Profile of Rodolphe Barrangou

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CRISPR, the Instapot of genome editing tools, has its origins in a bacterial immune system that recognizes and slices the genetic material of invading phages. Rodolphe Barrangou, a professor of food science at North Carolina State University, demonstrated the original function of the characteristic repeating genetic sequences long before it became a household word. Barrangou is now turning CRISPR inward, using bacterial cells' own machinery to edit bacteria. "Unfortunately, bacteria do not typically have good DNA repair mechanisms, so self-targeting usually turns out to be lethal," explains Barrangou, who was elected to the National Academy of Sciences in 2018. In his Inaugural Article, Barrangou outlines how repurposing the existing type I-E CRISPR-Cas3 system of *Lactobacillus crispatus* and inserting repair templates can enable targeted editing of this common member of the human microbiome (1).

Tinker, Taste, Smell, or Try

Born in France in 1975, Barrangou found his appetite for science relatively late, when he decided to major in chemistry at the Université René Descartes in Paris. After earning a bachelor's degree in 1996, he pursued a master's degree in biological engineering at the Université de Technologie Compiegne. Barrangou could not envision spending "10 to 20 years working on 1 molecule, 1 project," as an organic chemist, and engineering was not quite the right fit either. A microbiology class on fermentation propelled him toward a second master's degree in food science and the field he has helped shape for the past 2 decades. "The living part of microbes was a whole different dimensionality."

Barrangou found the shorter life cycle of projects in the food industry compelling and thinks some of the appeal is a nod to his French heritage. "You appreciate what you work on by tasting or trying it." You can "tinker and translate it into something practical." In 1998, Barrangou entered the food science program at North Carolina State University. Having grown up in a large urban area, he looked for a place to settle with "more green and less grey" and found himself attracted to the weather, culture, food, and hospitality of the US Southeast. "It became home almost immediately."

Much of food production requires protecting food from contaminating microbes, but other types of food production, such as cheese or yogurt making, harnesses bacteria to transform raw ingredients. These helpful bacteria can themselves be destroyed by phages that infect bacteria. Barrangou studied how bacteria survive the phages naturally found in pickle brine (2) for his Master's degree before moving to dairy fermentations for his doctoral research. With advisor Todd Klaenhammer, he explored how human gut bacteria take up and break down plant-derived sugars that the human alimentary system cannot digest on its own. The confluence of food, nutrition, and microbiology appealed to Barrangou, who sequenced *Lactobacillus acidophilus*, using what he refers to as the "old school way," and characterized the sequences using "microarrays back in the day" (3, 4).

Photograph of Rodolphe Barrangou. Image courtesy of North Carolina State University/Marc Hall.

Weird Sequences

Barrangou pursued a career in industry after earning his doctorate. In January 2005, he and his wife Lisa, whom he had met at North Carolina State University, moved to Madison, Wisconsin, where Barrangou joined Danisco, a company soon acquired by DuPont. "I loved

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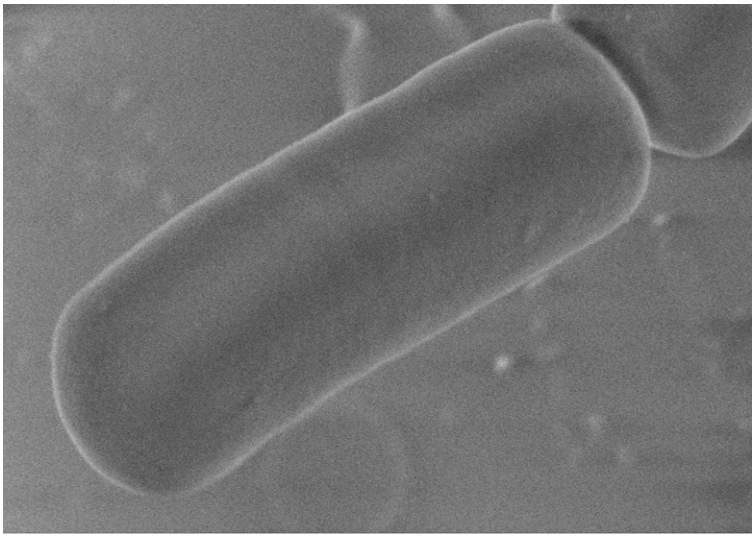
the balance between fundamental science and the application thereof."

The first task was to understand how the common yogurt starter culture bacterium *Streptococcus thermophilus* resists phage attack. Barrangou began by sequencing the genomes of a series of historical commercial starter cultures. At the time, in 2005, genome sequencing "wasn't trivial," he says. "It was very difficult to assemble." Part of the problem was the then-mysterious repeated sequences interspersed throughout the genome. These confusingly similar pieces of DNA made it hard to correctly overlap all of the shorter pieces of sequenced DNA into a cohesive assembly, like having puzzle pieces that fit the shape of another piece but not the colors of the overall design. To initiates, these sequences were known as CRISPR, for clustered regularly interspaced short palindromic repeats. "We didn't know what it was," Barrangou explains. "We just knew it was weird."

Work by other groups sequencing CRISPR elements in *Yersinia* and other prokaryotic genomes available at the time had recently shown that portions of the sequences showed homology to foreign genetic material, and a putative role in immunity had been proffered (5, 6). Barrangou had an additional clue to the puzzle: Access to sequences of phage genomes that had been causing failure of commercial starter cultures. A BLAST (Basic Local Alignment Search Tool) search, which looks for similar or related sequences in databases, confirmed that the CRISPR sequences in his *S. thermophilus* strains had two elements: One portion of the sequence consisted of superconserved repeats interspaced by seemingly random spacer sequences. Barrangou showed these spacer sequences indeed derived from specific phage genomes (7).

Barrangou and his colleagues also had access to historical research and development (R&D) strains that were selected for their phage resistance. Sequencing the strains, Barrangou observed that an *S. thermophilus* strain had evolved over time through acquisition of a new spacer sequence. "To show that CRISPR content actually determines phage resistance, we did some genetic engineering pre-CRISPR on CRISPR," says Barrangou. "In one of my favorite experiments of all time, we swapped the CRISPR content of two strains. It's like an immune system graft or transplant," he explains. "We showed for the first time that the CRISPR sequence content determines the ability of the bacteria to be resistant to phages" (7).

To look for the underlying mechanism of action, the group developed knockout strains missing one of the largest genes, which would later turn out to be Cas9. The knockout showed loss of phage resistance (7). These experiments confirmed that CRISPR is a type of adaptive bacterial immune system, with specificity conferred by the spacer sequences and the machinery contained in conserved Cas elements. Barrangou continued to work on characterizing the system, showing that transferring the CRISPR system of *S. thermophilus* to *Escherichia coli* confers immunity from the associated phage (8) and elucidating the molecular DNA cleavage mechanisms of Cas9 (9), while also keeping the big picture of CRISPR's evolutionary origins and population dynamics in mind (10). The extensive work characterizing CRISPR "laid the foundation for subsequent work on today's applications," says Barrangou. He notes that much of the original CRISPR work in dairy bacteria has been commercialized in the dairy industry, generating starter cultures with enhanced phage resistance (11, 12).



Probiotic bacterium *L. acidophilus* NCFM. Image courtesy of North Carolina State University/Courtney Klotz, Valerie Lapham, and Charles Mooney.

Beyond CRISPR

As work in the laboratory continued, Barrangou moved into management, eventually becoming the R&D director of genomics at DuPont. The business aspects of the company fascinated Barrangou as much as the science. He enjoyed "managing budgets and teams and understanding the impetus behind it" so much that he earned an MBA degree at the University of Wisconsin-Madison in 2011. "I was always seen as the CRISPR guy, the scientist," explains Barrangou. "When I moved up the ranks, I was seen as a technical advisor. That's why I went to business school, not just to get the degree but to really understand the big business picture."

As much as he enjoyed working at DuPont, the hectic travel took a toll on his work/life balance. Barrangou describes a "eureka moment" in his late 30's when he realized that he could "unleash the full power of CRISPR in academia." He joined the faculty of North Carolina State University early in 2013, just as the world of CRISPR exploded with the revelation that the bacterial machinery could be harnessed for gene editing in eukaryotes (13, 14). Barrangou maintains deep connections to the business world; he has founded 3 companies and sits on the board of several others. "I travel as much now, but I have control over where and when."

For the first 10 years of his CRISPR career, Barrangou had been using CRISPR to kill phages in bacteria. His work at North Carolina State University, where Barrangou

is able to pursue ideas and directions deemed impractical in a strategic business setting, has focused on using phages to deliver CRISPR that kill bacteria (15). "Like big companies, we fail early and move on to the next idea," he says. "I don't fish, but I love the imagery. We throw a bunch of hooks and look for signs of a legit bite." While the team must be strategic about which lines to pick up or let go, Barrangou says, unlike industry, they make decisions based on pure scientific interest or gut instinct.

Recently, Barrangou's focus has evolved to genome editing. "It's back to the beginning or back to the future, or maybe both at the same time," Barrangou notes, of working with CRISPR in probiotics once again. His team is using CRISPR to enhance host colonization (16) and exploring ways to use CRISPR to edit entire microbiomes. Currently, the laboratory is engineering probiotics with the ability to modulate the composition of bile salts in the gastrointestinal tract, employing CRISPR to alter the bile salt hydrolase enzyme pool in the human gut microbiome (17).

All in Good Pun

In his Inaugural Article, Barrangou combines CRISPR biology and probiotics with a touch of the contrarian, choosing to focus on the type I-E CRISPR-Cas3 system, which is more common in bacteria than the industrial favorite type II CRISPR-Cas9 system (1). Barrangou and his colleagues characterized and then repurposed the endogenous CRISPR-Cas system in *L. crispatus*, a common probiotic found in human vaginal flora and the intestinal tract of poultry. To drive this self-targeting–based genome editing system, they "provision the cell with a designed template to alter the sequence precisely at the site of the target" (1). The work "lays the foundation for type I systems to be repurposed," according to Barrangou.

In 2016, Barrangou was named the Todd R. Klaenhammer Distinguished Professor in Probiotics Research, an endowed chair named in honor of his former thesis advisor, who retired in 2018. "Being able to come back and work with Todd and continue the legacy of his program was a big part of the decision to come back to North Carolina. He was a great mentor and a great scientist, but also a great person and role model," Barrangou explains. "He told me that 'It's great to be a great scientist, but it's even better to be likable.'"

Barrangou, a father of 3, tries to keep perspective inside and outside the laboratory. He practices yoga 250 times each year, 5 days a week for 50 weeks, a commitment that he says allows him to stretch his body and mind and "keep calm and CRISPR on." Puns play a role in Barrangou's writing and communication. "If the right pun presents itself, I'm going to use it," he says. "I don't know if they all work. I don't know if they're all equally appreciated by the readership, but I like to keep it fun."

As a professor at a land-grant university, Barrangou explains that outreach and extension are a critical part of his work. In addition to keeping it fun, Barrangou looks forward to extending his realm of influence into communication, public engagement, and regulatory work. "Sadly, with CRISPR, the science has perhaps become the easier part."

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