

Banking on Microbiota

How one company searched and conserved the world's healthiest microbiomes to create condition-specific probiotic products

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Banking on Microbiota

How one company searched and conserved the world's healthiest microbiomes to create condition-specific probiotic products

Loss of biodiversity is one of the great threats and challenges of the 21st century. As many as one million plant and animal species are now at risk of extinction due to human activities. Climate change is only accelerating the problem, spurring nations to step up conservation efforts before it is too late.¹ But it's not just the macroscopic world that's under threat by industrialization and global development.

Thousands of different bacterial species live within the human gut. A growing body of research has shown that the microorganisms that comprise the microbiome are responsible not only for gastrointestinal health, but play important roles in immunity, pre- and postnatal development, cardiovascular health and more—even affecting mood and mental well-being. Other microbiome communities also exist on human skin, inside the oral cavity and elsewhere.

Similar to how urbanization is altering Earth's ecosystems, westernized diets and lifestyle are altering the human microbiome—leading to poor health.

For instance, obesity afflicts about 35.5% of the U.S. population and more than 600 million people worldwide. Scientists believe Western-

style diets high in fats and sugar profoundly impact the structure and function of gut microbiota, which is linked to poor health.

Research suggests that ultra-processed foods, for example, create a unique breeding ground for microbes that promote poor health. In addition, industrial-scale sanitation and antibiotic use have also shrunk the number of beneficial bacteria, such as lactobacilli and bifidobacteria, while potentially allowing harmful species such as enterobacteria to proliferate.²

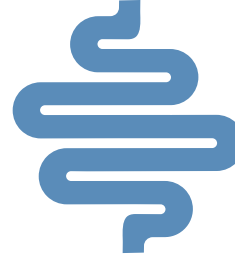
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Perhaps not surprisingly, studies comparing microbiomes from industrialized societies to those from rural and isolated populations that adhere to traditional diets find meaningful differences in microbiota diversity and poor health.

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A well-known 2015 paper, for example, contrasted the fecal microbiota of adults from





two non-industrialized regions in Papua New Guinea with those in the United States. The former harbor communities with greater bacterial diversity, far less individual variation between people, vastly different abundance profiles, and numerous bacterial lineages not found in U.S. microbiomes.³ Many other studies comparing gut microbiota between Westerners (mainly in the United States and Europe) and rural populations in South America, Africa and elsewhere suggest the strong role diet and lifestyle play in the health of a person's microbiome.^{4,5,6}

At the forefront of microbiota conservation

The scientific evidence linking a healthy microbiome to a healthy human being has catalyzed a recent movement to conserve beneficial bacterial species before they disappear, especially as the United Nations projects 70% of people will live in urban centers by 2050. Organizations and initiatives like the Global Microbiome Conservancy and the Microbiota Vault are working to conserve microbiota biodiversity by collecting and preserving microbes before they disappear

from human microbiomes entirely.

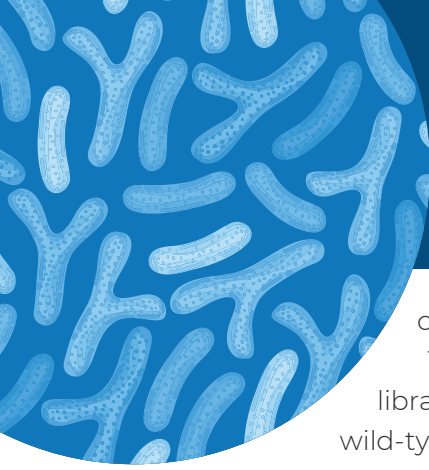
Nearly 20 years ago—before these mainstream movements emerged—A-B Biotics (a Kaneka company) conducted its own search for microbiomes untouched by westernized diets and lifestyle.

“When this work began, techniques to analyze microbiota composition—mainly metagenomics—were not completely developed and for this reason the characterization of the microbiota in indigenous or non-industrialized societies versus western societies was not a relevant topic at that moment,” said Mike Kolifrath, vice president of sales and marketing in the probiotics division at Kaneka.

The idea beginning back in 2004 was to create a collection of probiotic strains that would serve as a basis to screen and select probiotics with outstanding properties for different health applications, according to Kolifrath.

“Today, this is one of the most important and differentiating assets, as it is the starting point for any new product development. In addition, this platform is continuously being expanded with new strains that adds to the diversity of





The 2021 study showed that Baby Colic, a probiotic formulation of Bifidobacterium longum KABP™-042 and Pediococcus pentosaceus KABP™-041, went to work as quickly as the end of the first week of the three-week-long clinical trial.

our portfolio,” he said.
The result: A microbiota library of more than 1,000 wild-type strains and isolates belonging to different species, mainly in the bacterial genera Bifidobacteria and Lactobacillus.

“Most of our probiotic bacteria were isolated from rural or indigenous areas, from pristine environments or from human microbiota with little exposure to modern lifestyle and amenities,” Kolifraith said. “Other strains have been isolated from traditional fermented food samples.”

Clinical trials support strong health claims

Kaneka has invested millions of dollars in developing the most promising strains into its line of Floradapt probiotic products with specific health benefits, including for gut comfort, baby colic, cardio health, immune support, and mood and stress support. In each health category, scientists have overseen novel research that demonstrated the safety and efficacy of probiotics for widely different applications. These randomized, double-blind and placebo-controlled trials represent the “gold standard” in the scientific community by ensuring the probiotic-based intervention is directly connected to the outcome in a human population.

Take the example of a recent clinical trial involving 112 infants diagnosed with colic, a gastrointestinal disorder that affects an estimated 20% of infants aged five months or younger. The frequent GI discomfort causes the infants to cry and fuss, impacting early

quality of life for the child (and likely sleep deprivation and stress for the parent). The 2021 study showed that Baby Colic, a probiotic formulation of Bifidobacterium longum KABP™-042 and Pediococcus pentosaceus KABP™-041, went to work as quickly as the end of the first week of the three-week-long clinical trial.

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The final results were compelling:
About 83% of the probiotic group compared to just 36% of the placebo group responded to treatment with decreases in total duration of crying per day.
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In addition, the probiotic formula also decreased the number of crying and fussing episodes, as well as improved stool consistency by the third week.⁷

Another interesting example of the





company's ongoing research into probiotic health benefits is its Cardio Probiotic formulation, which combines three strains of *Lactobacillus plantarum*. Scientists screened about 550 strains before identifying a trio of lactic acid-producing bacteria with documented cholesterol-lowering properties.⁸ Developed more than 10 years ago, it was one of the first probiotics to go beyond more well-known categories like digestive health and immune support.⁹

"Today, research with Cardio Probiotic is still one of our priorities and very interesting results from new studies are expected to be public in the next few months," Kolifrath said.

Innovation for new market opportunities

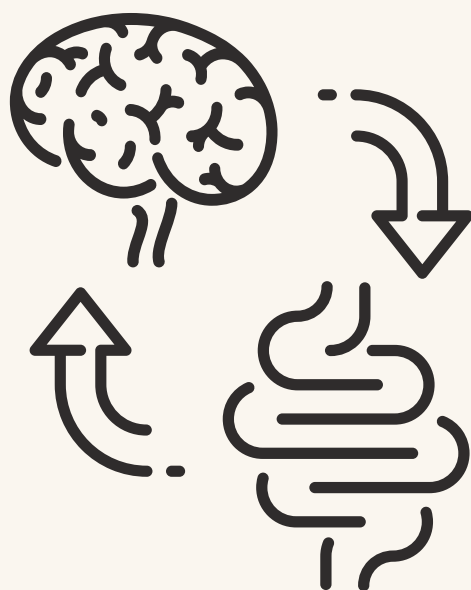
Kaneka remains at the forefront of investigating novel applications to expand or open new markets for probiotics. One category that has gained traction in recent years is brain health, leveraging the well-established gut-brain axis. This bi-directional communication network enables the brain to influence intestinal health and the gut to affect mood and cognition.¹⁰

Research on the topic has increased exponentially over the last decade: In 2011, just 32 published papers addressed the topic versus 730 publications in 2021. In addition, the Nutrition Business Journal expects supplements like probiotics to drive future growth in the brain health category¹¹—a

diverse market that includes aging Gen Xers trying to stave off cognitive decline and Millennials dealing with depression and anxiety.

For instance, a 2019 study showed that Kaneka's *Lactobacillus plantarum* DR7® strain used in its Mood & Stress Probiotic helped alleviate stress in adults during a 12-week clinical trial. Participants in the treatment group also showed improvements in memory and other cognitive capabilities.¹² *L. plantarum* DR7 works, in part, by enhancing serotonin pathways by increasing the expression of key enzymes like tryptophan hydroxylase (TPH1), which converts tryptophan to serotonin. In fact, about 90% of the body's available serotonin comes from the gut.

In addition, *L. plantarum* DR7 is one of the few probiotic strains studied in healthy humans for support of the overall health of the upper respiratory tract—again by modulating the metabolism of tryptophan. In a 2019 study involving 109 adults, DR7 helped strengthen the immune system to help participants cope with infections.¹³ A later study in 2021 also provided statistically significant results that DR7 supports overall upper respiratory health.¹⁴



In 2021, Kaneka announced that DR7 probiotic is now self-affirmed GRAS.

“The neurochemistry of the brain is one of the most exciting new playgrounds of probiotics. We have the most competent strain in the gut-brain axis field with *L. plantarum* DR7, based on its unique ability to impact the biosynthesis of serotonin,” Kolifrath said. “What’s unique about our gut-brain probiotic is its ability to impact immune health also.”

Indeed, not all probiotic bacteria are created equal. While many possess beneficial properties, they may not be able to survive the harsh environment of the gut or adhere to the cell wall to successfully colonize the intestine. Synergistic effects may also be lacking. That’s why Kaneka continues to investigate its unique library of conserved microbiota to support healthy lifestyles in novel ways.

“It is important to start with a big collection of probiotic bacteria if we want to be successful in the selection of the best candidates,” Kolifrath noted.

Kaneka Probiotics library consists of four culture collections:

Collection 1

554 bacteria isolates from microbiota (n=440) and saliva (n=114) samples collected from healthy children in rural areas.

Collection 2

47 vaginal strains collected from healthy women, which have been obtained from hundreds of isolates coming from vaginal samples.

Collection 3

6 strains isolated from human milk and breast-fed infant microbiota.

Collection 4

131 isolates from traditionally fermented milk products with anti-inflammatory properties.

Case Study on IGI Probiotic

Floradapt Intensive G.I. is more than a gut feeling

Kaneka is more than just a formulator of leading probiotic products for specific health applications. The company collaborates with its partners on every phase of product development, from initial R&D to market research and support.

“In the history of the company, most of the products in our portfolio have originated through contact and relationships with other pharma or food companies, which have provided us with insights to better understand the needs of brands and product developers, as well as healthcare practitioners,” said Mike Kolifrath, vice president of sales and marketing in the probiotics division at Kaneka.

One example of this collaboration is the formulation of the company’s flagship probiotic, Floradapt Intensive G.I. (IGI), which was initially developed in collaboration with a pharmaceutical company that was looking for a natural solution to support those experiencing gut discomfort.

Chosen from among 100 strains, the formulation includes the strains *L. plantarum* KABP022, *L. plantarum* KABP023 and *Pediococcus acidilactici* KABP021, based on their ability to survive the acidic environment of the stomach and intestines, as well as highly oxidizing conditions that can occur with gut discomfort. In addition, all three showed an excellent ability to adhere to the intestinal cell wall for successful colonization.¹⁵

More importantly, these strains demonstrated different but synergistic benefits, including supporting the inhibition of pro-inflammatory cytokines and bad bacteria such as *E. coli* in healthy individuals.* They also produce short chain fatty acids like acetate, which serve as a nutrient source for the cells of the intestine, and help strengthen the gut barrier.^{16,17}

The true breakthrough came from human clinical studies that showed the improvements Floradapt IGI had on gut health and comfort.* For instance, a 2014 multicenter, randomized, double-blind, placebo-controlled trial involving 83 subjects found statistically significant changes in the quality of life for groups that took low and high doses of the probiotic formulation compared to placebo. In addition, there was a statistically significant change in gut-related anxiety in healthy participants who received probiotics for six weeks.¹⁸ *

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[A more recent study confirmed the ability of Floradapt IGI to support the improvement of quality of life for healthy individuals living with gut discomfort.¹⁹](#)
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Today, Floradapt Intensive G.I. is distributed in more than 60 countries around the world, with more than two dozen possible structure-function claims.

Endnotes

- 1 Plumer B. "Altering the Natural World at an 'Unprecedented' Pace." The New York Times. May 6, 2019.
- 2 Rook GA, Martinelli R and Brunet LR. "Innate immune responses to mycobacteria and the downregulation of atopic responses." *Curr Opin Allergy Clin Immunol* 2003;3:337-42.
- 3 Martínez I et al. "The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes." *Cell Rep*. 2015 Apr 28;11(4):527-38.
- 4 Blaser MJ et al. "Distinct cutaneous bacterial assemblages in a sampling of South American Amerindians and US residents." *ISME J*. 2013 Jan;7(1):85-95.
- 5 Yatsuneneko T et al. "Human gut microbiome viewed across age and geography." *Nature*. 2012 May 9;486(7402):222-7.
- 6 De Filippo C et al. "Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa." *Proc Natl Acad Sci U S A*. 2010 Aug 17;107(33):14691-6.
- 7 Chen K et al. "Infantile Colic Treated With Bifidobacterium longum CECT7894 and Pediococcus pentosaceus CECT8330: A Randomized, Double-Blind, Placebo-Controlled Trial." *Front Pediatr*. 2021 Sep 10;9:635176.
- 8 Bosch M, et al. "Lactobacillus plantarum CECT 7527, 7528 and 7529: probiotic candidates to reduce cholesterol levels." *J. Sci. Food Agric*. 94.4 (2014): 803-809.
- 9 Fuentes, MC et al. "A randomized clinical trial evaluating a proprietary mixture of Lactobacillus plantarum strains for lowering cholesterol." *Mediterr. J. Nutr. Metab*. Vol. 9, no. 2, pp. 125-135, 2016.
- 10 Appleton J. "The Gut-Brain Axis: Influence of Microbiota on Mood and Mental Health." *Integr Med (Encinitas)*. 2018 Aug; 17(4): 28-32.
- 11 2021 Condition Specific Report. Nutrition Business Journal.
- 12 Chong HX et al. "Lactobacillus plantarum DR7 alleviates stress and anxiety in adults: a randomised, double-blind, placebo-controlled study." *Benef Microbes*. 2019 Apr 19;10(4):355-373.
- 13 Chong HX et al. "Lactobacillus plantarum DR7 improved upper respiratory tract infections via enhancing immune and inflammatory parameters: A randomized, double-blind, placebo-controlled study." *J Dairy Sci*. 2019 Jun;102(6):4783-4797.
- 14 Altadill T, Espadaler-Mazo J and Liong MT. "Effects of a Lactobacilli Probiotic on Reducing Duration of URTI and Fever, and Use of URTI-Associated Medicine: A Re-Analysis of a Randomized, Placebo-Controlled Study." *Microorganisms*. 2021 Mar 4;9(3):528.
- 15 Espadaler M et al. "Probiotic composition for use in the treatment of bowel inflammation." US patent 2013/101566 A1. April 25, 2013.
- 16 Ibid.
- 17 AB-Biotics. "In vitro evaluation of the capacity to produce acetylcholine by i3.1 strains." Internal report, AB-Biotics, Dec 16, 2018.
- 18 Lorenzo-Zúñiga V, et al. "i. 31, a new combination of probiotics, improves irritable bowel syndrome-related quality of life." *World J Gastroenterol* 20.26 (2014): 8709-16.
- 19 Barraza-Ortiz DA et al. "Combination of a Probiotic and an Antispasmodic Increases Quality of Life and Reduces Symptoms in Patients with Irritable Bowel Syndrome: A Pilot Study." *Dig Dis*. 021;39(3):294-300.