

## Healthcare industry BW

### Bacteria – a treasure box for genetic engineers

**Bacteria have developed a versatile defence system to protect themselves against viral infections. One of these defence tools, known as CRISPR/Cas9 system, is currently hogging the headlines as it promises to revolutionise the way genetic material can be modified. Prof. Rolf Backofen from the Institute of Bioinformatics at the University of Freiburg has managed to classify the defence system of all bacterial species sequenced to date. This will certainly facilitate the search for a new generation of powerful genetic engineering tools.**



Prof. Dr. Rolf Backofen  
© Rolf Backofen/IIF

Scientists have long assumed that only vertebrates have an immune system to fight off pathogens such as bacteria and viruses. However, just ten years ago it became clear that they were wrong. Prokaryotes, i.e. bacteria and archaea, were also found to have an immune system that they use to protect themselves against bacteria. The mechanism that they use for this purpose is called CRISPR.

CRISPR stands for clustered regularly interspaced short palindromic repeats. These are segments of DNA containing palindromes, i.e. short repetitions of base sequences where the sequences reading 5' to 3' match the sequences reading 5' to 3' on the complementary strand. In other words, a sequence of characters that reads the same backwards and forwards like the name Hannah. "45 percent of all sequenced bacteria and 83 percent of sequenced archaea have at least one CRISPR structure," says Rolf Backofen from the Institute of Bioinformatics at the University of Freiburg.

The first time that bacteria are attacked by a bacteriophage, a virus that exclusively attacks bacteria and archaea, they are unable to defend themselves. Phages are around 1000 times smaller than bacteria and inject their genetic material into the bacterial cell where they multiply. If they are successful, they multiply endlessly and the bacterial cell eventually bursts.

## Bacteria have developed an anti-virus programme

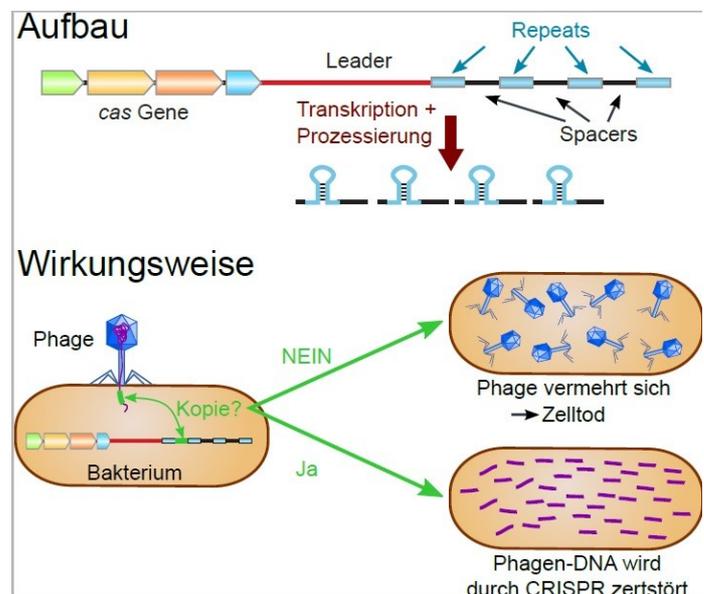
If the bacterium survives the infection, it memorises previously encountered attackers and is able to protect itself against further attacks. It does so by inserting short phage DNA pieces into an array of CRISPR repeats (spacers). The viral DNA that is framed by the repeats now becomes a distinctive mark and is used to target and destroy the nasty virus upon new infections.

The bacterial defence mechanism involves CRISPR as well as several proteins of the Cas family (Cas stands for CRISPR-associated). The Cas proteins translate the incorporated phage DNA into an RNA molecule that pairs with the matching DNA segment upon new infection. This activates the nuclease enzyme of the Cas protein complex, which then cuts the viral DNA into small pieces. "However, it is not yet known in detail how this defence system works," says Backofen.

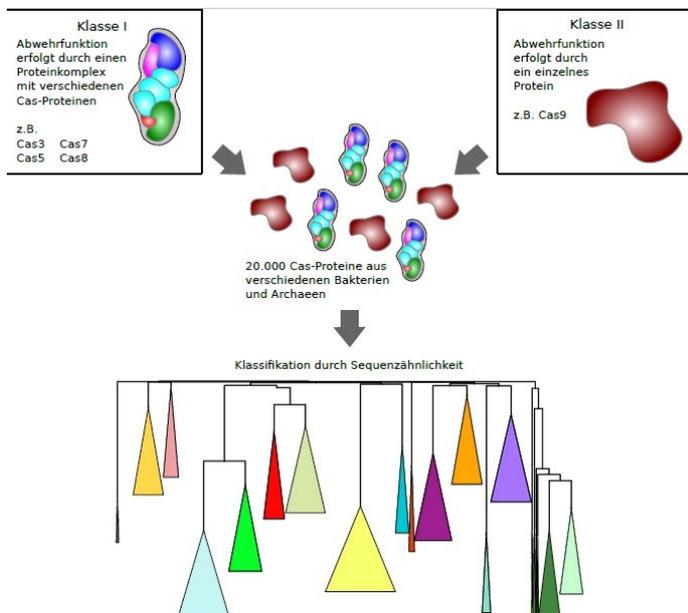
What is known is that the CRISPR/Cas system is widespread among bacteria and archaea and occurs in numerous different variants. CRISPR DNA can contain up to 250 palindromes of different lengths (up to 50 base pairs) and can be associated with between six and 20 Cas proteins. Bacteria exchange their adaptive immunity, i.e. their CRISPR/Cas system, by way of what is known as horizontal gene transfer. They kind of mutually "vaccinate" themselves. This means that a prokaryote can contain several different CRISPR structures.

## Structuring the versatile protection against viruses

This variability initially made the classification of the CRISPR/Cas system rather difficult. However,



Structure and mode of action of the CRISPR/Cas system in bacteria and archaea  
© Backofen/IFF, Sita J. Saunders and Omer Alkhanbashi/IFF



Classification of all known CRISPR/Cas systems: Class 1 systems build defence complexes in which they use several proteins, while class 2 systems only use one protein. The classification is based on the sequence similarity of groups of proteins.

© Backofen/IIF

Backofen and his team developed software that takes into account the modular structure of the bacterial defence system and rapidly analyses vast amounts of data – more than 20,000 protein sequences in five minutes.

The bioinformaticians from Freiburg found that the defence system of known prokaryotes can be divided into two classes, five types and 16 subtypes. The classification is based on Cas protein similarities and CRISPR repeat similarities. “Based on machine learning, our programme can automatically classify any new defence system,” says Backofen who does not exclude the possibility that other, previously unknown, variants exist.

The classification has several advantages: first, it can be used to analyse metagenomic data: “We can sequence bacterial communities from the stomach or from the skin and determine the bacteria’s bacteriophage immunities, i.e. CRISPR

systems,” says Backofen.

## Applications in research, industry and medicine

The researchers hope that this information can be used in the long term in the fight against pathogens or other harmful bacteria, in the food industry for example. If the immune defence system of bacteria can be understood in detail, it can therefore be specifically manipulated. For example, by switching off the CRISPR system of pathogens and making bacteria susceptible again to their natural enemies, or by vaccinating bacteria used in the dairy industry in order to protect them against certain viruses.

The classification of the CRISPR/Cas9 system also facilitates the search for new methods that can be used in biotechnology. These are the reasons that make the CRISPR/Cas9 system an excellent candidate for a new generation of powerful genetic engineering tools. The renowned journal *Science* called the method the scientific “Breakthrough of the Year 2015”. CRISPR/Cas9 can be easily reprogrammed into highly precise DNA scissors that can be used to cut any DNA segment whatsoever. DNA segments can be excised and others reinserted. The technique, therefore, has the potential, at least in theory, of being used to heal or cure diseases for which there are currently no remedies.

---

### Article

15-Feb-2016

Juliette Irmer

© BIOPRO Baden-Württemberg GmbH

---

## Further information

Prof. Dr. Rolf Backofen

Tel.: +49 (0)761 203-7461

E-mail: backofen(at)informatik.uni-freiburg.de

► [Chair for Bioinformatics  
Freiburg](#)

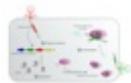
---

## The article is part of the following dossiers



Evolutionary research - from classical biology to molecular phylogeny

---



CRISPR/Cas – genome editing is becoming increasingly popular

sequencing

virus

microbiology

bacterium

genetic engineering

immune system

drug application

defence mechanisms

CRISPR/Cas