" published in 1907. He asserted that some of the bacterial organisms present in the large intestine were a source of 'toxicants', toxic substances that contributed to illness and aging. He 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" [1]. To test the hypothesis on the health benefit of consuming lactic acid bacteria, Metchnikoff drank sour milk every day until his demise at the ripe age of 71 years in 1916.

* Corresponding author: e-mail: kanukam@uwo.ca, anukamkc@yahoo.com Tel: 519-646-6000 X61547 Fax: 519-646-6031

Historical development of Probiotics.

At the time of Metchnikoff's scientific demonstration of lactic acid bacteria benefits, Henry Tissier, a French paediatrician, working independently observed that children with diarrhea had in their stools a low number of bacteria characterized by a peculiar, Y shaped morphology. These "bifid" bacteria were, on the contrary, abundant in healthy children [2]. He suggested that these bacteria could be administered to patients with diarrhoea to help restore a healthy gut flora.

The pre and post-world war discovery of antibiotics may have triggered abandoning Metchnikoff's concept, as apart from the launch of Yakult in Japan in the 1930s, and ongoing studies in the Soviet Union, there is little evidence to indicate that Metchnikoff's concept was taken seriously, at least from a commercial standpoint. Indeed, between 1908 and 1964, little or nothing was heard of microbial therapy in Western countries. In 1965, the term 'probiotics' was first used by Lilly and Stillwell [3] in a different context to represent 'substances secreted by one organism which stimulate the growth of another'. After nine years, Parker [4] described probiotics as "organisms and substances which contribute to intestinal microbial balance".Fifteen years later, Fuller [5] proposed that probiotics were 'live microbial supplements which beneficially affects the host animal by improving its microbial balance. This was followed by Salminen et al., [6] who defined probiotics as 'foods containing live bacteria which are beneficial to health'.

As research in probiotics become more visible and in confirming the validity of evidence on probiotics, the United Nations Food and Agriculture Organization and the World Health Organization (FAO/WHO) in 2001, sponsored an Expert Consultation following a request from the Argentinian government. During the consultation which was chaired by Dr. Gregor Reid, the Director of the Canadian Research and Development Centre for Probiotics, a consensus definition of probiotics was adopted as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [7]. This is now the widely used and accepted definition as it embraces all applications of live microbes, not just those for intestinal benefits.

Probiotics beyond fermented dairy products

Many microbial types are used around the world to ferment milk, plant food, meat and other products. Two of the most widely known and characterized are *Lactobacillus delbreuckii* subsp. *bulgaricus* and *Streptococcus thermophilus.* They were reported to positively influence the microbiota of the gastrointestinal tract, thereby decreasing toxic microbial metabolic activities [8]. However, much progress has been made since in terms of the fermentation of dairy products. In this regard most probiotics fall into categories of lactic acid-producing bacterial organisms, including *Lactobacillus* and *Bifidobacterium*, but also non-lactic acid organisms (Table 1).

Probiotics are most often incorporated in yogurt and fermented milk, but other food lines are now available and numerous products are sold in tablet, capsule, and powder forms. The dairy version require refrigeration in the distribution channels, outlets and homes (unless the products are eaten within a day of purchase), and these may not always be available. Some dried formulations, can survive without refrigeration, as long as they are retained in proper vials with appropriate desiccants, and kept in a cool, dry location suitable for the developing countries with tropical temperatures [9].

Table 1: Proven probiotic microorganisms.

Lactobacillus strains	**Bifidobacterium strains**	**Other lactic acid bacteria**	**Non-lactic acid bacteria**
L. rhamnosus GG (LGG)	*B. lactis* Bb 12	*Lactococcus lactis* L1A	*Escherichia coli* strain nissle
L. rhamnosus GR-1	*B. infantis* 35624		
L. reuteri RC-14	*B. breve* strain Yakult		*Saccharomyces boulardii* lyo

L. casei DN114001	*B. animalis* DN 117-001		
L. acidophilus LA-1	*B. lactis* HN019		
	B. longum BB536		
L. reuteri SD2112			
L. plantarum 299v			
L. casei Shirota			
L. acidophilus LB			
L. rhamnosus HN001			
L. salivarius UCC118			
L. acidophilus NCFM			
L. fermentum VRI003			
L. johnsonii Lj-1			
L. paracasei F19			

Effects on healthy human consumers

A large number of consumers, who regard themselves as being otherwise 'healthy', use various probiotic products. They do so, on the assumption that probiotics can maintain their health and well-being and potentially reduce their long-term risk of diseases of the gastrointestinal, urogential tracts, kidney, respiratory tract and cardiovascular tract. However, although healthy people are the common target for these new functional food products, the number of clinical trials proving efficacy has until recently been quite limited. In one sense, unless products are available, then there is nothing to take or to test clinically; yet on the other hand, when there is a plethora of products calling themselves probiotics, but not clinically proven, the consumer or healthcare provider is unsure which to take and what to expect from their use.

Due to the inability of probiotic organisms to colonize the host, it soon became apparent that they needed to be taken regularly so that the benefits they confer can be accrued daily. Testing this concept, a study in Finland showed that in day care centres regular use of *L. rhamnosus* GG probiotic reduced the incidence of respiratory infections and days absent due to ill health [10]. In another study, dietary deprivation of fermented foods in healthy volunteers caused a fall in innate immune response, with a significant decrease in faecal lactobacilli and concentration of short chain fatty acids. Moreover, a decrease in phagocytic activity in leukocytes was observed after two weeks of restricted fermented food diet that might affect the capacity to respond against infections [11]. A randomized, double blind, placebo-controlled human clinical trial involving 30 healthy adults was performed to investigate the effect of a fermented product containing *Lactobacillus gasseri* CECT5714 and *Lactobacillus coryniformis* CECT5711, on several blood and fecal parameters, most of them related to the host intestinal function. The volunteers were randomly distributed into two groups, one receiving a standard yogurt and the other a similar dairy fermented product in which the *L. delbreuckii* subsp. *bulgaricus* yogurt strain had been replaced by a combination of the probiotic strains *L. gasseri* CECT5714 and *L. coryniformis* CECT5711. The volunteers who received the probiotic strains reported no adverse effects and the strains were isolated from their feces at a relatively high level. In fact, the concentration of fecal lactic acid bacteria significantly increased in the probiotic group. Additionally, the oral administration of the probiotic strains led to an improvement of parameters such as the production of short chain fatty acids, the fecal moisture and the frequency and volume of the stools. As a result, the volunteers assigned to the probiotic group perceived a clear improvement in their intestinal habits [12]. More recently, Alvaro et al., [13] showed a significant decrease in the level of enterobacteriaceae and increase in galactosidase activity in yogurt consumers than non-yogurt consumers. These are but a few examples of regular use of probiotics in healthy subjects. In the future, it would be worthwhile to have longer term studies to determine if longevity truly is affected by consumption of probiotics.

Effects on infectious diarrhea, antibiotic-associated diarrhea and traveler's diarrhea.

The use of probiotics to treat diarrheal diseases of children is becoming a mainstay among some health care providers, further to successful double-blinded, randomized, placebo-controlled studies [14], and several well-conducted meta-analyses [15]. The success of *Saccharomyces boulardii* in treating patients with acute watery diarrhoea and reducing the frequency of episodes of diarrhoea in subsequent two months has been documented [16].

There is some evidence of efficacy in the prevention of community-acquired and nosocomial diarrhea. More solid evidence of efficacy is found in the treatment of sporadic, infectious diarrhea, where several probiotics, and especially *L. rhamnosus* GG, have been found capable of reducing by approximately 1 day, the duration of diarrhea, shortening the initial phase of watery stools, and reducing hospital stay in developed countries [17]. The effect is best documented in viral diarrheas where probiotics appear to increase antibody secretary IgA and decrease viral shedding [18]. Isolauri et al., [19] demonstrated previously that *L. rhamnosus* GG performed effectively whether in fermented milk or freeze-dried powder, compared to a placebo-containing pasteurized yogurt, tested in infants with acute rotavirus infection

A recent study investigated the preventive effect of a milk drink fermented with multi-strain probiotics on antibiotic associated diarrhoea (AAD). In the double-blind placebo controlled study, probiotics prevented four of five cases of AAD in adult hospitalized patients [20].

Traveler's diarrhea (TD) is a common health complaint, with rates ranging from 5% to 50%, depending on the destination. Randomized controlled trials conducted since 1977 and 2005 have shown that probiotics exhibit significant efficacy [21]. In short, the data on redcucing the duration of illness is strong, while the use of probiotics to prevent diarrhea has mounting evidence.

Inflammatory bowel disease (IBD) and beyond

The etiology of inflammatory bowel disease (IBD) disorders is unknown, but is known for chronic and recurrent intestinal infection or inflammatory-based problems. Ulcerative colitis (a relapsing inflammatory disorder of the colon), Crohn's disease (a chronic IBD occurring anywhere from the mouth to the anus), and pouchitis (a non-specific inflammation of the ileal reservoir) are generally included in the IBD grouping. The mechanisms responsible for onset of IBD are not clear, but some people assume that it results from dysfunction of the host response (both innate and acquired immunity) towards normal GI microbiota or from a defective mucosal barrier [22]. Controlled clinical studies have demonstrated the efficacy of probiotics in the maintenance of remission of pouchitis, prophylaxis of pouchitis after the formation of an ileoanal reservoir, and maintenance of remission of ulcerative colitis, but successful treatment of Crohn's disease has so far been illusive. [23, 24, 25].

Many other applications of probiotics to the intestine have been assessed, mostly for lactose intolerance [26, 27], irritable bowel syndrome, reduction of colorectal cancer risk [28], and gastric ulcers [29]. These topics are outwits the scope of this brief review, but many publications provide insight into their strengths and weaknesses.

Extra-intestinal uses of probiotics.

When Elie Metchnikoff put forth the probiotic theory, he was not unmindful of the fact that the benefits can transcend the intestinal environment, hence his suggestion of disease-free state and subsequent longer live for the population consuming fermented products. The best example of this, is the use of probiotics for urogenital health, namely to prevent and in some cases treat urinary tract infection and bacterial vaginosis [30, 31, 32].

Allergic diseases, on a rapid increase in developed countries, such as atopic eczema have been the target for probiotic intervention in pregnant women and newborns [33], however failure to repeat these

findings means that the extent of this intervention remain somewhat controversial. With obesity, diabetes, heart diseases and stroke on the increase, there are some isolated human studies showing that elevated blood cholesterol levels can be reduced by consumption of probiotic-containing dairy foods [34]. This reduction is not as high as pharmaceutical statins, but the side effects are minimal.

The use of probiotic preparation VSL#3 and experimental *Oxalobacter formigenes* have shown that it is possible to influence urinary oxalate excretion and potentially reduce urinary super-saturation levels and the formation of kidney stones [35]. In further studies in animals, oral administration of specific *L. acidophilus* strains has been shown to induce the expression of μ-opioid and cannabinoid receptors in intestinal cells and mediate analgesic functions in the gut, similar to the effects of morphine [36]. While this effect has not yet been tested in humans, and is not extra-intestinal per se, the feeling of pain relief would be perceived in the brain, and thus offering possible extra-intestinal relief.

Genomic sciences and probiotic mechanistic actions

In the last few years, with the unraveling of the complete sequences for several probiotic organisms, accompanied by the development of bioinformatic tools for nucleic acid and protein analysis, it is now possible to use comparative genomics to reveal important similarities and differences in strains, species, and genera. In time, this will uncover mechanisms responsible for the beneficial properties ascribed to probiotic organisms. Practical genomics will allow better selection of desirable phenotypes, improve culture stability by stress preconditioning, provide opportunities for metabolic engineering, and for ways of optimizing the targeting of strains to tissues sites [37].

Complementary approaches using host cells, *in vitro* systems together with animal models [38] and human studies, will reveal specific tissue cell responses to probiotics, hopefully disclosing the bacterial and host effector molecules and pathways by which probiotics are able to modulate human health [39]. It will also form the platform for microarray and proteomic technologies that allow real-time analysis of RNA and protein expression in the bacterial cell as it travels through the host. Investigation of probiotic organisms with these new and potentially powerful tools will facilitate the development of bacteria as therapeutic agents, and provide the mechanisms to produce advanced probiotic strains.

Emergence of probiotics advocates

While research efforts continue to reveal the potential of probiotics, it is timely that groups of scientists are getting together to promote high standards in scientific excellence, adherence to reputable guidelines and standards, and appropriate product labeling and claims. The formation of the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2002 is a case in point. ISAPP is a non-profit organization (www.isapp.net) comprised of international scientists whose intent is to strongly support and improve the levels of scientific integrity and due diligence associated with the study, use, and application of probiotics and prebiotics [40]. ISAPP has endorsed the FAO/WHO guidelines for what constitutes probiotics [41], namely strain designation, efficacy/effectiveness and safety [42].

Probiotics guidelines

1. Probiotic organisms must be living;
2. Identify the organism(s) to species level;
3. Have proven safety data;
4. Show physiological benefits when using a defined viable count of probiotics in a defined delivery vehicle (food, capsule or whatever) in a defined patient population, controlled by a placebo and/or standard therapy option if the end outcome is treating a disease.

Probiotics origin and identity

(i) The origin of the probiotic strain is not relevant if it confers a health benefit.
(ii) Strains should be speciated using DNA-DNA hybridization or the equivalent molecular method.
(iii) Each strain should have a number or letter designation

Safety of Probiotics

(i) Properly tested and prepared probiotic strains are, for the most part, extremely safe for human oral and vaginal use.
(ii) New strains and products wishing to be designated as probiotics, should be proven in human studies to be safe.
(iii) Clear labeling is recommended when a strain, for example *S. boulardii (S. cerevisiae),* has some limitations in its use, such as for patients with a leaky gut or at risk of bloodborne infection.

Probiotics Efficacy and Effectiveness

(i) Carefully designed, and sized, placebo-controlled, statistically significant human efficacy or effectiveness studies are required to prove that probiotic strains in their product formulation confer specific disease reduction or clinical treatment benefits.
(ii) Regulatory agencies need to develop new criteria that allow food and dietary supplement products to make health claims that are informative and useful for consumers, and that can be substantiated by appropriately designed and completed clinical studies

Future directions for probiotics:

With the recognition and endorsement by the United Nations and World Health Organization, the call that "Efforts should be made to make probiotic products more widely available, especially for relief work and populations at high risk of morbidity and mortality" is yet to be ratified by government agencies and multinational probiotic companies. Probiotics, such as Yakult has been available, in Japan since 1935 and various forms are equally accessible in Europe, Australia, USA but no such well-documented dairy product is available in Africa.

If the private sector is unable to find a suitable business model to reach Africa, then universities, non-governmental agencies and others must make strides in taking this concept and these products to the people in that region. This may require education of government agencies in Northern countries. For example, after the Tsunami, the Danish government provided a plane filled with relief supplies. When Chr Hansen offered to send probiotic products that could help alleviate the diarrheal plaque that was imminent, the offer was refused and the plane left with sugar and salt!

A group of scientists, social scientists, staff and students at the University of Western Ontario have provided a good example of the power of the individual. They formed Western Heads East, and with donated time and money, they set up a community kitchen in Mwanza Tanzania, teaching local mothers to produce probiotic yogurt. Using locally produced milk, the kitchen feeds 80 AIDS patients per day and over 100 family members. A preliminary analysis has shown that diarrhea and yeast infections are down and energy levels are up with several months use of the probiotic. (http://www.westernheadseast.ca). The project will soon be widened within Tanzania and to at least Kenya.

The success of probiotics in recent times, fueled by failure of pharmaceutical agents (antibiotics, statins, anti-inflammatories), consumer demands for natural products and scientific validity of the concept, has led to the entrance of products onto the market that are not close to meeting the standards

set by the FAO/WHO. Hopefully, in the near future either these companies will cease selling their products (some with outlandish and doubtful claims), or change the name of their product to live microbes or another term not probiotics (doubtful), or be taken off the market by regulatory agencies (doubtful as these agencies themselves do not seem to understand what a probiotic actually is), or do the necessary studies to show the probiotic effects of their product (hopefully this is what will happen). Pressure from scientists, physicians and consumers is needed to force this issue, and determine the depth and breadth and limitations of probiotics, and their mechanism of action.

It would not be surprising if in the near future, laboratories test human blood, stool, saliva and tissues to determine how 'healthy' we are, then replenish beneficial bacteria absent from certain body sites (e.g bifidobacteria in the colon, lactobacilli in the vagina, streptococcus in the oral cavity). In addition, it will be possible to quantify in some way, the status of the immune system then select specific probiotics to modulate recovery from, or prevention of, or adjunctive treatment of certain illnesses. In terms of treatment of conditions, probiotics for allergy, cholesterol, oxaluria, cancer and infection may be the cusp. As knowledge of human-bacteria genomics, nutrigenomics, proteomics and metabolomics emerges and becomes intertwined; the mechanisms and functionality of probiotics will become uncovered. Genetic technology will equally lead to the development of new strains and with the combination of basic and industry-based research, personalized probiotic products will eventually get to the consumer in the near future.

The Metchnikoff Prize

In recognition of Metchnikoff's place in the probiotic realm, the International Dairy Federation (IDF) created in 2007 'The IDF Elie Metchnikoff Prize 2007' to recognize outstanding scientific discoveries in the fields of microbiology, biotechnology, nutrition and health with regard to fermented milks. The inaugural winners awarded in Moscow in 2007, illustrate the depth and excitement of probiotic research.

Professor Tomatari Mitsuoka from Japan won the prize in Microbiology category for his outstanding contribution to progress in intestinal bacteriology and his commitment to providing the first developing culture and research methods for intestinal flora, and subsequently discovering, classifying, and naming many lactic acid bacteria and intestinal anaerobes. The scientific work of Prof. Mitsuoka paved the way for application of the theory of intestinal flora balance to measures for the maintenance of human health and prevention of disease, as well as for the development and assessment of functional foods.

In the biotechnology category, Prof. Larry Lee McKay from USA was noted for a series of discoveries focused on mobile genetic elements in dairy lactococci. This work created an entirely new field of research—the genetics of lactic acid bacterial starter cultures—a field that today engages over a thousand researchers, both academic and industrial, around the world. Prof. McKay's seminal discovery was that lactococci harbour a diversity of plasmids that encode the key traits necessary for their function as dairy starter bacteria.

In the category of nutrition and health, the work of Prof. Gregor Reid and Prof. Andrew Bruce from Canada showing that probiotics could be ingested but influence the vagina, has implications for the well-being of women around the world. They showed that probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 could be delivered to the vagina via oral intake and natural passage through the intestine and along the perineal and vulval skin. Their findings have since been confirmed by scientists in other parts of the world.

Without knowing him in person, it is hard to know what Metchnikoff would have seen as the ultimate prize. He would likely have been disheartened that it took the scientific world so long to expand upon his ideas. But, ultimately as a man with a passion for enquiry and novelty, he would likely feel satisfied that great progress is now being made in an area that promises to provide tangible benefits for many people the world over.

Acknowledgement. The support by Ontario Neurotrauma Foundation, Ontario Ministry of Agriculture and Food, AFMnet and NSERC is gratefully appreciated.

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