Business Plan

Biosynthesis of natural UV absorber gadusol by *Saccharomyces cerevisiae*

2021igem team of
Jiangnan University

Shiyu Yu
Team leader
Jiangnan University, 1800 Lihu Avenue, Binhu District, Wuxi City, Jiangsu Province

Phone: 15852712918
Email: 1024180213@stu.jiangnan.edu.cn

Tingan Zhou
Advisor
Jiangnan University, 1800 Lihu Avenue, Binhu District, Wuxi City, Jiangsu Province

Phone: 15006102723
Email: 1023180225@stu.jiangnan.edu.cn

Table of Contents

1 Executive Summary

1.1 The Quick Pitch

Our team hopes to use *Saccharomyces cerevisiae* to biosynthesis gadusol and use gadusol as the core component of sunscreen to produce marine environment-friendly sunscreen on a large scale.

1.2 The Problem

Economic and ecological value of coral:

Coral reefs are mainly distributed in Southeast Asia, northern Australia, Central America, Malay islands and other sea areas(Fig. 1). The area of global coral reefs is about 284300 square kilometers. The value of products and resource services provided to mankind every year exceeds US $100 billion, especially the annual net economic benefit to global tourism is about US $10 billion.

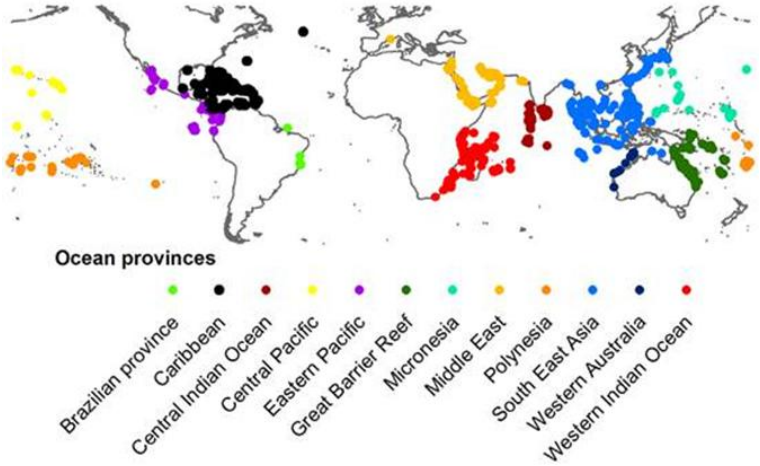


Fig. 1 main distribution of coral reefs

Take Hawaii, the most representative coral reef resort, and the Great Barrier Reef in Australia as examples: in 2015, the number of overnight tourists in Hawaii exceeded 8 million, a year-on-year increase of 8%; The Great Barrier Reef also brings more than a $3 billion (about 14.5 billion yuan) to the local area every year.

In addition to stimulating the economic benefits brought by tourism, coral also has extremely important ecological value. Most marine organisms are difficult to survive in the tropical seawater called "blue desert" lacking nutrients such as nitrogen and phosphorus. Coral reefs are like oases in the desert, providing shelter for many marine organisms (Fig. 2). Although coral reefs account for less than 0.1% of the total ocean area, they may live as many as 9 million species and more than 25% of marine life 、 It is the area with the highest marine species diversity.

Fig. 2 coral reefs provide shelter for many marine organisms

The bleaching of coral reefs will lead to the extinction of a large number of fish and marine organisms, and then lead to the collapse of marine ecosystems.

The seriousness of coral bleaching:
In seawater, low concentrations of chemical sunscreen are enough to cause a series of coral diseases and coral bleaching (Fig. 3). Among them, the main ones are aucylin, hydroxybenzophenone, octyl methoxycinnamate and their similar derivatives.

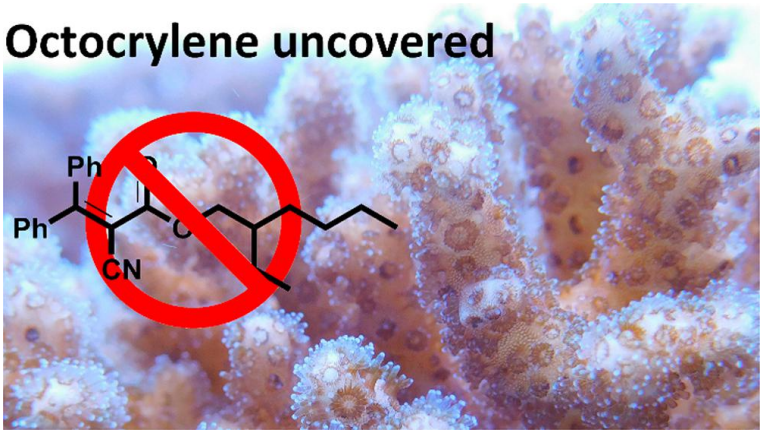


Fig. 3 coralline induced mitochondrial dysfunction

About 14000 tons of sunscreen are washed out and stored in the sea every year, about 10% of which are sunscreen ingredients such as hydroxybenzophenone, far exceeding the minimum concentration leading to coral bleaching, making about 15% of the world's coral reefs and about 40% of the coastal coral reefs fatally threatened by chemical sunscreen (Fig. 4).

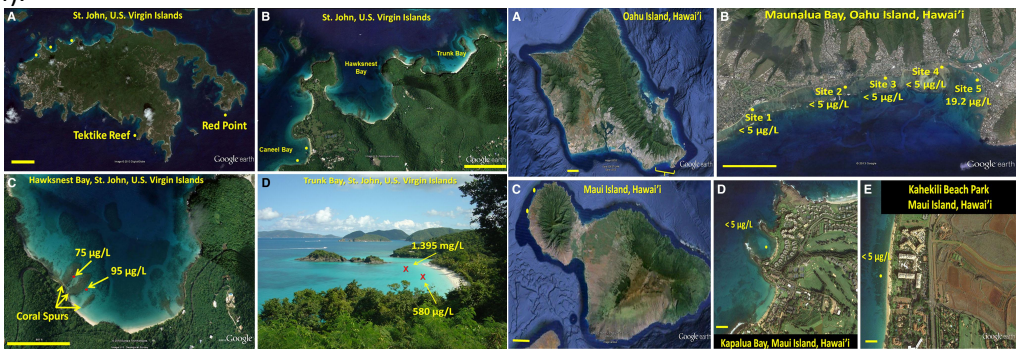


Fig. 4 harmful chemical sunscreen components detected in seawater in many places in the United States

Deficiencies of other coral reef protection methods:
Take 2020 UNSW_ Taking Australia and other iGEM teams as an example, at present, it is mainly through metabolic transformation of zooxanthellae to produce stress resistant substances such as glutathione, so as to help corals survive under adverse conditions such as active oxygen enrichment and high temperature. However, according to the information we obtained from Professor Liu Sheng of the South China Sea Institute of Oceanography, Chinese Academy of Sciences, we learned that coral transplantation is needed if the stress resistance of coral is enhanced by metabolic transformation of zooxanthellae. The artificial cultivation of coral requires a lot of human and material resources, and the scale is also greatly limited. The protection cost will be further increased by means of transplantation, which is difficult to realize.

Therefore, the current protection methods based on the transformation of zooxanthellae to enhance the stress resistance of coral have some defects and are difficult to achieve.

1.3 The Solution

Gadusol is a kind of natural ultraviolet absorber. Its natural source is zebrafish eggs. This substance naturally exists in coral reef ecosystem. Compared with other sunscreen ingredients, gadusol has more reliable safety performance.

The mainstream sunscreen on the market is aimed at protecting against ultraviolet rays of specific wavelengths. Ultraviolet rays can be divided into UVA, UVB, UVC and UVD. Among them, UVB with a wavelength of 280 ~ 320 nm is the most threatening and thoroughly studied to human beings, which is also the main target of sunscreen products. In order to verify the UV absorption capacity of our biological sunscreen core component gadusol, we analyzed its UV absorption spectrum (FIG. 5). The results show that gadusol has good absorption in UVB band and can be added as an ideal core functional component of sunscreen.

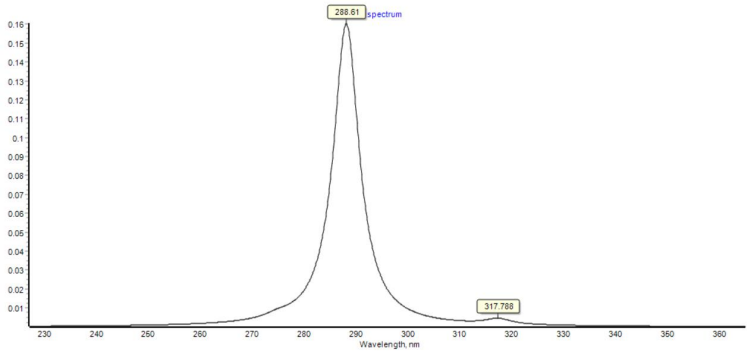


Fig. 5 gadusol ultraviolet absorption spectrum

However, the natural yield of gadusol is very low: only 10 ~ 20mg gadusol can be extracted from 1000 fish eggs, which not only limits its large-scale production and application, but also the way of extracting products from fish eggs is very inhumane. Therefore, using the modified *Saccharomyces cerevisiae* strain to produce gadusol is a feasible and relatively low-cost industrialized way.

1.4 Highlights

Since gadusol, the substance we want to produce, is naturally derived from zebrafish eggs, we first excluded the prokaryotic host and chose the eukaryotic host. And because we hope to use it in cosmetics production, we chose *Saccharomyces cerevisiae* as the starting strain after consulting ***China's measures for the safety management of genetic engineering***.

Gadusol is synthesized by two-step enzyme catalyzed biosynthesis of Sedum heptanone 7 phosphate (s7p) through eevs and MT ox (Fig. 6). We first need to introduce the synthetic pathway of gadusol into *Saccharomyces cerevisiae*. In our experiment, we first optimized the codon of *EEVS* and *MT-ox* genes for *Saccharomyces cerevisiae* to make them better expressed in *Saccharomyces cerevisiae*. Then, the recombinant *EEVS* gene and *MT-ox* gene were cloned into the high copy plasmid pYEP352 for effective expression. Finally, pYEP352 vector containing *EEVS* and *MT-ox* genes was transferred to *Saccharomyces cerevisiae* BY4743 for subsequent fermentation production.(see Fig. 7 for free plasmid design)

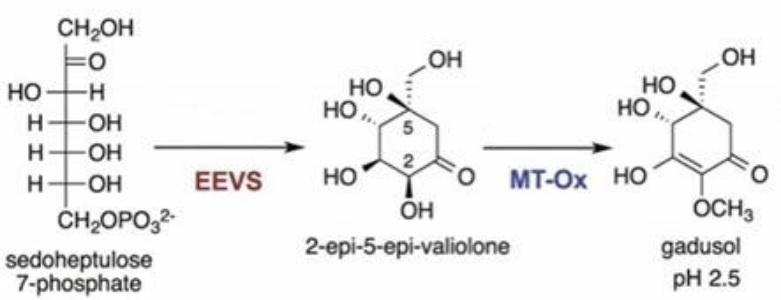


Fig. 6 gadusol synthesis pathway

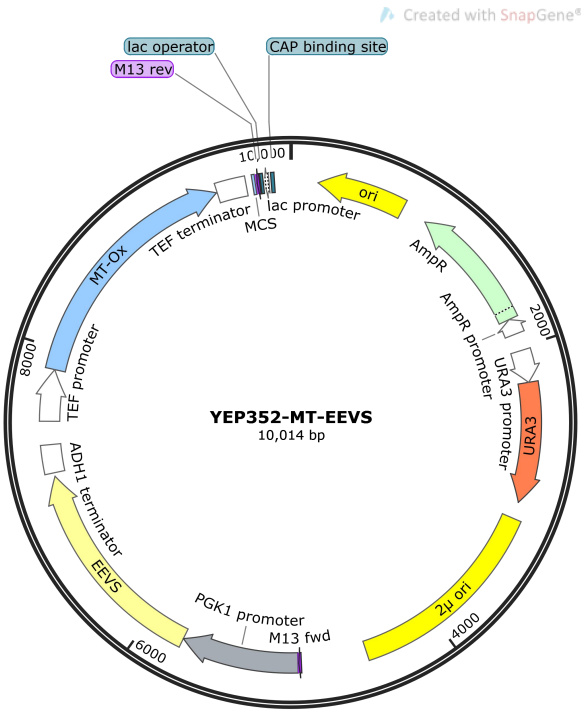


Fig. 7 gadusol synthetic plasmid

1.5 Keys to Success

Only introducing the genes of key enzymes in gadusol synthesis pathway into *Saccharomyces cerevisiae* BY4743 can not meet our expected output, so we further improved the experiment. Firstly, the accumulation of sedulose 7-phosphate (s7p), the precursor of gadusol, in *Saccharomyces cerevisiae* is very low. In order to improve the yield of gaduosl, the content of s7p must be increased through appropriate regulation strategies. Therefore, we propose to introduce xylose as a more efficient carbon source for gadusol production, because xylose can effectively improve the supply of s7p, eliminate the cofactor imbalance caused by the expansion of s7p production, and has more economic potential than glucose. In the XR / XDH pathway, xylose reductase (XR) will first convert xylose to xylitol, and then xylitol dehydrogenase (XDH) will convert xylitol to xylose. Xylose can be transformed into xylose-5-phosphate (X5P) under the action of xylose kinase (XKS) of *Saccharomyces cerevisiae*. X5P is also an intermediate in HMP pathway and will continue to be transformed into s7p under the action of transketase (tkl1). Therefore, the introduction of XR / XDH pathway is an effective strategy to improve s7p accumulation.

However, considering the low promoter strength and low enzyme expression of xylose kinase (XKS) in Saccharomyces cerevisiae, xylose accumulation and insufficient X5P may occur after the introduction of XR / XDH pathway, resulting in small increase in gadusol production and metabolic disorder of Saccharomyces cerevisiae (xylose has certain cytotoxicity), we decided to carry out heterologous expression of XK at the same time (see Fig. 8 for the transformation approach).

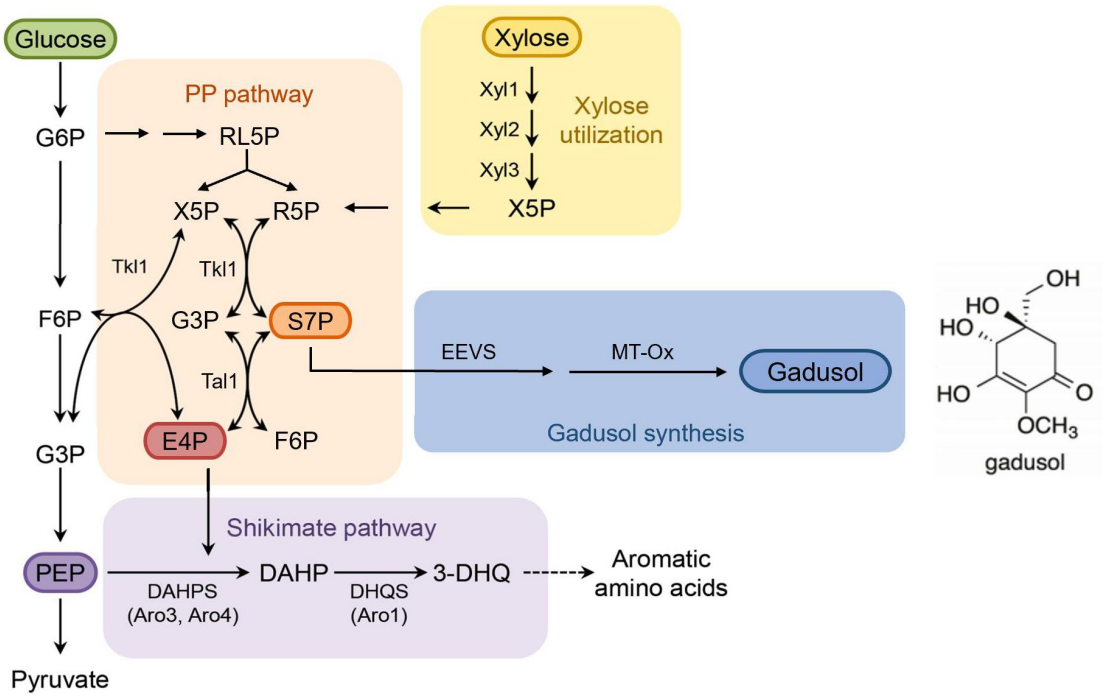


Fig. 8 metabolic diagram of gadusol synthesis from xylose
(XYL1, xyl2 and xyl3 represent XR, XDH and XK respectively)

At present, we have completed the construction of all *Saccharomyces cerevisiae* strains required for gadusol biosynthesis. We found that the yield of *Saccharomyces cerevisiae* strains with only two genes of *EEVS* and *MT-ox* was very low (FIG. 9), while the product yield increased after the introduction of *XR* and *XDH*, and further increased after the introduction of three xylose consumption related genes of *XR*, *XDH* and *XK* (FIG. 10).

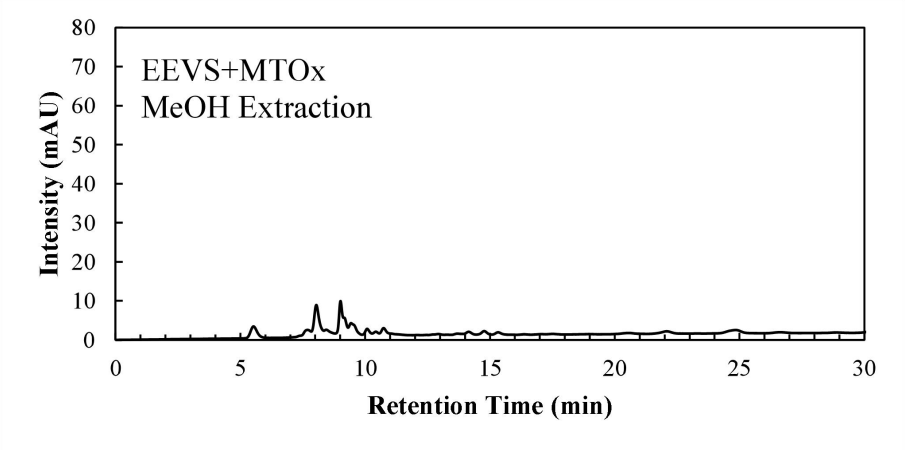


Fig. 9 Very low yield after only introducing *EEVS* and *MT-ox*

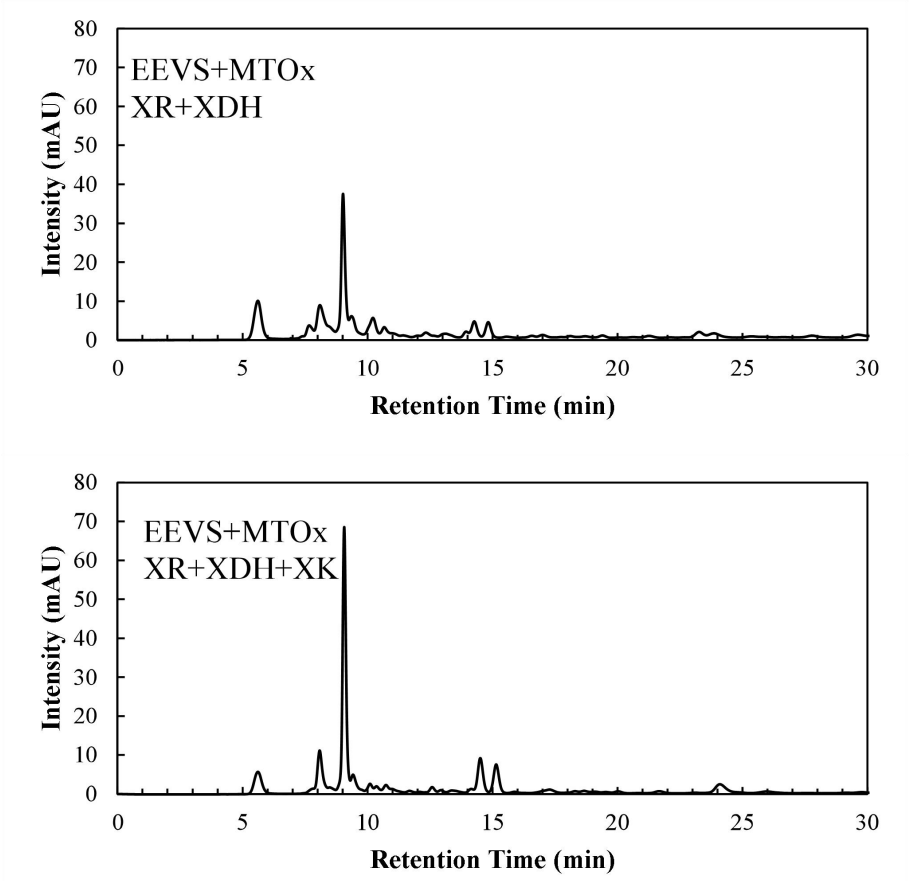


Fig. 10 After the introduction of xylose consumption, the yield increased significantly

Due to the impact of the epidemic, gadusol has just completed the qualitative work of biosynthesis, and there is no specific data on the output. According to the preliminary experiment, the preliminary rough measurement yield can reach more than 100mg / L, which is significantly higher than the yield of less than 20mg / L reported by eLife.

2 Our Team & Organization

2.1 Partnership

The school of bioengineering of Jiangnan University is the birthplace of the discipline of fermentation engineering in China. It has established China's first national key discipline of fermentation engineering and the training system of undergraduate, master and doctoral talents. It is one of the most influential and competitive higher education bases in the field of Industrial Biotechnology (especially fermentation Engineering). "Light industry technology and Engineering" ranked first, second and a + in the discipline evaluation of the Ministry of education in 2009, 2012 and 2017, and was selected as the "double first-class" construction discipline in China in 2017. The research team is established by the school of bioengineering of Jiangnan University. The research content will be guided and helped by the school in metabolic engineering and fermentation engineering. In addition, the Key Laboratory of industrial biotechnology of the Ministry of education and the National Engineering Laboratory under the school of bioengineering of Jiangnan University have complete pilot workshops, which will help the future development of the project.

The formulation of cosmetics is very important for cosmetics, as is sunscreen. Important indicators such as film-forming ability are closely related to the composition ratio. The research team will rely on the Key Laboratory of cosmetics quality research and evaluation of the State Food and Drug Administration of the school of chemistry and materials engineering of Jiangnan University to measure and optimize a series of quality parameters required by the products.

Although gadusol exists in the natural marine environment, the concentration is very low. We still need to conduct enrichment experiments with high concentration gadusol to verify that high concentration gadusol will not have a significant negative impact on corals. The research team has reached a cooperation intention with the teams of the school of Oceanography of Guangxi University and China coral reef research center. The other party can provide relevant experimental facilities for environmental confirmatory experiments.

The research team has cooperated with Friends of Nature (Wuxi group), the largest environmental protection NGO in China, and the non-governmental organization committed to marine protection, Better Blue. It has completed many publicity cooperation, and has completed preliminary public publicity for our coral friendly biological sunscreen.

2.2 Management & Team

Team profile:

Jiangnan University has been participating in iGEM since 2018. This year is the fourth year of participating in iGEM. In the iGEM competition in the first three years, Jiangnan University set up six teams and won five gold and one silver. In 2019, JNU-China was nominated by Best Manufacturing and Best Education and Public Engagement.

Jiangnan_ China, iGEM team of Jiangnan University in 2021, is composed of students from the school of bioengineering, the school of chemistry and materials engineering, the school of pharmacy, the school of design and the school of Internet of things engineering. It is committed to the production of coral friendly sunscreen to realize the protection of coral and marine ecological environment. Relying on Professor Li Qi's brewing science and Engineering Laboratory of Bioengineering College, the team has received the construction funds of double first-class disciplines of light industry technology and engineering of Jiangnan University and by Frederick Gardner Cottrell Foundation sponsored iGEM impact grant.

Principal of the team:

Shiyu Yu, a 2018 undergraduate majoring in biotechnology in the school of bioengineering of Jiangnan University, is the leader of iGEM team in 2021 and is responsible for all experimental design of the project. Once undertook a national innovation training program for college students, and the project results were selected into the national

finals of the National College Students' bioscience competition (innovation and Entrepreneurship). In May 2021, Shiyu Yu published a paper on *Microbial Cell Factories* (SCI, Q1, if = 5.328) as the second author. The tutor is the instructor who won two individual nominations of iGEM in 2019, Associate Professor Xu Guoqiang. At the undergraduate stage, Shiyu Yu has many scientific research and practice experiences, such as the laboratory of functional molecules and health, the laboratory of systematic fermentation and pharmaceutical engineering, and the Pasteur Institute of the Chinese Academy of Sciences.

Ting'an Zhou, a 2018 undergraduate majoring in biotechnology in the school of bioengineering of Jiangnan University, is the second leader of iGEM team in 2020 and advisor of iGEM team in 2021. Starting from July 2019, scientific research and training will be conducted in the functional molecule and health research laboratory under the Key Laboratory of sugar chemistry and Biotechnology of the Ministry of education. The tutor is Professor Rao Yijian, a national talent. In May 2021, he published a paper on *Microbial Cell Factories* (SCI, Q1, if = 5.328) as the first author, and a related national invention patent entered the substantive examination stage.

Instructor:
Jinjing Wang, associate professor, master supervisor and assistant dean of Jiangnan University, doctor of genetics, Institute of Microbiology, Chinese Academy of Sciences.She has long been engaged in beer yeast metabolic engineering, beer nutrition, safety and flavor improvement, breeding of brewing microbial strains, development and research of new colored beer products, and mainly undertakes the teaching of brewing technology and food fermentation technology. In recent years, a total of 26 high-level research papers have been published, with a cumulative impact factor of 53.81, and one monograph has been published; 23 invention patents were applied for and 9 invention patents were authorized; Presided over 3 provincial and ministerial scientific research projects including 863 and National Natural Science Foundation of China. He is now a member of the Asian Biotechnology Association (afob), a consultant expert of the China Council for the promotion of agriculture, a member of the Chinese society of Biochemistry and molecular biology, a holder of the three-level certificate of the British WSET, and a judge of the CCBA refined beer competition. Reviewers of Applied Biochemistry and biotechnology, scientific report, European food research and technology and other journals.

2.3 Company Goals and Objectives

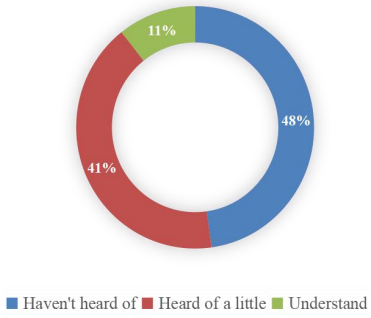
Using gadusol as the core component of sunscreen to produce environment-friendly sunscreen on a large scale, especially ocean friendly sunscreen, so as to replace the mainstream chemical sunscreen in the market and achieve both environmental protection and economic benefits.

3 Market Analysis

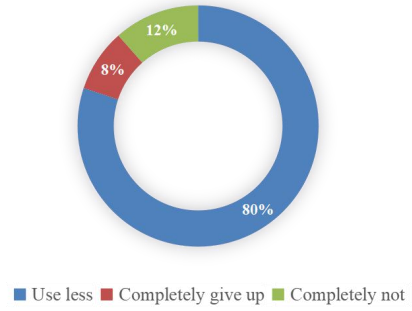
3.1 Market Summary

In view of the idea of producing coral friendly biological sunscreen, we first conducted a preliminary market survey questionnaire.

Q1: Do you know the negative effects of chemical sunscreen on coral?

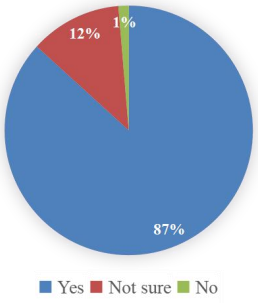


Q2: If we guide the negative impact of chemical sunscreen on coral, will it change the use habit of sunscreen?

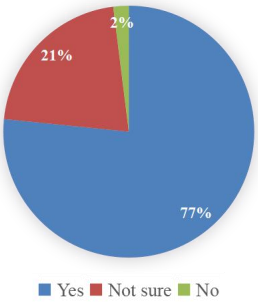


Through the answers to Q1 and Q2, we learned that most of the public (80%) are only willing to reduce the use of sunscreen even after they know that chemical sunscreen will cause a series of fatal problems such as coral mitochondrial dysfunction, rather than give up the use of sunscreen solely for environmental protection purposes. This survey result preliminarily proves that people have a strong demand for sunscreen, which has existed as a necessity of life.

Q3: Are you willing to buy biological sunscreen produced by genetically engineered strains?



Q4: If the performance of biological sunscreen is slightly lower than that of chemical sunscreen, are you willing to buy it?



Through Q3 and Q4 questions, we confirmed consumers' acceptance and tolerance of biological sunscreen. According to the data, even if the performance of biological sunscreen is slightly lower than that of chemical sunscreen on the market, most consumers still have consumption intention.

According to the data of China beauty.com, the sales of a large number of cosmetics and skin care products decreased during the epidemic, while the sales of sunscreen products increased instead of decreased (Fig. 11). This also shows the broad market prospect of sunscreen products in the future.

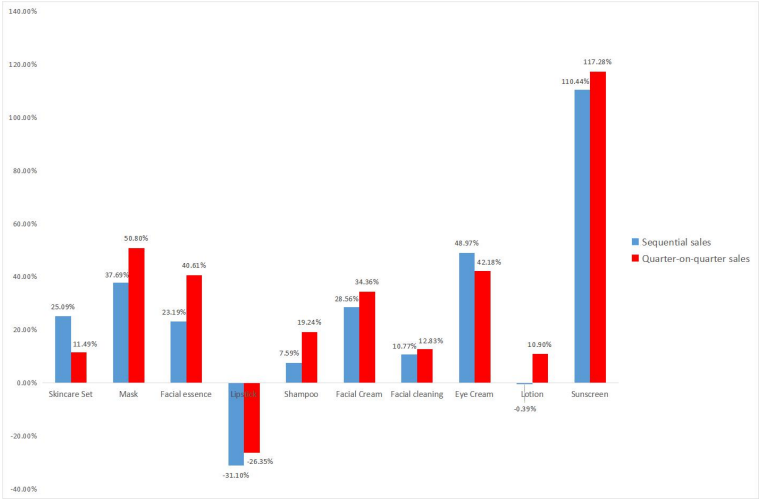


Fig. 11 sales of sunscreen increased instead of decreased during the epidemic

In addition, we visited Guangzhou longhui Trading Co., Ltd., a cosmetics raw material supplier focusing on the R & D and sales of special cosmetics raw materials, and Lubrizol Management (Shanghai) Co., Ltd. and had some exchanges with the sales managers of the two companies on the development of sunscreen ingredients and natural products and synthetic biological cosmetics. We also learned from the interview that the popularity of natural products cosmetics is increasing. As our core functional UV absorber, gadusol comes from natural sources such as fish eggs, which will also add luster to our product sales and promotion.

3.2 Customers

Our target customers are tourists, divers and surfers.

Tourist:

According to a sunscreen report released by *China beauty.com* in 2018, the use of high-power sunscreen products is increasing year by year. Nocole tyrimou, a beauty and personal care analyst at Euromonitor International, predicted that due to the rapid development of China's economy, more and more people go out on vacation. About 74% of Chinese consumers believe that they live in an area that needs sunscreen products, and 91% of consumers say they are willing to spend money on sunscreen products. Allard Marx, one of the founders of the Aquatic Conservation Association, said: "About 10% of the destruction of coral reefs is caused by sunscreen globally."

This further reminds us that with the increase of seaside tourism, a large number of sunscreen will enter the sea and bring serious pollution to the aquatic environment. In the subsequent questionnaire survey, we found that a large part of the

respondents were unwilling to give up on using the sunscreen products, but were willing to use environment-friendly sunscreen. Therefore, we believe that tourists will be our main users.

Divers and surfers:
Studies have shown that the sea is one of the important destinations of sunscreen, among which bathing beach swimming, diving, surfing and other marine recreational activities are the most direct way for sunscreen to enter the marine environment . In an interview with Susu, the head of China's first public welfare organization focusing on divers' participation in marine environmental protection, we learned that among the current audience of high-power sunscreen, surfers are a group that can not be ignored. Because surfers stay at sea for a long time, they often apply a lot of sunscreen to prevent themselves from sunburn. If these sunscreens enter the sea, they will pose a serious threat to aquatic organisms such as corals. Therefore, we believe that surfers are also the target group of environmentally friendly sunscreen.

3.3 Competition

There has been no shortage of bio friendly sunscreen on the market, but it is mostly added as an additive rather than a core functional component.

In addition, most of the natural sunscreen ingredients currently on the market, such as sulforaphane, ferulic acid, carotenoids and tetrahydro lycopene, are from plants. As a biological sunscreen committed to environmental protection, its impact on the ocean should be an important measurement factor. Many plant derived substances do not exist in the ocean, so it is difficult to explain whether they have an impact on coral and marine environment. As a natural substance in a variety of fish eggs and coral reef ecosystems, gadusol has more reliable safety performance than natural sunscreen from plants.