Directed Evolution of EPIs

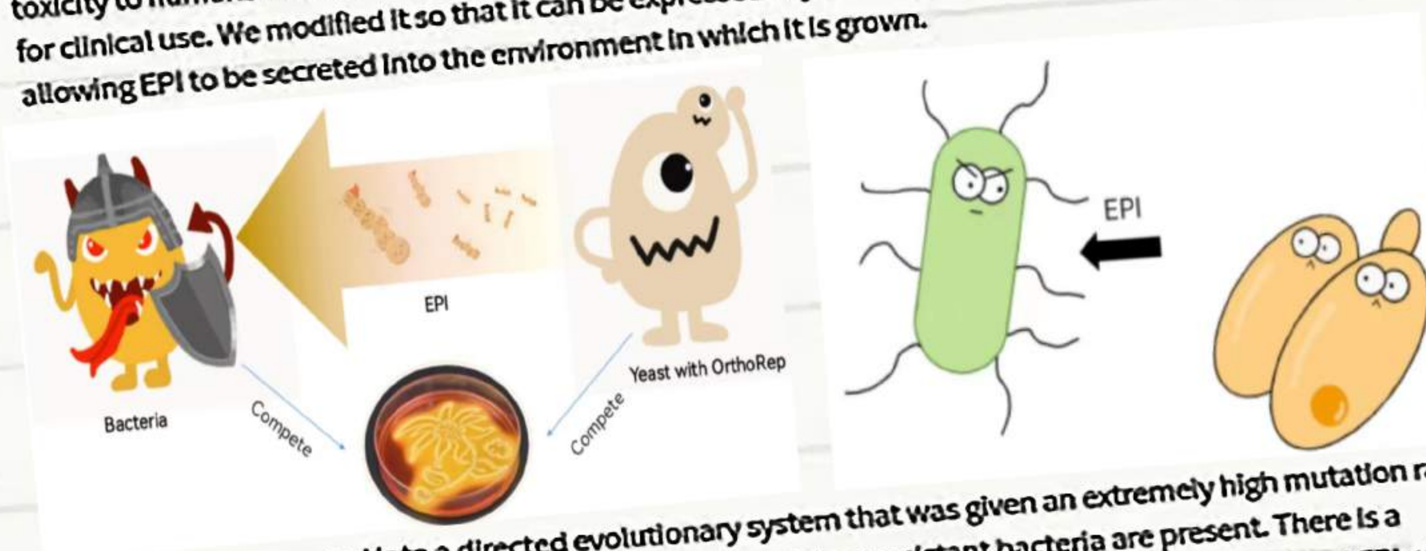
Description & Design

Nowadays, the antibiotic crisis is severe.There are now many ways to deal with the antibiotic crisis, such as phage therapy, immunotherapy, population-sensing modulation, and so on. Among these approaches one has attracted our attention: efflux pump inhibitors (EPI).

Our world faces a similar dilemma to the Oxford policeman, called the "antibiotic crisis".

There are many mechanisms of bacterial resistance, and mutation and high expression of the efflux pump is one of the most widespread and important ones. EPI targets this resistance mechanism by inhibiting the function of the efflux pump and allowing antibiotics to work again.

We chose a synthetic peptide EPI with special modifications as the basis of our project. Because of its low toxicity to humans and broad spectrum of action, it is the most promising of the various EPIs now available for clinical use. We modified it so that it can be expressed in yeast. And integrated it with a secretion system allowing EPI to be secreted into the environment in which it is grown.



It was finally integrated into a directed evolutionary system that was given an extremely high mutation rate. Yeast is cultured in an environment where antibiotics and drug-resistant bacteria are present. There is a competitive relationship between the growth of yeast and drug-resistant bacteria, and the mutated EPI with good efficacy of efflux pump inhibition can inhibit the growth of drug-resistant bacteria.

Yeast expressing such EPI can gain growth advantage and achieve directed evolution of EPI. Ultimately, we expect to obtain an EPI with significant efficacy of efflux pump inhibition, no toxicity to humans, and capable of large-scale fermentation production.

Proof of Concept

There are four basic parts in our directed evolution system:

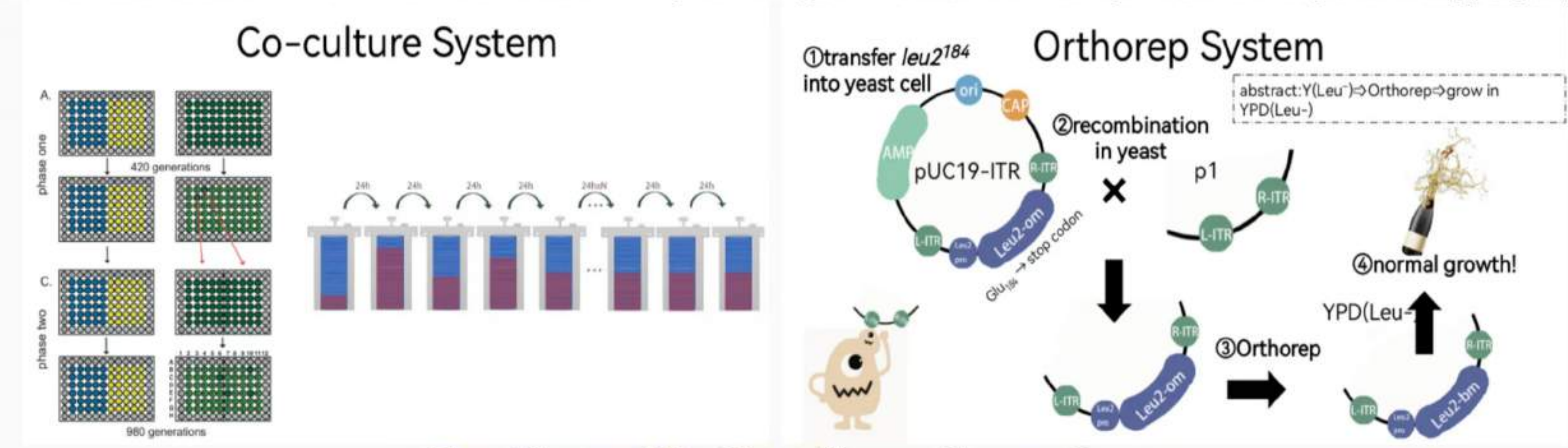
The acquisition of stable co-culture strains; Verify the functions of the Orthorep system. Function verification of secretion system and function verification of TM1/TM8 prototype peptide.

Co-culture:

Through daily subculture, the growth state of bacteria and yeast is recorded to screen stable co-culture system .

Orthorep:

Function verification: Through the homologous recombination of PUC19-ITR and P1 plasmid, the Leu2 gene mutated at position 184 was transferred into yeast and δ DNAP was transferred, and whether there was leucine-deficient supplement was observed in continuous subculture. If a backfill occurs, a recovery mutation has occurred, i.e. the Orthorep is working properly.

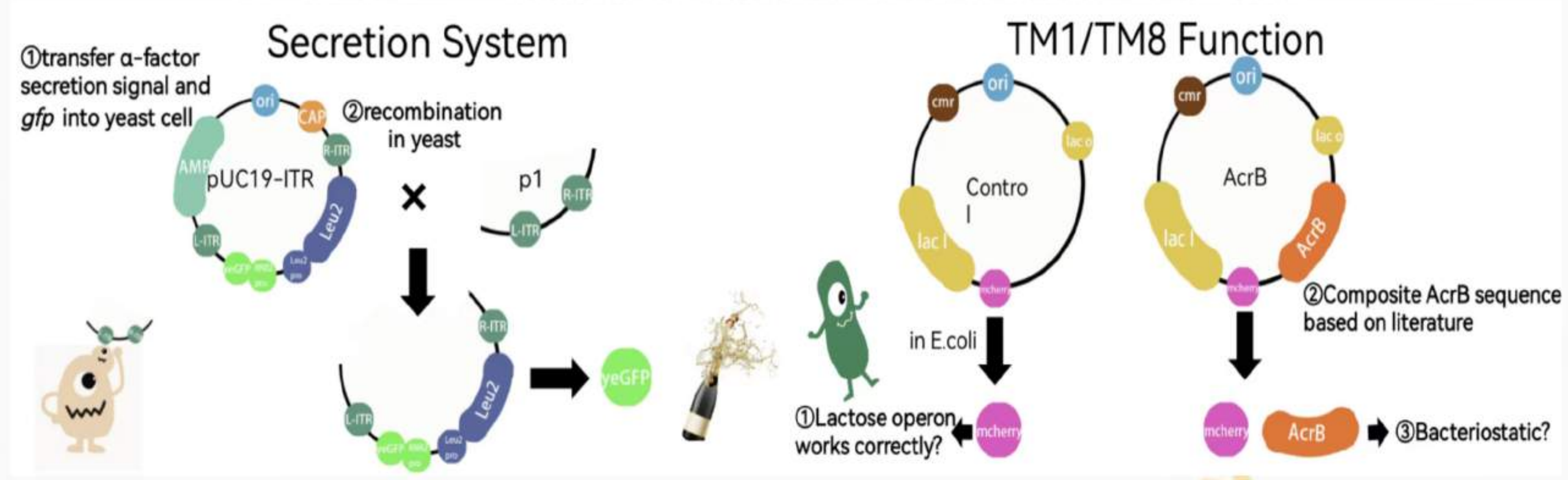


Function verification of secretion system:

The GAP-α signal peptide secretion system was selected as the structural basis for the final secretion of EPI, and the working ability of the secretion system was verified by detecting the expression intensity of fluorescent protein in the medium and downstream.

Functional verification of TM1/TM8:

We replaced the non-natural amino acids in the original EPI in the literature and converted them into natural amino acids. In the presence of specific antibiotics, TM1/TM8 prototype peptide was added to observe the changes in its growth, and the effect of the prototype peptide was preliminarily verified to provide a starting point for directional evolution.



Modeling

The main idea behind the co-culture modeling of bacteria and yeast is that the two will compete for nutrients, and conventionally, bacteria with the advantage of growth rate will quickly displace the ecological niche of yeast in the competition. However, in our project, yeast has the ability to secrete EPI, and bacterial growth will be inhibited by EPI, thus allowing the two to coexist in a co-culture environment.

$$\frac{dc}{dt} = -(u_b g_b[B] + u_y g_y[Y])c$$

$$\frac{d[B]}{dt} = g_b c[B] - d_{bb}[B] - d_e[EPI][T]$$

$$\frac{d[EPI]}{dt} = a[Y] - b_e$$

$$\frac{d[Y]}{dt} = g_b c[Y] - d_{yy}[Y]$$

Our modeling also starts from this, considering the efficiency of yeast to secrete EPI and the effect of bacteria to be inhibited by EPI, as well as the ability to compete for nutrients in the medium, and thus modeling the growth curves of bacteria and yeast.

HP&Education

Human practice

1.Medical Staff Interview — We tried to get in touch with doctors, nurses and patients in Peking University Hospital, learned about the clinical diagnosis and treatment of antibiotic resistance with their help, and asked for their suggestions in our proposed project plan.

2.Market Research — Before the project begin, we begin to research the global market for drugs to treat bacterial resistance. Based on the epidemiological overview of bacterial resistance, we predicted the market size of drugs for bacterial resistance in China and the world, and tried to judge the global development trend in the future.

3.Questionnaire Survey — In order to guide our education and plan making, we conducted a questionnaire survey on the knowledge of bacterial resistance and its treatment among the general public. After the survey, we send the respondents a well-made popular science pamphlet to help them deepen their understanding of bacterial resistance.

Education

1.Life Code — In recent years, various domestic streaming media platforms have launched podcasts, and more and more people choose to make it. We hope to establish a public radio station to educate the public about the mysteries of life and to introduce some of the basics of synthetic biology.

2.Bioculture Workshop — Collaborate with other iGEM teams to organize biocultural workshops focusing on bio-philosophical thinking, and jointly collect donations to contribute to biodiversity or disability groups by designing innovative games that match the project and releasing prizes.

3.Bacteria Company — Design board games and other puzzle games, while promoting to target schools, try to establish cooperative relationships, and popularize disease prevention knowledge in various scenarios to children by means of teachers releasing online practice tasks in summer vacation

4. The Old Man and the Medical Care — Design board games and other puzzle games, while promoting to target schools, try to establish a cooperative relationship, and popularize disease prevention knowledge in various scenarios to children with the help of card games by the way of teachers releasing online practice tasks in summer vacation

5.iGEM Dreamers Program — Launch the iGEM Dreamers Program and connect with past teams to work together on a biotechnology workshop for future iGEMer or high school students interested in exploring synthetic biology issues.

6. Metaverse Virtual Sales — As a new thing in the frontier field, the meta-universe has shown a strong attraction to the information industry and traditional industries, with the potential that everything can be meta-universe. We will use the technical support provided by the metasverse to hold virtual online sales meetings.