COM ON!

Cau_China 2022

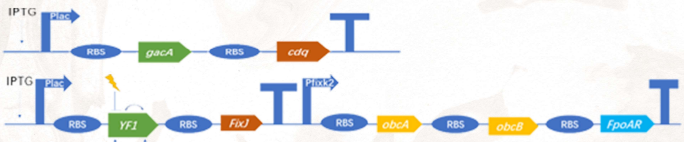
Members
Jiang Naqi, Liu Muhua, Zhao Yulin, Gong Wei, Zou Xueran, Li Jiayi, Dong Lehan, Ning Hongshu
Hu Siyuan, Zhou Yunlu, Li Jiahui, Guo Yun, Qu Minghao, Ma Yu, Liu Daiyuan, Peng Aoyang

Open-air stone cultural relics are faced with severe problems of long-term weathering and corrosion. Inspired by the natural calcium oxalate protective film formed on the stone tower of Linyin Temple in Hangzhou, CAU_China decided to transform pseudomonas fluorescens 2P24 to produce EPS and oxalic acid, thus forming calcium oxalate(COM) protective film.

DESIGN

The oxalic acid we need can be produced by two genes, obcA and obcB from Burkholderia glumae BGR1, while the protein expressed by Fomitopsis palustris FpOAR is oxalate transporter. We plan to introduce above-mentioned genes into our chassis bacteria, Pseudomonas fluorescens 2p24, so it will be equipped to secrete oxalic acid. The excreted oxalate can combine with the calcium ion, forming calcium oxalate that resists erosion.

In order to ensure the strength of COM film and avoid the formation of calcium oxalate poly-hydrate, which is harmful to cultural relics, we are going to enhance the global regulator gacA and the quorum sensing factor c-di-gmp of Pseudomonas fluorescens 2P24 stain to produce large amounts of EPS. So the bacteria can grow more biofilms, this is helpful to form a dense COM film on the surface of the stone relics. Besides, this action also play a important row in our product by enhance antibacterial ability and reduces the motor capacity of bacteria.t



In practical use, our efforts are being made to develop a medium with both water retention and viscosity. This is our bacteria's carrier, and its outside is a layer of PVA sponge, which can also hold water inside the medium. Such device have several uses: it can provide hydration and nutrition for engineering bacteria, also, it is a binder to make sure the P.f stay on the surface of that cultural relic. As an ingredient, IPTG (isopropyl-β-D-thiogalactoside) will be added into the medium, this can induce the expression of biofilm-related genes, we can also add other regulators into it in future modifications. The device is also able to create a dark environment, this can initiate the secrete of oxalic acid by YF1 mediated photoregulatory pathway. Then after the protection service, the medium will be teared off, and the sunlight will stop the whole reaction.

TARGET

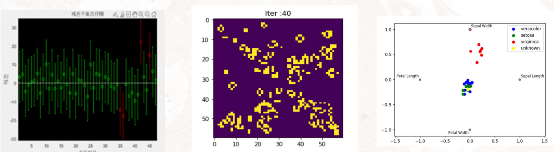
Our final target is to produce a kind of microorganism that can discharging oxalic acid and generate biofilms, so it can form a dense COM film to protect stony antique.

Our team is currently committed to communicating with microbial experts, and modelling on the work efficiency problem to enrich our idea about the product step by step.

MODEL

The core goal of our modeling was to verify that the weathering rate of engineered bacteria treated stone was lower than that of untreated stone. The main problems to be solved and the mathematical model we used are:

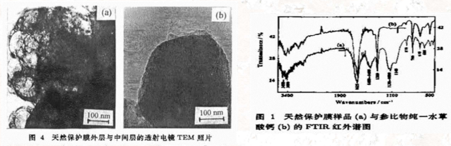
1. Verify that oxalic acid can be produced and discharged in engineering bacteria, and predict oxalic acid yield: The model used is Hill equation.
2. Verify the yield increase of biofilm in engineering bacteria and predict the yield of biofilm: the model used is cellular automata.
3. Verify that calcium oxalate monohydrate film can be generated: the model used is precipitation solution equilibrium.
4. Prediction of stone weathering rates under different treatment conditions: the models used are empirical formulas.
5. Predict the time required for each link of the technological process: use BP neural network.



BACKGROUND

Stone relics are precious historical cultural heritage left for us. Some stone relics have been moved to the museums, and have been well restored and protected, but there are still many stone relics outdoors like statues and large stone tablets. Suffering from acid rain, salts corrosion and weathering, the characters and pat-terns on these stones are ertased or even vanished as time flows.

The White Pagoda in Lingyin Temple, Hangzhou, was built in 907 AD. Though it has a history of more than a thousand years, some scripture carvings on it are still clear and complete, and even the tool marks of these years are faintly visible. Scientists found that the intact reserved carvings are covered with a naturally formed crystalline calcium oxalate monohydrate (COM) film, which acts as protective layer.



Inspired by the natural calcium oxalate protective film, CAU_China set out from the principle of biomineralization, improving Pseudomonas fluorescens 2P24 stain by means of synthetic biology, enable it to produce a dense COM protective film on the surface of stone relics.

HUMAN PRACTICE

First, we consulted Xi'an Forest of Stele Museum and learned that no good method for weathering protection of sttone cultural relics has been developed so far. Before carrying out the experiment, we consulted Professor Zhang Liqun to understand the characteristics of the chassis bioluminescent Pseudomonas 2P24 we selected in terms of biosafety and genetic operation. We then consulted a number of heritage institutions to understand how specific conservation work is carried out and their attitudes towards the use of genetically modified microorganisms for heritage work.

Collaboration

We participated in several IGEM online meetings. First of all, we had an online meetup with ZJU-China, who is also engaged in cultural relics protection and restoration. We got to know each other's project with XJTLU-China and provided them with biosafety design schemes. Meanwhile, XJTLU-China helped us contact Nanjing Institute of Cultural Heritage Protection for our subsequent humtan practice. We also participated in large online meetups held by iBown-China and GXU-China to learn about the projects of various teams.

Communication and Education

We considered that people are more likely to get information from short videos than from lengthy online courses, so we made a series of videos of no more than 2 minutes to introduce synthetic biology. In the future, we plan to offer lectures for different groupsof people, so that they can better understand synthetic biology based on their own knowledge.

Primary PI: Wang Na
Email: 393811901@qq.com
Secondary PI: Tian Changfu
Instructor: Yang Jinshui