中国海洋大学“东升本科生发展基金”申请表

（ 2017 年 ）

申请人情况	姓名	陈宇卿	性别	女	民族	汉族
	学院	海洋生命学院	专业	生物技术	学号	15050031004
	电话	17806276755	邮箱	ykkchen@outlook.com		
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竞赛整体情况	**国际基因工程机器大赛，International Genetically Engineered Machine competition，简称 iGEM。是合成生物学领域的国际顶级大学生科技赛事，也是涉及数学、计算机、统计学等领域交叉合作的跨学科竞赛。由美国麻省理工学院于 2003 年创办，2005 年发展成为国际赛事，于每年 10、11 月在麻省理工学院进行最终角逐。iGEM 竞赛本次参赛队伍全世界共 313 支，立陶宛维尔纽斯大学 Vilnius-Lithuania 获得了总冠军 Grand PrizeWinner，美国威廉玛丽学院 William and Mary 获得亚军 1st Runner Up，德国海德堡大学 Heidelberg 获得季军 2nd Runner Up。中国地区共有 98 支 iGEM 团队参赛（含退赛），包括中国大陆地区 83 支，香港特区 5 支，台湾地区 10 支。与 2016 年相比，大陆地区增加了 20 支队伍，增幅惊人，也远远高于全球参赛队伍数量的增长率，比赛规则较往年更加严格。中国地区，共有 79 支队伍（含港台地区）获得了奖牌，其中大陆地区 66 支，香港特区 4 支，台湾地区 9 支。中国大陆地区的 83 支团队中，有 12 支得到了单项奖或单项提名奖。我团队位列其中，与北京大学等高校齐名。Best New Basic Part 的筛选来自于全球 313 支队伍提交的近 3000 个标准化的基因元件中，全球获得此奖项的队伍共 8 支。**			
当年获奖情况	（1）获国际金奖 （2）【单项奖】Nominated Best New Basic Part 奖：中国大陆地区的 83 支团队中，有 12 支得到了单项奖或单项提名奖。我团队位列其中。Best New Basic Part 的筛选来自于全球 313 支队伍提交的近 3000 个标准化的基因元件中，全球获得此奖项的队伍共 8 支。		申请额度（万元）	5 万元		

参赛费用支出情况及获得经费支持

项目类型	具体内容	支出金额（元）
实验经费	试剂购买、基因合成、引物合成、测序等	45750.5（海洋生命学院获得补助 35000 元，校外赞助 10000 元）
参赛经费	队伍注册费、jamboree 门票（6 张）	55807.53（暂未获得补助）
国际差旅	12 名队员国际差旅及住宿费用	71612.9（暂未获得补助）
国内差旅	与国内其他高校（北京大学、福建农林大学）交流、与山东大学，北京化工大学教授交流，参加上海合成生物学学者论坛	25575（暂未获得补助）
日常花费	签证办理及日常打印、队服等	14700（暂未获得补助）

指导教师审核意见	同意推荐 指导教师（签字）刘娟 年 月 日	学院审核	同意推荐 分管教学副院长（签章）：刘娟 年 月 日
管委会意见			

1. 竞赛介绍包括竞赛主办方、比赛内容、参赛队伍数量、奖项设置及竞赛影响力等；
2. 获得经费支持情况包括学校支持经费额度、学院支持经费额度、教师科研经费支持额度及其他经费支持；参赛费用支出情况包括出境参赛人数、国际差旅费支出、国内差旅费支出、实验材料支出及其他费用支出；
3. 中国海洋大学“东升本科生发展基金”管理委员会办公室制表。

iGEM 2017

International Genetically Engineered Machine Competition

Yuqing Chen

OUC-China

Giant Jamboree
November 9 - November 13, 2017
Boston, Massachusetts

iGEM 2017

INTERNATIONAL GENETICALLY ENGINEERED MACHINE COMPETITION

TIAN SHUO

OUC-CHINA

GIANT JAMBOREE
NOVEMBER 9 - NOVEMBER 13, 2017
BOSTON, MASSACHUSETTS

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Rong Mu

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Giant Jamboree
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Boston, Massachusetts


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PROJECTS

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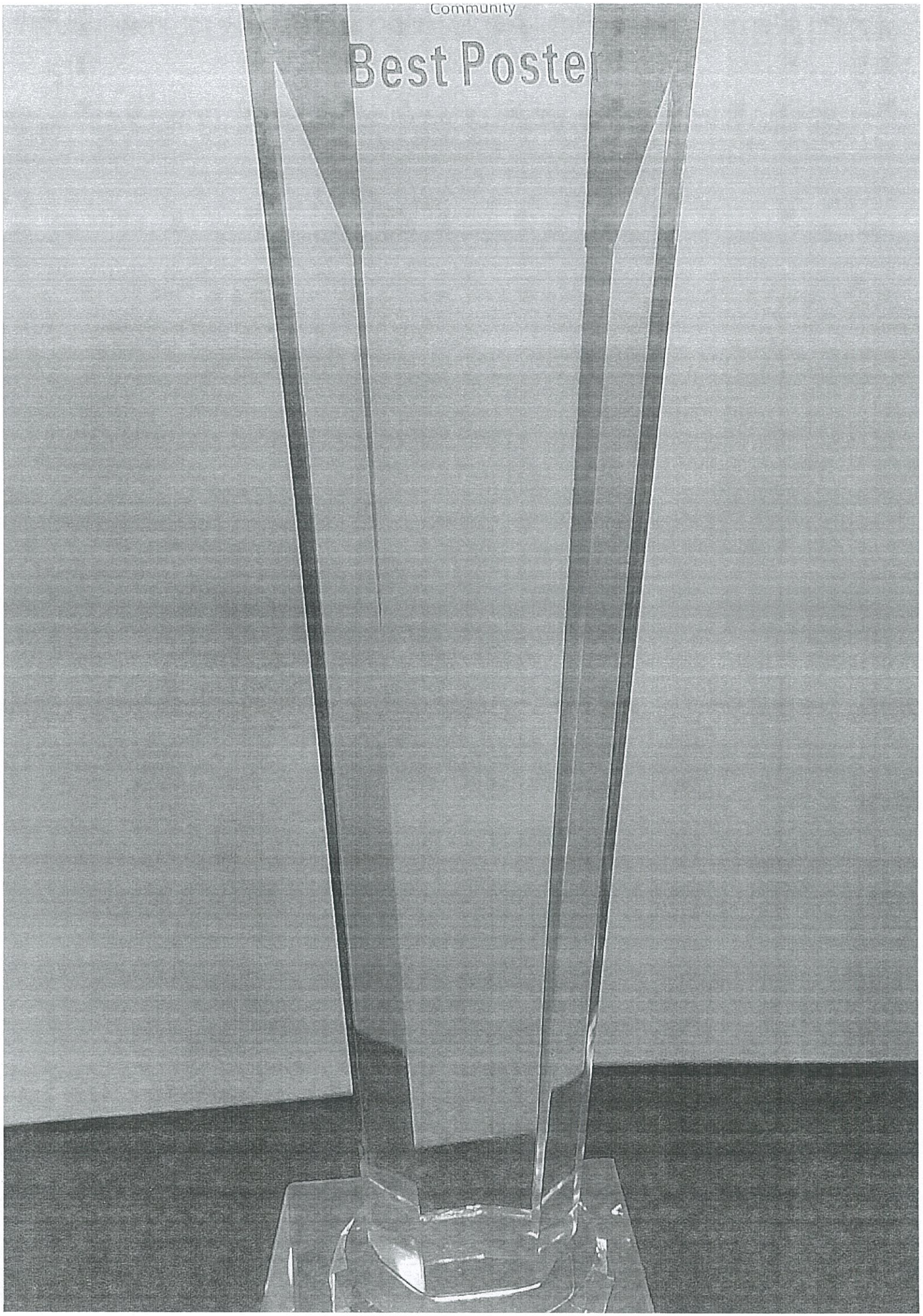
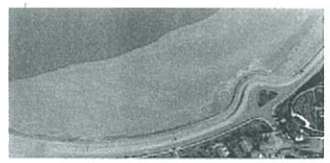

Community
Best Poste

With a worldwide increasing disposal of garbage and sewage, nutrients level of coastal waters is rising considerably, resulting in more and more marine disasters such as red tide and green tide, whose residual greatly threatens aquatic creatures' health and water environment. As a team of a university featuring marine biology, this year we focus on residual of *Enteromorpha* outbreak in Qingdao, hoping to offer a universal solution to utilize these algae residual effectively.
Currently, the huge amount of superfluous *Enteromorpha* is poorly utilized, with only polysaccharides extracted, leaving a second-round residue as direct fertilizer for farmland. However, compared with traditional terrestrial plants like straw, *Enteromorpha*, as a kind of green algae, possesses great advantages in biofuel production. It has low lignin content and softer structure making it easier to ferment. Additionally, its aquatic habitat means there is no need of extra land source for its growth. So this year, we decide to make use of *Enteromorpha* more efficiently by turning its residue into ethanol in a synthetic biological way.



The outbreak of Enteromorpha along the coastline in Qingdao

Over all, we make use of *Enteromorpha* residue and turn it into ethanol in a synthetic biological way:

Firstly, we use engineered two *S. cerevisiae* strains to realize waste utilization and ethanol production.

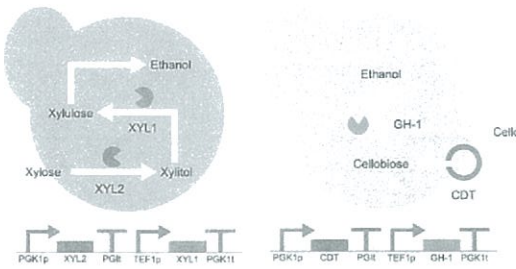
Secondly, we constructed an adhesion platform between heterogeneous cells, making *E. coli* the surface-display system of yeast to realize extra functions.

Thirdly, we introduced a set of minimal promoter and terminator in yeast called MINI-GRE, which as a basic research in our project, will have more extensive applications in the future.

Ethanol from Alage

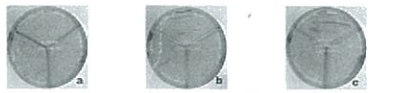
Turning algal bloom into clean energy

In regard of glucose repression, we ferment xylose & cellobiose from pretreated *Enteromorpha* powder with two separated *S. cerevisiae* strains, so that the intracellular hydrolysis of cellobiose minimizes glucose repression of xylose fermentation. For yeast A, we introduced cellobiose transporter and β-glucosidase genes, and in yeast B we expressed xylitol dehydrogenase and xylose reductase.
These two strains that can live on either cellobiose or xylose and produce ethanol through their own metabolic pathways, which means they can ferment cellobiose and xylose synergistically. It will be a big step further since the yeast can take the place of chemicals and fulfill this meaningful task.



We ferment xylose & cellobiose from pretreatment of *Enteromorpha* powder with two recombinant *S. cerevisiae* strains we constructed. Compared to wild type yeast, they can live happily on xylose & cellobiose as sole carbon source. We characterized their ability by measuring (i)the amount of biomass, (ii) the concentration of xylose or cellobiose, (iii) the production of ethanol over time with plate reader, HPLC or SBA.

Proof 1 Qualitative Validation



2% xylose | 2% cellobise | 2% xylose &2% cellobiose

Proof 2 Quantitive Validation

- *Increase in ethanol*
- *Increase in biomass*
- *Decrease in xylose&cellobiose*

> XYLOSE

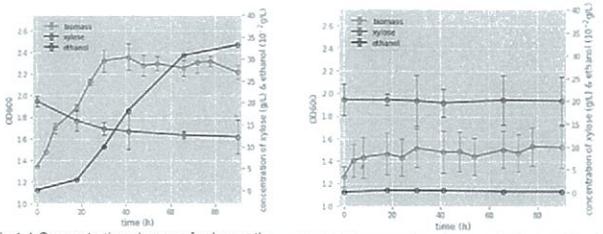


Fig 1.1 Concentration change of xylose, ethanol and biomass (n=4) of engineered strain with xylose-utilizing circuit

Fig 1.2 Concentration change of xylose, ethanol and biomass (n=4) of WT strain

> CELLOBIOSE

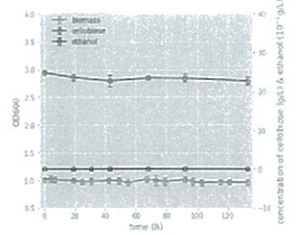


Fig 2.1 Concentration change of cellobiose, ethanol and biomass (n=4) of engineered strain with cellobiose-utilization circuit..

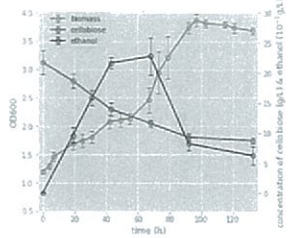
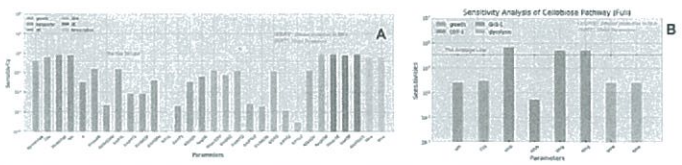


Fig 2.2 Concentration change of cellobiose, ethanol and biomass (n=4) of WT strain

Model



In xylose pathway and cellobiose pathway model, we describe the kinetic system in some ordinary differential equations (ODEs), and explore the rate-determined steps (RDS) of producing ethanol in both pathways by sensitivity analysis.

Figure (A) Numerical integration of sensitivities of kinetic parameters in xylose pathway. Results illustrate that the RDS in xylose pathway are the reaction rate of XK (xylulokinase) and the transportation of the transporter. Figure (B) Numerical integration of sensitivities of kinetic parameters in cellobiose pathway. Results show that the RDS in cellobiose pathway is the transportation of the transporter.

Hightlights

We successfully constructed two strain of *S. cerevisiae* that can either utilize xylose or cellobiose from *Enteromorpha* residue. With the natural metabolic pathway in *S. cerevisiae*, these two carbohydrates can be transformed into ethanol, which is a widely used clean energy.

Achievements

Experiment:
1.We successfully converted *Enteromorpha* residue into ethanol with yeasts for first time!
2.We successfully built the adhesion platform between heterogeneous cells!
3.The MINI-GRE we built is short but strong, with a good modularity, and is able to work in different yeast strains with a good modularity.

Attribution:
We made a detailed description on what is done by ourselves and what supported by others with precise attribution.

Interlab:
We participated in the Interlab Measurement and submitted our result.

Part Submission:
We submitted 11 new Biobrick Parts designed by ourselves which play significant roles in our project.

Collaboration:
We communicated and collaborated with nine teams, which functions as a crucial support to each other's project!

Human Practice:
1.We carefully confirmed that our work is safe and do not harm to the environment and society and dig deep into the society for inspiration.The human practice work feedback to our wet lab.
2.We spread iGEM spirits and promoted the development of synthetic biology in China through popular science brochure, synthetic biology lecture, summer camp, and social media.

Improvement:
We improved Part BBa_J23108 by adding a 5'UTR sequence and enhanced the expression level of RFP reporter by 1.5 times!

Modeling:
We built pathway models for both xylose and cellobiose and agent-based models for adhesion platform. Moreover, we defined the ANRC to analyze the simulation results in ABMs.

Adhesion platform

Engineering E.coli the surface-display system of Yeast for more potential

When dealing with cellulose decomposition, it is natural to think of cellulosome, which functions as a surface-display scaffold and helps express relevant enzymes. However, the advantages of synergistic effect and proximity effect are largely limited by its complicated structure, which leads to a huge metabolic burden.
By adhering *E. coli* to *S. cerevisiae*, this substituted 'surface-display system' can reserve these advantages as well as broaden the potential functions of the yeast as a model organism rather than a scaffold.

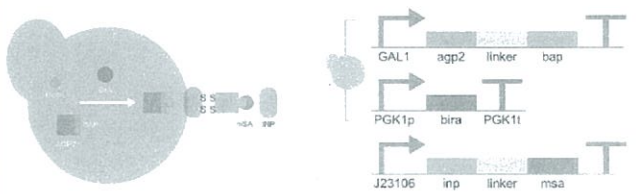
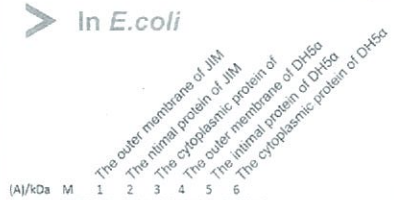


Fig 1: The Sketch of adhesion platform.

Proof 1 Validation of Protein Expression

> In *E.coli*



Engineerd *E.coli* Negative strain

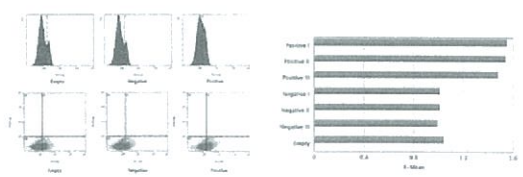
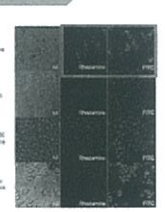


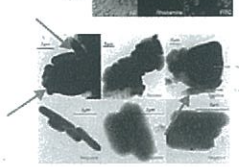
Fig 6: the FCM result for BAP-biotin expressing *S.cerevisiae*
Under the same CV, the mean fluorescence of positive sample is 1.5 times higher than that of negative sample.

Proof 2 Validation of Protein Function



Co-existence of *E. coli* and *S. cerevisiae* even after rinsing---ADHESION VALIDATION!

Fig.7 The result of confocal laser scanning microscopy



Successful adhesion!

Fig.8 Observation of Transmission Electron Microscopy

Model

The aim to the adhesion platform is binding the *E.coli* to *S.cerevisi*

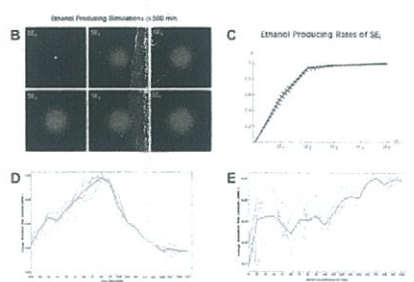


Figure (A) The diagram of binding-dissociation model. Figure (B) Ethanol producing simulations of SEi (i=0, 1, 2, 3, 4, 5) in 500 min. The white circle represents a S.cerevisiae cell, and blue dots represent ethanol particles. Figure (C) Normalized ethanol production rate. Results show that binding *E.coli* to *S.cerevisiae* could increase the ethanol production rate of single cells. Figure (D) ANRC values of different initial concentrations of cells. Figure (E) ANRC values of different initial ratios of *S.cerevisiae* and *E.coli*. The aim to the adhesion platform is binding the *E.coli* to *S.cerevisiaes* to form a microbial collaboration platform utilizing streptavidin-biotin interaction, and further enhancing the ethanol production. Therefore, we first investigate the binding-dissociation process to gain insight of binding conditions, and then explore the ethanol production rate of different types of cells. In the end, we combine both factors to define a standard equation (ANRC) to analysis the quality of adhesion platform under different conditions, and higher ANRC values means better performance.

Hightlights

Mini -GRE

Minimal promoter &terminator elements for regulating gene expression

In the progress of circuit construction, we found that the native fungal promoters and terminators are usually significantly longer than those of Bacteria , with quite a few unnecessary regions for engineered circuits. However, their big size limits large-scale synthetic biological applications in yeast. In previous study, the behavior of promoter-terminator pair in yeast is difficult to be predicted by quantitative data from promoter or terminator respectively.
Therefor, we not only constructed minimal promoters and terminators, but also further combined them into minimal promoter-terminator pairs (minimal genetic regulatory elements, MINI-GRE), hoping to achieve similar or higher transcription output

> Structure of MINI-GRE(Minimal Genetic Regulatory Element)

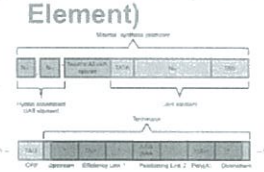


Fig 1 The structure of MINI promoter(top) and MINI terminator(botom).

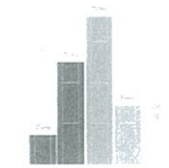


Fig 2 The length of promoters and terminators used in our project

> Circuit Construction -- Different combinations of PMIMI,PCYC1,TMINI,TCYC1

Fig. 3 The circuit construction

> Strength Detection -- Compare the difference intensity of four combinations

Reference
[1]Umel, M. A. et al. "Physico-chemical characterization and enzymatic functionalization of Enteromorpha sp. cellulose." Carbohydrate Polymers 135(2016):274-279.
[2]Artz, L. E. A. Bayer, and S. Morais. "Cellulosomes: bacterial nanomachines for dismantling plant polysaccharides." Nature Reviews Microbiology 15.2(2017):83.
[3]Fan, L. H., et al. "Engineering yeast with tetrafunctional miniscaffoldin and cellodextrin pathway for co-utilization of cellulose-based sugars." Biotechnology for Biofuels 9.1(2016):137.
[4]Tanaka T, Matsumoto S, Ishii J, et al. Displaying non-natural, functional molecules on yeast surfaces via biotin-streptavidin interaction[J]. Journal of Biotechnology, 2010, 145(1):75-83.
[5]Park M, Jose J, Thommes S, et al. Autodisplay of streptavidin [J]. Enzyme & Microbial Technology, 2011, 48(4):307-311.
[6]Klose, Edwin Van, et al. "Decorating microbes: surface display of proteins on Escherichia coli." Trends in Biotechnology 29.3(2011):79.
[7]Redden H, Alper HS. The development and characterization of synthetic minimal yeast promoters[J].Nature Communications, 2015,6 : 7810.
[8]Curran KA, Morse N J, Markham K A, et al. Short Synthetic Terminators for Improved Heterologous Gene Expression in Yeast[J]. Acs Synthetic Biology, 2015, 4(7):824.
[9]Fan, Li Hai, et al. "Self-surface assembly of cellulosomes with two miniscaffoldins on Saccharomyces cerevisiae for cellulosic ethanol production." Proceedings of the National Academy of Sciences of the United States of America 109.33(2012):13260.