# Case Study

ETERNAL

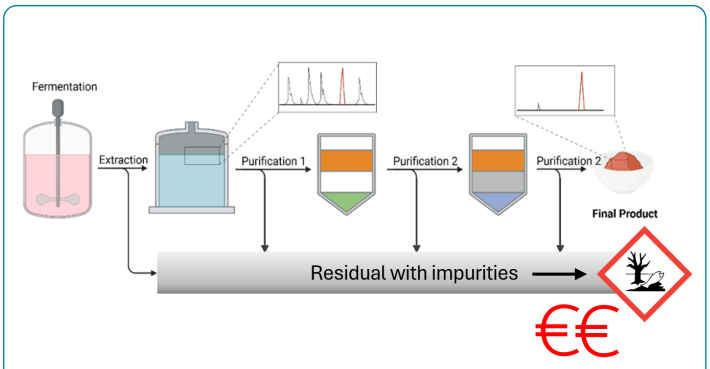
# STRAIN ENGINEERING FOR GREENER BIO-MANUFACTURING

## TARGETED GENOME ENGINEERING

Microorganisms have naturally evolved over millennia to produce an array of bioactive secondary metabolites. These have been successfully harnessed since the advent of penicillin to provide many medically important drugs like antibiotics, immunosuppressants, and vaccines.

Under industrial fermentation conditions however, many microbial producer strains will naturally produce multiple by-products alongside the intended target compounds. Some of these untargeted compounds may be bioactive and it would hardly be surprising if some of them at least were implicated with issues of ecotoxicity, leading to harmful effects on non-target organisms and ecosystems.

The production of particular compounds is known to be associated with specific parts of the bacterial DNA. If the parts encoding non-targeted secondary metabolites can be identified and selectively deleted from the bacterial genome without impacting upon the part responsible for making the desired product, then a pathway opens up to safer, more streamlined and sustainable pharmaceutical bio-manufacturing processes using genetically engineered versions of the strains to produce only the intended active compounds.




Conventional microbial based bio-synthesis presents manufacturers with an uncomfortable choice: either add to process capital and operational costs by investing in and maintaining expensive effluent treatment systems or risk the release of bioactive / cytotoxic compounds into the environment. Even if manufacturers' processes are scrupulously managed, concerns remain about uncontrolled end of life drug disposal and the ability of hard pressed public water treatment infrastructures to protect watercourses, and all life that depends upon them, including humans, from the consequences.

## Context

**Microorganisms are well-known, producers of bioactive compounds. Microbially derived medicines include antibiotics, immunosuppressants, and vaccines.**

## Challenge

**Many microbial producer strains produce multiple by-products alongside their target compound. These may exhibit bioactivity, including cytotoxicity, raising environmental concerns in the event of their release during production or disposal, and making downstream purification processes more challenging. Preventing the expression of these by-products by removing the parts of the bacterial DNA responsible for their synthesis opens the way to safer, more streamlined and sustainable pharmaceutical bio-manufacturing processes.**

## Innovation

**A simple and straightforward genome editing platform for bacteria has been used to precisely delete targeted DNA regions in a producer bacterium used for making the immunosuppressant drug rapamycin. The method is potentially adaptable to any microbial system.**

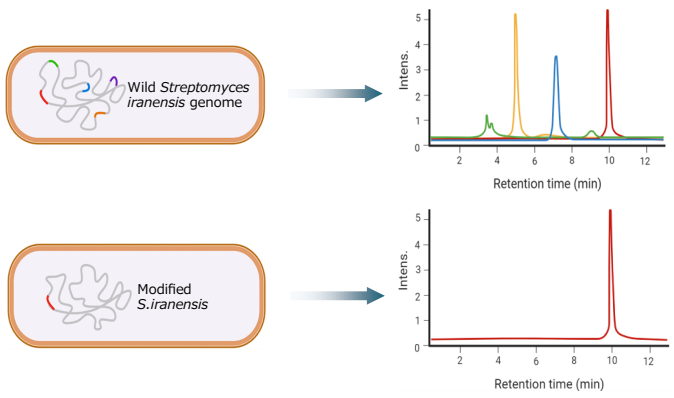
## Next Steps

**With five identified DNA target regions already successfully removed, work continues on five more. In parallel, fermentation conditions are being optimized for the modified producer strain and fine tune the production media for cost-effectiveness and scalability.**

# Features and Advantages

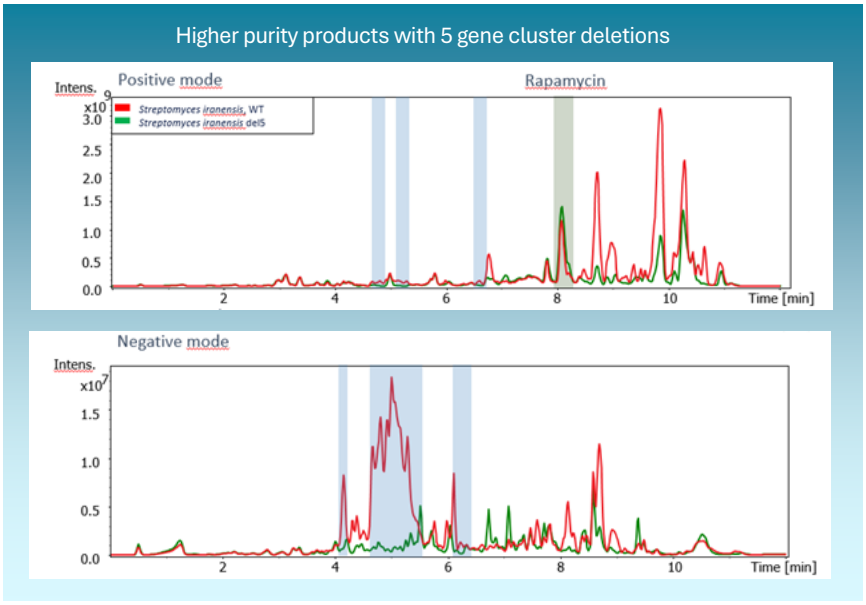
The University of Saarland's innovation focuses on precise genome editing to remove specific parts of biosynthetic gene clusters within the genetic make-up of the producer strain responsible for the production of toxic by-products. To enable this, a genomic library has been constructed from fragmented chromosomal DNA of the rapamycin-producing strain, allowing for efficient and targeted genome modifications. Additionally, a DNA delivery method using E. *coli–Streptomyces* conjugation has been optimized , significantly improving the efficiency of genetic editing in the target strain.

Genetically engineered bacteria can produce active pharmaceutical ingredients like rapamycin with fewer toxic by-products. This makes the manufacturing process safer, simplifies waste management, and significantly reduces the costs associated with purification and disposal.




Precise genome editing of the wild producer strain makes it possible to remove the specific parts of biosynthetic gene clusters responsible for producing toxic by-products. With fewer impurities to remove and less of those that do remain, processes complexity and costs associated with purification and disposal simply disappear along with the non-target peaks in the analytical results.

## *Fewer toxic by-products, a safer process, simpler waste management, and lower costs*




LC-MS chromatograms comparing the metabolite profiles of *Streptomyces iranensis* wild-type (WT, red) and mutant strain **Δ5** (green).

The terms "positive" and "negative" modes here refer to the ionization polarity used during mass spectrometry detection. Some metabolites are more efficiently ionized, and thus better (or even exclusively) detected, in one mode or the other. Both are therefore required to have a full picture.

Peaks in the blue-shaded regions correspond to by-products synthesized by the wild type but not by the mutant strain, indicating successful producer gene deletion. The rapamycin peak (green-shaded area) remains unchanged, confirming that the desired compound is still produced after all genetic manipulation.

# Results and Benefits

The ETERNAL team at Saarland have identified ten DNA regions to delete in order to reduce unwanted by-products and improve strain performance. They have already successfully deleted five regions, leading to a marked reduction in unwanted by-products. The latest variant strain displaying a significantly cleaner metabolic profile compared to the original, with work continuing on further deletions, and on optimizing the fermentation conditions to enhance production yield and economics.

Genome editing designs quality into API bio-manufacture, eliminating the issue of by-products at source. This reduces the need for complex downstream purification, lowers waste generation, and minimizes environmental risks. It can help manufacturers comply with ever stricter environmental and safety regulations and future proof microbial biomanufacturing. Most importantly, it can contribute to lowering production costs, ensuring that life-saving medicines like rapamycin become more affordable and accessible to patients around the world.

ETERNAL is contributing to the sustainable development of pharmaceutical manufacture, use and disposal, by using and promoting full life cycle approaches covering design, manufacture, use, and disposal through

- application-industry oriented R&D and scale-up;
- clear pathways to compliance;
- new scientific knowledge on the environmental fate and eco-toxicological effects of pharmaceuticals; and
- behavioural change for safe use and disposal.

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**Find out more at: www.eternalproject.eu**