Biography of Todd R. Klaenhammer

Nearly a century ago, Elie Metchnikoff of the Pasteur Institute in Paris proposed that lactic bacteria in fermented milk could promote the development of healthy intestinal microbiota. The consumption of probiotic lactic acid bacteria is useful in maintaining gastrointestinal health and has been used to preserve intestinal integrity and mobility and treat diarrheal diseases. Recent evidence from *in vitro* systems, animal models, and clinical studies suggests that lactic acid bacteria, primarily those from the *Lactobacillus* and *Bifidobacterium* species, can enhance both specific and nonspecific immune responses, possibly by activating macrophages, altering cytokines, increasing natural killer cell activity, and/or increasing levels of immunoglobulins (1, 2). Recognition of *in vivo* and immunomodulatory roles for probiotic bacteria are now promoting opportunities for use of these microorganisms for delivery of biotherapeutics, such as vaccines, to targeted regions of the intestinal mucosa (3).

Todd R. Klaenhammer, William Neal Reynolds Distinguished Professor of Food Science, Microbiology, and Genetics in the College of Agriculture and Life Sciences at North Carolina State University (Raleigh, NC), has devoted his career to studying lactic acid bacteria used in food bioprocessing. "I always found it inspiring to think that Louis Pasteur was a food microbiologist," he says. In his Inaugural Article in this issue of PNAS (4), Klaenhammer and his group present the complete genome sequence of *Lactobacillus acidophilus* NCFM (North Carolina Food Microbiology). His team identified several genetic regions within the *L. acidophilus* genome that could contribute to the organism's survival and interactions within the gastrointestinal tract.

The Science of Food

Klaenhammer began his scientific career by earning a bachelor's degree in microbiology at the University of Minnesota (Minneapolis, MN). During his senior year, he worked as an undergraduate researcher in the laboratory of Russell Johnson, whose research at the time was focused on spirochetes, such as *Leptospira* and *Treponema*. One of Johnson's postdoctoral students, Russell Bey, encouraged Klaenhammer to consider graduate studies and to speak with a new professor in Food Science, Larry McKay. McKay discovered plasmid DNA elements in dairy lactic acid bacteria and "was doing some interesting work with the genetics of bacterial cultures used to make cheese," says Klaenhammer. McKay became Klaenhammer's graduate advisor at the University of Minnesota. McKay's laboratory pioneered efforts to correlate the presence of plasmids with properties critical to the industrial performance of lactic acid bacteria, such as lactose fermentation (acid production), proteolytic activity (flavor development and milk coagulation), and bacteriocin production (inhibition of pathogens). Working with McKay piqued Klaenhammer's interest in food microbiology, microbial genetics, and bacteria used in dairy and food fermentations.

Todd R. Klaenhammer

Klaenhammer earned his master's and doctoral degrees in Food Science at the University of Minnesota, with minors in Biochemistry and Genetics. In 1978, immediately after earning his Ph.D., Klaenhammer moved to the Food Science Department at North Carolina State University to work with Marvin L. Speck, then a William Neal Reynolds Professor of Food Science and Microbiology. "[I] consider myself fortunate to [have started] out in a well-recognized food microbiology program with some really talented graduate students who set the bar high," says Klaenhammer. Speck's research focused on preservation methods for dairy starter culture bacteria, and he and his team developed Sweet Acidophilus milk, using the bacterium *L. acidophilus* NCFM. This culture is now distributed widely in dietary adjuncts and dairy foods.

Over the years, Klaenhammer and his colleagues at North Carolina State University have systematically developed genetic tools for genetic modification of lactic acid bacteria and intestinal *Lactobacillus* species, including DNA transformation and, more recently, a plasmid-based integration system for inactivation of targeted chromosomal genes (5). The recent availability of genome sequence information, combined with genetic access to the organism, has established the base on which the predicted functions of *L. acidophilus* can be investigated. "It was paralyzing 5 years ago," says Klaenhammer. "We did not have the tools to transform or genetically manipulate these organisms." Today, however, genetic tools are readily available and are being used to systematically investigate metabolic pathways, identify cell surface proteins that mediate attachment to intestinal epithelial cells, and construct organisms that may be used to deliver biotherapeutics.

Fructooligosaccharide Metabolism

In his research, Klaenhammer has also sought to characterize the metabolic pathways and enzymes responsible for transport and catabolism of complex sugars in lactobacilli. Two years ago, he described a gene locus in *L. acidophilus* involved in transport and catabolism of fructooligosaccharides (FOS), which can promote competition of beneficial microorganisms in the human gastrointestinal tract (6). FOS comprise one of several groups of nondigestible prebiotics that selectively stimulate the growth and/or activity of probiotic strains residing in the host intestine. A wide range of FOS is present in the human diet in foods such as wheat, onions, artichokes, bananas, and asparagus. Although FOS had been shown to increase the population of bifidobacteria and lactobacilli within the lower gastrointestinal tract, little had been known about the metabolic pathways and enzymes responsible for transport and catabolism of such complex sugars in lactobacilli. A locus within the *L. acidophilus* NCFM genome was identified *in silico*, indicating the presence of a gene cluster encoding proteins potentially involved in prebiotic transport and hydrolysis.

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 3906.

Klaenhammer and his colleagues computationally identified this specific gene cluster and employed a functional genomic approach, using gene knockouts, to determine how FOS were metabolized by *L. acidophilus.* By using a membrane-associated transporter, the organism was found to first internalize larger oligosaccharides and then catabolize the molecule into monosaccharides for glycolysis via an intracellular fructosidase. The ability to capture oligosaccharides was hypothesized to provide a competitive edge to *L. acidophilus* during competition for sugars in the human gastrointestinal tract.

Resisting Phage Attack

A long-term research effort of Klaenhammer's has focused on understanding how fermentation cultures resist attack by viral bacteriophages. Bacteriophages appear constantly in milk fermentations, infect and kill the lactic starter culture, and often slow or stop the fermentation. Thus, protecting fermentation bacteria from bacteriophage attack is a chief concern to the dairy and other bioprocessing industries. Between 1980 and 1986, Klaenhammer's laboratory showed that some strains of *Lactococcus lactis* harbored a collection of plasmid-encoded defense systems that could protect the organism from bacteriophage attack.

In one of his earliest papers, published in 1986 (7), Klaenhammer and colleagues directed the transfer of plasmids encoding phage defense systems into industrial starter culture strains using conjugation, a natural mating process used by bacteria. This work was performed in collaboration with scientists at Marschall Laboratories (currently Danisco, Inc., Copenhagen, Denmark) and represented one of the first examples where food bioprocessing cultures were genetically improved via a gene transfer technology. "This was a really exciting period for our research group," says Klaenhammer, "because these phage-resistant strains were used as starter cultures industrially, and we could follow their performance under the most dynamic and challenging environment for phage evolution and adaptation." Later, his group stacked different defenses together, providing the cultures with more powerful and complementary defenses. Klaenhammer's strategies were instrumental in providing fast acid-producing, phage-resistant *L. lactis* starter culture strains for the dairy industry. "These approaches serve as a model for other bioprocessing industries that are threatened by bacteriophage attacks," says Klaenhammer.

Klaenhammer's group has also studied how bacteriophages evade natural defenses and evolve new virulent types in dairy plants. One study (8) identified how a virulent phage evolved spontaneously in an industrial cheese processing plant and threatened to wipe out the cultures in use. The cultures had been modified by using Klaenhammer's strategies to carry two defense systems—one a restriction and modification (R/M) system that recognizes and hydrolyzes incoming phage DNA, and the other an abortive system that stops the phage genome from replicating. Klaenhammer and his group discovered that the phage had acquired the functional domain of the methylase from the R/M system through a genetic exchange. Sequence analysis demonstrated that the identical region, 1,273 bp in length, was present in both plasmid and phage, indicating a recent genetic exchange from plasmid to bacteriophage. As a result, the new virulent phage was able to methylate its DNA during replication in any host and thereby protect itself from digestion by the restriction enzyme. With the newly acquired protection mechanism, the phage could propagate and potentially put a halt to the factory's production.

"The one cell that commits suicide also entombs the virus and altruistically saves the rest of the population."

With this and other discoveries on how phages evolve by genetic exchange during industrial processes, Klaenhammer's group has found ways to close the genetic routes the viruses use to evolve new virulent types (9). These strategies are covered by seven patents in the United States, and the approaches are currently used worldwide in the fight against bacteriophages that infect food and dairy fermentations.

Another major milestone in Klaenhammer's research career was the development of a novel defense strategy against bacteriophages that involved triggering bacteria to self-destruct after infection (10). This approach contrasted with previous strategies that focused on engineering bacteria to resist infection. The problem with previous approaches was that new phage strains continually surfaced that could overcome bacterial defense mechanisms. Klaenhammer and his group therefore investigated ways to prevent the proliferation of an emerging phage after infection, rather than preventing infection in the first place. They focused their efforts on a variety of abortive infection mechanisms, natural self-imposed suicide systems that function in bacteria. These suicide systems tend to switch on as a last resort after the host machinery has been irreversibly redirected toward phage functions.

Klaenhammer established a genetically engineered form of an abortive infection mechanism, thus developing a novel approach with the potential to control phage infection in industrial bioprocesses. Specifically, Klaenhammer and his colleagues engineered a phage-specific inducible promoter to detect infection and trigger expression of a lethal gene. Expression of this gene was designed to kill the host and the infecting phage simultaneously in a process that mimicked a natural abortive infection mechanism. "When you have 100 million bacterial cells per milliliter, the one cell that commits suicide also entombs the virus," explains Klaenhammer, "and, in so doing, altruistically saves the rest of the population."

Sequencing of *L. acidophilus* NCFM

In his Inaugural Article, Klaenhammer and his team report the complete genome sequence of *L. acidophilus* NCFM (4). The genome of *L. acidophilus* NCFM is relatively small and, consistent with its auxotrophic nature, lacks some important biosynthetic pathways. On the other hand, the organism encodes many transporters, permeases, peptidases, and glycolases for internalization and catabolism of sugars and amino acids; these features likely reflect the organism's adaptation to the nutrient-rich environment of the upper human gastrointestinal tract.

Klaenhammer identifies several genetic regions within *L. acidophilus* that may be important in the organism's functioning and survival within the gastrointestinal tract. For example, the bacterium encodes a variety of cell surface proteins implicated in adherence to epithelial cells and signaling with immune cells. Also, Klaenhammer and his group identified a number of regulatory systems that may be important in the organism's ability to sense environmental changes.

Technology and the Future of Probiotics

Klaenhammer's genetic study of *L. acidophilus* NCFM increases the understanding of the biological mechanics of probiotic bacteria. Clinical evidence "supporting the beneficial roles of pro-

biotic cultures is accumulating rapidly," says Klaenhammer, but the specific microbial features, metabolic pathways, and properties responsible for cause-and-effect relationships have yet to be fully explained.

Having spent nearly a quarter century studying lactic acid bacteria, Klaenhammer says he has seen technological advances dramatically influence the direction of his research. "Initially, the task was to search one by one for genes that might enhance the performance of lactic acid bacteria in either their bioprocessing or probiotic roles," he says, "but the development of high-throughput DNA sequencing now allows analyses of the entire genome of the organism and related organisms." Consequently, understanding the genetic networks underlying the industrial performance of these beneficial bacteria is now possible. "With the explosion of genomic information and the tools for genetic modification, the potential benefits of lactic acid bacteria in bioprocessing and promotion of human health are very exciting," he says.

Despite his wide-ranging scientific accomplishments, Klaenhammer says he is most proud of his graduate students, whom he says continue to bring excellence to his laboratory and the university. From 1983 through 2000, six of his students have been recognized with North Carolina State University's Kenneth R. Keller Research Award, which is given annually to the graduate student deemed to have the most outstanding doctoral dissertation. According to Klaenhammer, working with graduate students is the most gratifying part of his job. "There is no greater experience than working with talented young people and being part of their accomplishments."

Emma Hitt, *Freelance Science Writer*

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