Inspiration

- As the interplanetary“farmer”, if we want immigrate to other planet, the utilizing and the remolding of the bacteria seems to be the indispensable part.
- What we need is the bacteria which can transform the earth in anaerobic way. However, if we grow plants, the content of the oxygen will be unsuitable to these anaerobic bacteria.
- So we need to engineer them in order to let them survive from the “great Oxidation Event”.

2022 iGEM

NJTech_China

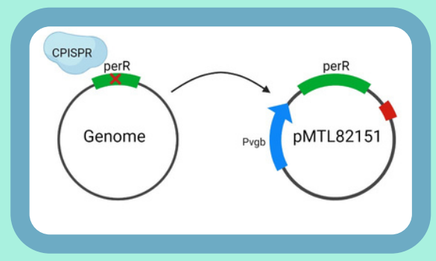
Contact way： njtech_china_2022@outlook.com

We warmly welcome your cooperation.

Experiment

Experiment introduction

- Clostridium tyrobutyricum, an obligate anaerobe, was used in our project. The goal of our project is to engineer C. tyrobutyricum to resist oxygen toxicity, then achieve aerobic growth.
  - *PerR* is a peroxide repressor widely distributed in Gram-positive bacteria. It could inhibit the activity of its corresponding enzyme by binding to a conserved sequence on the promoter of that regulated gene, which would cause the bacteria failing to activate its own oxidative stress defense system to clear out oxygen or reactive oxygen species(ROS), and cannot survive in an aerobic environment.
    - It has been proved that the deletion of a PerR-homologous protein could result in prolonged aerotolerance and rapid consumption of oxygen from an aerobic environment.Thus, we knocked out the perR gene from the genome of C. tyrobutyricum by the method of endogenous Type I-B CRISPR-Cas, finding out that the engineered bacteria could be resilient to aerobic conditions.



- However, in further fermentation we found that the bacteria without *perR* could not grow as well in anaerobic conditions as before. So how could we reduce the impact on the bacteria growing in their original anaerobic conditions is a problem that needs to be solved.
  - In our project, a plasmid of PerR expressed by Vitreoscilla hemoglobin promoter Pvgb was constructed in a perR-knockout C. tyrobutyricum. Promoter Pvgb is a microaerobic induced promoter, which is expected to express PerR under aerobic conditions and reduce its expression under aerobic conditions. In this way, we expect our engineered bacteria to grow well under both aerobic and anaerobic conditions.

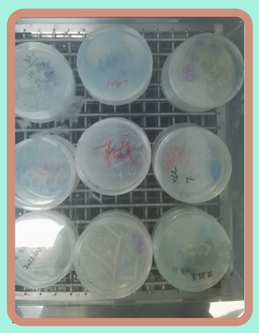
Experiment grogress

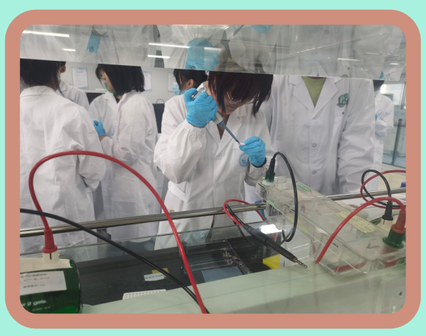
- Plasmids constructed with an endogenous Type I-B CRISPR-Cas system knock out the perR gene on the genome of C. tyrobutyricum.Construct the PerR overexpressed plasmid using pMTL82151 as the basic plasmid to express PerR using the constitutive promoter Pthl.The Pvgb promoter is a microaerobic induced promoter, which expression ability under anaerobic conditions is several times higher than when oxygen is present. It has microaerobic induction characteristic so it could be used as an oxygen suppressor promoter, we expect it to express PerR under anaerobic conditions and reduce the expression under aerobic conditions.
  - First, promoter Pvgb is used to express BS2， a kind of flavin mononucleotide-based fluorescent protein that could be used as a marker for gene expression in anaerobic conditions. By this way, the effectiveness of the promoter Pvgb in C. tyrobutyricum could be verified by the fluorescence intensity.Then， we will construct a new PerR expression plasmid, using promoter Pvgb to replace the constitutive promoter Pthl.
  - In this case, parameters related to the growth of engineered bacteria in the presence of oxygen are detected. Finally the promoter P*vgb* will be engineered for better expression of *PerR,* and the expression capacity of *PerR* under different conditions will be adjusted. Finally, a well engineered promoter that enables bacteria to grow well under both aerobic and anaerobic conditions will be obtained.

Human practice

- We attended Nanjing iGEM association which was held by NJU-China and the association meeting held by hubei university of technology. During these meetings, teams from different cities converged, shared their research process and their daily study, in order to deal with the problems we met during the iGEM competition.

- We held a summer camp which was aimed to help the senior high school students to enrich their biology knowledge and let them enjoy the interests of biology. During the 5-day-long summer camp, we arranged several interesting biological games and experiments which might help students to learn meaningful knowledge during playing and practice.





- According to the performance of the students and the quality of their experiment report , we prized them the certificate of honor in the camp ceremony.