

Abstract

Model

Product

Human practice

Due to the rapid development of society, both young and old people have a very serious bad diet. An unhealthy diet will affect the level of low-density lipoprotein (LDL) and triglyceride in the body, which will lead to the occurrence of a series of cardiovascular diseases(CVD) such as hypertension.

The HZAU-China team hopes that by modifying Escherichia coli Nissle 1917, the intestinal content of TMA (trimethylamine), the precursor of TMAO (trimethylamine oxide) and an important factor inducing thrombosis, can be efficiently reduced from two aspects: inhibiting the production of TMA and degrading the TMA already existed, so as to reduce the possibility of thrombosis. At the same time our engineered bacteria will also release some relief substances that are beneficial to vascular protection.

The project aims to develop an edible probiotic powder, which is expected to be used for the treatment of patients with cardiovascular disease caused by poor diet. It provides a new idea for cardiovascular disease treatment by intestinal drug therapy of low side effect and high universality.

Design

Our engineered bacteria consists of two main functional modules, the cure module and remission & health-care module. When people suffer from CVD after eating too much high-fat food, if taking the probiotic powder containing engineered bacteria only, the engineered bacteria will turn on health functions to do the cardiovascular protection. And If the theophylline is ingested, it will enter the cure module which to reduce the TMA concentration. The entire process has an alleviative component that remains to function.

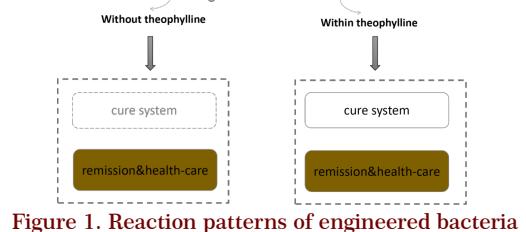


Figure 1. Reaction patterns of engineered bacteria

Cure system module

As mentioned above, TMA is an important factor for thrombosis, and the core idea of our treatment is to reduce the content of TMA in the intestine and prevent excessive TMA from entering the blood circulation and being oxidized into TMAO in the liver, so as to achieve the purpose of cardiovascular disease treatment. Here, efficient reduction of TMA concentration is the key.

In our project, there are two different versions for the cure system. In the cure system 1.0, degradation module enhances the conversion of the TMA already exited into other substances. In the cure system 2.0, inhibition module is added to inhibit the synthesis of the TMA, which reduces the risk of reverse synthesis of TMA from degradation products due to the uncertainty of intestinal microenvironment , resulting in failure of therapeutic. The updated cure system is designed to efficiently cure the CVD patients by reducing the intestinal TMA level.

The core of our modeling is to verify the feasibility of the project and to optimize the project by validating it with the experimental results. In the modeling part, the degradation of TMA is the core of the model and we first simulated the degradation effect of TMA (Figure 6).

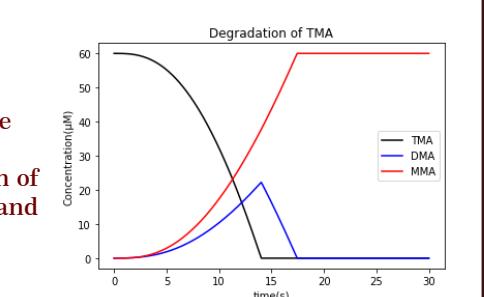
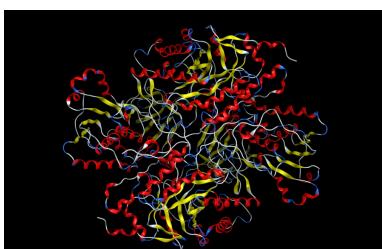


Figure 6. The simulation of degradation of TMA



In order to obtain higher degradation efficiency, the key enzymes will be identified and point mutase optimization will be performed by analyzing the decisive steps of TMA degradation process (Figure 7).



Figure 8. 3D structure of the active site of CutC

In addition, we want to reduce the TMA content by inhibiting the conversion of choline to TMA by CutC protein, so the modeling part will try to predict a dipeptide inhibitor to achieve bioinhibition of CutC protein (Figure 8).

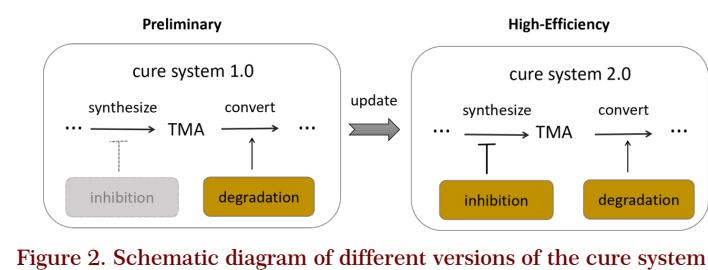


Figure 2. Schematic diagram of different versions of the cure system

Degradation module

When only taking probiotics, the alleviating module is expressed, while the degradation module is not expressed. When thrombosis related to diet occurs and the concentration of TMA in the patient's intestines increases, they can take probiotics with theophylline tablets. The riboswitch that senses theophylline molecules in probiotics is on making the cyclization recombination enzyme expression activated, reversing the sequence (a constitutive promoter) between loxP sites. Then, the probiotics will express tmd (trimethylamine dehydrogenase), dmd (dimethylamine dehydrogenase), fdhA (formaldehyde dehydrogenase) respectively, which gradually degrades TMA into less toxic monomethylamine and formic acid.

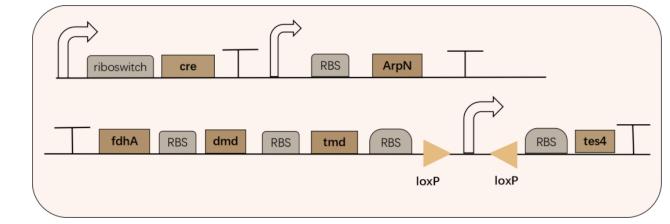


Figure 3. The circuit of degradation module

probiotic powder

We plan to turn our project into a probiotic powder that can help patients cure diseases with less pain. We hope that our probiotic powders can have the advantage of having fewer side effects and integrating therapy into life.

Software(app)

WeChat applet: Psychological Care

Because this project is based on the alleviation of cardiovascular disease, in order to better serve the people with cardiovascular disease, realize heart to heart communication, and also to popularize science about thrombosis and publicize this year's project, we plan to make an Wechat applet to add functional modules such as "about us", "science about thrombosis", "project introduction", "message board", "product publicity". Message board lies in the communication between patients to achieve psychological care and help cardiovascular patients recover as soon as possible. We expect this Wechat applet to be online and help most patients with cardiovascular disease.

Inhibition module

The main source of intestinal TMA is synthesized by choline utilization TMA lyase system(CutC/D). In this module, we hope to predict some small peptides by modeling and further generate a large number of simulated dipeptides by expressing L-amino acid ligases to inhibit the enzymes' activity, reducing the TMA level finally. The HZAU-China team members are under the discussion on the specific experimental scheme. We are also looking forward to communication and guidance from other teams in this part.

Remission & health-care module

After people take in our engineered bacteria, nattokinase (ArpN) in this module is constitutively expressed. Fibrinolytic (anti-clotting) capacity of nattokinase has been recognized by scientists and nattokinase helps decrease the risk of a variety of cardiovascular diseases without producing any adverse side effects. The other substance in this module is butyric acid, which is expressed in the absence of theophylline and stopped to be expressed when theophylline is taken. Butyric acid keeps colon cells stable, which prevents or suppresses cancer, regulates intestinal flora and boosts immunity.

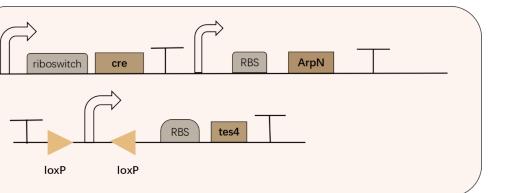


Figure 4. The circuit of remission&health-care module

kill switch

In this part we decided to use the same circuit as our previous project. We designed two different suicide schemes based on temperature. In the first scheme, the expression of HepT would be inhibited by heat-repressible RNA thermometer in people's guts, whereas it could be continually expressed in vitro and make the bacteria commit suicide. In another plan, we imported the HepT/MntA toxin-antitoxin system and continued to produce HepT. Instead of directly controlling HepT, we applied a heat-inducible RNA thermometer to activate the expression of MntA and neutralize HepT in the intestine. Both schemes could execute the same expected suicidal function, even if we applied different RNA thermometers with opposite effects. In addition, the specific suicide scheme could be adjusted appropriately by changing the selection of RNA thermometers to address different environmental temperatures in different regions when it comes to practical application.

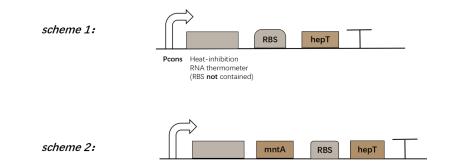


Figure 5. The circuit of suicide module

Public education

Biological Health Week

In June, considering that thrombosis is closely related to lifestyle, we launched the biological health week publicity activity of "Cardiovascular disease and Healthy Life". Through the WeChat Public Platform to carry out science popularization of healthy life, at the same time, answer activities and a questionnaire survey of students were also held. Nearly 200 people participated in the questionnaire, and we found that many students lacked the knowledge of cardiovascular diseases, which guided our next publicity activities.

At the same time, we held the "Healthy life and South Lake Run" exercise activity during the biological health week. With the help of the Red Cross society of Huazhong Agricultural University, nearly 300 students participated in the exercise to promote students to live a healthy life through the South Lake Run.



Figure 9. Students participate in "Healthy life and South Lake Run" exercise



Figure 10. iGEMers organizes the questionnaire happily

Stakeholder

In the future, we will carry out this work from two aspects: 1. visit the cardiovascular disease experts and participate in the Cardiovascular Disease Summit Forum of Tongji University in Hubei Province to obtain suggestions and opinions on our vascular disease treatment program from all aspects of society; 2. Investigate the probiotics powder industry, explore the feasibility and market demand of probiotics powder production, broadening the connotation of the project.

At present, we are interviewing related cardiovascular disease experts and looking forward to win-win cooperation with teachers. Welcome teams with relevant ideas to communicate with us and let's help each other.

Collaboration

Communication among iGEM teams can often inspire each other, so we participated in 2022 iGEM China Summit Meetup in June. In the exchanges, all teams were actively discussing the difficulties encountered in the progress of the project and giving each other suggestion. We have not only benefited but also enjoyed a lot. Further we also plan to take part in China Central Regional Meetup in July and Conference of China iGEMer Community in August. Our team hopes to exchange different thoughts and ideas with other iGEM teams more often!



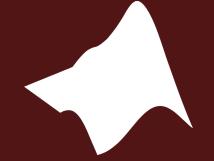
Reference

- [1] Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arús F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Solà JV, Martínez JA, Martínez-González MA; PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med*. 2013 Apr; 368(14):1279-90.
- [2] Peng J, Xiao H, Hu M, Zhang X. Interaction between gut microbiome and cardiovascular disease. *Life Sci*. 2018 Dec; 1-214:153-157.
- [3] Hoess RH, Abremski K. Interaction of the bacteriophage P1 recombinase Cre with the recombinase site loxP. *Proc Natl Acad Sci U S A*. 1984 Feb; 81(4):1026-9.
- [4] Brown JM, Hazen SL. Microbial modulation of cardiovascular disease. *Nat Rev Microbiol*. 2018 Mar; 16(3):171-181.
- [5] Lifschitz AS, Salvano MA, Lucchesi GI. *Pseudomonas putida* A ATCC 12633 oxidizes trimethylamine aerobically via two different pathways. *Arch Microbiol*. 2010 Jun; 192(6):471-6.
- [6] Yang CC, Packman LC, Scrutton NS. The primary structure of Hyphomicrobium X dimethylamine dehydrogenase. Relationship to trimethylamine dehydrogenase and implications for substrate recognition. *Eur J Biochem*. 1995 Aug 15; 232(1):264-71.
- [7] Zhang W, Chen S, Liao Y, Wang D, Ding J, Wang Y, Ran X, Lu D, Zhu H. Expression, purification, and characterization of formaldehyde dehydrogenase from *Pseudomonas aeruginosa*. *Proteins Expr Purif*. 2013 Dec; 92(2):208-13.
- [8] Topp S, Reynoso CM, Seeliger JC, Goldlust IS, Desai SK, Murat D, Shen A, Puri AW, Komeili A, Bertozzi CR, Scott JR, Gallivan JP. Synthetic riboswitches that induce gene expression in diverse bacterial species. *Appl Environ Microbiol*. 2010 Dec; 76(23):7781-4.
- [9] Jing F, Cantu DC, Tvaruskaova J, Chipman JP, Nikolau BJ, Yandeu-Nelson MD, Reilly PJ. Phylogenetic and experimental characterization of an acyl-ACP thioesterase family reveals significant diversity in enzymatic specificity and activity. *BMC Biochem*. 2011 Aug 10; 12:44.
- [10] Hoyne O'Connor A, Hinman K, Kirchner L, et al. De novo design of heat-repressible RNA thermosensors in *E. coli*[J]. *Nucleic acids research*, 2015, 43(12): 6166-6179.
- [11] Weng Y, Yao J, Sparks S, Wang KY. Nattokinase: An Oral Antithrombotic Agent for the Prevention of Cardiovascular Disease. *Int J Mol Sci*. 2017 Feb 28; 18(3):523.

sponsors



華中農業大學
HUAZHONG AGRICULTURAL UNIVERSITY



MathWorks®

GenScript
Make Research Easy



SnapGene



C.V.D

Online Education
Our team investigated and popularized the causes and treatment of thrombosis. The team mainly used the WeChat Public Platform to publicize the initial background of the project, from the cause of thrombosis and harm of thrombosis, as well as the treatment methods we mainly use now and the defects of them. We hope that through this education, we can help people reduce the risk of cardiovascular disease.

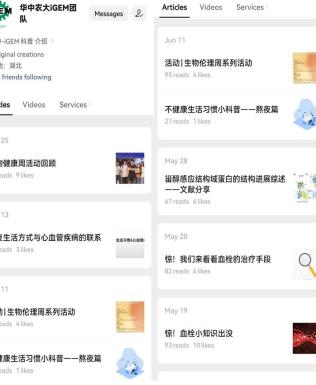


Figure 13. The Wechat public platform of our team