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In 2007, Philippe Horvath and his colleagues explained how bacteria protect themselves against viruses at Danisco, a Danish food company, in Dangé-Saint-Romain, France. Horvath and his team worked to improve the lifespan of bacteria cultures for manufacturing yogurt and ice cream. Specifically, they focused on bacteria’s resistance to bacteriophages, or viruses that infect bacteria. Horvath and his colleagues found that the bacteria used to culture yogurt, Streptococcus thermophilus, has an adaptive immune system that can target specific viruses that have previously infected the bacteria. The immune system is called the CRISPR/cas system, or the clustered regularly interspaced short palindromic repeats/CRISPR associated protein system. Horvath and his colleagues explained how bacteria use CRISPR/cas as an immune system to target viruses and protect themselves from infection. The discovery informed the development of CRISPR/cas as a gene editing tool to modify bacterial, animal, and human genomes.

The research was funded by Danisco, part of the DuPont Nutrition and Health in Dangé-Saint-Romain, France. Horvath, a molecular biologist, joined Danisco to develop molecular biology techniques for working with bacteria used in the manufacture of dairy products. Christophe Fremaux, Hélène Deveau, Melissa Richards, Patrick Boyaval, Sylvain Moineau, and Dennis A. Romero were research scientists involved with Horvath’s project at Danisco. As the research and development director of genomics at Danisco, Rodolphe Barrangou studied how bacterial genes worked and collaborated with Horvath on the experiment. Together, the group of researchers aimed to understand why some bacteria cultures used to produce yogurt and other dairy products were resistant to viruses and why others were not.

All organisms and viruses carry information in a genetic code in the form of deoxyribose nucleic acid of DNA or ribonucleic acid or RNA. Cells translate the genetic code of DNA and RNA to make different proteins. CRISPR sequences are repeated DNA sequences found in the genomes of bacteria and archaea, which is a domain of single-celled organisms that resemble bacteria. The CRISPR sequences are involved in protecting bacteria and archaea cells from viruses that have infected the cells before. When a virus infects a cell belonging to bacteria or archaea, the cell copies a part of the virus’s DNA. The copied viral DNA are then inserted between CRISPR sequences to make spacers. The spacers are pieces of viral DNA added into the genome between CRISPR sequences and provide the cell with a history of the viruses that have infected it before. The cell can use the spacers to recognize viruses with DNA or RNA sequences that match the spacer sequences. Once a cell recognizes a virus, it can inactivate the DNA or RNA of the virus, thereby fighting off the virus. Horvath and his research team in 2007 confirmed that bacteria use the CRISPR/cas 9 system to defend against viruses and described its mechanism.

Before Horvath and his colleagues discovered how bacteria use the CRISPR/cas system, scientists had noted the existence of CRISPR sequences and their relationship with viruses. In the late 1980s, Yoshizumi Ishino from the Osaka University in Osaka, Japan, was one of the first to describe a CRISPR sequence after he found a distinct organization of a repeated DNA sequence in bacteria. In 2002, Francisco Mojica and Ruud Jansen at the University of Alicante in Alicante, Spain, observed the same distinct organization of a repeated DNA sequence within a single region of the genome in archaea. Mojica and Jansen coined the term CRISPR in their publication. Additionally, Jansen discovered genes associated with CRISPR sequences, which he called cas, or CRISPR associated. He found that the DNA sequences coding for the cas genes were always found near CRISPR sequences within bacteria and archaea genomes. By comparing the gene sequences to known gene sequences, he found that cas genes coded for helicase, an enzyme that breaks DNA strands apart, and nuclease, an enzyme that cuts DNA strands. Jansen and Mojica’s findings suggested that cas genes are involved with gene editing because they code for proteins that manipulated DNA strands.

In 2005, scientists showed that DNA sequences, or spacer content, between repeated CRISPR sequences in bacteria matched perfectly with DNA sequences from viruses. The correlation indicated that CRISPR regions in genomes likely have a significant role in bacteria’s interactions with viruses, as bacteria do not normally carry viral DNA in their genomes. Mojica’s group published data showing that CRISPR sequences derived from viruses that had previously infected the bacterial cell. They hypothesized that CRISPR/cas played a role in microbial immunity to protect bacteria from viruses because the spacer content sequences matched DNA sequences from viruses. Bacteria used RNA target sequences to recognize DNA or RNA from viruses. Recognizing viruses by their DNA or RNA sequence then allowed the cell to protect itself from the virus. However, the exact mechanism for how CRISPR/cas protected bacteria from viruses remained unknown.
In 2007 Horvath and his colleagues validated the hypothesis that bacteria cells used the CRISPR/cas system to protect against viruses and described the mechanism of the CRISPR/cas system. They studied different strains of *Streptococcus thermophilus*, a species of bacteria used for yogurt culture. They wanted to understand why some strains of bacteria were resistant to viruses and why other strains were infected and killed by viruses. Virus-resistant bacterial strains were optimal for yogurt culture. Because scientists previously discovered that some bacteria have DNA sequences from viruses in CRISPR regions in the bacteria genome, Horvath and his team hypothesized that virus-resistant bacterial strains would have viral DNA in their genomes.

Horvath and his colleagues first aimed to determine the CRISPR sequences of the bacteria before virus infection to identify how CRISPR sequences change with infection of viruses. To do that, Horvath and his colleagues analyzed the DNA sequences and identified the CRISPR sequences of the *S. thermophilus* strains. Then, to test how virus infection would impact the CRISPR sequences, they infected bacterial strains with two different viruses, individually or simultaneously. Those infected bacterial strains were called parental strains because they were the first generation of the bacterial culture. Daughter bacterial strains were any following generations replicated from the parental bacterial strain. The research team replicated those parental bacterial strains after virus infection. Then, they analyzed how the CRISPR sequences changed after infection and whether or not those changes were inherited in the bacterial strains.

Upon analyzing the DNA of the bacteria, Horvath and his colleagues found that the bacterial daughter strains replicated after virus infection had an increased number of CRISPR sequences and spacers, sequences contained between CRISPRs. Also the DNA sequences of the spacers matched the viral DNA of the viruses used to infect the bacteria. Because the number of CRISPR sequences and spacers increased in response to virus infection and the spacer sequences matched the DNA of the specific viruses that infected the bacteria, the researchers confirmed CRISPR’s involvement in protecting bacteria from viruses.

To determine how the increase in spacer and CRISPR sequences impacted virus-resistance of the bacteria, Horvath and his colleagues infected the bacterial daughter strains with a second virus infection. The researchers found that the bacteria survived better. They suggested that having an increase in the number of spacers that mimicked the virus’s DNA increased bacteria’s chance of survival. Horvath and his group showed that bacteria with more spacers were less affected by viruses. The results suggested that the bacterial cell incorporated DNA from the virus as spacer sequences into the bacteria genome and became resistant to the virus. By incorporating DNA from viruses into their genomes, bacteria prevented a second infection by viruses they had already encountered. Furthermore, Horvath and his colleagues found that each bacterial daughter strain was resistant only to the virus that had infected its parent. The specificity of the acquired resistance suggested that bacteria integrated virus DNA into their genomes to acquire resistance against that virus.

To further determine the role of the CRISPR spacer content in bacteria’s viral resistance, Horvath and his colleagues tested virus resistance of bacteria after adding or deleting spacers from the bacteria’s genome. Spacers are pieces of viral DNA that are added into the bacterial genome in between repeated CRISPR sequences and they are what bacteria use to identify viruses. First, the research team modified a virus-resistant bacterial strain by deleting spacer content from its genome. Then, they exposed the modified bacterial strain and unmodified bacterial strain to the virus whose DNA matched the deleted spacer content. As the researchers predicted, the virus infected the modified bacterial strain with deleted spacers, while the unmodified bacterial strain with spacers remained resistant to the virus. Because bacteria with spacers showed viral resistance and bacteria with deleted spacers did not, Horvath and his colleagues concluded that spacers provided bacteria immunity against viruses and that only bacteria with spacers were resistant to viruses.

Next, the research group showed that adding spacers into bacterial genomes could provide immunity against viruses. The group used a bacterial strain that did not have resistance to a particular virus. They added spacer content particular to that virus into the bacterial strain and infected the modified bacteria with the virus. The bacteria gained resistance and was unaffected by the virus infection. Through deleting and adding spacer content in the bacteria cells and demonstrating loss or gain of virus resistance, Horvath and his research team established that the spacers between CRISPR sequences provided bacteria immunity against viruses.

In the same set of experiments, Horvath and his colleagues also discovered the involvement of cas genes in bacterial immunity. They used cas 5, an enzyme known to cut DNA. The researchers inactivated cas 5 in the bacteria, meaning the enzyme could no longer cut DNA. By inactivating the cas 5 gene in virus-resistant bacteria, Horvath and his colleagues found that the bacteria lost its resistance. They noted that the cas 5 protein recognized a virus, which the bacteria already had immunity to, and cut the virus DNA to inactivate it. However, because the cas 5 in the experiment was not active, it could not cut the viral DNA, and the bacteria were infected by the virus. Because bacteria without functional cas 5 were infected by viruses, Horvath and his colleagues suggested that cas 5 protein played a direct role in recognizing or inactivating the virus.

Horvath and his colleagues also investigated the effect of inactivating cas 7, an unknown gene. Again, they inactivated cas 7 in a virus-resistant bacterium, and tested the bacterium’s resistance by exposing it to the virus. Horvath and his colleagues found that bacteria with inactivated cas 7 remained resistant to the virus. Therefore, they hypothesized that cas 7 was used in adding or
making new spacer content rather than recognizing and inactivating viruses. So even with an inactivated cas 7 gene, the bacterium could inactivate a viral DNA it already had stored in its genome. Through establishing the link between CRISPR spacer content and virus resistance and investigating the role of cas genes, Horvath and his colleagues demonstrated that the CRISPR/cas system provides acquired immunity for bacterial cells.

Horvath and his colleagues’ experiments uncovered how the CRISPR/cas system operates in bacteria and informed future research on the CRISPR/cas system, including research about its abilities to edit genes within the human genomes. In 2015, Horvath received the Massry Prize, awarded to significant contributors in biomedical sciences. Horvath and Barrangou were awarded the 2016 Canada Gairdner International Award for their research on the CRISPR/cas system.

Horvath and his colleagues’ establishment of the CRISPR/cas system as an acquired immune system for bacteria helped lead to the development of CRISPR/cas-9, a gene editing tool that Jennifer Doudna and Emmanuel Charpentier created. CRISPR/cas-9 has enabled scientists to modify the genomes of bacteria, plants, and animals, including human stem cells, or cells that can give rise to any kind of cell for that organism. Furthermore, DuPont and other food production companies have used CRISPR/cas technology to modify bacteria and plants for food production.

Sources


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