# Oral Probiotics

ZJUT China

## Intro

Oral problem is a high-impact and wide-ranging health problem. Most oral health conditions are largely preventable and can be treated in their early stages.In this project, we developed oral probiotics based on *Bacillus subtilis* to seek for a low-cost and convenient way to prevent oral diseases. We designed multiple genetic circuits to achieve the purpose of detecting oral hygiene and preventing dental caries, as well as achieving convenient feedback of test results. The end result was a combination of scientific research, computer modeling and extensive experimental data to develop a reliable and convenient oral probiotic.

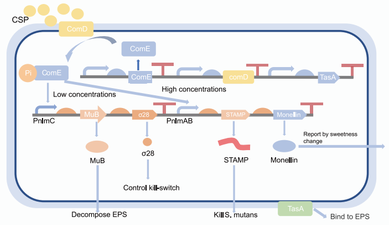
## Background

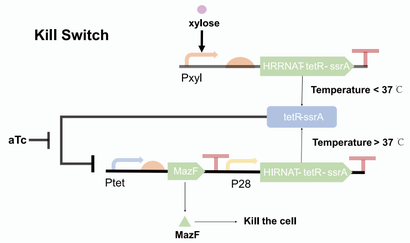
Research continues to show： Oral health is a key indicator of overall health, well-being and quality of life.It encompasses a range of diseases and conditions that include dental caries, periodontal (gum) disease, tooth loss, oral cancer, oro-dental trauma, noma and birth defects such as cleft lip and cleft palate. Oral diseases, while largely preventable, pose a major health burden for many countries and affect people throughout their lifetime, causing pain, discomfort, disfigurement and even death.The Global Burden of Disease Study 2019 estimated that oral diseases affect close to 3.5 billion people worldwide, with caries of permanent teeth being the most common condition. Globally, it is estimated that 2 billion people suffer from caries of permanent teeth and 520 million children suffer from caries of primary teeth. According to the literature, *S. mutans* causes caries by producing an extracellular matrix (EPS) that protects it and provides an attachment site for other oral strains to form plaque. It and other acid-producing bacteria metabolize lactic acid, which can demineralize teeth and lead to cavities.

## Gene circuit

We used a two-component system derived from *S. mutans* to sense the signaling molecule competence-stimulating peptide (CSP) of *S. mutans*. According to the literature, its concentration is positively correlated with the number of *S. mutans*. CSP binds to and phosphorylates the ComD protein on the cell surface, which in turn phosphorylates intracellular ComE. Phosphorylated ComE will bind to promoters nlmC and nlmAB and enhance promoter expression. Because nlmC has a higher affinity for phosphorylated ComE, it preferentially binds to nlmC to express MuB and σ28 when *S. mutans* is less abundant (low CSP concentration). Among them, σ28 controls the suicide switch, which be described in detail later.

MuB is a glucanase that degrades EPS produced by *S. mutans* and destroys dental plaque. When the number of *S. mutans* is high (high CSP concentration), nlmAB expresses STAMP and Monellin. STAMP is a specific targeted antimicrobial peptide that can target and kill *S. mutans*, thereby inhibiting the growth of *S. mutans*. And Monellin is a plant sweet protein , which we used as a reporter molecule to enable users to perceive oral hygiene status by changes in sweetness.




*Bacillus subtilis* is able to express the TasA protein, which gives *B. subtilis* the ability to attach to the EPS surface, thus enabling our engineered bacteria to target *S. mutans* more efficiently. Also, considering biosafety, we designed a suicide switch to prevent the leakage of engineered bacteria. When our engineered bacteria are not used, the xylose we added to the bacteriawill induce the expression of promoter xyl and then express tetR-ssrA protein to repress promoter tet and the engineered bacteria survive. When the engineered bacteria were used and entered the oral cavity, the HRRNAT-tetR-ssrA secondary structure changed due to the high temperature and could not express tetR-ssrA. σ28 induced promoter P28 to expresses the tetR-ssrA to inhibitor promoter tet. If the engineered bacteria are leaked, the HRRNAT-tetR-ssrA secondary structure changed due to the lower temperature and lack of xylose. tetR-ssrA could not be expressed, while mazF was expressed normally,which results in the death of engineered bacteria. In addition, a suicide switch could also be initiated to kill the engineered bacteria by using diluted anhydrous tetracycline (aTc).

## Parts

ComDE: Derived from the population-sensing system of *S. mutans,* it regulates gene expression through competence-stimulating peptide (CSP, a population-sensing signaling molecule whose concentration is positively correlated with the number of *S. mutans*). The ComD protein on the membrane can phosphorylate itself after sensing CSP, then in turn phosphorylates the ComE protein, and the phosphorylated ComE protein (P-comE) initiates the expression of the corresponding promoter.



PnlmC/PnlmAB: The corresponding promoter of ComDE system PnlmC initiates at low CSP concentration, while PnlmAB initiation requires higher CSP concentration.

STAMP: It consists of three parts: target peptide structural domain, junction, and antimicrobial peptide structural domain. T he target peptide structural domain enables the antimicrobial peptide to specifically bind to the target strain to make the antimicrobial peptide have the ability to kill specifically.





Monellin: A lecithin sweetening protein that possesses hundreds of times the sweetness of sucrose and is used to report oral hygiene.
MuB: α-1,3-glucanase, since the main component of EPS is α-1,3-glucan (and α-1,6-glucan, therefore, glucanase can be used to degrade EPS.

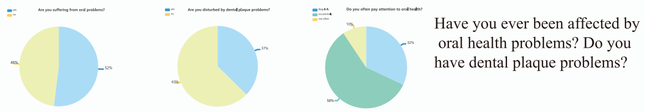
Ptet: promoter, which is inhibited by TetR.
TetR-ssrA: Tetracycline resistance blocking protein with added ssrA tag, which can be rapidly degraded.


MazF: A cytotoxin from Mycobacterium tuberculosis divergent bacilli which acts as a suicide executor in the suicide switch.

P(xyl): a xylose promoter that can be initiated by xylose modulation.
P(28): a promoter from E. coli that can be initiated by σ28 regulation.

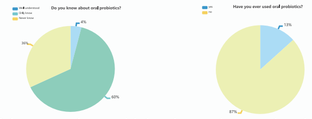

## Human Practise

According to research,prevalence and harm degree of oral disease that has an impact on the overall health are high. The presence of *S. mutans* and dental plaque are important factors leading to oral problems. Our project target at plaque removal or targeted *S. mutans* for different users' oral conditions, and strive to make low-cost and convenient oral disease prevention products. We hope to make a contribution to oral health through synthetic biology. In order to promote the progress of HP's social practice and improve our project, we did the preliminary survey questionnaire, and invited the public to participate in the answer. Here, we analyze and present some of the research results.



Have you ever been affected by oral health problems? Do you have dental plaque problems?

About 52 percent of those surveyed had oral problems, and about 37 percent had plaque problems.
Do you pay attention to oral health and take active care to prevent oral problems?
Nearly 90% of the people surveyed would be concerned about their oral health, with 32% of them frequently concerned about oral health issues, and the vast majority would take care of their mouths on a daily basis by brushing their teeth and rinsing mouthwash.
Do you know about oral probiotics and their related products, and do you think oral probiotic products can have better results in oral health?



About 60% of the respondents said they knew about oral probiotics and 13% had used oral probiotic-related products. The vast majority of people believe that oral probiotic products will be beneficial to oral health.
What features would you expect from oral probiotic products and what kind of help and effect do you want them to have?
Most people want oral probiotic products to be safe, effective, convenient, odorless, non-irritating, and low-cost.
The chosen form is a carrier form that people can easily use; it can play a role in balancing oral flora and maintaining a healthy and stable oral environment; It can be effective in the short term and can meet the long-term use.
The results of the previous research show that people are more concerned about oral health and expect safe, effective, convenient, odorless, non-irritating and low-cost oral probiotic products, which is in line with our topic.

## Application

Oral health is an important consideration in the management of the critically ill patients. Studies have suggested benefit in the reduction of respiratory complication such as Ventilator Associated Pneumonia associated with effective oral health care practices. However, at present there is no consensus as to the best way of providing optimal oral health care in the critically ill. Further research is needed to standardise oral health assessment and care practices to enable development of evidenced based personalised oral care for the critically ill.

The project's *Bacillus subtilis*-based oral probiotic chewing gum allows for safe, effective, convenient, odorless, non-irritating, and low-cost prevention of oral diseases. Also the diverse use carriers of oral probiotics make this project hopeful to provide a better way of oral health care for patients with severe symptoms.