
iGEM 2024 team of IISER Kolkata. From left to right: Shamba Chakraborty, Anushka Mishra, Sharanya Chatterjee, Ayantik Kundu, Utkarsh Aryan, Gitanjali Das, Smrutimayee Prusty, Fathima Anjum EC, Sampoorn Pandey, Divyanshu Kumar

# Engineering Solutions for Foot Care: IISER-K's *E. coli*-Powered Foot Insole Bags Gold at iGEM

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The IISER Kolkata iGEM 2024 team addresses the widespread problem of foot infections by developing a self-sustaining, GMO-based insole that can both treat and prevent fungal growth. Using engineered E. coli to produce linalool, a natural antifungal and antibacterial compound found in lavender, the insole continuously protects against fungal and bacterial growth, reducing discomfort and leaving a refreshing scent. The team, through complex control systems, such as quorum sensing and cell cycle arrest, ensured the bacteria became effective with time. Their interdisciplinary project won a gold medal and special award nomination for Best Fashion and Cosmetics.

For IISER Kolkata's iGEM 2024 team, their journey began with a common but persistent issue—foot infections. Athlete's foot, diabetic ulcers, and other fungal infections cause discomfort, itching, and unpleasant odours for millions around the world. The team wondered whether biotechnology could offer a solution? Their answer was a **GMO-powered foot insole designed to treat and prevent foot fungal infections**. Their project stood out among global entries, earning the team a **gold medal and a nomination for the Best Fashion and Cosmetics Project category**. Through genetically modified organisms (GMOs), the project transforms everyday foot care into a scientific marvel. This interdisciplinary approach, typical of iGEM, integrates biology, chemistry, and mathematical modelling to address critical societal challenges like health and hygiene.

**iGEM educates the workforce and leaders of the synthetic biology industry**. The iGEM Foundation is an independent, non-profit organisation dedicated to advancing synthetic biology, fostering education and competition, and building an open, collaborative, and cooperative community. Synthetic biology often originates from simple, everyday problems, and iGEM's mission is to provide a platform for teams like IISER Kolkata to find innovative, impactful solutions.

Mentors always play a key role in projects like this, guiding young and enthusiastic students through complex challenges, ensuring that **interdisciplinary collaboration** leads to **innovative results**. This year, Prof. Supratim Dutta, Prof. Tapas Kumar Sengupta, Prof. Susmita Roy and Prof. Anindita Bhadra helped the students in refining their designs, offering crucial insights from their own expertise.

## The Innovation: A GMO-Powered Foot Insole

At the heart of their idea lies **Escherichia coli** (*E. coli*) - **a genetically modified bacterium that produces linalool**, a fragrant monoterpene(compounds found in the essential oils extracted from many plants, including fruits, vegetables, spices and herbs). Found in lavender, linalool boasts antifungal, antibacterial, and pain-relieving properties, making it perfect for foot care applications. This naturally occurring compound isn't just about making your feet smell good—it actively combats the microbes responsible for common infections, while also reducing discomfort.

The engineered insole nurtures the growth of these bacteria, using a **multi-layered structure** for protection and functionality. A non-porous bottom layer ensures durability, while the bacteria thrive in a nutritive medium held in a biocompatible polymer. The semi-permeable upper layer allows the beneficial compounds to diffuse, reaching the foot while keeping the bacteria contained.

As **Prof. Tapas Kumar Sengupta** puts it, this system offers a unique advantage: it transforms foot care from something you have to do into something that happens naturally and continuously, all thanks to the insole. Importantly, teamwork among experts from various fields—biologists, engineers, and modellers—is essential for iGEM projects like this one, enabling the fusion of disciplines to create well-rounded, innovative solutions.

But why linalool, you might ask? **Its antifungal properties help fight infections such as athlete's foot, while its antibacterial effects ward off other common pathogens.** The analgesic and anti-inflammatory benefits reduce pain and swelling, providing relief from the discomfort often caused by these infections. Plus, its pleasant lavender-like scent adds a touch of freshness to the mix, eliminating foot odour—a common symptom of microbial infections.

However, it's not just about choosing the right compound. The real genius of the project is in how the bacteria are engineered to consistently produce linalool. This is where the magic of synthetic biology comes into play.

## The Engineering: Using Synthetic Biology to Power the Insole

The team didn't just slap some bacteria onto an insole and call it a day. **They carefully designed a system where the bacteria could live and thrive, ensuring long-term production of linalool**. The main challenge they faced was controlling the bacterial growth to ensure that it continued

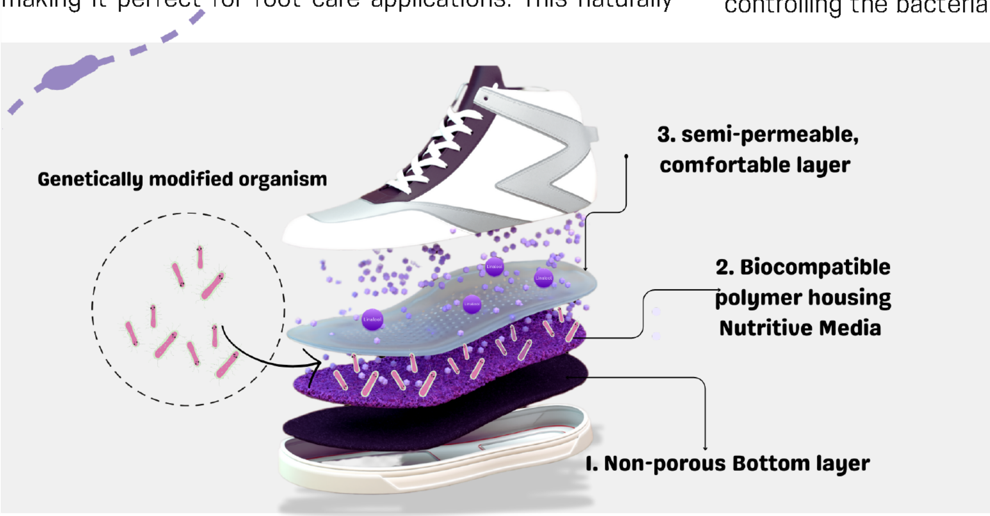


**Fig 1**.The GMO-powered foot insole is structured with multiple protective layers. A biocompatible polymer layer containing the GM bacteria sits at the core, providing nutrients for the bacteria. This polymer layer is sealed on the underside with a non-porous layer to prevent bacterial leakage, while a porous upper layer allows only the targeted volatile compounds to escape, keeping the bacteria contained within the insole. [IISER Kolkata iGEM 2024 Wiki]

Population Control Module: Ensures regulation of population

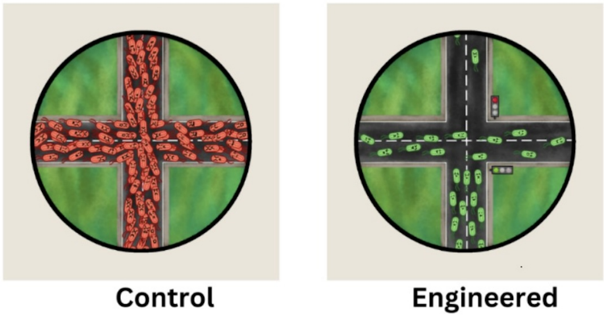


**Fig 2**. The system operates similarly to traffic control, managing bacterial "flow" to maintain efficient functioning. Bacteria can multiply as needed, but once the population hits an optimal level, the system temporarily "stops" further growth until it's necessary again. This method keeps the bacterial population balanced and efficient. [IISER Kolkata iGEM 2024 Wiki]

producing linalool without exhausting its resources or multiplying unchecked.

**Lactobacillus rhamnosus** is a well-established probiotic known for its safety and effectiveness in treatments for humans. It has gained recognition for its beneficial role in various therapeutic applications, making it a reliable choice in bioengineering projects aimed at improving health outcomes. One of its key advantages lies in its antifungal and antimicrobial properties, which complement the production of linalool—a compound with antibacterial and antifungal effects. Together, they create a robust defence against harmful microorganisms, enhancing the protective qualities of products in which they are incorporated.

Moreover, Lactobacillus rhamnosus is **compatible with the skin microbiome**, meaning it can **outcompete harmful pathogens without disrupting the natural microbial balance on the skin**. This compatibility is crucial, as it ensures that beneficial bacteria thrive, thereby supporting skin health rather than compromising it. Beyond its protective capabilities, Lactobacillus rhamnosus also offers **immunomodulatory potential**, aiding in **reducing inflammation** and **improving overall skin health**. This probiotic, therefore, provides a multifaceted approach to skin care, making it an invaluable asset in biotechnology applications focused on health, hygiene, and sustainability.

**The solution was a combination of quorum sensing and cell cycle arrest** (CCA). Quorum sensing is a **natural process where bacteria communicate and regulate their behaviour based on their population density**. This allows the team to control when and how much linalool is produced, ensuring that the bacteria don't consume all the nutrients too quickly or overpopulate, leading to their own demise.

The **cell cycle arrest mechanism** (CCA), powered by antisense RNA, regulates the growth of the bacterial colony. Essentially, the bacteria can "pause" their growth to focus on producing linalool, preventing them from growing too quickly and running out of resources. These two mechanisms, quorum sensing and CCA, work together to ensure the bacteria remain in a productive phase for longer, keeping the insole functional and effective over extended periods.

The complex interaction between bacterial growth, nutrient availability, and linalool production required detailed mathematical modelling. Using ordinary differential equations (ODEs), the team predicted how the bacteria would behave under different conditions. These equations helped them simulate population growth, nutrient consumption, and the timing of linalool release.

The **mathematical models were key to fine-tuning the design**. For instance, if the bacterial population grows too quickly, it could run out of nutrients, halting linalool production prematurely. By **adjusting the quorum sensing and CCA mechanisms**, the team was able to **predict and control the lifespan of the bacterial culture, ensuring sustained production of linalool without exhausting the insole's resources**. Mathematical modelling, alongside biology and outreach, plays an integral role in an iGEM project's success, contributing to the overall balance and precision of the design.

## Safety First: Ensuring Biosecurity

The idea of putting genetically modified organisms (GMOs) into a consumer product naturally raises concerns about safety. The IISER Kolkata team tackled this issue head-on by incorporating several safety mechanisms. They used an **auxotrophic strain** of *E. coli* that requires specific nutrients to survive—nutrients that are only provided within the

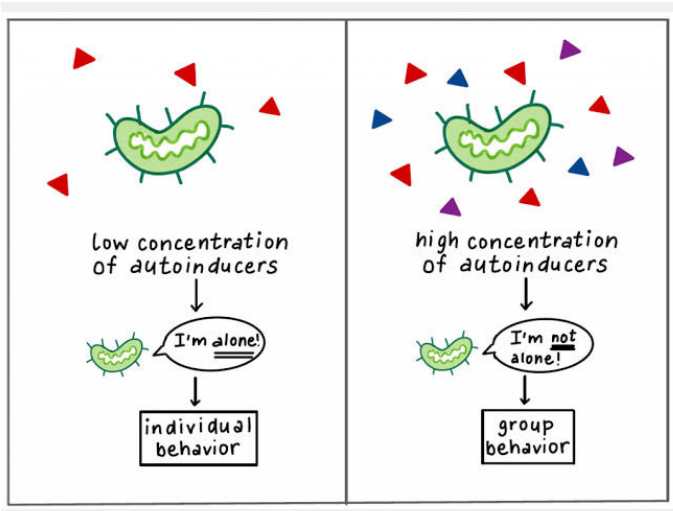


**Fig 3**. Live Application treatments often suffer from short activation periods, lowering effectiveness and requiring frequent reapplication. This system regulates GM bacteria to oscillate around a set population, enhancing linalool output and media conservation, extending product usability over time. [IISER Kolkata iGEM 2024 Wiki]

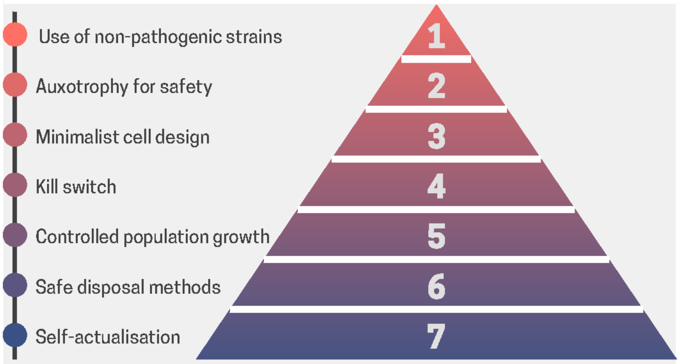


**Fig 4**. The team employed a large number of safety measures (see main text). These layered safety protocols guarantee that the microbial system is both efficient and safe for extended use in footbed applications, safeguarding both the user and the environment. [Wiki]

insole's environment. By requiring these specific nutrients, the bacteria are confined to the footbed, preventing any unintended growth in uncontrolled areas. Additionally, the **bacteria are genetically streamlined**, containing only the essential components needed to produce beneficial compounds like linalool, reducing the risk of genetic interactions or unintended behaviours. This minimalist design reduces the likelihood of unpredictable outcomes, ensuring a safe and controlled microbial system.

Lactobacillus rhamnosus, the selected bacteria for the footbed, is a **non-pathogenic, safe probiotic commonly found in the human microbiome**, minimising health risks while performing its intended function. This strain has been carefully chosen for its safety profile, ensuring that it poses no threat to human health while fulfilling its role within the footbed. The footbed is **designed with a physical barrier to contain the bacteria and prevent unintended release into the environment**, adding an extra layer of safety. This barrier ensures that the microbial system remains confined and secure, further reducing any potential risks to the user or the surroundings.

To further enhance safety, the system includes a **kill switch** to deactivate the bacteria under certain conditions, such as exposure to the environment or the end of the product's lifespan. The kill switch provides a failsafe to ensure the bacteria are rendered inactive if needed, such as at the product's disposal or after use. **Population growth is regulated through quorum sensing**, controlling cell division and preventing overgrowth, while disposal protocols ensure any remaining bacteria are neutralized, preventing environmental contamination. These combined measures ensure the bacteria do not pose any lasting risk once the footbed is no longer in use.

## Contribution to Synthetic Biology

The impact of this project goes beyond just foot care. The IISER Kolkata team has made significant advancements in using quorum sensing and CCA mechanisms to regulate bacterial production of bioactive compounds. By contributing new plasmid designs (the intentional engineering and modification of **plasmids**, which are small, circular pieces of DNA found in bacterial cells) and models for the continuous production of bio-manufactured molecules, they've provided valuable tools to the synthetic biology community.

Their approach to sustaining the production of a medicinal molecule like linalool can be adapted for other applications as well. This opens up new avenues for creating sustainable, bio manufactured products that could benefit a wide range of industries, from pharmaceuticals to agriculture.

In the long run, **iGEM teams that address real societal issues can have a lasting impact on improving the quality of life**. Such projects can pave the way for marketable products, especially with institutional support or links to industries. Teamwork, mentoring, and interdisciplinary collaboration are the cornerstones of such success stories.

## Going Green: A Sustainable Solution

In a world where sustainability is more important than ever, the team's solution is a breath of fresh air. Unlike traditional foot care products, which often rely on synthetic chemicals and come with a high environmental cost, this insole uses naturally occurring biological processes to achieve the same goal.

**Most foot creams and sprays are not only laden with chemicals, but they also require constant reapplication**. They wash off easily, and many contain formaldehyde derivatives and other harmful substances. The team's insole, by contrast, offers a self-sustaining solution. The bacteria continuously produce linalool, providing long-term protection with minimal environmental impact. There's no need for large-scale manufacturing of chemical compounds or complex supply chains, making this approach far more

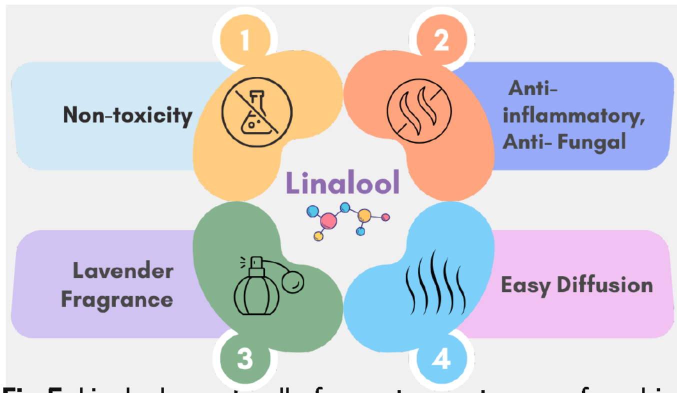


**Fig 5**. Linalool, a naturally fragrant monoterpene found in plants like lavender, offers a range of benefits ideal for foot care. It possesses strong antifungal and antibacterial properties that help combat harmful microbes responsible for infections. Linalool has pain-relieving qualities that can reduce discomfort and is known for its pleasant, soothing scent, which helps to eliminate unwanted odours.

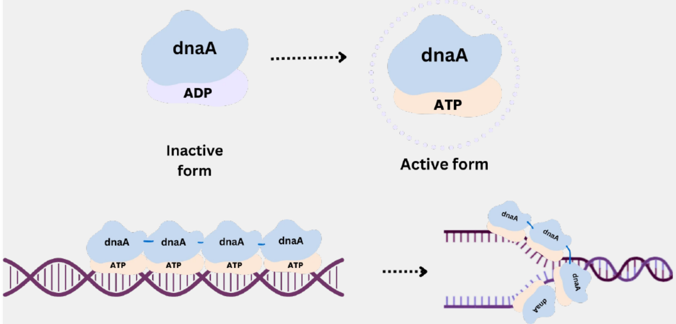


**Fig 5**. In order to limit bacterial population growth without stopping it indefinitely, the team used DnaA, a protein in bacteria, particularly *E. coli*, that plays a key role in initiating DNA replication. It binds to the origin of replication, called the oriC region, recognizing specific DNA sequences and unwinding the DNA to allow replication machinery to begin copying the bacterial genome. DnaA's activity is carefully regulated to ensure replication occurs only once per cell cycle, as over- or under-activation can lead to cell growth issues or genomic instability.

eco-friendly.

The beauty of this project lies in its **practicality**. Foot infections are a common problem, especially for athletes, soldiers, and people with diabetes. These individuals face higher risks of fungal infections, and traditional treatments can often fall short. By offering continuous protection through a simple insole, the team's product could make a huge difference. **The insole doesn't just treat infections after they occur—it helps prevent them from developing in the first place**. This proactive approach is a game-changer for foot care.

## Bridge Between Academia and Industry

This project promises to revolutionise foot health for all our diabetic relatives suffering right now who require foot insoles to be at least a centimetre thick. While the current focus is on linalool production for foot care, the platform the team has developed has much broader applications. **The same technology of live application could be used to produce other medicinal compounds**, by modulating a specific gene of interest, opening up possibilities in fields like wound care, hospital infection prevention, and more.

For example, the team envisions creating wound dressings that continuously produce antimicrobial compounds, helping prevent infections in patients with open wounds and burns. Another possible pursuit could be production of room fragrance with population-regulated, live-application of bacteria, producing certain fragrant molecules. **The concept of in-situ production, where the compound is made directly at the site where it's needed, could revolutionise the way we approach medical treatment**.

The IISER Kolkata iGEM 2024 team has already started conversations with academic institutions and industry players to explore commercialising their product. While the project is still in its experimental phase, the team's long-term goal is to **develop a prototype that can be pitched to companies specialising in foot care and biotechnology**. They've also been actively engaged in outreach and collaboration within the iGEM community, sharing their designs, models, and insights to help others build on their work.

## A Game-Changing Solution

IISER Kolkata's iGEM project is a testament to the power of synthetic biology, **representing a shift in how we approach healthcare and sustainability**. Returning from Paris after winning a gold medal, the team brought back more than just accolades; they demonstrated the potential for biotechnology to address real-world challenges. Their biologically powered foot insole offers continuous protection, eco-friendliness, and convenience, showcasing a sustainable, bio-based approach to healthcare. This innovation is not just a novel solution for foot infections—it's a glimpse into the future of biomanufacturing and sustainable healthcare, setting the stage for synthetic biology to change the world.

*InScight heartily thanks the members of IISER Kolkata 2024 iGEM team and the principal investigators of the project for fruitful discussions and for their feedback on the article. InScight is also grateful to the team for allowing us generous use of figures and information from their wiki.*

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