

内容由8所高校IGEM团队编写

2024 E.coli Nissle1917 白皮书

白皮书

主编

PekingHSC NJTech- China

GXU-China NJTech- China-A
LZU- Medincine-China

Table of contents

- 1.EcN nouns explain... 1
- 2.Introduction and usage history of ECN... 2
- 3.Application scenarios of EcN... 8
- 4.EcN's research group overview... 11
- 5.Related modification method of EcN... 13
- 6.EcN related projects in iGEM in the past five years... 20
- 7.Current ECN clinical medication in development and progress... 46
- 8.Analysis of EcNdeliverystrategy... 47
- 9.Comparison of partial bacterial therapeutic modification and EcN... 50
- 10.Policy on bacteriotherapy... 60
- 11.Overview of key companies in EcN industry market... 62
- 12.Forecast of EcN development prospects in China... 64
13. Side effects and biosafety prospects of EcN related therapies..... 67
- 14.Introduction of teams that use EcN projects... 69
- 15.Author list... 78
- 16.Legal statement... 79

1. EcN noun explanation

As one of the most important industrial production strains. *Escherichia coli* has a long research history. It has clear genetic background and is convenient for gene manipulation, and has been used in scientific research and industrial production. However, most *Escherichia coli* strains are opportunistic pathogens, and their application is limited to a certain extent.

The discovery of *Escherichia coli* Nissle 1917 (EcN) has provided more possibilities for the study of *Escherichia coli* [1].

EcN, the full name *Escherichia coli* Nissle 1917, is named after its inventor Alfred Nissle. EcN is a non-pathogenic *Escherichia coli*, which is sensitive to serum and easy to be sero-cleared.

Studies have confirmed that EcN has the characteristics of probiotics such as lack of virulence factors, production of special adaptive factors, and strengthening the natural barrier of the gut [2], and it has been widely used in the prevention of infectious diarrhea and immune regulation.

2. Introduction and usage history of EcN

The discovery of EcN dates back to World War I, when Alfred Nissle, a German physician and bacteriologist, isolated a strain of non-pathogenic *E. coli* from the feces of two soldiers who were not affected by an outbreak of dysentery (Shigellosis) at the Dobruk front [3]. The strain was able to live in the gut and inhibit the colonization of pathogenic bacteria to maintain intestinal health. The strain was named after Dr. Alfred Nissle and because the year of its discovery was 1917, the strain was named Nissle 1917. EcN is sensitive to serum and does not produce any enterotoxins or cytotoxins associated with pathogenic *Escherichia coli* strains. EcN has functions such as inhibiting pathogenic microorganisms, biofilm formation and regulating the immune system. It can be used not only for infectious diseases, but also for the treatment of gastrointestinal diseases such as diarrhea and ulcerative colitis [4]. In the past few decades, the microbial characteristics and molecular genetic background of EcN have been elucidated in detail.

1. Microbial characteristics

1.1 The LPS in EcN has a special structure

EcN is a typical Gram-negative bacterium, which contains lipopolysaccharide (LPS) in the outer cell membrane structure. Molecularly, the LPS of EcN is distinct from all other types of *Escherichia coli*. For example, the O6 polysaccharide side chain of LPS in EcN is very short and contains only a repeating unit consisting of oligosaccharide building blocks of O6 antigen; Modification of the *E. coli* LPS was found in the oligosaccharide core fragment of the molecule. The special structure of LPS may explain the immunomodulatory effect of EcN rather than the immunotoxic effect [5].

1.2 EcN has serum sensitivity

EcN is serum sensitive. There are so-called "capsule" structures in many extraintestinal pathogenic *Escherichia coli*, which can protect them from the attack of non-specific defense components of serum, so that bacteria can acquire resistance to serum, thereby prolonging their survival time in blood and enhancing the virulence of pathogens [6]. On the contrary, EcN strains are rapidly killed due to their own serum sensitivity when they are present in human serum or other mammals (bovine and pig serum) [7].

1.3 The fixed factor in EcN

EcN will produce specific fixative factors. Pathogenic bacteria contain virulence factors encoded by chromosomal specific DNA sequences (pathogenic islands [8], referred to as PAIs), which improve the infection ability of strains. Different fixed factors are also detected in EcN. Their role is to enhance the competitiveness of EcN with other strains in the gut, improve their survival in the gut, and promote effective communication with the host. These factors are regulated by chromosomal specific DNA sequences (gene islands [9], These factors are encoded by genomic islands (GEIs).

1.4 Metabolic features of EcN

As a typical *E. coli*, the EcN strain was biochemically analyzed for its unique metabolic capacity and fermentation characteristics, and a series of biochemical tests were performed. The results showed that EcN possessed typical *E. coli* metabolic characteristics except arginine metabolism. The waste culture supernatant was analyzed by gas chromatography [10], and the results showed that under both aerobic and anaerobic growth conditions, EcN produced short-chain fatty acids as the metabolic end products of carbohydrates.

1.5 The toxicological potential and biosafety of EcN

Escherichia coli includes both enteropathogenic and extraintestinal, so the Nissle strain has received special attention in terms of toxicology and biosafety. According to the principles of probiotic selection for Enterobacteriaceae, probiotic candidates should not exhibit pathogenic adhesion factors, nor should they form enterotoxins and cytotoxins or invade epithelial cells, nor should they carry typical virulence factors of extraintestinal pathogenic *E. coli*, such as serum resistance or hemolysin, nor should they exhibit uropathogenicity or cause immunotoxic effects. EcN strains do not produce any of the toxins associated with pathogenic *E. coli* strains, such as heat-labile enterotoxins (H-LT), heat-stable enterotoxins (H-ST), cytotoxins (e.g., cytotoxic necrosis factor, CNF 1), or Shiga toxoid (SLTI, SLTII). Studies have shown that EcN does not have the genetic information to produce the above-mentioned toxins [11].

2. Molecular genetic background

2.1 The genome structure of EcN

There are different chromosomal regions in non-pathogenic *Escherichia coli*, enteropathogenic *Escherichia coli* and extraintestinal pathogenic *Escherichia coli*, while five large genomic islands and some small genomic islands have been detected in EcN, carrying genomic islands encoding special fixed factors in EcN [12]. The main reason for the probiotic characteristics of EcN may be the lack of virulence factors, including α -hemolysin, P-pilus adhesin, and semi-rough lipopolysaccharide phenotype that binds to the expression of fixation factors [13].

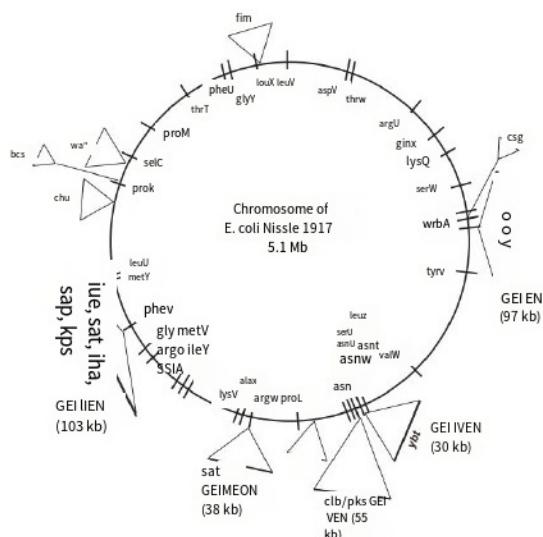


Figure1. Functional genome map of *Escherichia coli* Nissle 1917 (EcN) [12].

2.2 Plasmid characterization of EcN

EcN contains two small invisible plasmids, designated pMUT1 and pMUT2^[14]. Both are genetically stable and not transferable to other *Escherichia coli*, and have been fully sequenced^[15]. At present, these two invisible plasmids have not been found in other *Escherichia coli* and can be used as a method to identify EcN strains. Although the function of these invisible plasmids in EcN is not clear, there is evidence that the invisible plasmids themselves may play a role in protecting the carrier bacteria from the attack of mobile genetic elements such as bacteriophage, thus affecting the genetic stability of the strain^[16].

3. History of the use of EcN

In 1917, Alfred Nissle isolated EcN from the feces of a German soldier and found that it showed strong antagonistic activity against different pathogenic *Escherichia coli* through experimental determination, which was the first time that EcN entered the scientific field of research. In the past 100 years, EcN strains has been used in Germany and several other countries sales of a licensed drug drug active ingredient^[17]. In recent decades, with the deepening of EcN research, scientists have found new probiotic activities in EcN, and the application of EcN in the field of probiotics has been expanding, and it has also been used in many important applications in the treatment of diseases.

3.1 Alfred Nissle and the Discovery and Use of EcN

After Nissle first discovered and isolated EcN strains, the collected strains were cultured in the laboratory. He grew the strains on large AGAR plates and then used them in gelatin capsules sealed with wax or paraffin for the treatment of individual patients. In 1917, he applied for and received a patent for the trademark Mutaflor from the then existing Imperial Patent Office in Berlin. The term Mutaflor, derived from the Latin for mutant and strain, demonstrates the ability of EcN to affect the gut microbiome through antagonism. To mass-produce the drug to meet the needs of more patients, Nissle first selected G. Pohl, a company with special experience in the production of gelatin capsules, for the production of strain preparations. In 1932, Nissle transferred the production license of the strain to the Berlin-based Hageda company. In 1944, the production facility was destroyed by bombs during the war. After the war, Hageda repaired and rebuilt the production facility and resumed the production of Mutaflor. Until his death in November 1965, Nissle provided subcultures of EcN strains as starter cultures for Mutaflor preparations. Later, the EcN strain was deposited in the industrial use strains of the German Microbial and Cell Culture Collection under the name *E.coli* DSM 6601.

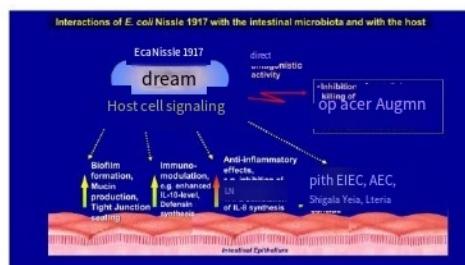


Figure2. Interaction of *Escherichia coli* Nissle 1917 with gut microbiota and host^[17]

From 1930s to 1940s, EcN was commercialized and popularized, especially in Germany and other European countries, and EcN related products were gradually commercialized. With the deepening of research on EcN, scientific research began to frequently discuss the benefits of EcN on intestinal health as an early example of probiotics, and it has been recognized in clinical application. In the late 20th century, researchers intensively studied and developed standard clinical trials. In the 1970s and 1980s, the number of controlled clinical trials on EcN began to increase, and researchers confirmed its positive role in digestive diseases (such as IBD, constipation and diarrhea) [17].

In the 21st century, the function and mechanism of EcN have attracted more attention, and researchers have explored its potential efficacy in immune regulation, severe diarrhea and other gastrointestinal diseases. As one of the representatives of probiotics, EcN has been included in a variety of health foods and dietary supplements for mass market. The researchers also found the potential utility of EcN in vaccine development, mainly in inducing immune responses. When an attenuated bacterium or virus is used as a vaccine, it triggers a protective immune response by presenting the immune system with the necessary antigen, and the use of genetically modified probiotics could, in theory, enhance this effect. EcN is also widely used in diagnostics and drug development. Since EcN possesses tumor-specific colonization properties in mice, it has been used to diagnose solid tumors in clinical studies. In recent studies, researchers have attempted to engineer EcN to enhance its ability to induce immune responses and improve its utility in diagnostic and therapeutic applications [18].

4. The use of EcN in intestinal diseases

Since the 21st century, the research on the function and mechanism of EcN has been constantly improved. Based on this, researchers have turned their attention to the immunomodulatory properties of EcN and applied it in related intestinal diseases. The main task of intestinal inflammation research is to develop new treatments that can precisely target the intestinal mucosa. Transgenic bacteria can be used as a good carrier for local antigens to enter the intestine, but its biological safety is also crucial. Studies have confirmed that EcN has the potential to be a safe carrier of recombinant proteins targeting to the intestinal mucosa. The investigators tested whether EcN had an effect on the migration, clonal expansion, and activation status of specific CD4+ T cells in a well-defined and sensitive immune system. The results showed that EcN did not affect these processes either in healthy mice or in animals with acute colitis. The excellent colonization properties of EcN strains make ECN strains ideal vector candidates for gut focused *in situ* synthesis of therapeutic molecules.

4.1 The use of EcN in neoplastic diseases

In recent years, the research of EcN in cancer targeted therapy has gradually become a hot spot, which is expected to bring new hope for cancer treatment. Traditional cancer treatment methods such as chemotherapy and radiotherapy have improved the survival rate of patients to a certain extent, but there are still many problems, such as large toxic side effects, poor targeting, easy to develop drug resistance and high recurrence rate. By specifically colonizing the solid tumor environment, tumor-targeting bacteria can continuously synthesize and release anticancer drugs in the tumor environment under the condition of genetic engineering, and improve the selectivity of drugs to tumor tissues. Compared with chemotherapy, tumor-targeting bacteria can also reduce the drug damage to the tissue. The hypoxic and immunosuppressed tumor microenvironment creates conditions for some bacteria to proliferate in it. Currently, obligate anaerobes

Such as *Bifidobacterium infantis*, *Clostridium novyi*

As well as facultative anaerobes such as *Salmonella typhimurium* and *Escherichia coli* have been studied for tumor targeted therapy. EcN has a good tumor targeting ability, and the sero-sensitive lipopolysaccharide (LPS) present in its outer membrane ensures that it can complete its "suicide" in vivo and be eliminated from normal organs [5]. In addition, EcN does not produce any enterotoxins and cytotoxins related to pathogenic strains, and its genetic background is clear and convenient for genetic manipulation. Therefore, EcN has become an ideal living drug for tumor targeted therapy.

Genetic modification of EcN to directly express therapeutic factors is one of the important strategies for tumor targeted therapy. EcN directly expresses cytotoxic drugs, prodrug convertase, angiogenesis inhibitor protein and other therapeutic factors to achieve targeted therapy for tumors, and has good therapeutic effects. EcN can also be used as an adjuvant to chemotherapy, radiotherapy, immunotherapy, phototherapy and other therapeutic methods to treat tumors, injecting new vitality into the traditional treatment of tumors. The key challenge in tumor bacterial therapy is to improve the targeting, controllability and safety of bacteria [19]. Studies have confirmed that the main strategy to improve the tumor targeting of EcN is to strengthen the recognition of EcN in tumor hypoxic and acidic microenvironment and promote its binding to tumor cell surface antigens. As a multifunctional probiotic, EcN has become an excellent chassis strain for tumor bacterial therapy. With the development of technology and further research, EcN will become an effective weapon in the treatment of cancer [20].

References:

- [1] 汤佳冰, 张颖, 叶佳微, et al. 大肠杆菌Nissle 1917 的多组学分析及其产微菌素的功能验证. 微生物学报[J]. 1-19.
- [2] 温杨, 万朝敏. 益生菌大肠埃希菌EcN 研究进展. 中国实用内科杂志[J]. 2012, 32 (09): 714-6.
- [3] OZEN M, DINLEYICI E C. The history of probiotics: the untold story [J]. Beneficial Microbes, 2015, 6(2): 159-65.
- [4] KRUIS W. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine [J]. Gut, 2004, 53(11): 1617-23.

- [5] GROZDANOV L, ZA HRINGER U, BLUM-OEHLER G, et al. A Single Nucleotide Exchange in the *wzy* Gene Is Responsible for the Semirough O6 Lipopolysaccharide Phenotype and Serum Sensitivity of *Escherichia coli* Strain Nissle 1917 [J]. *Journal of Bacteriology*, 2002, 184(21): 5912-25.
- [6] Taylor P W. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria[J]. *Microbiological reviews*, 1983, 47(1): 46-83.
- [7] Hughes C, Phillips R, Roberts A P. Serum resistance among *Escherichia coli* strains causing urinary tract infection in relation to O type and the carriage of hemolysin, colicin, and antibiotic resistance determinants[J]. *Infection and Immunity*, 1982, 35(1): 270-275.
- [8] Hacker, J., & Kaper, J. B. (2000). Pathogenicity islands and the evolution of microbes. *Annual Reviews in Microbiology*, 54(1), 641-679.
- [9] Hacker, J., & Carniel, E. (2001). Ecological fitness, genomic islands and bacterial pathogenicity: A Darwinian view of the evolution of microbes. *EMBO Reports*, 2(5), 376 -381.
- [10] Zijlstra J B, Beukema J, Wolthers B G, et al. Pretreatment methods prior to gaschromatographic analysis of volatile fatty acids from faecal samples[J]. *Clinica Chimica Acta*, 1977, 78(2): 243-250.
- [11] Blum, G., Hacker, J., & Marre, R. (1995). Properties of *Escherichia coli* strains of serotype O6. *Infection*, 23(4), 234-236.
- [12] SONNENBORN U, SCHULZE J. The non-pathogenic *Escherichia coli* strain Nissle 1917 –features of a versatile probiotic [J]. *Microbial Ecology in Health and Disease*, 2009, 21(3-4): 122-58.
- [13] DOBRINDT U. (Patho-)Genomics of *Escherichia coli* [J]. *International Journal of Medical Microbiology*, 2005, 295(6-7): 357-71.
- [14] YU X, LIN C, YU J, et al. Bioengineered *Escherichia coli* Nissle 1917 for tumour-targeting therapy [J]. *Microbial Biotechnology*, 2019, 13(3): 629-36.
- [15] Blum-Oehler, G., Oswald, S., Eiteljörge, K., Sonnenborn, U., Schulze, J., Kruis, W., & Hacker, J. (2003). Development of strain-specific PCR reactions for the detection of the probiotic *Escherichia coli* strain Nissle 1917 in fecal samples. *Research in microbiology*, 154(1), 59-66.
- [16] FELDGARDEN M, GOLDEN S, WILSON H, et al. Can phage defence maintain colicin plasmids in *Escherichia coli* [J]. *Microbiology*, 1995, 141(11): 2977-84.
- [17] SONNENBORN U, ROBERTSON L. *Escherichia coli* strain Nissle 1917—from bench to bedside and back: history of a special *Escherichia coli* strain with probiotic properties [J]. *FEMS Microbiology Letters*, 2016, 363(19).
- [18] OU B, YANG Y, THAM W L, et al. Genetic engineering of probiotic *Escherichia coli* Nissle 1917 for clinical application [J]. *Applied Microbiology and Biotechnology*, 2016, 100(20): 8693-9.
- [19] WEBER W, FUSSENEGGER M. Emerging biomedical applications of synthetic biology [J]. *Nature Reviews Genetics*, 2011, 13(1): 21-35.
- [20] 李雨桐, 崔天琦, 张海林, et al. 肿瘤靶向细菌 *Escherichia coli* Nissle 1917 在癌症治疗中的研究进展. *中国生物工程杂志*[J]. 2023, 43(06): 54-68.

3. Application scenarios of EcN

1. Cancer detection and treatment

Gene circuits expressing various anticancer drugs were constructed in EcN to improve their killing ability to tumor cells. Butyrate has the biological activity of reducing carcinogenesis. To achieve targeted cancer therapy, Chiang C J et al. developed bacterial cancer therapy (BCT) with butyrate as a payload. Through metabolic engineering, the recombinant Escherichia coli Nissle 1917 (EcN) was used to synthesize bio-butanoate, named ECN-BUT. The strategies employed include the construction of biobutanoate synthesis pathways and metabolic pathways to increase the production of biobutanoate at the expense of ethyl acid. In tumor-bearing mice, injection of EcN-BUT showed tumor-specific colonization and a significant 70% reduction in tumor volume [1]. To improve the controllability, a synchronous lysis circuit is constructed to achieve self-triggered release of drugs, or external signals (such as small molecules, light, heat, and ultrasound) are used to precisely control the time, location, and dose of drug release. Immune checkpoint inhibitors targeting programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have revolutionized the cancer immunotherapy landscape, resulting in tumor regression in several cancers. Leveraging advances in the fusion of immunotherapy and synthetic biology, a probiotic EcN can be engineered for local and controlled release of PD-L1 and CTLA-4 antagonists. Specifically, it is to combine immunotherapy expression with an optimized lysis mechanism, so that probiotics carry nanobodies back to the necrotic tumor core, grow to a critical density, and lyse to effectively continuously release therapeutic drugs within the tumor microenvironment (TME) [2].

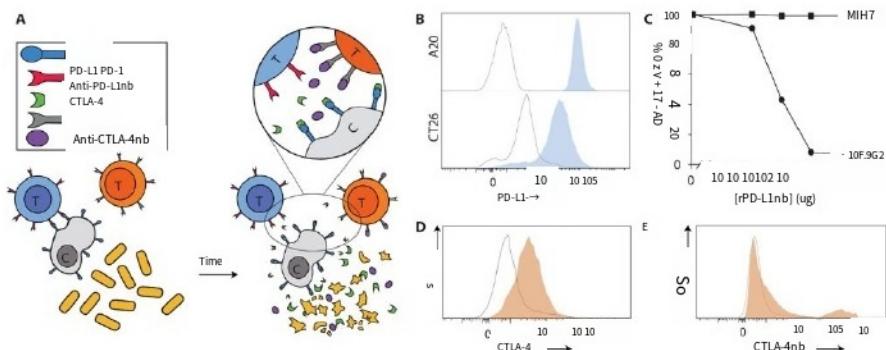


Figure1. Design and Characterization of a Probiotic Cancer Therapy System for the Release of Functional PDL1- and CTLA-4-Blocking Nanobodies. [2]

Nguyen, DH et al. enriched bacterial functions by expressing enzymes, antibodies (such as CD47 antibody, PD-L1 antibody, CTLA-4 antibody), cytokines, etc. to assist traditional cancer therapy, such as improving tumor hypoxic microenvironment to improve the effectiveness of radiotherapy, and producing immune activators to improve the effect of immunotherapy [3].

Mohamad H. Abedi et al. designed a therapeutic bacterium that is controlled by focused ultrasound, a form of energy that can be noninvasive applied to specific anatomical sites, such as solid tumors. This control is boosted by a temperature-driven genetic state switch

For the switch in brief application focused ultrasound after heat treatment have a lasting output, through the combination of reasonable design and high throughput screening, optimized the engineered cells switch circuit, and will release its activity and immune checkpoint inhibitors. In clinically relevant cancer models, ultrasound-activated therapeutic microbes successfully turned on in situ and induced significant inhibition of tumor growth. This technology provides a key tool for the spatiotemporal targeting of effective bacterial therapy in a variety of biological and clinical situations [4].

STING (INterferon gene stimulator) agonists are emerging as a new approach to cancer therapy due to their effect of enhancing immune responses within tumors. As a potential means of delivering STING agonists directly to tumors, Leventhal and colleagues developed SYNB1891, an EcN variant that uses a hypoxia-induced promoter to generate high levels of cyclic AMP. SYNB1891, like SYNB1618, is trophic for thymine and diaminopimelic acid. Phagocytosis of SYNB1891 by phagocytes can trigger the secretion of interferon, which can be used to treat tumors [7].

The SLIC strain (using QS switch) constitutively expressing anti-PD-L1 Nb (nanoantibody), anti-CTLA-4 Nb, and granulocyte-macrophage colony stimulating factor (GM-CSF) has the ability to effectively promote anti-tumor immunity and tumor growth [7].

EcN has been designed to improve the availability of the phytochemical sulfathionine, an anti-tumor compound derived from glucosinolates in cruciferous vegetables. Ho and colleagues engineered Eda-11-HlpA to constitutively secrete horseradish melanistin myrosinase, which is fused to the YebF secretion sequence. The strain was also designed to express HlpA on its outer surface, which promotes interaction with heparan sulfate proteoglycan on the surface of colorectal tumors. To display HlpA on the surface of EcN, it was fused to the ice nuclease protein. When administered with a cruciferous vegetable diet, mice receiving the resulting strain Eda-11-HlpA exhibited smaller and fewer colorectal tumors in a chemically induced colorectal cancer (CRC) model [7].

The reduced availability of L-arginine in tumors limits T cell responses, and Canale and colleagues developed L-Arg EcN (an EcN variant optimized for the conversion of ammonia to L-arginine), which promotes T cell activation and inhibits tumor growth [7].

Article [8] designed EcN to produce a small molecule of salicylic acid. Oral administration of the strain resulted in increased urinary levels of salicylic acid, which has therapeutic effects on rectal cancer, in adenoma-bearing mice compared with healthy controls. Basic method is the base for mbti, irp9, menF, entC cloning on coding pchB plasmid, and then to the plasmid cloning EcNATT Δ clbA - lux (EcNATT), the bacteria including genome integrated aroG, tktA and talB genes. These genes are involved in shikimate and pentose phosphatase pathways, through which salicylic acid is synthesized [8]. Then the article [8] designs an EcN, it delivers blocking PD - L1 and CTLA 4 targets of nano antibody and cellular factor gm-csf, also found that it has inhibitory effect of tumor (oral this strain can reduce the burden of adenoma about 50%) [8].

In the spleen and liver, compared to remove EcN systemic application to a tumor-burdened mice reveals its preferential accumulation in the tumor. Here, Stritzker, Jochen et al. compare the tumor-specific colonization efficiency strains of several different microorganisms.

All strains colonized and replicated efficiently in the tumor, with each strain producing more than 1×10^{-8} CFU per gram of tumor tissue. Compared with salmonella typhimurium, using EcN. When the spleen and liver engraftment rate is significantly reduced, and the pathogenic strains, home didn't have these organs. Further studies with E. coli Nissle1917 showed that no significant differences in colonization and expansion were observed when immunocompetent and immunocompromised animals were used, and Stritzker, Jochen et al. were able to demonstrate that E. coli Nissle1917 replicates at the edge of living tumors and necrotic tumor tissue. Stritzker, Jochen et al. also demonstrated exogenously applied L-arabinose dependent gene activation in colonizing tumors in living mice. These findings will pave the way for bacteria-mediated delivery of the controlled proteins to solid tumors^[11].

Escherichia coli Nissle 1917 (EcN) is able to penetrate barriers and proliferate at the interface between the living tumor and necrotic areas. By creating nanoscale microcells through genetic engineering of EcN, Zhang, YL targeted delivery of chemotherapeutic agents to hypoxic regions of tumors for cancer treatment.^[12]

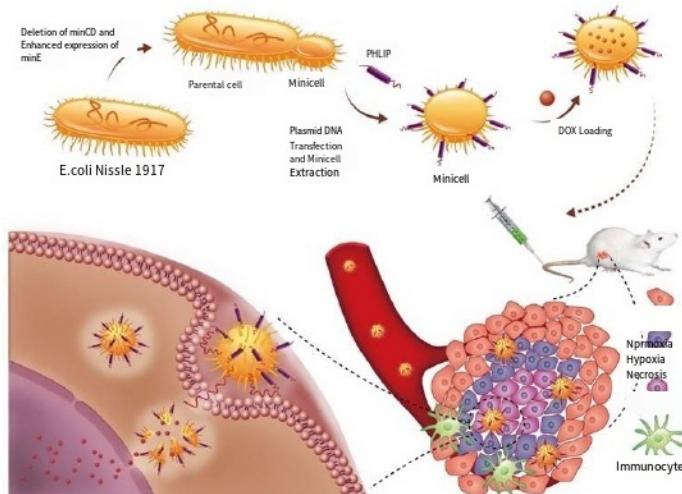


Figure2. Schematic depiction of the construction of microcellular pHLIP for targeted delivery of chemotherapy drugs to hypoxic regions of solid tumors

Domain to kill cancer cells.^[12]

4 . EcN's research group overview

1 Qinglian Hu Research Group - Probiotics modified with polyphenol nanoparticles for targeted therapy of microenvironment reconstruction

Inflammatory bowel disease (IBDs) refers to a multifaceted disorder of intestinal microenvironment and microbiota homeostasis. Given the wide range of biological activities and high compatibility of polyphenols, there is considerable interest in developing a polyphenol-based collaborative platform to reshape the IBD microenvironment and modulate the microbiota. Here, we demonstrate the collaborative assembly of nanostructured polyphenols to modify probiotics and simultaneously deliver drugs for IBD treatment. Inspired by the unique structure of tannic acid (TA), we fabricated a nanostructured pBDT-TA using self-polymerized aromatic dithiol (BDT) and TA, which exhibited excellent antioxidant and anti-inflammatory properties *in vitro*. Therefore, we layered pBDT-TA and sodium alginate (SA) onto the surface of *Escherichia coli* Nissle 1917 to construct a collaborative platform EcN@SA-pBDT-TA. The enhanced resistance of the modified probiotics to oxidative and inflammatory stress resulted in better colon accumulation and retention in IBD model mice. In addition, EcN@SA-pBDT-TA can alleviate dextran sulfate sodium (DSS)-induced colitis by controlling inflammatory response, repairing intestinal barrier and regulating intestinal microbiota. Importantly, EcN@SA-pbdt-ta mediated IBD drug delivery can improve the therapeutic efficacy of DSS model mice. Considering the availability and functionality of polyphenols and prebiotics, we expect that nanostructured polyphenol-modified probiotics provide a solution for the development of a collaborative platform for IBD treatment.

2 Yenifer Olivo Martinez Group -Regulation of serotonin-related genes by probiotic 1 β 1917 extracellular vesicles in a model of intestinal epithelial cell inflammation

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder involving dysregulation of the immune response and imbalance of the intestinal microflora in genetically susceptible individuals. Current treatments for IBD often have significant side effects and limited success, prompting the search for new therapeutic strategies. Microbiome-based approaches aim to restore the balance of the gut microbiota to achieve anti-inflammatory and mucosal healing properties. Extracellular vesicles (evs) derived from beneficial gut microbes are emerging as potential post-biotics. Serotonin plays a crucial role in gut homeostasis, and its dysregulation has been linked to the severity of IBD. Using an IL-1 β -induced inflammation model in Caco-2 cells, our study investigated the effects of evs from the probiotic bacterium Nissle 1917 (EcN) and commensal *Escherichia coli* on gut serotonin metabolism under inflammatory conditions. We found that the strain specificity effect. Specifically, EcNev up-regulated SERT expression by down-regulating miR-24, miR-200a, TLR4, and NOD1, which reduced free serotonin levels. In addition, EcNev reversed IL-1 β -induced changes in tight junction proteins and markers of oxidative stress. These findings highlight the potential of postbiotic interventions as a therapeutic approach for IBD and related pathologies, with EcNev showing promise in regulating serotonin metabolism and maintaining intestinal barrier integrity. This study for the first time confirmed that the probiotic derivative of ev in miR - 24 and miR - 200 - a regulatory role.

3. Ouvs derived from probiotic *Escherichia coli* Nissle1917 regulate gut microbiome in obese and diabetic mice and enterohepatic metabolism in the host

Obesity and diabetes mellitus are common chronic metabolic disorders, which can lead to imbalance of gut microbiota and gut-liver metabolism. Several studies have shown that probiotics, including *E. coli* Nissl 1917 (EcN), promote microbial balance and metabolic health. However, how EcN outer membrane vesicles (EHN-OMVs) affect gut microbiota and metabolic disorders in obesity and diabetes has not been investigated. Methods In the present study, we evaluated the effects of EcN-OMVs on high fat diet (HFD) -induced obesity and HFD + streptozotocin (STZ) - induced diabetes. Results ecn-omv reduced body weight, decreased blood glucose and increased plasma insulin in obese mice. Similarly, EcN-OMVs treatment changed the ratio of Firmicutes to Bacteroidetes in the gut, increased the intestinal short-chain fatty acid (SCFA) -producing flora, and affected the intestinal SCFA content. In addition, the intestinal metabolites ornithine and fumaric acid, liver ω -6 unsaturated fatty acids and SCFAs were significantly increased after EcN-omv treatment. In summary, the present study suggests that EcN-OMVs may act as bioactive factors to regulate enterohepatic metabolism and improve the pathophysiology of obesity and diabetes.

4 O 'Neill Group -Escherichia coli Nissle 1917 inhibits biofilm formation and reduces toxicity of Pseudomonas aeruginosa

To find ways to mitigate bacterial virulence, cell-free supernatants (CFS) from 25 human commensal and related bacteria were tested for activity against *Pseudomonas aeruginosa*. Among them, *Escherichia coli* Nissle 1917 CFS significantly inhibited biofilm formation, dispersing the extant *Pseudomonas* biofilm without inhibiting the growth of plankton bacteria. eDNA in the biofilm was reduced after exposure to *E. coli* Nissle CFS as observed by confocal microscopy. E. also showed significant protection in the larval virulence assay of *E. coli* 24 h before challenge with *Pseudomonas aeruginosa*. No inhibition against *Pseudomonas aeruginosa* was observed against other *E. coli* strains. According to proteomic analysis, several *Pseudomonas aeruginosa* proteins downregulated by *E. coli* Nissl CFS are involved in motility (flagellar secretion chaperone FliSB, flagellin type b turnover, PilB type IV pilus assembler) and quorum-sensing (laser and rhlR, a HTH-type quorum-sensing regulator). This is associated with biofilm formation. The physicochemical characteristics of the hypothesized antibacterial film compound (s) suggest that a heat-labile protein factor with a molecular size of more than 30 kDa is involved.

5. Related modification method of EcN

Escherichia coli Nissle 1917 (EcN) is a non-pathogenic Escherichia coli isolated by Alfred Nissle in feces in 1917. It does not express P-pilus adhesin, synthesize semi-rough lipopolysaccharide, and secrete α -hemolysin, that is, it does not produce known virulence factors and has biological safety. The use of EcN in the treatment of intestinal diseases and biological fermentation production has unique advantages.

EcN has two cryptic plasmids, pMUT1 and pMUT2, which are genetically stable and non-transferable and are only found in EcN. pMUT1 carries a COLE1-type replication system, and pMUT2 contains a ColE2-like replication system and other replication systems, but no other open reading frames with known functions are found in either plasmid [1]. These two cryptic plasmids may be related to the defense against phage infection. Blum-Oehler et al. constructed a plasma-free variant of Escherichia coli DSM 6601 (EcN) to lay the foundation for gene expression and modification using cryptic plasmids.

The genetic modification of EcN is mainly focused on disease treatment, cell factory, CRISPR tool development and gene wiring, and the genetic modification operation related to EcN is also relatively perfect.

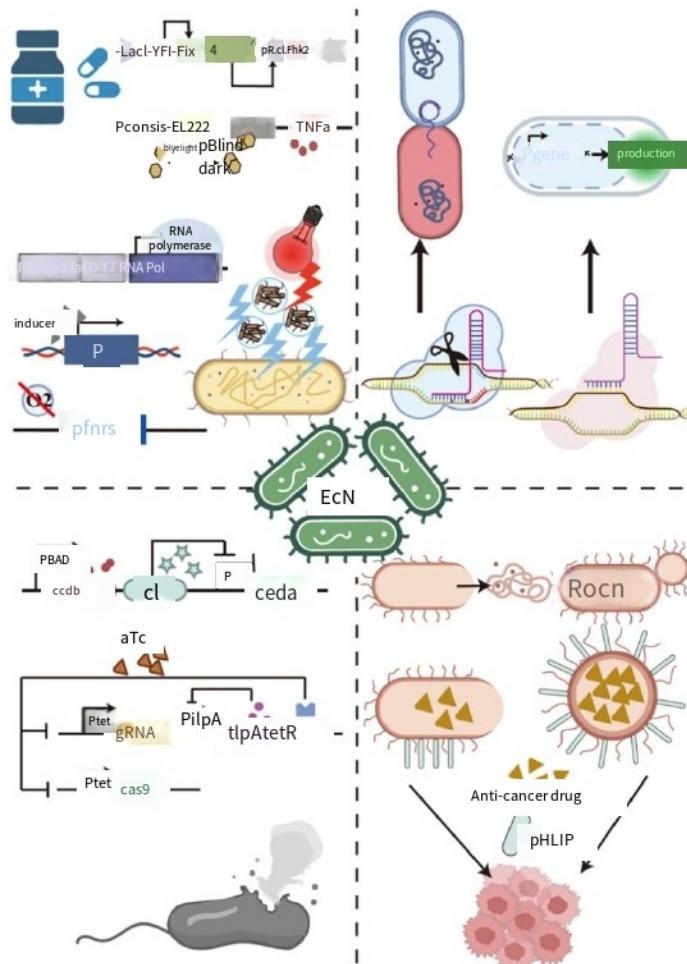


Figure1. EcN related genetic modification and application [2]

1. Disease treatment

As intestinal probiotics, EcN prevents pathogen infection by strengthening the intestinal mucosal barrier and stimulating the immune system. In recent years, genetically engineered EcN has been further applied to treat inflammatory bowel disease, colorectal cancer and metabolic diseases.

In the treatment of IBD, Wang et al. secreted and expressed immunomodulatory protein Sj16 in EcN through α -hemolysin secretion system to alleviate colitis and promote intestinal antioxidant capacity^[2], and further increased Treg cells and reduced Th17 cells through butyrate to alleviate immune response^[3]. Praveschotinunt et al. developed a probiotic PATCH therapy system to relieve inflammation by displaying the fusion self-assembling protein subunit CsgA and TFF3 on the surface of EcN, which is a factor that promotes mucosal healing. This genetic circuit is expressed through an arabinose dependent promoter, and this EcN variant is named PBP8 CsgA-tff3^[4].

Yu et al. transferred the gene encoding the lipase ABC transporter of Erwinia into the EcN chromosome to recognize and secrete transforming growth factor beta (TGF- β 1) and promote mucosal healing^[5].

Cui et al. designed blue-light-responsive expression of Ag43, an adectin that promotes biofilm formation and enables bacteria to form "bio-glue", thereby improving the survival rate of bacteria in the GI tract^[6].

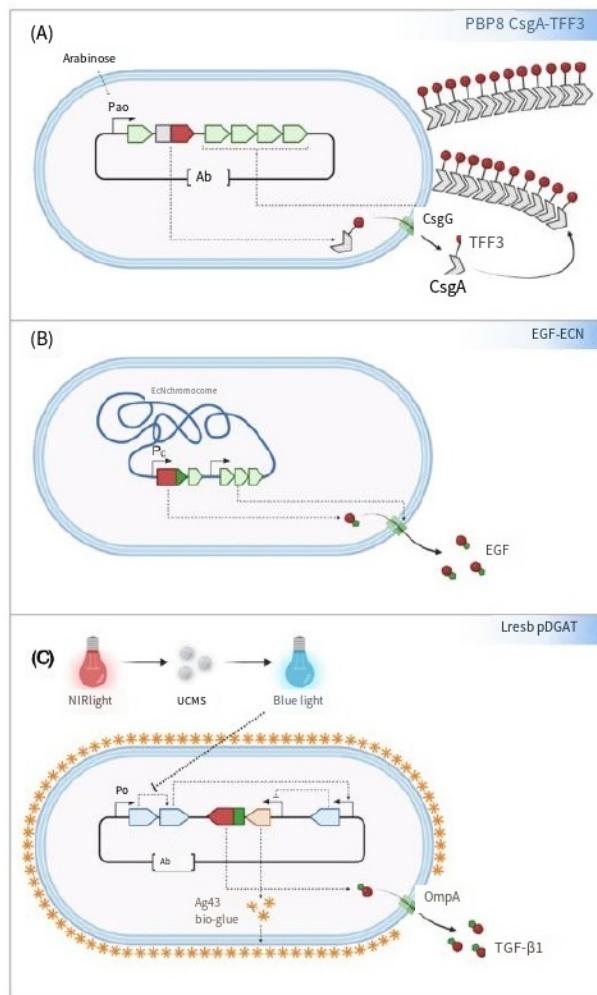


Figure2. Application of EcN modification to promote mucosal healing [21]

At the same time, engineered EcN can also be used as an anticancer agent to treat cancer. The current main ideas include improving the targeting and lethality of EcN and giving it diagnostic ability. Leventhal developed an EcN variant, SYNB1891, that expresses Listerial diadenylate cyclase through the Pfnrs hypoxia-inducible promoter, producing high levels of cyclic AMP. Uptake of SYNB1891 by phagocytes can trigger interferon secretion. It can promote its clearance and induce the development of immune memory, thereby preventing tumor recurrence [7].

Table 1. EcN tumor therapy Related research [22]

Feature	Principles	Implementation
Improve the targeting of EcN	<p>Micro in response to tumor low pH Environment</p> <p>HlpA and tumor markers Bind to specific targeted proteins</p> <p>EcN produces tumor-killing eggs Expression of tumor therapeutics</p> <p>albumin Antiangiogenic proteins</p> <p>Block tumor-derived nutrient source Tum-5 with tumor-killing eggs Improves tumor killing of EcN force</p> <p>Apopsin and cells Facilitating drug entry into the tumor Tat co-expression</p> <p>PD-L1 inhibitors and tumors Restore T cell immune activity PD-L1 binding</p> <p>Trzl can sense glucose Responds to the concentration of glucose and displays different concentrations</p> <p>EcN is endowed with tumor diagnostic capabilities force</p> <p>Analysis of urine color Decipher LuGal expression, urine</p> <p>Color change</p>	<p>EcN-ca-dox at low pH Dox is released in the tumor environment</p> <p>pHLIP wraps around EcN targets To the tumor environment</p> <p>HSPG binding</p> <p>EcN produces tumor-killing eggs Expression of tumor therapeutics</p> <p>albumin Antiangiogenic proteins</p> <p>Block tumor-derived nutrient source Tum-5 with tumor-killing eggs Improves tumor killing of EcN force</p> <p>Apopsin and cells Facilitating drug entry into the tumor Tat co-expression</p> <p>PD-L1 inhibitors and tumors Restore T cell immune activity PD-L1 binding</p> <p>Trzl can sense glucose Responds to the concentration of glucose and displays different concentrations</p> <p>EcN is endowed with tumor diagnostic capabilities force</p> <p>Analysis of urine color Decipher LuGal expression, urine</p> <p>Color change</p>

According to the characteristics of low pH in tumor microenvironment, Li et al. connected the acid-labile linker of tumor killing drug doxorubicin and cis-aconitine anhydride to the surface of EcN to form ECN-CA-DOX targeted drugs, so that doxorubicin can only be released in the tumor environment [8]. Similarly, Zhang et al. expressed the insertion peptide pHLIP in EcN in response to low pH and anchored it on the surface of EcN cells to enhance the targeting effect [9].

Gurbatri et al. constructed a PD-L1 inhibitor in EcN and used quorum sensing effect to lyse EcN cells, releasing PD-L1 inhibitory protein to bind to PD-L1 on the tumor surface and activate T cells to continuously kill tumor cells [10].

In view of the oral cavity is difficult to diagnosis early, constructed based on lactic acid markers, such as Hamilton extracellular lactic acid reaction fluorescence EcN report gene engineering, including a response transcription factor LIdR lactate and LLD promoter expression of green fluorescent protein, when loss of lactic acid, When lactate is absent, LIdR protein represses the LLD-controlled promoter and blocks GFP expression [11].

It is very promising to modify EcN by synthetic biology technology and apply it to the treatment of diseases. Using EcN as chassis cells to assemble biochips, etc., can further improve the response and killing ability of EcN to tumors, and take advantage of its natural anti-inflammatory, targeting and anti-tumor properties, becoming a new powerful weapon for the treatment of inflammation and cancer.

2. EcN cell factory

EcN in culture can be achieved in the commonly used microbial strain equivalent cell density matrix, the chassis is ideal for high density fermentation.

Hu et al. carried out metabolic modification of EcN to produce β -alanine, and the yield reached 11.9 g/L after fed-batch fermentation [12]. Meanwhile, Datta et al. achieved the production of 3 g/L heparan glycan by high-density fermentation and using glucose as carbon source [13]. EcN is also in the production of bacterial cellulose good microbial chassis. The natural producer of bacterial cellulose, sweet and vinegar Bacillus xylosa, grows slowly and is not suitable for industrial production. Sajadi et al. constructed a bacterial cellulose metabolic pathway in EcN and achieved a fermentation yield of 1.9 g/L in shaking jar [14]. Omega-3 fa, including eicosapentaenoic acid and docosahexaenoic acid, is involved in fetal development, immune and the dietary composition of alzheimer's disease, through the expression of EPA and DHA gene cluster in the EcN, shake flask production reached 31.36 mg/L [15].

Table 2. EcN Cell factory Related Research [21]

metabolite	Action	Implementation	Yield
β -ALA	As a pharmaceutical intermediate Strengthen fumaric acid to L-ASP's	Expression of engineered aspartic acid transfer transferase 4 Increase the supply of fumaric acid	11.9 g/l

		way
		Promote oxaloacetic acid to L-ASP
		The conversion of
L-Arg (L-ARG)	Take NH3 and convert it to L-Arg	Delete the negative regulator gene Insert the feedback resistance enzyme gene
		Increase T cells
L-Arg (levorin)	The number of T cells L-Arg and PD-L1 inhibitors	L-arginine (L-argG) 3 Delete the arginine repressor gene
		The synergistic effect of PD-L1
Butyric acid	Butyric acid 2 Improve anti-inflammatory properties	Allogeneic BCD and BUT genes 297.3± 34.25 Mg/L
		Integration from the glucose pathway
butyrate	Cell cycle arrest in G1 phase induces mitochondrial apoptosis pathway	Butyryl Coenzyme A 20 mm Convert butyryl CoA to butyl Acid gene
		Overexpression encoding 5-ALA
5-ALA	As a component of PDT	Genes of components 300 mg/l Inhibit downstream pathway LA
		Regulate energy intake and
GLP-1	Depletion of neuropeptides	GLP-1 overexpression
		Expression
TFF	Mucosal healing	Construct the synthetic curli operon Coding plasmid

3. EcN gene editing tool and gene circuit construction

The development of EcN gene editing tools and the construction of gene circuits will further facilitate the modification and utilization of EcN. Fiege et al. integrated T7RNA polymerase into the EcN genome and constructed EcN::T7 strain. The engineered EcN strain can recognize the T7 promoter and realize the massive expression of heme-dependent proteins^[16]. It was found that the leaky expression of T7 promoter was more serious in EcN than in BL21, and replacing the lacI promoter with the constitutive promoter significantly increased EcN

The strictness of T7 expression system in T7 [17]. The near-infrared light response system can also be applied to EcN. Due to the poor tissue permeability of blue light, the near-infrared induction system EcN (ROEN) has been constructed, which contains a chimeric light sensor Cph8, which is inactivated under red light at 635nm, so that cl expression is inhibited and p λ controlled cleavage gene expression is initiated [18].

The CRISPR toolbox related to EcN has been gradually developed and used. Previous studies have used related technologies to remove cryptic plasmids in EcN and edit their genomes to improve the production capacity of cell factories.

Pan et al. constructed a suicide system composed of L-arabinose inducible promoter, λ promoter, λ promoter inhibitor and toxin-antitoxin system. The toxin protein-coding gene (ccdb) and cI are controlled by the pBAD promoter, and the antitoxin protein-coding gene (ccda) is controlled by the p λ promoter. When supplemented, cI is highly expressed and inhibits ccda transcription, thereby inducing the death of EcN [19].

More gene circuits can be developed in combination with quorum sensing systems. Hwang et al. constructed a suicide system in response to a quorum sensing signal (N-acylhomoserine lactone). When Pseudomonas aeruginosa grows to a threshold cell density, increased N-homoserine lactone triggers the expression of an engineered EcN cleavage gene. As a result, sepsis S5 and DspB were produced, which inhibited Pseudomonas aeruginosa [20].

References:

- [1] Decanio, M. S., Landick, R. & Haft, R. J., The non-pathogenic Escherichia coli strain W secretes SslE via the virulence-associated type II secretion system beta. *BMC MICROBIOL* **13** 130 (2013).
- [2] Wang, L. *et al.*, rSj16 Protects against DSS-Induced Colitis by Inhibiting the PPAR-alpha Signaling Pathway. *THERANOSTICS* **7** 3446 (2017).
- [3] Wang, L. *et al.*, An engineered probiotic secreting Sj16 ameliorates colitis via Ruminococcaceae/butyrate/retinoic acid axis. *Bioeng Transl Med* **6** e10219 (2021).
- [4] Praveschotinunt, P. *et al.*, Engineered E. coli Nissle 1917 for the delivery of matrix-tethered therapeutic domains to the gut. *NAT COMMUN* **10** 5580 (2019).
- [5] Yu, M., Kim, J., Ahn, J. H. & Moon, Y., Nononcogenic restoration of the intestinal barrier by E. coli -delivered human EGF. *JCI INSIGHT* **4** (2019).

- [6] Cui, M. *et al.*, NIR light-responsive bacteria with live bio-glue coatings for precise colonization in the gut. *CELL REP* **36** (2021).
- [7] Leventhal, D. S. *et al.*, Immunotherapy with engineered bacteria by targeting the STING pathway for anti-tumor immunity. *NAT COMMUN* **11** 2739 (2020).
- [8] Xie, S. *et al.*, Doxorubicin-conjugated Escherichia coli Nissle 1917 swimmers to achieve tumor targeting and responsive drug release. *J CONTROL RELEASE* **268** 390 (2017).
- [9] Zhang, Y. *et al.*, E . coil Nissle 1917-Derived Minicells for Targeted Delivery of Chemotherapeutic Drug to Hypoxic Regions for Cancer Therapy. *THERANOSTICS* **8** 1690 (2018).
- [10] Gurbatri, C. R. *et al.*, Engineered probiotics for local tumor delivery of checkpoint blockade nanobodies. *SCI TRANSL MED* **12** (2020).
- [11] Hamilton, S., Shea, D., Ibsen, S. & Brasino, M., On-chip dielectrophoretic recovery and detection of a lactate sensing probiotic from model human saliva. *ELECTROPHORESIS* **44** 442 (2023).
- [12] Hu, S. *et al.*, Development of probiotic E . coli Nissle 1917 for 尾-alanine production by using protein and metabolic engineering. *APPL MICROBIOL BIOT* (2023).
- [13] Datta, P., Yan, L., Awofiranye, A., Dordick, J. S. & Linhardt, R. J., Heparosan Chain Characterization: Sequential Depolymerization of E. Coli K5 Heparosan by a Bacterial Eliminase Heparin Lyase III and a Bacterial Hydrolase Heparanase Bp to Prepare Defined Oligomers. *BIOTECHNOL J* **16** (2021).
- [14] Sajadi, E. *et al.*, Increased cellulose production by heterologous expression of bcsA and B genes from Gluconacetobacterxylinus in E . coli Nissle 1917. *BIOPROC BIOSYST ENG* **42** 2023 (2019).
- [15] Amiri-Jami, M., Abdelhamid, A. G., Hazaa, M., Kakuda, Y. & Griffiths, M. W., Recombinant production of omega-3 fatty acids by probiotic Escherichia coli Nissle 1917. *FEMS MICROBIOL LETT* **362** (2015).
- [16] Fiege, K. & Frankenberg-Dinkel, N., Construction of a new T7 promoter compatible Escherichia coli Nissle 1917 strain for recombinant production of heme-dependent proteins. *MICROB CELL FACT* **19** (2020).
- [17] Effendi, S. S. W. & Ng, I., Reprogramming T7RNA Polymerase in Escherichia coli Nissle 1917 under Specific Lac Operon for Efficient p -Coumaric Acid Production. *ACS SYNTH BIOL* **11** 3471 (2022).
- [18] Fernandez-Rodriguez, J., Moser, F., Song, M. & Voigt, C. A., Engineering RGB color vision into Escherichia coli. *NAT CHEM BIOL* **13** 706 (2017).
- [19] Pan, H. *et al.*, Engineered NIR light-responsive bacteria as anti-tumor agent for targeted and precise cancer therapy. *CHEM ENG J* **426** (2021).
- [20] Hwang, I. Y. *et al.*, Engineered probiotic Escherichia coli can eliminate and prevent *Pseudomonas aeruginosa* gut infection in animal models. *NAT COMMUN* **8** (2017).
- [21] Yu, M., Hu, S., Tang, B., Yang, H. & Sun, D., Engineering Escherichia coli Nissle 1917 as a microbial chassis for therapeutic and industrial applications. *BIOTECHNOL ADV* **67** (2023).
- [22] 王彦雯, 叶静怡& 王鹏超, 合成生物学在E.coli Nissle 1917 靶向治疗癌症中的应用. 中国生物化学与分子生物学报**37** 20 (2021).

6. EcN related projects in iGEM in the past five years

2019

3081 Peking

<https://2019.igem.org/Team:Peking>

E. coli host cells, and its general physiology will also show significant changes over time, these physiological function can be directly or indirectly affect the synthesis of part of the "expectations" function. Many of the challenges could be addressed if we could better control the growth rate of our cells. However, previous methods have many drawbacks for growth rate control. Therefore, we developed a novel system to achieve precise growth rate control by targeting the origin of DNA replication using dCas9.

Solution: directly to the growth of genome replication control toolbox

In Escherichia coli, genome replication starts at a single site, oriC. Formation of DnaA protein filaments on the DnaA cassette within oriC accurately regulates replication bubble opening and subsequent helicase loading. Here, we managed, primarily based on CRISPR/dCas9 competition with DnaA cassette arrays, to block DnaA binding.

So their approach: a novel approach for prokaryotic genome replication interference (CRISPRri), similar to CRISPR-interfered transcriptional repression [5]. The CRISPRri system can well control the growth of E. coli strain Nissle 1917.

Considering the oriC escherichia coli Nissle sequence is the same as the oriC sequence in the top 10, we want to prove that the new e. coli Nissle CRISPRri system function the same as in the top 10.

They transformed the CRISPRri system into Escherichia coli Nissle 1917. Using the microfluidic imaging system, we observed that the bacterial proliferation of the experimental group (whose sgRNA was targeted on the M box in the oriC region) was significantly slower than that of the control group (polyA) under 0.25% arabinose treatment. Quantitative results showed that the average doubling time of cell number in the experimental group (M+) was more than 2 hours, while it only took about 40 minutes to complete cell division in the control group (polyA). The results indicated that CRISPRri system could well control the growth of EcN.

3036 BNU-China

<https://2019.igem.org/Team:BNU-China>

Obesity can cause many diseases and become a serious problem worldwide. They propose the development of a synthetic slim-promoting gut microbe (SLIM) that colonizes the human gut and promotes catabolism of fat (both assimilated and unassimilated fat) through two separate pathways. One of the growing human consumption of excessive high fatty acid of beta oxygen, and the other leads to acetic acid (as a signal to promote human consumption of white adipose tissue) of overproduction. The latter of these is placed under the control of the sensing module so that it is activated only at the optimal time, thereby preventing interference with the digestion caused by acidification.

They chose Escherichia coli Nissle 1917 as the chassis for the final product. Because E. coli nissle1917 is a probiotic strain derived from the human gut, it doesn't easily elicit an immune response. In addition, to prevent the engineered bacteria from being excluded, they boosted their ability to proliferate, making them more competitive. BNU-China 2018 has demonstrated that strains can gain a significant proliferation advantage by overexpressing glucose dehydrogenase (GDH), a key enzyme in the pentose phosphate pathway. The same enzyme can be used to promote microbial colonization.

On the other hand, they don't want the engineered bacteria to disrupt the natural microbiome. So we included quorum sensing systems in our design that are orthogonal to natural microbes. Due to the limitations of the quorum sensing mechanism, the proliferation rate of the engineered bacteria is slowed once a certain population threshold is reached, thus ensuring that it does not interfere with the gut microbiome.

3245 Fudan

<https://2019.igem.org/Team:Fudan>

Lactose intolerance is a common disease in China, which is irrelevant for adults but crucial for infants. We used E.coli Nissle 1917 as our chassis and added several modules to improve its viability and lactose digestion ability so that it could stably resolve lactose intolerance.

The first is an acid-resistant module. To enable bacteria to survive the highly acidic environment of passing through the stomach, we improved the acid tolerance system by knocking out the key gene hns in the acid tolerance system 2 (AR 2) of E.coli Nissle 1917. Therefore, the acid tolerance of E.coli Nissle 1917 was significantly improved and it did not suffer great loss when passing through gastric acid.

The second is the antimicrobial peptide module. microcin B17 (Mcc B17) was used to moderately inhibit the propagation of other bacterial groups, thereby improving the competitiveness of E.coli Nissle 1917 by secreting antimicrobial peptides. Despite intense competition with gut microbiota, it can multiply to a certain number.

The third is the secretion module. To make lactose break down more efficiently, we chose the latter between increasing lactase activity and expression. Fusion of luxpR and high-intensity promoter J23100 significantly increased lacZ gene expression.

We used a Quorum sensing system (QS system) to control our independent modules. When our engineered bacteria reach the stomach, they can survive due to the acid-resistant module. When they're in the gut, the antimicrobial peptide modules work, so our bacteria have an advantage and multiply quickly. When the population reaches a certain level to trigger the QS system, turn off the antimicrobial peptide module and start secreting lactase. In this way, we reduce the burden on the bacteria, allowing them to multiply before they secrete lactase. And when there aren't enough, they can start reproducing again, repeating the last cycle and keeping their population stable.

2967 NEU_CHINA

https://2019.igem.org/Team:NEU_CHINA/homepage.html

We treat IBD by using gut microbiota transplantation. E.coli Nissle 1917 (EcN), a probiotic in the human gut, acts as a chassis cell. We tried to put our plasmid into EcN and characterization of the EcN.

1. Considering the sensitivity of EcN, we optimized the NO sensor from last year's team and successfully reduced the leakage rate of the sensor with additional regulatory protein binding sequences. In addition, we built a new NO sensor, and carries on the characterization, to obtain a better choice.

2. We found a feasible system for *Pseudomonas syringae* and designed a tunable gain amplifier. We set up a fixed gain amplifier model of leakage, to further explore the tunable amplifier.

3. We characterized the therapeutic compounds IL-10 and myrosinase using immunoblotting. To confirm the activity of these compounds, we tested the activity of the immune cytokine IL-10.

4. With the toxin-antitoxin system mazEF, we significantly improved cell viability and precisely controlled the expression of antibacterial toxin at different temperatures.

All of this work and optimization contributed to the creation of ECNs that can precisely colonize inflamed areas of the GI tract; Successfully expressing and secreting anti-inflammatory proteins and acting in the gut with little biological harm and minimal side effects.

We used E.coli Nissle 1917(EcN) as a competent cell to express inflammation-sensing proteins. EcN was selected not only because it is an E. coli strain (capable of rapid growth, easy availability of components, and easy stimulation to transform into competent cells to absorb foreign DNA), but also because it is a clinically established harmless probiotic that lacks virulence factors and is used to promote intestinal health and has been confirmed as a safe strain for treatment. In addition, EcN has several unique functions, including microcin synthesis and different iron absorption systems, providing EcN with the advantage of intestinal colonization. In addition, recombinant EcN had no deleterious effects on migration, clonal expansion and immune cell activation in immunocompetent hosts. Therefore, E.coli Nissle 1917 May be an ideal bioengineering strain for in situ synthesis of inflammation-sensing proteins and anti-inflammatory molecules associated with the gastrointestinal tract.

5313 NEFU-China

The team used Escherichia coli Nissle 1917 to design a novel, controlled system that releases anti-tumor drugs in response to changes in uric acid levels.

The project is based on in vivo bacteriological therapy, selecting a bacterial vector that can survive in human tissues without causing an intolerable inflammatory response. The system is mainly divided into four core design modules: tumor microenvironment sensor module, uric acid regulation module, orientation and self-regulation module, and plasmid protection module.

To ensure that the treatment system can only work when it reaches the tumor cells, the team selected hypoxia and high lactate levels, characteristics of the tumor microenvironment, as the detection indicators of the sensor module. By sensing these two parameters, the sensor module activates the expression and secretion of a mutant form of tumor necrosis factor- α (mTNF- α), thereby enhancing the activity of killing tumor cells. In order to prevent these

Bacteria harmful to normal tissue, the team used cytosine deaminase (CD) as part of the targeted and self adjusting module, it can be 5 - fluorine cytosine (5 - FC) into 5 - fluorouracil (5 - FU) to kill bacteria. The team also designed ftnA Nissle1917 e. coli expression - M, this is ferritin gene mutations in form, it can effectively in the accumulation of iron ions in fine bacteria, this will make the bacteria when exposed to magnetic fields to locate at the tumor site to enhance tumor cell killing. Tumor site of low levels of uric acid, bacteria will release antitumor drugs. However, when uric acid reaches a certain level (threshold), bacteria can slow the release of antineoplastic drugs, to express the uric acid enzyme to reduce uric acid levels. To kill the tumor target, at the same time, avoid the occurrence of acute uric acid nephropathy, thus mildly and safely treat cancer patients. In order to avoid the loss of plasmid after injected into mice, the team in the e. coli genome type besides alr and dadX gene, once the alr or dadX gene coding sequence into the carrier, the bacteria can only use this carrier to survive. As a result, the bacteria are not lost this carrier.

The team that using e. coli Nissle1917 to delivery and release of anticancer agent, at the same time prevent cancer treatment of uric acid in the process of accumulation, this will lead to eliminate cancer cells and prevention of kidney disease.

2997 NCKU

The team engineering Nissle1917 is used to reduce the accumulation of cresol in the human body, thus the treatment of chronic kidney disease (CKD). The project of escherichia coli Nissle1917 engineering design, make its have two specific function. First, transfer the original on the cresol, by converting the precursor of cresol tyrosine to beneficial substances to the body. The team designed the escherichia coli Nissle to express TyrP and TAL, to convert intestinal excess tyrosine to coumaric acid, beneficial to human. By reducing the amount of tyrosine precursor (BHT), can reduce other of cresol content produced by intestinal flora. Second, from the commonly used probiotic bacteria (CBM - B), can reduce other clostridium strain of cresol producers (main). The team has designed three kinds of building body, one is the total length of CBM - B and e. coli secretory protein (yebF) fusion, two kinds of CBM - B and the C terminal of the yebF structure domain (for some clostridium strain bactericidal activity), with two different connection: a GS connect child (three glycine serine repetitive sequence: GSGSGS) and a TB connection (thrombin cracking site: LVPRGS).

For biological safety reasons, the team by removing canned implemented induced killer switch genes, to ensure that the e. coli Nissle1917 can only survive in the human gut. Canned gene knockout, e. coli cannot spread out and leads to cell death in CO₂ before fast enough to convert the CO₂ into bicarbonate (metabolic substrate). E. coli in the bowel, live, CO₂ concentration is high enough, allowing CO₂ spontaneous transformation into bicarbonate ions. However, when he left the body of e. coli, the concentration of CO₂ lowers will lead to its death. With team also by removing DapA (4 - hydroxy - 4 hydrogen double peptide formic acid synthase, necessary for cell wall synthesis enzymes), bacteria will have to rely on exogenous 2 amino g acid ester (DAP) in cell wall synthesis and growth, making it a kill switch, because the bacteria will die in the absence of DAP supply.

Pseudomonas has the ability of perception of cresol. The team with fluorescent pseudomonad (PC24) as the center, it has a positive induction operon gene cluster of pchR, the operon to perception of cresol and promote the downstream areas of transcription in degradation of BHT. PchR coding and revulsant (p - Cresol) combined with activated protein and lead to protein conformation changes. Therefore, activator - cresol compounds will combine to located in the downstream of the pchR BHT induction and start transcription. The team are replaced with GFP downstream genetic regions, so it can sense of cresol and glow.

3096 Tuebingen

The team developed a based on e. coli Nissle 1917 probiotics, it secretes Exendin - 4, a bowel to promote insulin analogue, response to glucose. Agents are secreted intestinal probiotics promote insulin analog through oral capsule on patients with intestinal tract. This makes it easier to treatment, in the meantime, do not need to take medication regularly.

The system through carbon catabolite suppression system, the system will start the tetR transcription. Exendin - 4 is upstream of TetR inhibits the promoter, ensures Exendin - 4 only in the presence of glucose is transcribed. In addition, Exendin - 4 and N end secretion labels and C end coupling cell penetrating peptides (CPP). Secrete a label to ensure Exendin - 4 into the intestines, and the CPP ensure Exendin - 4 is absorbed into the blood, thus improve bioavailability as a whole.

In order to ensure its safe use as genetically modified organisms, the team integrated with CRISPR/Cas3 Kill Switch to use our probiotics. Once the termination of the switch is activated, Cas3 nuclease degradation bacterial DNA, from and prevent the spread of genetically modified organisms to the environment. Once probiotics from the designated area, can inhibit stop switch was abolished, the CRISPR/Cas3 system is activated. Stop switch is a can, therefore, through the exchange of drugs and/or conditions for many kinds of therapy.

3282 Lund

The team of probiotics - e. coli Nissle 1917 (EcN) for genetic modification, in order to increase its ability to absorb and accumulation of lead and arsenic.

By taking some genes from Candida metallifera CH34 and inserting them into the probiotic chassis EcN, the team was able to improve the ability of EcN to accumulate arsenic and lead. Then, the bacteria as probiotics intake, due to the innate ability to EcN, it adapt to gut microbes group. There, it will be your local accumulation of arsenic and lead. Soon, the metal-filled bacteria will be expelled from your body, reducing the amount of toxic metal in your body.

With PbrT (a pilot transporters) and PbrD (a hypothetical pilot binding protein) of lead cumulants. Turn gene in e. coli expression ArsR (ars a regulatory proteins in operon) can make the 5-60 times higher arsenic accumulation ability, therefore the proteins as an accumulation of protein is included.

2 020

3121 IISER_Bhopal

The team USES the renovation engineering probiotics e. coli Nissle 1917 protein expression in intestine in situ treatment, promote the intestinal crypt cell transdifferentiation, and insulin in the body for the treatment of diabetes.

The team on EcN ternary system based on an innovative: sensors, such as NO or thiosulfate) secrete peptide (CsgA) - therapeutic proteins. When the intestinal environment changes, such as inflammation, the sensor will trigger the EcN, lead to therapeutic protein secretion, promote the intestinal cells repair and functional recovery. In order to enhance the secretion of EcN ability, the team special blend of CsgA secrete peptide, ensure effective release for the treatment of protein.

Because people with diabetes may exist in intestinal adhesion invasive e. coli (AIEC), the team introduced Microcins production capacity. Microcins as a set of peptide can inhibit the growth of AIEC secretion, with AIEC membrane atpase combined to play a role. In order to achieve this, EcN endowed with mature e. coli CA46 micro expression system, make its can restrain AIEC in intestine, to help restore the balance of intestinal flora.

To solve the problem in the process of conveying EcN conditions faced by the digestive tract environment, the team used the PCS (chitosan down product poly (dopamine) technology. PCS can form a layer of protective film on the surface of the EcN, through the way of chemical self-assembly, as environmental protect EcN from the digestive tract.

In the end, through the engineering EcN encapsulated into a capsule, iBETA innovative products can be obtained, it provides a kind of non invasion into the way of treatment, patients can be treated with oral capsules. This way is expected to reduce dependence on external insulin injections in diabetic patients, convenience and improve treatment compliance, at the same time reduce the economic burden of diabetes worldwide.

3661 CPU_CHINA

The team in engineering probiotic e. coli Nissle 1917 (EcN) as the core, genetically engineered to give the two main functions: one is the secretion of specific bacteria had lower intestinal abnormally high density in enterococcus, restore the balance of intestinal flora. Secondly, anti-inflammatory effects have been secreted cytokines IL - 22, to alleviate the liver inflammation, which is developed for the treatment of alcoholic liver disease (ALD) innovation.

The team's EcN design adopted similar to the treatment of inflammatory bowel disease (IBD) ternary system: sensors (such as alcohol metabolites) secrete peptide (CsgA) - therapeutic proteins. When they tested the biomarkers of ALD, EcN is triggered, secrete therapeutic protein to the intestinal tract, promotes intestinal health and anti-inflammatory. At the same time, the team chose a special CsgA secrete peptide in order to enhance the ability of therapeutic proteins are secreted by EcN.

Reference in IBD treatment strategies, the team introduced cutting-edge research, discovered the dung enterococcus (e. faecalis) in the important role in the development of ALD. In the subsequent design, the team used EcN through secretion bacteriocin JM79, PLW alpha, beta, PLW specificity to reduce the density of dung enterococcus, and control of its expression through quorum sensing system, enhance the specificity of treatment. At the same time, the team used the alcohol induced promoter palcA, to control the expression of IL - 22, to ensure that it exists in alcohol to be activated, which play an anti-inflammatory role in liver. This design is similar to IBD treatment using sensors in inflammation and trigger protein secretion.

In addition, with the Alcoholic Anonymous communication in the community, the team realized that the existing limitations of drugs, alcohol, and on the basis of optimized design scheme, by focusing on the EcN to control the balance of intestinal flora and anti-inflammatory support, rather than to simply acetaldehyde decomposition.

3475 Tongji_China

The team used engineering probiotics e. coli Nissle 1917 to generate the micro element and therapeutic proteins.

The team used a ternary system innovation design: inflammation of the sensor (such as nitric oxide NO or thiosulfate) - auxiliary secreted peptide (CsgA) - therapeutic proteins. When inflammation, the sensor can quickly detect inflammatory markers, trigger a therapeutic protein secretion, and through the auxiliary CsgA, the protein to the intestinal effectively, promote the healing of the damaged parts.

Aiming at common adhesive in IBD invasive e. coli (AIEC), by using Microcins, namely a secretion produced by certain strains of e. coli peptide, can make its specificity combined with AIEC ATPase and inhibit its growth, will not affect the EcN strains which can produce them at the same time. In order to enhance the function of the EcN, the team will mature e. coli CA46 micro expression system integrated into the EcN, can produce functional Microcins, effectively restrain IBD patients with intestinal AIEC, promote the recovery of intestinal flora.

For the stability problem of engineering bacteria in the digestive tract, the team used the PCS technology (chitosan codeposition of poly (dopamine)). Through chemical self-assembly, make the material in EcN form a layer of protective film on the surface of cells to protect bacteria from the destruction of enzymes, at the same time improve its stability and durability in the gut.

3482 UNI Australia

The team in engineering probiotic e. coli Nissle 1917 (EcN) as the core, by genetically engineering make it have the ability to produce anticancer peptide azurin, achieves the effect of the treatment of colorectal cancer.

The team treatment based on the ternary system: inflammation of the induction sensors (such as nitric oxide NO or thiosulfate) - (CsgA) - the anti-cancer peptides secreted peptides (azurin). When the colorectal cancer cells release inflammatory signal engineering EcN to the newsletter

Velocity response, with the help of CsgA secrete peptide, azurin anti-cancer peptides secreted into the intestine, directly and effectively inhibit the growth of its role in tumor cells, and promote the healing of the damaged intestinal tissue at the same time.

In order to ensure stability and treatment efficiency of the EcN in the gut, the team used the PCS technology (chitosan codeposition of poly (dopamine), form a protective film in EcN cells. The membrane is not only to protect EcN from the destruction of the gastrointestinal tract environment, and enhance its persistence in the gut, ensure the continuity and the effectiveness of treatment.

For positioning problem in engineering bacteria in the gut, the team on the EcN FliC gene knockout, using Lambda Red restructuring and I - SceI cutting technology, effectively eliminate the initiative in the EcN athletic ability, to improve the positioning accuracy in the gut.

In terms of biological safety, the team has designed two kinds of kill switch mechanism, ensure the EcN can safely after complete treatment from a patient's body to clear. Internal kill switch by the E protein expression induced by arabinose, quickly eliminate any EcN in the gastrointestinal tract. External end switches with temperature sensitive RBS and RelE - RelB system, trigger when the environment temperature is lower than the body temperature, effectively kill EcN may leak into the environment.

3121 IISER_Bhopal

The team USES the renovation engineering probiotics e. coli Nissle 1917 protein expression in intestine in situ treatment, promote the intestinal crypt cell transdifferentiation, and insulin in the body for the treatment of diabetes.

The team on EcN ternary system based on an innovative: sensors, such as NO or thiosulfate secrete peptide (CsgA) - therapeutic proteins. When the intestinal environment changes, such as inflammation, the sensor will trigger the EcN, lead to therapeutic protein secretion, promote the intestinal cells repair and functional recovery. In order to enhance the secretion of EcN ability, the team special blend of CsgA secrete peptide, ensure effective release for the treatment of protein.

Because people with diabetes may exist in intestinal adhesion invasive e. coli (AIEC), the team introduced Microcins production capacity. Microcins as a set of peptide can inhibit the growth of AIEC secretion, with AIEC membrane atpase combined to play a role. In order to achieve this, EcN endowed with mature e. coli CA46 micro expression system, make its can restrain AIEC in intestine, to help restore the balance of intestinal flora.

To solve the problem in the process of conveying EcN conditions faced by the digestive tract environment, the team used the PCS (chitosan down product poly (dopamine) technology. PCS can form a layer of protective film on the surface of the EcN, through the way of chemical self-assembly, as environmental protect EcN from the digestive tract.

In the end, through the engineering EcN encapsulated into a capsule, iBETA innovative products can be obtained, it provides a kind of non invasion into the way of treatment, patients can be treated with oral capsules. This way is expected to reduce dependence on external insulin injections in diabetic patients, improve the treatment of convenience and compliance, and relieve diabetes treatment by worldwide

Economic burden.

3661 CPU_CHINA

The team in engineering probiotic e. coli Nissle 1917 (EcN) as the core, genetically engineered to give the two main functions: one is the secretion of specific bacteria had lower intestinal abnormally high density in enterococcus, restore the balance of intestinal flora. Secondly, anti-inflammatory effects have been secreted cytokines IL - 22, to alleviate the liver inflammation, which is developed for the treatment of alcoholic liver disease (ALD) innovation.

The team's EcN design adopted similar to the treatment of inflammatory bowel disease (IBD) ternary system: sensors (such as alcohol metabolites) secrete peptide (CsgA) - therapeutic proteins. When they tested the biomarkers of ALD, EcN is triggered, secrete therapeutic protein to the intestinal tract, promotes intestinal health and anti-inflammatory. At the same time, the team chose a special CsgA secrete peptide in order to enhance the ability of therapeutic proteins are secreted by EcN.

Borrowing from IBD treatment strategies, the team introduced cutting-edge research and discovered the important role of E. faecalis in the development of ALD. In the subsequent design, the team used EcN to specifically reduce the density of E. faecalis by secreting the bactericins JM79, plw α , and plw β , and to control their expression through a quorum-sensing system to enhance the specificity of the treatment. At the same time, the team used the alcohol induced promoter palcA, to control the expression of IL - 22, to ensure that its exist in alcohol to be activated, which play an anti-inflammatory role in liver. This design is similar to IBD treatment using sensors in inflammation and trigger protein secretion.

In addition, with the Alcoholic Anonymous communication in the community, the team realized that the existing limitations of drugs, alcohol, and on the basis of optimized design scheme, by focusing on the EcN to control the balance of intestinal flora and anti-inflammatory support, rather than to simply acetaldehyde decomposition.

3475 Tongji_China

The team used engineering probiotics e. coli Nissle 1917 to generate the micro element and therapeutic proteins.

The team used a ternary system innovation design: inflammation of the sensor (such as nitric oxide NO or thiosulfate) - auxiliary secrete peptide (CsgA) - therapeutic proteins. When inflammation occurs, the sensor can quickly detect inflammatory markers, trigger the secretion of therapeutic proteins, and with the assistance of CsgA, these proteins are effectively delivered to the intestine to promote the healing of the injured site.

Aiming at common adhesive in IBD invasive e. coli (AIEC), by using Microcins, namely a secretion produced by certain strains of e. coli peptide, can make its specificity combined with AIEC atpase and inhibit its growth, will not affect the EcN strains which can produce them at the same time. In order to enhance the function of the EcN, the team will mature CA46 e. coli

Micro expression system integrated into the EcN, can produce makes functional Microcins, effectively restrain IBD patients with intestinal AIEC, promote the recovery of intestinal flora.

For the stability problem of engineering bacteria in the digestive tract, the team used the PCS technology (chitosan codeposition of poly (dopamine)). Through chemical self-assembly, the material forms a protective membrane on the surface of EcN cells to protect the bacteria from destruction by digestive enzymes, while improving its stability and persistence in the gut.

3482 UNI Australia

The team in engineering probiotic e. coli Nissle 1917 (EcN) as the core, by genetically engineering make it have the ability to produce anticancer peptide azurin, achieves the effect of the treatment of colorectal cancer.

The team treatment based on the ternary system: inflammation of the induction sensors (such as nitric oxide NO or thiosulfate) - (CsgA) - the anti-cancer peptides secreted peptides (azurin). When colorectal cancer cells release inflammatory signal, engineering EcN can response rapidly, with the help of CsgA secrete peptide, azurin anti-cancer peptides secreted into the intestine, directly and effectively inhibit the growth of its role in tumor cells, and promote the healing of the damaged intestinal tissue at the same time.

In order to ensure stability and treatment efficiency of the EcN in the gut, the team used the PCS technology (chitosan codeposition of poly (dopamine)), form a protective film in EcN cells. The membrane is not only to protect EcN from the destruction of the gastrointestinal tract environment, and enhance its persistence in the gut, ensure the continuity and the effectiveness of treatment.

For positioning problem in engineering bacteria in the gut, the team on the EcN FliC gene knockout, using Lambda Red restructuring and I - SceI cutting technology, effectively eliminate the initiative in the EcN athletic ability, to improve the positioning accuracy in the gut.

In terms of biological safety, the team has designed two kinds of kill switch mechanism, ensure the EcN can safely after complete treatment from a patient's body to clear. Internal kill switch by the E protein expression induced by arabinose, quickly eliminate any EcN in the gastrointestinal tract. External end switches with temperature sensitive RBS and RelE - RelB system, trigger when the environment temperature is lower than the body temperature, effectively kill EcN may leak into the environment.

3562 WHU-China

The team design modification probiotics Nissle 1917 combined with pathogenic bacteria, and promote the immune system. Nissle1917 as one of the most suitable chassis cells, the reasonable design of gene modules for gram-negative quorum sensing system.

Probiotics are considered to be the next generation in the field of biomedical chassis, proved by animal model by nasal or oral method to protect the host from respiratory infections. And guess the probiotic bacteria can secrete chemicals to stimulate and activate the immune system.

The team with pseudomonas aeruginosa as a pathogen, the design of probiotic includes four main aspects, namely the quenching module, sensor module

Block, safety measures and no quorum sensing cells.

Quenching enzyme build quenching module based on groups. Involving the AHL acylation enzyme and AHL esterase, biodegradable gram-negative bacteria of the main communication tools - N - acyl homoserine lactones (AHLs) (affect the pseudomonas aeruginosa quorum sensing network), with the decreasing of the concentration of AHLs, gram-negative bacteria cannot express virulence. In order to degrade AHLs with different acyl chain lengths, the quorum sensing kinetic model can be used to select enzyme combinations.

Sensing module based on immune cell chemotaxis build. Aims at in the same degree of infection (or natural chemotactic degree), probiotics on the role of immune cells more sensitive than pathogens. Through strong type of promoter in host a homologous factor PqsR to capture PQS (the main contributors to virulence, its revulsant pseudomonas quinolone signal (PQS) is characteristic of the genus pseudomonas molecules). Using phage to amplify the signal derived system, so as to realize the steady downstream restructuring chemokine expression, these chemokines including the SEC label DsbA for discharge. Several specific pathogens killer immune cells chemotaxis to the eradication of pseudomonas aeruginosa.

The team designed two measures to guarantee the safety of probiotics. The first of the security measures is to develop a flexible mechanism to control the secretion of chemokines, which involves TEV protease expression, in order to solve the upstream PQS promoter of possible leaks, and by cutting off the SEC TAB to adjust the secretion of chemokines, in case of cytokine storm. Design a model to use mathematical language to describe the process. The second security is added in the important module toxin - antitoxin system, in order to prevent the gene level. Transfer plasmid loss will lead to engineering of probiotics to suicide. Finally use the MazE and MazF toxin - antitoxin system to test its efficiency.

2021

3771 NCKU_Tainan

The team's goal was to engineer a probiotic for chronic stress-induced depression (CSID) that catalyzes the production of taurine in response to increased stress biomarkers in the gut. Taurine is a half essential amino acids, can be used as nerve protectant, chassis cells choose to use escherichia coli Nissle 1917 is based on the biological safety, as a kind of pathogenic e. coli strains. Resistance genes were removed to eliminate concerns about the spread of antibiotic resistance.

The team designed for e. coli Nissle 1917 two sensing system, they are made up of stress biomarkers interferon gamma (IFN - gamma) and reactive oxygen species (ROS) induced. (is strongly associated with inflammation and oxidative stress, depression and CSID detection difficult, ROS and IFN - gamma set as CSID two potential biomarkers.) When these two biomarkers of concentration increases, the enzyme needed for the taurine can express, and coordinate to L - cysteine into taurine (both have anti-inflammatory effect, also have clear the role of ROS).

Oxidative stress sensing system: using soxRS adjustment, for the most effective and most sensitive promoter, e. coli of superoxide reaction, can be used as a transcription factor, stimulate various antioxidant gene expression. For oxidative stress in human intestine, small changes is vital for the generation of taurine synthetase. In e. coli, SoxR mainly on superoxide, soxS gene soxRS regulation are an integral part, oxidation of SoxR can be induced by distorting soxS promoter soxS expression. In front of the soxS promoter added soxR promoter and soxR coding sequence, in the opposite direction, and it is located in e. coli chromosome in the same position. With the SoxR increases, the formation of the higher expression of return can promote CSAD soxS promoter.

Interferon - gamma (IFN - gamma) sensing system, build a OmpA/OprF chimeric protein. OmpA/OprF chimeric protein used to detect the human body in the gut IFN - gamma. OmpA protein from e. coli (responsible for maintaining the stability of the bacterial membrane) and from a small part of the pseudomonas aeruginosa OprF protein (the ability to combine with IFN - gamma). Both structure. Homology, so can each protein cell outer ring can be build.

In addition, the team design containing e. coli Nissle bubble of 1917, through the induction to high levels of IFN - gamma produced taurine can take to relieve the CSID and oxidative stress. Its main ingredients are sodium alginate, edible, can resist enough at low pH value of environment degradation, such as hydrochloric acid in gastric juice. In the environment of the pH value is higher, for example, small intestine, Menbles to our engineering release bacteria into the surrounding environment, and start producing taurine.

3733 HZAU-China

The team's goal is to early detection of pet IBD. Divided into test and report, anti-inflammatory, health care, health care, suicide die block. Nissle 1917 as its chassis cells.

Detection and report module to feel the intestinal abnormalities in and report to the outside. Based on two kinds of TCS (NarXL and ThsSR) and an enzyme (ScGS). NarXL is endogenous two-component system, e. coli ThsSR is from ocean shiva bacterium of two-component systems. They can be induced nitrate and thiosulfate concentration respectively, to influence the downstream circuits. When two TCS is activated, from Streptomyces A3 (ScGS) gesomin synthase expression, and release the smelly odor material soil element. Because IBD when it happens, the concentration of nitrate and thiosulfate in intestinal will increase obviously. So choose nitrate and thiosulfate as biomarkers of IBD occurred. When the concentration is increased at the same time, think IBD has occurred in the gut, and smelly report inform pet owners.

"Anti-inflammatory" module will gradually began to reduce inflammation. When IBD outbreak of lipopolysaccharide (LPS) as the antigenic determinant of bacteria trigger toll-like receptor 4 (TLR4) result in the breakout of IBD. LL - 37 (one of the antimicrobial peptide (AMP) and LPS to block the way. The module can be activated in zi chan gave birth to the small peptide LL37 - LTA to ease the intestinal inflammation, LL37 - LTA can kill some bacteria may cause inflammation. Pet owners found after smell, will get a pet

Go to hospital for further treatment.

Health care module is active all the time. When IBD occurs, the module of further deterioration of the azure can prevent inflammation, can also be stable p53 protein and p53 protein is a tumor suppressor gene expression by protein, produced by certain anti-cancer effect. Long-term inflammation may lead to bowel cancer. Azurin have anti-cancer effect, can stabilize p53. E. coli Nissle 1917 of cancer cells have certain anti-cancer effect, it is used as express Azurin chassis, can further enhance the cancer. In order to prevent leakage into the environment engineering bacteria, for engineering bacteria added a "suicide" system. If engineering bacteria into the external environment, "suicide" system will be activated. Considering the dog's intestinal temperature is generally higher than room temperature, decided to kill himself, let bacteria in low temperature thermometer USES RNA and HepT toxins (and MntA antitoxin) to build the module.

3838 SZU-China

The treatment of IBD

Chassis cell selection: choose Nissle 1917 as the main carrier of e. coli cells, its belong to the enshine fungi don't. It is a pathogenic, can colonize. To some extent, it can enhance the intestinal immune regulation and the protection of the intestinal epithelial barrier mucus ability. It can also express microbial element, can inhibit the growth of other microorganisms, such as salmonella typhimurium and e. coli o157 in intestinal inflammation. It itself has the potential for the treatment of IBD. Because it belongs to e. coli, so it is more than the lactic acid milk coccus is experimental maneuverability.

The design of the project: the cocktail therapy. SOD: to improve the stress state of peroxide of excessive reactive oxygen metabolites play a role in mediating IBD intestinal injury. Will contain superoxide dismutase (sod) of exocrine expression plasmid into engineering bacteria. TES: kind of intestinal beneficial to people. In patients with IBD intestinal, butyric acid bacteria proportion reduced. From the bacteroidetes (Bacteroides polymorpha) select the sulfur esterase, and will contain its gene expression vector plasmid to EcN. BSH: bile acid metabolism intervention in patients with IBD microbial enzyme activity damage can result in impaired bile acid metabolism, characterized by bile salts can't hydrolysis, transformation and desulfurization. Good hope expressed in engineering bacteria heterologous coding representation of BSH enzyme genes, and make it settled in the intestinal tract, and provide a high level of enzyme activity in local, to supplement the BSH caused by IBD genetic lack of abundance. LL37: target decomposition of endotoxin. In UC patients, intestinal flora imbalance, mucous membrane barrier function and permeability changes, gram-negative bacteria translocation and into the lymph and blood system. LL to 37 is the body's immune cells secrete antimicrobial peptide, inhibit helicobacter pylori and harmful bacteria such as staphylococcus aureus, it has strong resistance to bacterial endotoxin features.

Hope engineering bacteria can direct synthesis LL37 as a treatment for components, and use the exocrine carrier in exocrine expression as the supplementary material, participate in LPS immune regulation and targeted inhibition. Will contain LL37 exocrine expression plasmid into engineering bacteria.

3875 BUCT

Treatment of obesity

The team hope that through oral, the probiotics will enter your intestines, adipose decompose into glycerol and fatty acids, help regulate diet balance, absorbing oil and reduce the risk of diseases such as high blood pressure. Then, use them to produce small molecular precursor synthesis of new materials - gamma-aminobutyric acid (GABA) and 5 - hydroxy tryptophan (5 - HTP). We can not only slow weight increase, the disintegration of e. coli can also produce the GABA and 5 - HTP, can help you to reduce anxiety, depression prevention, promote the formation of melatonin, improve sleep quality, and suppress appetite.

The first part is EcN and lack of fadR fadL & fadD expression to increase the consumption of fatty acid. The second part is through the wild type gadB mutation forms for mutations, mutant gadB protein has active in a wide pH range, in order to gain better GABA production. In e. coli, chorion is a branch of the synthesis of aromatic amino acids, the third section, the team into the four related genes, these genes to promote the four steps of the material to the 5 - HTP transformation. Four hydrogen element bacterium (MH4) is used as the coenzyme P4H and also added the expression of PCD, to create artificial MH4 circulation, and make sure that the synthesis of 5 - HTP. Finally, considering the influence of biological safety, we chose the toxin - antitoxin system, the system is set up to engineering bacteria in the environment of the low oxygen, lactose free survival, and suicide in aerobic conditions. Our upstream placed a lack of oxygen in antitoxin gene promoter phyb, to ensure that the e. coli under aerobic conditions when no resistance to toxin of death.

3784 BNUZ-China

The treatment of chronic kidney disease (CKD)

In e. coli Nissle 1917 for chassis, the team developed a new type of engineering bacteria of colonization in intestinal tracts, the bacterium e. coli knock out original tryptophan metabolism genes TnaA produce indoles, glucose dehydrogenase and overexpression of engineering bacteria to enhance its competitiveness, so as to achieve the aim of alternative intestinal escherichia coli, so as to eliminate the generation of indole from the source. At the same time, expressed the catalysis of tryptophan in the engineering bacteria produce indoles propionic acid gene cluster, indole propionic acid can enhance intestinal mucous membrane barrier. In addition, the engineering bacteria will be expressed through a beta, galactose glucoside enzyme catalytic formation of low poly galactose, lactose to promote the growth of other probiotics. Through the above method, can be eliminated at the source enterotoxin indole phenol sulfate, at the same time promote the proliferation of intestinal probiotics, repair the intestinal mucosal barrier, ultimately achieve the goal of the treatment of chronic kidney disease (CKD).

3903 St Andrews

A kind of probiotics, sustainable sunscreen, won't cause harm to the Marine environment

Selects the EcN to express shinorine, so the bacteria when applied to human skin surface can provide users with uv protection. Using Nissle 1917 e. coli cells is very important to our project, because the bacteria strains

Can cause disease, approved by the FDA, and probiotics, that would mean the synthetic biology engineering theory is applied to the human skin is a completely safe process.

Shinescreen (coral reef safe sunscreens) will use shinorine -- molecules that non-toxic uv-a protection, has been produced by red algae and blue-green algae and other Marine creatures natural. Probiotic strain due to the synthetic project, approved by FDA (namely Nissle e. coli 1917) will be excessive production Shinorine: in the final product, customers will be able to rub the engineering bacteria on the surface of the skin, so as to realize uv protection.

Will have a way to participate in shinorine, all of the genes (DHQS, O - MT, ATPG and NRPS) cloning into the plasmid, the expression plasmid into EcN cells, the last four of enzymes involved in produce shinorine expression. Then, our team have to confirm EcN to generate A sufficient number of synthetic shinorine, to ensure the uv-a protection.

3924 Tsinghua

The team used engineering probiotics e. coli Nissle 1917 to generate the micro element and therapeutic proteins, respectively against intestinal flora composition, the healing process of mucus.

In order to heal damaged by IBD intestinal, the team used in situ expression of e. coli Nissle 1917 (EcN) in the treatment of protein. The design is based on the ternary system: sensor (NO sulfur or sulfuric acid) secrete peptide (CsgA) - therapeutic proteins. When the intestinal inflammation, NO/thiosulfate will be engineered Nissle 1917 present and testing, lead to therapeutic proteins secreted into the intestine, promote wound healing. 1917 such as e. coli Nissle bacteria are usually don't have the ability to actively secreted protein engineering. The team the secretion of fusion is a kind of special peptide (CsgA) to help therapeutic protein secretion. Adhesion invasive escherichia coli (AIEC) can adhere to and invade the intestinal epithelial cells and macrophages, the cause of inflammation reaction in the gut. Microcins is a set of secretion produced by certain strains of e. coli peptide, can with other strains of e. coli (including AIEC) on the plasma membrane atpase, thereby inhibit its growth. Because of the existence of immune genes produce tinge strain of itself will not be affected. Chassis bacteria e. coli 1917 has produced MccH47 endogenous Nissle micro expression system, but it is only under the condition of certain nutrients consumption will open and mature micro drug. So the team will be mature e. coli CA46 micro drug expression system is introduced into e. coli Nissle, in 1917 to produce functional micro, make its can inhibit IBD patients with intestinal AIEC, help the recovery of intestinal flora.

In addition, one of the biggest problems of engineering bacteria conveying lies in the gastrointestinal environment. The team chose the PCS (chitosan codeposition of poly (dopamine), because of its good effect in the EcN. Poly (dopamine and chitosan can be achieved by chemical self-assembly deposition on the cell surface, form a layer of film on the cell surface, thus protecting our engineering EcN from the influence of the digestive tract environment.

Cells, the team chose Nissle 1917 as chassis design contains probiotics used to produce the ADH ADH genes and used to generate ALDH ALD gene, solve the problem from the root. Compared with existing products, the team by making the probiotics can synthesis of ADH in the stomach and intestines and ALDH to reduce the damage of alcohol on the liver, so as to fundamentally solve the problem. In addition, in order to ensure that provide the reaction energy is sufficient, and enzyme activity to maintain a high level, the team added nadE and nox gene, in order to maintain the balance between the coenzymes NAD + and NADH.

3932 UI_Indonesia

The team design three systems, of which two are engineering, biological membrane dispersion and helicobacter pylori eradication, and the other one is the natural rendering: chemotaxis.

The team use the AI - 2 as EcN chemical attractant, AI - 2 is the only known helicobacter pylori secretion quorum sensing signal, and e. coli lure chemical signals.

The team used protease - lambda dispersed mainly composed of protein helicobacter pylori of biofilm. Protease - lambda synthesis under the control of the arabinose dependence pBAD promoter, and production is done by E7 cracking protein autolysis, E7 cracking protein triggered by ammonium induced by glnAp2 promoter.

The team to design a system, through a proven effective antimicrobial peptide (AMP) to the eradication of helicobacter pylori, PGLa - AM1. Because of PGLa - AM1 toxicity effect on e. coli as the host, so the team by the SUMO proteins with the AMP connection with inactivated form construction of AMP, namely fusion AMP (FAMP). In the time of controlled system, the SUMO - the AMP is then Ulp proteases cut, PGLa - AM1 is activated. Then, holin - antiholin system has been activated and cracking of e. coli to secrete we activate PGLa - AM1. ,

3984 Think_Edu_China

Using enzyme catalyst under different conditions of temperature and pressure environment with low energy consumption, strong stability, simple operation steps, etc. Whole-cell biocatalyst Ecn - IL can be a better solve the problem of the abuse of antibiotics. Using cell surface display technology, derived from pleurotus ostreatus Laccase genes lacc6 e. coli can show the probiotics Nissle 1917 (EcN) surface, and the mechanism of membrane formation, decomposition of antibiotic residues.

The team is committed to research for the degradation of the antibiotic sulfadiazine, choose from pleurotus ostreatus laccase genes Lacc6 can make use of the cell surface technology in probiotic Nissle e. coli 1917 cells, and named as EcN - IL to degrade antibiotics. Subsequently, the whole-cell catalyst preparation for producing strain, and applied to the animal waste.

The team through the ice ribosome protein used in cell surface display technology. Ice into nucleoprotein (INP) is a kind of surface protein secretion, in a bacterial cell surface display system is widely regarded as a carrier protein, and is used to show the different kinds of exogenous protein

质。

3986 LZU-HS-China

The team used *e. coli* Nissle as host strains of cell biological catalyst, 1917 by the method of synthetic biology, using reduction selenium engineering bacteria in the intestine, improve the bioavailability of selenium, and explore more effective ways of supplement. Different forms of selenium supplements bioavailability, nanometer selenium (SeNPs) than inorganic selenium (such as selenite) has lower toxicity, also with antioxidant and immune regulating function, and because of its small size, are more likely to be absorbed by the body. In order to bring in the gut of selenium and sodium selenite reduction for nano improve the selenium content of organisms, team designed a new kind of cell biological catalyst EcN as-is. Use of microbial cell surface display system, selenium reductase SerV01 show in EcN cell surface. This system by combining the specific function of exogenous protein anchor protein with, make the fusion protein expression can directly in the host cell surface. Fusion proteins build section, team chose the ice ribosome protein (INP - N) as a carrier protein, and its n-terminal sequence and biological reductase SerV01 fusion peptide sequence, formed to anchor in the EcN on the cell's outer membrane fusion peptide INP - SerV01. In order to improve the stability of passenger protein and utilization, the team through high copy plasmid transformation of homologous and heterologous protein expression, reduce the cost and improve the efficiency of the expression of the protein.

To optimize expression system, the project is used in the iGEM pSB1A3 plasmid in data repository, and insert the strong promoter and long sequences as interval area, in order to ensure the high quality of the subsequent protein expression.

In addition, the surface shows the fusion protein as a catalyst can be recycled, compared with the high cost of recycling immobilized enzyme, higher cost-effectiveness.

4060 NYCU-Taipei

The team focused on cardiovascular disease, especially in deep vein thrombosis (DVT), which is a serious health problems may caused by blood clots. They came up with an innovative solution, including the development of family support test suite to test D - dimer concentration in saliva, and designs a capable of producing modified *e. coli* of natto kinase Nissle 1917 living biological treatment products (LBP), the product can through the remote photoinduced system control the production of natto kinase.

The team design Nissle *e. coli* 1917 living creature treatment products (LBP), the first cloned from *bacillus subtilis* natto kinase gene (aprN), then aprN connected with pET - 21 a (+) to produce a cloning vector, Using *e. coli* Nissle 1917 production of natto kinase, and try to improve its activity by mutation.

In order to help the bacteria in the small intestine wall adhesion, prolong the retention period of LBP in the gut, the team developed a kind of adhesive proteins. The system by the outer membrane of transmembrane protein OmpA, GS would and adhesion of fimbriae fimH encoding genes.

In order to protect EcN from stimulating of gastrointestinal tract environment and deliver to the small intestine, the team of oral drug delivery system is designed. EcN freeze-drying to ensure long-term storage first, and then by loading the freeze-dried EcN in acid HPMC capsules, so oral probiotics can help in the face of low pH, bile salts, stomach disorders such as host immune system and competition.

The team also USES light genetics technology, through near infrared remote control EcN natto kinase gene expression. And designed based on MazE - MazF toxin - kill switch of antitoxin system, through the control of temperature variation and chemical induction of EcN.

2022

4169 hzau-china

Unhealthy diet affects the body low density lipoprotein (LDL) and triglyceride levels, this will lead to a series such as blood clots Cardiovascular disease (CVD). The team plans to develop a kind of edible probiotics Nissle e. coli 1917, down from the development of TMA and degradation of existing TMA two aspects, reduce the possibility of thrombosis. At the same time, make the engineering bacteria release many preventive substances in favour of blood vessel protection.

Project mainly design the three function modules and modules, a suicide three function module are respectively the degradation and inhibition and ease. The three function module through a simple small molecules theophylline connection, theophylline as a kind of sensor material, can open the switch of engineering bacteria in patients with specificity. This avoids only needed to express the ease module is the expression of the degradation and inhibition of module. The team provides the main functions of the two different scenarios loop design.

Expression of trimethylamine, dimethylamine, and formaldehyde dehydrogenase gene clusters was induced when patients were given theophylline containing probiotics. If the patient only taking probiotics, probiotics will only show the blood vessels to protect and short chain fatty acids beneficial intestinal mucosal repair.

In suicide in the module, the team designed a hot inhibiting RNA. Followed by thermometer with toxin proteins. Sensing the environment temperature and play a role in the control switch is closed and opened, it will affect subsequent path, while the latter can represent HepT toxin protein, as RNase, degradation of mRNA transcription in eventually lead to cell death. Through this design, to ensure that the engineering bacteria in vitro suicide, minimize the environmental pollution.

4156 lzu-china

The team using e. coli Nissle 1917 as host strains, genetic editor probiotics to targeted therapy of colorectal cancer (CRC).

Organs in order to build able to distinguish between the unique environment of bacteria biosensor system, the team will be oxygen, pH and lactic acid as a unique index of the colorectal cancer tumor tissues, and use the special micro environment characteristics of colorectal cancer tissue, which can identify three is designed

Operon to specific identification of low oxygen and low pH and lactic acid, including: low oxygen induced promoter (pPepT), activated by transcription factor (FNR) regulation; L - lactic acid induction system (lldPRD operon), pLldP promoter drive interested in gene expression, LldR repressor protein when not in combination with lactic acid inhibition of reporter gene expression; PH sensitive promoter (pCadC), activated by membrane bound proteins (CadC) regulation, the higher activity of acid medium.

However, the current clinical application of biosensor system faces many challenges, such as signal processing ability is limited, unable to integrate a variety of biomarkers for accurate diagnosis, response time and need a quick result of diagnosis is not compatible. In order to make the living cells that can perform complex signal processing operations, using a serine integrase enlarge genetic switch and Boolean logic gates in biosensor system design. The team designed the tumor cell adhesion module, using HlpA HSPG on the surface of the protein and tumor, improve the ability of engineering bacteria penetrating colorectal cancer cells.

Treatment part use the hemolysin E, CCL21 and CDD - iRGD (and tumor cell death domain fusion of perforin egg white Bit1), activate the host's immune response. Introduced the phage cracking gene phiX174 (E), fine cracking and deaths in expression, releasing stored within the strains of therapeutic agents.

For security reasons, the team introduced the Arabian sugar induced killer switch, as an additional protective barrier. Unsatisfactory due to kill switch the result of the experiment, also made of sodium alginate and chitosan microcapsule, ensure the safety and efficacy of treatment.

4205 NJMU-China

The team set up the first and second system to alleviate the mitochondria.

In the second system, the team's goal is to eliminate the heavy metal ions in people with autism intestinal, such as Hg²⁺ and Pb²⁺. Choosing escherichia coli as the chassis, 1917 pET - 28 (+) as a carrier.

The team at the DNA level pET28a - MT plasmid are constructed and transformed into e. coli Nissle 1917, and took the plasmid to prove successful. First of all, by adding the restriction endonuclease SphI and Xhol enzyme digest authentication for double enzyme digestion and run agarose gel. Secondly, through the design of primers verified the plasmid PCR amplification target fragment and run agarose gel. After enzyme digestion, samples from the extracted plasmid out close to 2000 bp band, which is consistent with the target strip (2399 bp). After plasmid PCR, extracted plasmid sample also ran out of the nearly 2000 bp band, this is consistent with the target (2399 bp) is the same. This further suggests that plasmid transformation was a success.

Follow-up of the experimental group and the escherichia coli Nissle 1917 electric conversion, the preparation of the cells and electric conversion. Used for reconstruction of e. coli Nissle1917, easy to build need plasmid, used for relief of mitochondria.

4387Uzurich

The university of Zurich team project is aimed at the treatment of inflammatory bowel disease (IBD). Using *E. coli* Nissle 1917 as a chassis, they designed a new genetic circuit to sense nitric oxide and produce and secrete anti-Tnf- α nanoantibodies to reduce inflammation.

ECN is designed to be able to perceive the intestines levels of nitric oxide, when detected a high level of nitric oxide, ECN will begin to produce and secrete anti TNF alpha nano antibody, this is a kind of biological molecules against inflammatory response.

The team has also developed a model to simulate the diffusion of ECN in the gut and role, and to evaluate the effectiveness of the treatment.

4244 Wageningen-Ur

To develop an in vivo diagnostic tool called "Colorectal" for the detection of colorectal cancer.

E. coli Nissle 1917 in this project is designed to combine to tumor cells, and detection of two different kinds of cancer biomarkers: lactate and matrix metalloproteinase 9 (MMP - 9).

When the marked increase in lactate level, ECN will produce a signal protein, and the existence of MMP - 9 can activate the signal protein in color. Colored signaling proteins are observed in feces only when both biomarkers are present.

The team also implements the biological safety circuits, to limit the ECN only work in the colon, and through the temperature and the dependence of the sticky protein to control the distribution, at the same time provides a mechanism to remove when there is a need for ECN.

4199 Nwu-China-A

The team Nissle *e. coli* 1917 chosen as the chassis. Through the cultivation of the bacteria in feces, cultivating and observing the color of the bacteria, can achieve the diagnosis of chronic lower gastrointestinal bleeding. The two recombinant plasmid pET28a (+) - ChuA - HrtR and pSB1C3 - HtrO - cjBlue into non-pathogenic consumption of probiotic Nissle 1917, used to make biological sensors used to detect the intestinal micro hemorrhage. When probiotics giving patients with small intestinal bleeding, it will be transported to the cells in the gut hemoglobin activation report gene expression in the bacteria, so as to realize the detection of micro on intestinal bleeding.

The team need to enter human body bowel to play a role of sensor, so the selection of *e. coli* Nissle 1917 strains as expression, rather than escherichia coli BL21. Since there are no T7RNA polymerase in escherichia coli Nissle 1917, the team plans to use instead of T7 has promote J23119 promoter.

Considering suicide blu-ray activation is required, it is not available in the ordinary families. Through the literature, the team found another switch, a simple suicide namely knock out a particular gene in *e. coli*.

4183-HS-China

The team put forward a method of syn - bio to solve lactose intolerance, exploitation and utilization of the *e. coli* strains of *e. coli*

Nissle 1917, can be safely transplanted into the intestine, and from the internal secretion lactase, break down any ill of lactose on the digestive system. This not only removes physical discomfort, but also releases the nutritional value of milk for people who were once unable to digest it.

The team design treatments are divided into three steps, first find a suitable chassis strains, and adjust the genetics to produce lactase. Then the chassis creature to safely transplant patients in the small intestine. Finally, to ensure that the strains in the intestinal environment medium and long term survival and effective operation. As a result, chassis strains to that in patients with abdominal catalytic decomposition of lactose enzyme as needed. First, using *e. coli* strains Nissle 1917 as chassis. *E. coli* strains has been one of the most popular chassis, with high productivity and operability. Our goal is to use the strains of *e. coli* in the small intestine of beta LacZ galactose glucoside enzyme, the enzyme can effectively breaks down lactose into glucose and galactose, both can be degraded in the human gut. The enzyme will be through the signal peptide are transported to the extracellular environment. This ensures that the bacteria itself does not produce gas intake of lactose, effectively prevent all forms of abdominal distension, discomfort or diarrhea. Second, plans to use enteric tablets to install the intake of freeze-dried bacteria. Swallowing, enteric tablets will not dissolve in the stomach, like ordinary pill and would only begin to decompose in the small intestine. This helps bacteria bypass acid chemical barrier, ensuring the security of the strains to the intestinal transport. Third, use the CAP (*e. coli* strains Nissle capsular polysaccharide around 1917) to create a protective film, to ensure that the bacteria safely into the intestinal environment without damage. Will CAP the gene encoding in bacteria, it will spontaneously. CAP help bacteria to adapt to a wider range of temperature and pH value, from harmful chemicals and toxins, and help them stick on the intestinal wall.

4331-LZU-HS-China-B

The team chose *Escherichia coli* Nissle 1917 as our chassis organism to address various diseases brought on by alcohol. EcN mechanism lies in its can colonize in the human gut, prevent pathogen attack the bowel glues the film, to has the protection and restoration of intestinal mucosa. EcN also participated in the host organism immunity, balance the secretion of immune factors and enhance the host immune ability, so as to relieve inflammation and treatment. By increasing the NOX enzymes to increase production of nad +, with the increase of number of nad + factor, alcohol dehydrogenase and aldehyde dehydrogenase efficiency is also improved. In the end, we hope the results can be achieved: increase ethanol into acetaldehyde conversion rate, reduce human damage by ethanol and acetaldehyde.

The design of the project initially followed the following steps. When people drink, alcohol can through the cell membrane, including NAD + promotion of ethyl alcohol dehydrogenase (ethanol dehydrogenase) into acetaldehyde (Aldehyde), Then the NAD + promote aldehyde dehydrogenase (aldehyde dehydrogenase) converts acetaldehyde into acetic acid (acetic acid), then these substances into acetyl coenzyme (acetv - CoA), and finally, they join the TCA cycle. We mentioned earlier NAD + is NADH through NOX conversion, helps to convert between ethanol and acetate. In these cells, the alcohol dehydrogenase can prevent people from drinking or some of the larger body flush on reaction time, and can help people faster and aldehyde dehydrogenase.

Decisions in order to test their idea, they created two kinds of engineering strains: pSB - AA and pSB - the AN. For pSB - AA, they only improved its degradation system; The pSB - AN accelerating system and degradation in the system were modified genes. To achieve this, they finally selected the alcohol dehydrogenase gene, the aldehyde dehydrogenase gene, the NAD⁺ synthase gene and the NADH oxidase gene. The reason is that they control ADH, ALDH, NAD⁺, and NADH, respectively, which all play crucial roles in accelerating and degrading systems.

4300-LZU-HS-Pro-A

In the team of the project, to genetically engineered *e. coli* Nissle 1917, make the bacteria can produce GABA and 5 - HTP, so as to achieve the goal of therapy for depression. We plan to the transgenic strains injected into the human intestinal tract, to alleviate symptoms of depression. We also designed an apoptosis system to kill the bacteria once they are out of the body and prevent contamination with the transgene.

Since scientists to prove the existence of the gut brain axis, they have studied the gut microbiota of nervous and mental effects on the brain. Many studies have shown that significant relationship between depression and gut microbiota. Gut microbes produce 5 - HTP and gamma aminobutyric acid (GABA) can effectively help depressed patients recovered from depression. Based on this study, we designed a kind of based on *e. coli* Nissle1917 5 - HTP and gamma aminobutyric acid production of synthetic biology.

Glutamic acid decarboxylase can promote bacteria GABA, tryptophan hydroxylase can promote the bacteria to produce 5 - HTP. We design the *e. coli* Nissle1917 to add a expression of the two genes, to produce large amounts of GABA and 5 - HTP. By these bacteria to the depression of the gut, we can help them to reduce or get rid of depression. We also need to solve other problems. In order to protect the environment and life from genetically modified (gm) pollution, we consider the design of a suicide system, in order to prevent the escape strains. Under normal circumstances, when there is arabinose, arabinose promoter to start the following expression of three enzymes to break down the sugar. Plan is inserted after the three enzymes to produce an antitoxin gene, and then express a toxin genes. The sugar exists in the small intestine, Arabia sugar decomposition of small intestine. So when *e. coli* in the small intestine, it can receive arabinose, in the small intestine in antitoxin genes expressed in the back, and on the back offset toxin genes, and bacteria can live normally. When he left the small intestine escherichia coli, no Arab sugar regulation of arabinose operon genes do not express the following antitoxin, only expressed genes. Bacteria will eventually kill themselves.

In addition, consumers after taking our probiotics, probiotics can produce GABA and 5 - HTP. In times of need, when we drink tea, tea can activate the pyrolysis system, bacteria after cracking, more GABA and 5 - HTP will release in the gut.

4410-Worldshaper-HZBIOX

The main idea of our project is the use of nonpathogenic bacteria *e. coli* Nissle 1917 production of L - arginine to promote colorectal

Bowel cancer immunotherapy. Since overcome arginine on inhibition mechanism, ArgR gene had ArgJ gene knock out and insert. Ensure that biological safety, we insert the EcN hemolysin gene into our design, make its have bacteria in their work. The team found that the gene engineering bacteria ENC1917, there are two genes with arginine, which has a far-reaching influence on production it is ArgJ and ArgR genes. Our project aims to provide a safe, effective and practical method, in solid tumors, especially in colorectal cancer, introduce and maintain a high level of L - arginine, in order to promote cancer immunotherapy. The main ideas of the project is to use the pathogenic bacteria EcN, it can be through the mouth into the digestive tract and the constant planting in the digestive tract tumor site. EcN in this project is designed to be able to through the use of the cancer cell metabolic product of ammonia as the substrate, continue to produce a large number of L - arginine in the tumor. After processing, the introduction of regulatory mechanism, eliminate the EcN system, assure safety.

Our project is composed of two main parts: used for arginine synthetic EcN engineering and high concentration of arginine preliminary validation of the colorectal cancer cell antitumor effect.

For local use of high concentrations of arginine coordinated immune therapy for colorectal cancer the antitumor function of the solid tumors, such as our EcN projects using probiotics as a treatment for the carrier, produces a large number of L - arginine in tumor. We add and delete argR argJ genes to transform to overcome arginine synthesis pathway, arginine inhibition. To ensure the safety of the creatures we insert the hemolysis protein gene engineering EcN, so that the bacteria cracking after finishing the work. Probiotics EcN have tumor targeting effect, can be accumulated in tumor tissue specificity, and play the role of targeted transporters. Our engineering probiotics can colonize quickly in the gut, taken by mouth for chee epithelial cells have good adhesion rate, can be long stay in the gut of tumor tissue, has great potential for the treatment of digestive system tumors, has a positive role. In addition, our probiotics can also be delivered by means of injection in experimental animals to targets, so the direction can be developed in future clinical application.

4762 NWU-CHINA-A

Super Probiotics: Intestinal Knight engineered bacteria beat IBD

Project description: the project is mainly by genetically engineered e. coli Nissle 1917 (EcN) to treat inflammatory bowel disease (IBD). In this study, the researchers tried to three kinds of beneficial protein genes, catalase (CAT), superoxide dismutase (SOD) and Elafin -- expressed in EcN at the same time, in order to enhance its ability to fight against IBD.

Express strains using ECN reason: ECN can colonize in the gastrointestinal (GI), and compared with other bacteria in the gut, showed better growth advantage, inhibiting pathogenic e. coli. More importantly, the EcN is pharmaceutical preparations Mutaflor active ingredients. Mutaflor is currently in some European countries are approved for use in human drug treatment of microbial drug intestinal diseases and disorders. In view of the person use long-term and safe delivery record, by putting a strain engineering into a living, industrial platform of drugs and drug delivery carrier. So choose EcN expression of target genes.

Experimental design: the first iteration, the researchers used pET carrier - 28 a (+) expressed the CAT and SOD genes respectively, to verify these genes expression and activity in escherichia coli BL21. In order to realize the second iteration: CAT and SOD expression at the same time, the researchers changed the carrier, add SOD gene sequence to the downstream of the CAT gene and promoter from T7 has changed to T5, so as to adapt to the expression in the EcN, because lack of T7 has RNA polymerase EcN. The third iteration: in order to further enhance the function of the EcN, the researchers introduced Elafin genes. In order to avoid problems with the plasmid incompatibility, chose different sources as Elafin pCDFDuet - 1 expression vector. Fourth iteration: researchers are included in the connect Elafin gene sequences to CAT and SOD gene carrier, construct a can at the same time these three protein expression vector. The fifth iteration: considering the food security, researchers will build a good carrier to probiotics EcN, in order to take advantage of the modified probiotics to treat IBD.

2023

4965 RDFZ-CHINA

A Double-stage Patch Set for Facial Acnes

Project overview: the study showed that p. the formation of acne and acne highly relevant, as it will release the extracellular enzyme products, facial sebum degradation of LCFA, cause inflammation. Therefore, we try to use while eliminating acne and they produce LCFA to solve the problem of inflammation. The team designed the e. coli Nissle 1917 (EcN), a kind of pathogenic e. coli strains, from facial LCFA absorption and degradation.

Chassis microorganisms using ECN reason: ECN with probiotic properties and the pathogenic nature, has a variety of adaptive factor, can effectively inhibit the growth of opportunistic pathogens, to ensure the safety of the product. In addition, the gene expression of EcN widely the transcription and translation of control, loop also got good understanding its metabolism.

Experimental design: design 1: remove and release the lipase degradation of free fatty acid acne, facial sebum degradation of LCFA, leading to inflammatory lesions. Therefore, we decided to design a kind of strong with T7 has starter, RBS and double finalizers FadD and FadL bacteria metabolic pathways, using FadL and FadD LCFA gene and decomposition, the two genes work of cooperation Can be thought of can complete LCFA metabolic function. Design 2: prevent scar formation design another EcN engineering strain, the strain has for the second patch production capacity of epidermal growth factor (EGF). Design 3: add label phoA phoA secretion is a kind of signal peptide, may guide the new synthesis of protein secretion pathway.

Although e. coli Nissle 1917 can secrete hEGF slowly growth factor, but the production rate is relatively low, the lack of effective for treating acne. Therefore, decided to add a label phoA secretion hEGF gene, promote hEGF secreted into the periplasmic space of e. coli. PhoA is a kind of signal peptide, synthesize a new protein secretion pathway. HEGF is negative

Human epidermal growth factor gene of hEGF synthesis. With fusion phoA - hEGF construct a plasmid gene, transformed into e. coli, and using ELISA and Western Blot verify its production and secretion.

5024 BZK

Gout "Firefighter" Probiotics

Description: uric acid is waste of purine metabolism of food, most of the uric acid through the liver. However, if the body cannot effectively excrete uric acid, uric acid levels will rise, the formation of uric acid crystals deposited in joints and the surrounding soft tissues, causing pain and inflammation, pain and swelling of the joints. The project aims to use probiotics Nissle 1917 to synthetase (UAO, allantoinase and allantoicase), these enzymes can digest uric acid to treat gout. Build the specific plasmid pGex - 4 t - 1 - UAO (BBa_K5024007) and pGex - 4 t - 1 - UAA (BBa_K5024008), and transformed into target probiotic Nissle 1917.

Chassis microorganisms using ECN reason: ECN with probiotic properties and the pathogenic nature, have a variety of adaptive factor.

Experimental design: plasmid construction: the team first designed and built three different plasmid, each plasmids containing a key enzyme genes. The plasmid pET28a based carrier, insert the uric acid oxidase (UAO), allantoin and allantoic acid enzyme enzyme genes. Replace the expression vector: preliminary build pET28a plasmid is for expression of these enzymes design in e. coli. In order to meet the needs of expression system for ECN, the target gene transfer to a more suitable for the carrier of ECN. As a result, the team chose pGEX - 4 t - 1 carrier, this is a suitable for a variety of foreign proteins expressed in bacteria of the carrier, with different resistance marker and the induced expression system.

4872 Canton-HS

Project Overview: This project aims to develop a dual vaccine that can provide immune protection against both norovirus and rotavirus. Two different plasmids were constructed to express two antigen genes of norovirus, GII.4-VP1 and GII.17-VP1, respectively. These plasmids were transformed into E. coli BL21 (DE3) strain for expression and purification. Based on this basic work, vaccines that can target both norovirus and rotavirus were further developed. Design and expressed a fusion protein, the protein is a combination of, such as virus GII. 17 - VP1 and rotavirus antigen of the RV VP7 antigen, and the coding of the fusion protein plasmid into e. coli BL21 (DE3) and Nissle 1917 strains, the protein expression and purification.

Inadequate: e. coli Nissle RV in 1917 - GII. 17 - the expression of VP1 is low, need to improve and optimize the protein expression method, in order to realize the RV - GII. 17 - mass expression of VP1.

Through the EcN crocin synthesis treatment of depression

4955-Japan-United

Using e. coli to produce the composition of antidepressants in saffron, saffron acid, saffron, saffron bitter element). These components are integrated into the cookies, as a new means of depression.

Design A new metabolic pathways (group A), the way in the biosynthesis of escherichia coli in 4 - hydroxy - 2,6,6 - third base - 1 - cyclohexene - 1 - formaldehyde (HTCC) (crocin precursors), as well as A new metabolic pathways (group B) enzyme, can be in in vitro from the biosynthesis of HTCC crocin. Introduce the enzyme A and enzyme group B BL21 (DE3) strains to crocin synthesis. Through the EcN testing soil information.

4916-WLC-Milwaukee

Based on EcN design a biological soil sensor to collect the balance of soil nutrient levels and maximize the information needed to promote crop growth.

Engineered e. coli (e. coli) using only available phosphate and by controlling the beta lactamase (bla) gene expression to perceive phosphate concentration. Finally using spectrophotometer colorimetric analysis, in order to accurately measure phosphate concentration in the soil samples.

4980-Jilin-China

Nissle 1917, an engineered nonpathogenic Escherichia coli strain, was used as a drug delivery system. Its ability to grow in the immunosuppressive environment of tumors and deliver anticancer drugs allows for targeted therapy.

Choose Nissle 1917 as chassis creatures. Based on anti-cancer protein melittin, we design a fusion peptide in order to achieve a targeted and effective treatment. Using the lambda phage TCI42 and pR, pL promoter, its target gene expression can be closed at room temperature, and expressed in 42 °C or more open. At the same time using the Axe - Txe system to ensure that the plasmid transfer correctly to the bacteria and control cell growth.

7. Current ECN clinical medication in development and progress

Purpose: Mutaflo was used in the treatment of ulcerative colitis (especially in remission) and constipation. It works by regulating the gut microbiome to restore gut balance and help relieve symptoms.

Status: in Germany, Canada, Australia and other countries as a drug or dietary supplement listed for many years.

2, Symbioflor® 2

Uses: Mainly used to treat chronic constipation and irritable bowel syndrome (IBS). It alleviates symptoms by enhancing intestinal barrier function and regulating immune response.

Status: Marketed in some European countries, it is commonly prescribed as a supportive treatment for intestinal health.

3, EcN-TNF α inhibitors

Purpose: Engineered EcN can secrete tumor necrosis factor- α (TNF α) inhibitors for the treatment of chronic inflammatory diseases such as inflammatory bowel disease (IBD). The drug is still in pre-clinical stage, has not yet been commercialized.

4, Tum 5-p53 (experimental drug)

Purpose: This is an experimental anticancer drug that has been genetically engineered to express the tumor suppressor protein p53 and the antiangiogenic protein Tum-5 in hypoxic regions of tumors. It is designed to directly attack tumor cells and inhibit their growth and angiogenesis.

5, Engineered E. coli for Nanobody Delivery

Usage: use of engineered e. coli Nissle 1917 as the carrier, therapeutic antibodies to bowel, nanotechnology used in the treatment of such as chronic diseases such as inflammatory bowel disease (IBD). The treatment is currently in the research phase, not yet in clinical.

8. Analysis of EcN delivery strategy

An in vivo delivery system for engineered bacteria EcN 1917

The use of engineered probiotics is suitable for detecting and producing key biomolecules and responding to a range of diseases, which is of great value in the prevention, diagnosis and treatment of diseases. Examples include inflammatory bowel disease, diabetes, multiple sclerosis, autism and cancer. Examples include delivery elements for cancer drugs designed to take advantage of the tumor-targeting behavior of bacteria. In addition, some engineered bacteria have the ability to stimulate and activate the adaptive immune system, which is used as an adjuvant immunotherapy. However, due to the bacteria heterogeneous biological characteristics (such as rapid proliferation and engraftment), in vivo applications of engineering bacteria often severely limited, such as bacteria itself or immunogenicity of normal tissue metabolites. For example, engineered ECN1917 has been shown to be effective in intestinal microbiota transplantation therapy, but its implementation has been largely limited due to the risk of intestinal epithelial barrier destruction. Considering the intrinsic defects of engineering bacteria, bacteria design special delivery systems used to enhance the accuracy of diagnosis and treatment is a feasible method, and to reduce potential safety problems.

1. Nano-coating

Surface decoration of engineered bacteria is a general way to modulate their biological behavior. Many with self-assembly and film-forming properties of functional molecules has been attached on the surface of the bacteria that based on genetically engineered, and alter the behavior of bacteria in the body, enhance its biological safety. For example, the red cell membrane bag was ECN1917 can escape the capture of the macrophages, to extend its remaining time in the organization.

There are also many other functional molecules that can be used to engineer bacterial surfaces for specific biological effects. Here are some specific examples:

1 Epitope display: Engineered bacterial surfaces can display specific epitopes for the development of vaccines or diagnostic tools. This approach can use bacteria as a carrier to effectively display epitopes on their surface and elicit an immune response from the host immune system. For example, researchers clone the genes of influenza virus, such as HA protein, into the expression vector of Escherichia coli and use the surface of E. coli to display influenza virus epitopes. This approach has been used to develop recombinant vaccines to enhance the immune response.

2 Drug delivery systems: The decoration of molecules with drug release functions on the surface of bacteria can enable targeted therapy. These decorative molecules can respond to specific physiological conditions or external stimuli to release drugs when needed, improving therapeutic efficacy and reducing side effects. Examples include polylactic acid (PLA) as well as polyvinyl alcohol (PVA). PLA is a biodegradable polymer that allows researchers to modify PLA onto the surface of bacteria, allowing it to release encapsulated drugs, such as antibiotics or cancer drugs, under certain conditions. This approach can be used for targeted therapies that reduce the impact on healthy cells. PVA is a kind of water-soluble polymer, studies show that PVA modification to the surface of bacteria, can release drug in certain pH value, to achieve targeted release

Put the effect.

3 Biosorption materials: the adsorption ability of biological materials attached to the surface of bacteria, for environmental treatment or pollutant removal. The engineering bacteria can specific chemical substances and metal ions adsorption, remove pollutants in water or soil. Researchers can the metal binding protein binding protein (such as cadmium, lead) gene cloning to bacteria, in the bacterial surface expression. These proteins to specific combination and remove the heavy metal ions in the water. In addition, Poly lysine (Poly real - L - lysine) is a kind of cationic polymer, can adsorption of anionic pollutants. By poly lysine modified to a thin surface of bacteria, the researchers were able to improve the ability of bacterial adsorption of anionic pollutants in water.

Through the nano coating surface design specific functions in bacteria can also according to the demand for programming the activation of bacteria, without having to rely on the promoter or recombinant protein strategy. Inhibition of methods that trigger bacteria within the nanocoating to reactivate on demand. The gut microbiome that is restricted in the nanocoating can respond to the GI environment and resume its function in the gut by ph-triggered dissolution of the nanocoating. In order to realize until after oral bacteria enter the intestinal bacteria in the process of security, at the same time in the gut to recover its therapeutic effect. Pingping, for example, Wang, the use of bacterial membrane such as calcium ions lead enteric polymer Eudragit L100-55 self-assembly ECN1917 surface engineering, and implement the corresponding drug release pH.

2. Nano-particles

To achieve richer biological functions and manipulability, it is an effective method to couple precisely designed nanoparticles, such as polymer particles, polymer tubes, and magnetic nanocomponents, to the surface of engineered bacterial membranes. This strategy can not only enhance the functions of bacteria, but also expand their potential applications in biomedicine, environmental governance, and synthetic biology. Here are some specific examples.

Researchers can use polymer microtubules as "transport vehicles" for bacteria, using them for directed movement of bacteria and drug release. This approach can control the rate and location of drug release in response to specific physiological circumstances or stimuli. The modification of polystyrene nanotubes on the surface of bacteria can improve the ability of bacteria to adsorb specific molecules. Such structures could be used to capture pollutants or biomarkers in the environment, allowing environmental monitoring or disease diagnosis. For example, engineered bacteria can trap specific toxic substances in water and detect their concentration by fluorescent signals.

The application of magnetic nanocomponents in bacterial surface decoration opens up new possibilities for drug delivery and targeted therapy. These magnetic nanoparticles are usually made of magnetic materials, such as iron oxide, cobalt or nickel, and are capable of directional movement, aggregation or drug release under the action of an external magnetic field. Examples include magnetic iron oxide, cobalt-based magnetic nanoparticles, and magnetic polymer nanoparticles. Magnetic iron oxide nanoparticles (such as Fe₃O₄) are widely used for bacterial surface decoration. Studies have shown that bacteria modified with these nanoparticles can target tumor cells and release anticancer drugs under the guidance of an external magnetic field. For example, bacteria modified with magnetic iron oxide nanoparticles can release chemotherapeutic drugs, such as Cisplatin, in the tumor microenvironment, improving the local area

Drug concentration and reduce systemic side effects. Cobalt-based magnetic nanoparticles similar to Fe₃O₄ have also been used for bacterial surface decoration to enhance their targeting and biocompatibility in vivo. These nanoparticles are able to be guided to specific locations by an external magnetic field, allowing precise control of drug release.

Magnetic polymers have more advantages in intelligent engineering of bacteria due to their more controllable particle size and surface structure. These composites not only possess magnetic properties, but also improve the drug loading capacity. For example, the researchers developed a magnetic polymer nanoparticles, surface modification can be found in bacteria, antibiotics targeting delivery as a carrier to bacterial infection. This approach effectively enhances the local concentration of antibiotics and reduces systemic side effects. For example Mukrime Birgul Akolpoglu will load a solar-thermal agent such as chemotherapy and molecular magnetic nanoparticles and nano liposome orthogonal tandem ECN1917 built on magnetic guide biological robot, to the applied magnetic field under the guidance of navigation in the biological matrix and engraftment in solid tumors, Under the stimulation of near-infrared light, the drug molecules can be released on demand, which greatly improves the intelligence of engineered bacteria.

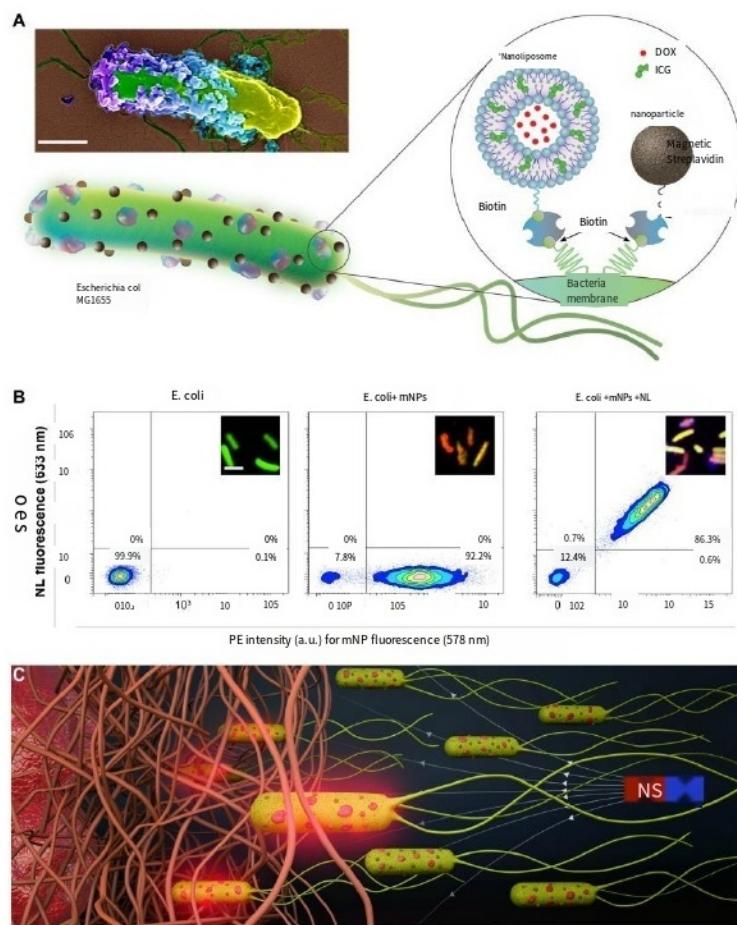


Figure1. Magnetile-guided engineered bacterial delivery system designed by Mukrime Birgul Akolpoglu et al

9.Comparison of partial bacterial therapeutic modification and EcN

1. Salmonella

1.1 Brief introduction

Salmonella (Salmonella) to American bacteriologist d. e. Salmon name, he for the first time in 1884 the bacteria was isolated from a pig intestines. [1] salmonella is a gram-negative, movement, produce hydrogen sulfide bacteria, belongs to the enterobacteriaceae. Bacteria salmonella is addicted to temperature, its optimal growth temperature is 35-43 ° C. At temperatures below 15°C, their growth rate is drastically reduced, while most Salmonella species are arrested at temperatures below 7°C. Salmonella is a facultative anaerobic bacteria can survive in hypoxic environment.

Salmonella is a kind of unstable acid facultative intracellular microbes, can infect humans or engraftment in humans, many have different clinical features of clinical infection, such as gastroenteritis, enteric fever, bacteremia and chronic carrier state, and cause cross infection between people and animals. Many animals are known carriers of salmonella, carrier one kind of animal is a chicken, not thoroughly cooked with lead to salmonella enteritidis, leading to inflammatory diarrhea. Salmonella can cause severe in immunocompromised patients focal infection. Overall, the world has more than 2500 kinds of salmonella serotype, enteric fever is caused by salmonella typhi and paratyphoid salmonella, and other salmonella strains is referred to as the typhoid strains. [2]

The tetrathionate respiration of Salmonella is closely related to its role in intestinal infections, especially in the context of inflammatory bowel disease (IBD). Intestinal inflammation in IBD for salmonella provides a beneficial to the growth environment. Salmonella can through its virulence factor invasion of intestinal epithelial cells and macrophages in mucous membrane of survive, cause acute intestinal inflammation. Inflammatory response of endogenous reactive oxygen species and lumen sulfur compounds (e.g., thiosulfate) reaction, even four sulfate to form new respiratory electron acceptor. Salmonella using this feature, growth in the inflammation of the intestinal, and with other rely on anaerobic fermentation microorganisms in the intestines competition advantage. [3]

1.2 Examples of applications

Through synthetic biology techniques, scientists are to gene editing of salmonella, knock out the pathogenic genes or introducing therapeutic genes, which makes it a potential biological treatment tool. For example, attenuated salmonella VNP20009 by knockout purI and msbB gene, reduced the toxicity and improve the effect of the tumor and tumor targeting therapy.

Salmonella VNP20009 is a gene of attenuated salmonella typhimurium strain subspecies, through the knockout purI and msbB gene. The strain is evaluated in phase I clinical trials for the treatment of human non reactive or renal cell carcinoma, metastatic melanoma and showed good safety and antibiotic sensitivity. Salmonella can pass flagella convert chemical energy into mechanical energy, the sports ability is 10 times more than e. coli. [4] VNP20009 under normal circumstances can whip

The hair bundle moves forward and the flagellar bundle opens when it encounters an obstacle, allowing VNP20009 to roll and reposition to avoid the obstacle. [5] as a carrier of the antigen delivery, this feature enables the VNP20009 advantages, compared with the traditional passive nanometer carrier, it has higher efficiency. In addition, the flagellum consists of flagella protein, has proven to be a highly effective and clinical safety of adjuvants, by combining toll-like receptors can increase the immunogenicity of antigen. [6]

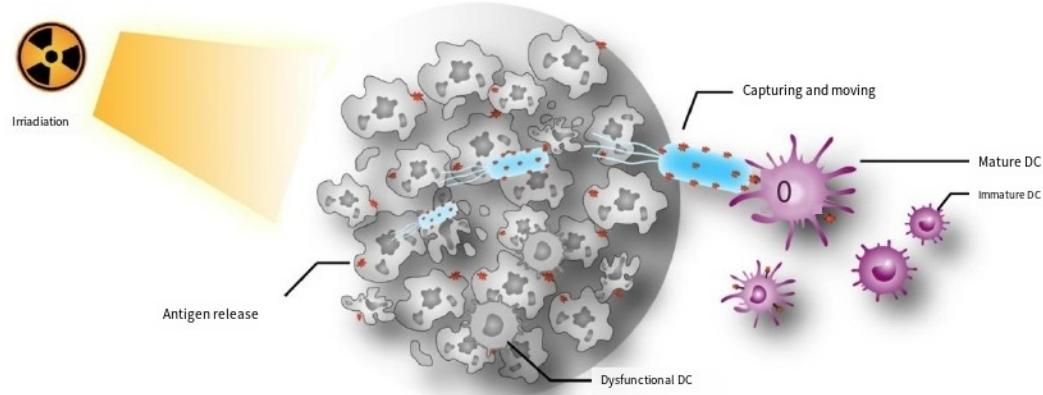


Figure1. After radiotherapy, tumor injection of adsorption of cationic polymer nanoparticles coated with antigens genetic attenuated salmonella strains accumulated in tumor surrounding tumor antigens. This enhances the crosstalk between antigen and dendritic cells, and lead to the activation of ovalbumin specificity of dendritic cells in vitro and antitumor effect of greatly increased, and prolong the lifetime of a variety of tumors in mice model, including the model of the metastasis and recurrence. [7]

1.3 compared with advantages and disadvantages of EcN

Salmonella can pass flagella convert chemical energy into mechanical energy, the sports ability is 10 times more than e. coli. [4] at the same time, for tumor-specific engraftment in live mice, tissue distribution and gene induction of related studies have shown that compared with salmonella typhimurium, using e. coli strains of the spleen and liver engraftment rate significantly reduced. [8]

However, because of its specific tumor targeting and facultative parasitic cells, salmonella more used in cancer treatment, rather than invasive EcN application scope is more extensive, such as, cytokines and other material has been used as a vaccine delivery carrier. EcN for different pathogenic escherichia coli showed a strong antagonist activity. In addition, the application of salmonella VNP20009 faces a clinical dilemma, VNP20009 after intravenous injection, tumor response rate is low and a dose-dependent toxicity problems.

2. Listeria

2.1 Brief introduction

Listeria (Listeria) is a kind of bacillus in the shape of a gram-positive facultative anaerobic bacteria, widely in the nature in escrow. Listeria include a diverse genus, has four branches and 15 different species. In the 15 different species, known only mononuclear cell hyperplasia listeria and Ivan listeria will cause disease in humans and animals. rees

Teriomycosis is a very dangerous disease that is usually contracted by eating food contaminated with Listeria monocytogenes. [9] hyperplasia of monocyte Listeria, Listeria monocytogenes, Lm) because of its known to infect humans and produce a variety of symptoms, including gastroenteritis, meningitis, and encephalitis. In general, the body's immune system can produce effective congenital and adaptive immune response, can control the Lm infection. Therefore, Lm is rare severe infection, usually limited to the elderly, pregnant women or immunocompromised patients. [10]

2.2 Application Example

Lm has many characteristics, make its become the carrier of cancer immunotherapy of attractive. Lm infection caused only modest of the humoral response to block infection [11] again, this allows the carrier according to the need to repeat application based on Lm to enhance T cell responses of patients. In addition, compared with DNA or peptide vaccine, Lm strong induction of congenital and adaptive immune responses. Bacteria, in fact, activate innate immune mechanism and the ability to cause antitumor response received for the first time in more than 100 years ago, when William colli (William Coley) observed for bacterial skin infections sarcoma patients with spontaneous cancer away back [12]. In addition to innate immune mechanisms, compared with salmonella and other bacteria carrier, Lm is effective cytotoxic t cell and cell mediated immune stimulants [11]. In more than 50 years ago, George McCandless (George Mackaness) further proved that mice exposed to the lethal dose Lm produces long life, antibody dependent immune response, the immune response to prevent future with lethal dose for Lm to attack [13]. These observations and other observations eventually led to the exploration of Lm as vaccine carrier, the purpose is to induce similar cell-mediated immune response against foreign antigens. Paterson and colleagues used express Lm beta galactose glucoside enzyme antigen to induce the cytotoxic t cell responses, thus kill death express beta galactose glucoside enzyme of cancer cells [14]. Since the pioneering papers, published 25 years ago, have developed many Lm vaccine used in the treatment of various cancers.

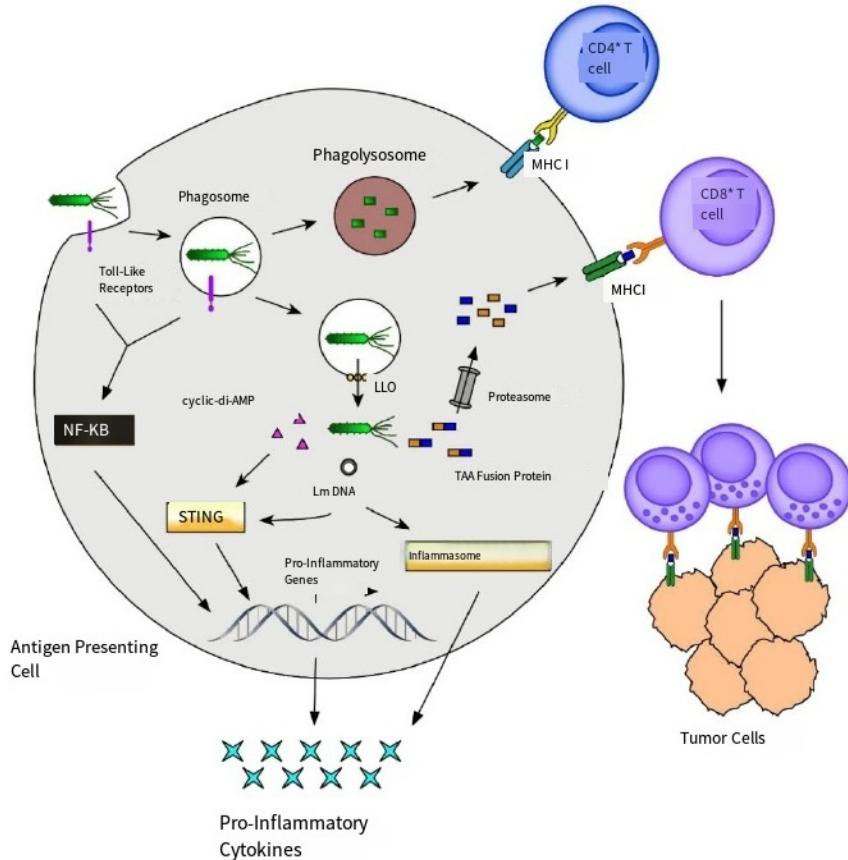


Figure 2. The restructuring of listeria congenital and adaptive immune response.

Monocytes hyperplasia of listeria monocytogenes (Lm) is internalized into the phagosome antigen-presenting cells. In the process of entering, the Lm perception by toll-like receptors, leading to the activation of NF kappa - B predominate in the synthesis of proinflammatory genes. Then the phagosome formation may merge with lysosomes devouring lysosome, including Lm can be killed, led to its loaded to MHC class II antigen by activated CD4 helper T cells. Or, Lm can be expressed by the hole of toxins listeria hemolysin O (LLO) to punch phagosome and into the cytoplasmic sol. Once in cytoplasmic sol, restructuring Lm will tumor associated antigen (TAA) as a fusion protein with Lm antigens secreted into the cytoplasm sol, where they can be proteasome degradation and loaded into the MHC

I class, to activate the TAA specific CD8 cytotoxic T lymphocytes. In addition, the cytoplasmic Lm triggers further induction of proinflammatory pathways through the secretion of cyclic dinucleotide cyclic-di-AMP and detection of Lm DNA. The secretion of cyclic - di - AMP directly stimulate the STING pathways and negative control the nf-kappa B inhibitors RECON (not shown), the existence of Lm and genome DNA can lead to STING and inflammatory corpuscle pathways

Activation, both of which contribute to the transcription of proinflammatory genes and cytokines.^[15]

Considerable effort has been spent to improve Lm safety because wild-type Listeria is pathogenic and not suitable for clinical use. The ideal Lm strains will minimize pathogenic, and at the same time maximize the immunogenicity of the target antigen. For this, have developed a variety of strategies to reduce the Lm, develop attenuated liszt strains for vaccination. It is the lack of virulence genes, a kind of widely used less Lm strategy involves knockout responsible for Lm tropism and genetic transmission between cells. Such as knock out two virulence factor genes Lm Δ actA / Δ inlB strain, also known as lack of live attenuated double (LADD), show some antitumor response^[16], is the foundation of many vaccine clinical trials. The second is to use the free replacement virulence genes or

Metabolism genes. Three is to develop inactivated but metabolically active (killed but metabolically active, KBMA) strains. [15]

Fusion of TAA with Listeria antigen enhances anti-tumor responses. Design Lm expression TAA alone can be used to produce therapeutic immune response, but almost all Lm vaccine will be TAA to merge with natural Lm antigen chimeric protein expression. Preliminary study of Lm vaccine will TAAs Lm fusion protein with high secretion increased, as a kind of TAAs to a host cell cytoplasm sol delivery method [17]. Now, however, it has been fully aware that when some Lm protein with TAA, also can be used as adjuvant, improve the effect of treatment. TAA partners including the two most common fusion LLO and ActA variant. [15]

2.3 compared with advantages and disadvantages of EcN

As mentioned above, Lm has many properties that other strains, such as EcN, do not possess that make it an attractive vehicle for cancer immunotherapy, such as strongly inducing innate and adaptive immune responses and being a potent stimulator of cytotoxic lymphocytes and cell-mediated immunity. Compared with salmonella, parasitic Lm has a unique cell processing, the ability of oral antigens, therefore is considered to be a good tumor vaccine vectors. [18]

But Lm vaccines are still in their relative infancy, and further clinical testing is still needed. At the same time, based on Lm invasive, Lm more for the treatment of immune related and retrofit, and EcN relatively wider application range. Lm is originally a pathogenic bacterium, so it should be focused on the development of attenuated engineering, while EcN, as a probiotic derived from feces, does not produce any toxins related to pathogenic E. coli strains, as mentioned above. Such as heat stable enterotoxin (H - LT), thermal stability of bowel poison element (H - ST), cytotoxic necrosis factor (such as cell toxicity, CNF 1) or shiga toxoid (SLT I, SLTII). Studies have shown that EcN does not have the genetic information to produce the above toxins, so it does not need to be attenuated, and its application is more convenient.

3. Microalgae

3.1 Brief introduction

In the strictest definition, Microalgae are unicellular eukaryotic microorganisms that perform photosynthesis and often have an aquatic lifestyle. Although cyanobacteria are prokaryotes and thus not true algae, they share similar physiology and ecology with eukaryotic microalgae and share many biotechnology applications. [19]

Although oxygenic photosynthesis, by which algae convert inorganic carbon (CO_2) into biomass, is an important feature in the definition of microalgae, it is not necessarily a common denominator, as the loss of photosynthetic capacity has occurred several times in several microalgal lineages. These species rely on heterotrophic (that is, the use of organic carbon as an energy source) rather than photosynthetic autotrophic growth, and are thus able to grow in total darkness. Other species, combines the two strategies are often referred to as hybrid biological nutrients. [19]

3.2 Application Example

Due to their high exponential growth rates, microalgae achieve higher area biomass productivity compared to terrestrial crops, and microalgae biomass can be used almost entirely as it is very low in fiber and rich in protein, lipids and/or other carbohydrates. Despite the interest in the large-scale production of microalgae for biofuel or bulk protein, microalgae are still unable to compete with agricultural commodity crops. Microalgae are expensive to produce compared to conventional crops.

[19]

Because microalgal lineages diverged long before land plants, they have high metabolic diversity. Indeed, since the 1970s, several companies worldwide have commercially produced microalgae (*Arthrospira* and *Chlorella*, for example), not for the production of biofuels or bulk proteins, but for high-value applications such as nutritional supplements, where several species of microalgae accumulate high concentrations of carotenoids. Many microalgae contain high levels of omega-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, which are important for cardiovascular health and development of the central nervous system. Other examples of unique and promising metabolites that are currently unexploited include toxins produced by blot-forming phytoplankton, some of which are so potent that they can be used to control tumor growth in targeted chemotherapy. Other substances, including a variety of polysaccharides and phytohormones, can be used as plant biostimulants to optimize nutrient use efficiency and improve crop tolerance to environmental stress. Microalgae are also emerging feedstocks for the production of bioplastics. [19]

We can also take advantage of the unique metabolic pathways of microalgae by incorporating them into terrestrial crops. For example, genes derived from microalgae could be expressed in terrestrial crops to produce oils rich in omega-3 fatty acids. Vice versa, microalgae can be used as a platform to synthesize metabolites that are naturally present in other organisms. For example, microalgae hold promise for the manufacture and (orally) delivery of various biopharmaceuticals. [19]

Microalgae have attracted much attention in recent years for biomedical applications. Microalgae can be cultured in large quantities, and many of these species have been commercialized as nutritional and food supplements, demonstrating their utility and high biosafety as oral pharmaceutical preparations [20,21]. In addition, many studies show that microalgae in *in vitro* and *in vivo* targeted drug delivery has strong potential, because they can effectively adsorb active surface loading drug molecules,^{23,24} [22]. In view of this paradigm, the *Spirulina platensis* (SP) can be used as a possible drug carrier for oral drug delivery. The helical structure of SP may allow it not only to be more easily trapped by intestinal villi, but also to adhere to the intestinal wall, thereby prolonging the retention time of the drug in the intestine. In addition, the intrinsic fluorescent chlorophyll produced by SP allows non-invasive fluorescence imaging without the need for chemical modification^{25,26}, making the multifunctional SP particularly suitable for therapeutic and diagnostic applications. In this sense, the utilization of drug delivery systems based on microalgal biomass may provide innovative means to reduce costs, minimize toxicity, and improve therapeutic efficacy of oral administration.

A microalgae-based oral drug delivery system (SP@Curcumin) has been constructed to treat a variety of intestinal diseases. Curcumin, a US Food and Drug Administration-approved drug with a variety of pharmacological functions, such as anti-inflammatory and anti-cancer, is loaded into SP

In SP. The study demonstrated that SP@Curcumin can pass through the stomach while its structure remains unchanged. SP@Curcumin can then be captured by the intestinal villi and gradually degraded and released curcumin, resulting in a desired drug distribution in the gut without causing adverse reactions. In traditional radiotherapy for colon cancer, SP@Curcumin has shown synergistic therapeutic effects by combining chemotherapy and radiotherapy to inhibit tumor progression. At the same time, SP@Curcumin can also protect normal intestinal tissue during radiotherapy by eliminating reactive oxygen species (ROS) produced in normal cells and reducing ROS-induced DNA damage. In addition to its application in cancer therapy, the study also proved that the intestinal SP @ the anti-inflammatory ability of Curcumin, can reduce the levels of proinflammatory cytokines and relieve colitis mice inflammation related symptoms.

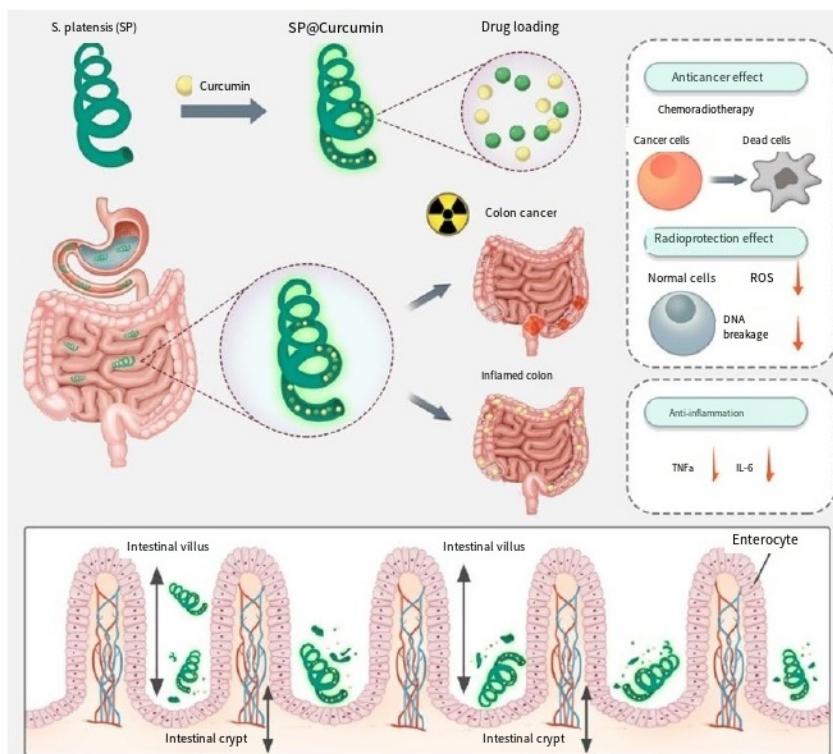


Figure3. Diagram of SP@Curcumin mediated chemoradiotherapy for colon cancer

It includes radiation protection by inhibiting tumor cells and normal cells, and anti-inflammatory effect on intestinal inflammation [27]

In addition, biodegradation-based microalgal carriers can also be used for targeted delivery and imaging-guided therapy of lung metastasis of breast cancer. High delivery efficiency, long-acting drug release and low systemic toxicity are effective weapons for drug delivery systems to win the battle against metastatic breast cancer. *S. platensis* can be used as a natural carrier to construct a drug delivery system for targeted delivery and fluorescence imaging-guided chemotherapy for the treatment of lung metastasis of breast cancer. The chemotherapeutic drug doxorubicin (DOX) can be loaded into spirulina in a single step, and the DOX-loaded SP (SP@DOX) can be prepared with ultra-high drug loading efficiency and ph-responsive drug release. The abundant chlorophyll gives SP@DOX excellent fluorescence imaging capabilities, which can be used for non-invasive tracking and real-time *in vivo* monitoring. In addition, the micrometer size and helical shape of the SP vector enabled the prepared SP@DOX to passively target the lung, thereby significantly improving the therapeutic efficacy against lung metastasis of 4T1 breast cancer. Finally, the undelivered vector can be biodegraded by renal clearance without significant production

Is toxic. SP@DOX is a novel biological hybrid strategy for targeted drug delivery and effective treatment of cancer metastasis.

[24]

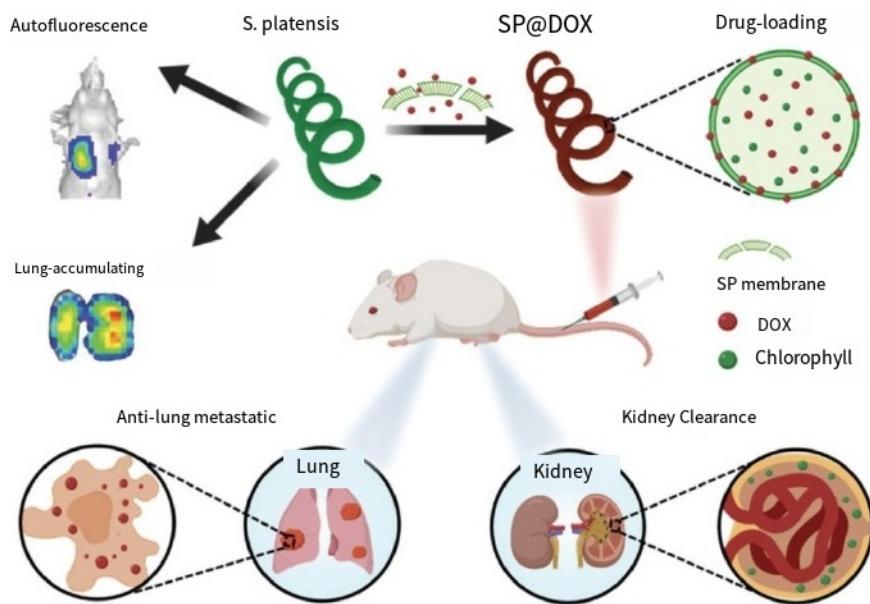


Figure 4. Schematic diagram of SP@DOX mediated lung-targeted drug delivery and fluorescence imaging-guided chemotherapy to inhibit lung metastasis of breast cancer. [24]

Comparison of advantages and disadvantages between 3.3 and EcN

Microalgae have high metabolic diversity relative to EcN, for example, several microalgae accumulate high concentrations of carotenoids, and many microalgae contain large amounts of omega-3 fatty acids that can be used as high-value nutritional supplements.^[19] Microalgae hold strong potential for in vitro and in vivo targeted drug delivery because they can efficiently load drug molecules through their active surfaces. The helical structure of some microalgae such as SP as described above may make them not only more easily trapped by intestinal villi, but also adhere to the intestinal wall, thereby prolonging the retention time of drugs in the intestine. The intrinsic fluorescent chlorophyll produced by SP allows non-invasive fluorescence imaging without the need for chemical modification. All these are the unique advantages of microalgae over EcN.^[27]

Microalgae production is growing rapidly worldwide, and companies are constantly developing new products. Nonetheless, the market success of novel microalgae products depends not only on a cost-effective production process, but also on consumer acceptance and demand (especially for food applications). In addition, all products must strictly comply with relevant regulations (for example, food and feed regulations), which can be complex and unsuitable for new microalgal products. For example, in the European Union, only a few microalgal species are currently authorized for the food market, and obtaining commercial authorization remains a bottleneck limiting the potential of microalgal products.^[19]

References:

- [1] Su LH, Chiu CH. (2007) Salmonella: clinical importance and evolution of nomenclature. *Chang Gung Med J.* 2007 May-Jun;30(3):210-9.
- [2] Andino A, Hanning I. (2015) *Salmonella enterica*: survival, colonization, and virulence differences among serovars. *ScientificWorldJournal.* 2015;2015:520179.
- [3] Winter, S., Thiennimitr, P., Winter, M. et al. (2010) Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature* 467, 426–429
- [4] Toley, B. J. & Forbes, N. S. (2012) Motility is critical for effective distribution and accumulation of bacteria in tumor tissue. *Integr. Biol.* 4, 165–176
- [5] Bhattacharjee, T. & Datta, S. S. (2019) Bacterial hopping and trapping in porous media. *Nat. Commun.* 10, 2075
- [6] Zhou, S., Gravekamp, C., Bermudes, D. & Liu, K. (2018) Tumour-targeting bacteria engineered to fight cancer. *Nat. Rev. Cancer* 18, 727–743
- [7] Wang, W., Xu, H., Ye, Q. et al. (2022) Systemic immune responses to irradiated tumours via the transport of antigens to the tumour periphery by injected flagellate bacteria. *Nat Biomed Eng* 6, 44–53
- [8] Jochen Stritzker, Stephanie Weibel, Philip J. Hill, Tobias A. Oelschlaeger, Werner Goebel, Aladar A. Szalay, (2007) Tumor-specific colonization, tissue distribution, and gene induction by probiotic *Escherichia coli* Nissle 1917 in live mice, *International Journal of Medical Microbiology*, Volume 297, Issue 3, 2007, Pages 151-162
- [9] Janet R. Donaldson, Kamil Hercik, Aswathy N. Rai, Sweetha Reddy, Mark L. Lawrence, Bindu Nanduri, Mariola Edelmann, (2015) Chapter 8 - *Listeria* and -Oomics Approaches for Understanding its Biology, Editor(s): Steven C. Ricke, Janet R. Donaldson, Carol A. Phillips, *Food Safety*, Academic Press, 2015, Pages 135-158
- [10] Paterson Y, Guirnalda PD, Wood LM. (2010) *Listeria* and *Salmonella* bacterial vectors of tumor-associated antigens for cancer immuno-therapy[J].*Semin mmunol*,2010,22(3):183-189
- [11] Wood, L.M.; Paterson, Y. (2014) Attenuated *Listeria monocytogenes*: A powerful and versatile vector for the future of tumor immunotherapy. *Front. Cell. Infect. Microbiol.* 2014, 4, 51.
- [12] Coley, W.B. (1893) The treatment of malignant tumors by repeated inoculations of erysipelas: With a report of ten original cases. *Am. J. Med. Sci.* 1893, 105, 487–511.
- [13] Mackaness, G.B. (1962) Cellular resistance to infection. *J. Exp. Med.* 1962, 116, 381–406.
- [14] Schafer, R.; Portnoy, D.A.; Brassell, S.A.; Paterson, Y. (1992) Induction of a cellular immune response to a foreign antigen by a recombinant *Listeria monocytogenes* vaccine. *J. Immunol.* 1992, 149, 53–59.
- [15] Flickinger, John C., Jr., Ulrich Rodeck, and Adam E. Snook. (2018) "Listeria monocytogenes as a Vector for Cancer Immunotherapy: Current Understanding and Progress" *Vaccines* 6, no. 3: 48.
- [16] Brockstedt, D.G.; Giedlin, M.A.; Leong, M.L.; Bahjat, K.S.; Gao, Y.; Luckett, W.; Liu, W.; Cook, D.N.; Portnoy, D.A.; Dubensky, T.W. (2004) *Listeria*-based cancer vaccines that segregate immunogenicity from toxicity. *Proc. Natl. Acad. Sci. USA* 2004, 101, 13832–13837.
- [17] Ikonomidis, G.; Paterson, Y.; Kos, F.J.; Portnoy, D.A. (1994) Delivery of a viral antigen to the class I processing and presentation pathway by *Listeria monocytogenes*. *J. Exp. Med.* 1994, 180, 2209–2218.
- [18] 汪舒颖,丁承超,马俊飞,等. (2019) 减毒单核细胞增生李斯特氏菌作为疫苗载体在肿瘤

治疗中的应用. *微生物学杂志*, 2019, 39(5):87-97.

- [19] Eli S.J. Thoré, Koenraad Muylaert, Michael G. Bertram, Tomas Brodin, (2023) Microalgae, *Current Biology*, Volume 33, Issue 3, 2023, Pages R91-R95
- [20] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, (2006) Commercial applications of microalgae. *J. Biosci. Bioeng.* 101, 87–96.
- [21] B. Da Silva Vaz, J. B. Moreira, M. G. de Morais, J. A. V. Costa, (2016) Microalgae as a new source of bioactive compounds in food supplements. *Curr. Opin. Food Sci.* 7, 73–77.
- [22] O. Yasa, P. Erkoc, Y. Alapan, M. Sitti, (2018) Microalga-powered microswimmers toward active cargo delivery. *Adv. Mater.* 30, 1804130.
- [23] X. Yan, J. Xu, Q. Zhou, D. D. Jin, C. I. Vong, Q. Feng, D. H. L. Ng, L. Bian, L. Zhang, (2019) Molecular cargo delivery using multicellular magnetic microswimmers. *Appl. Mater. Today* 15, 242–251.
- [24] D. Zhong, D. Zhang, T. Xie, M. Zhou, (2020) Biodegradable microalgae-based carriers for targeted delivery and imaging-guided therapy toward lung metastasis of breast cancer. *Small* 16, 2000819.
- [25] X. Yan, Q. Zhou, M. Vincent, Y. Deng, J. Yu, J. Xu, T. Xu, T. Tang, L. Bian, Y.-X. J. Wang, K. Kostarelos, L. Zhang, (2017) Multifunctional biohybrid magnetite microrobots for imaging-guided therapy. *Sci. Robot.* 2, eaaq1155.
- [26] D. Zhong, W. Li, Y. Qi, J. He, M. Zhou, (2020) Photosynthetic biohybrid nanoswimmers system to alleviate tumor hypoxia for FL/PA/MR imaging-guided enhanced radio-photodynamic synergetic therapy. *Adv. Func. Mater.* 30, 1910395.
- [27] Danni Zhong et al. , (2021) Orally deliverable strategy based on microalgal biomass for intestinal disease treatment. *Sci. Adv.* 7, eabi9265.

10. Policy on bacteriotherapy

name	Time of issue	Agency	Related content
13th Five-Year National Innovation Plan	2016	State Department	To develop advanced and efficient biotechnology, accelerate innovative breakthroughs and application development of leading technologies such as synthetic biology, and develop a number of innovative pharmaceutical biological products
The 13th Five-Year Plan for the Development of Biological Industry	2016	National Development and Reform Commission	It will accelerate the construction of an innovation system for the biofuturing industry, solve problems such as the design, synthesis, optimization and regulation of artificial organisms, and accelerate the creation and industrialization of new drugs, relying on advanced technologies to promote the development of translational medicine.
Guidance Catalogue of Key Products and Services for Strategic Emerging Industries (2016 edition)	2016	National Development and Reform Commission	Biotech drugs mentioned developing related technologies such as genetically engineered drugs.
Catalogue of Key Industries Supported by Intellectual Property (2018 edition)	2018	State Intellectual Property Office	Synthetic biotechnology and related technologies such as gene editing are mentioned in the advanced biological industry
General Introduction of Microecological Viable Bacteria Products (2020 edition) »	2020	Chinese Pharmacopoeia Commission	The concept of microecological live bacteria products, as well as related preparation requirements and detection methods are defined.
The 14th Five-Year Plan for Health and Health Science and Technology Innovation	2022	Ministry of Science and Technology of China	Accelerate the promotion of cutting-edge technological breakthroughs, accelerate technological innovation breakthroughs and applied research, and focus on malignant tumors in drug development.
Notice on the 13th Five-Year National Strategic Emerging Industry Development Plan	2016	State Department	To grasp the new trends in the in-depth development of life sciences, the wide application of new biological technologies and the integration of innovation, and promote the development of medical care toward precision medicine and personalized medicine.
Regulations on Clinical Application and Management of New Biomedical Technologies (Draft for comment) »	2019	National Health Commission	The approval of clinical research by the health administration department includes academic and ethical review, clarifies the responsibilities of various parties, technical access thresholds, etc., to promote innovation capacity, and accelerate the promotion of biological research and development technologies such as the creation of new drugs.
Guidelines on Promoting the Healthy Development of the Pharmaceutical Industry	2016	State Department	

14th Five-Year Plan for the Development of Biological Economy	2021	National Development and Reform Commission	The Commission will strengthen originality, lead basic research, and build a national strategic scientific and technological force in the biological field targeting cutting-edge fields such as clinical medicine and health management, new drug development, and synthetic biology.
Table of Reference for Classification of Strategic Emerging Industries and International Patent Classification (2021) (Trial)	2021	State Intellectual Property Office	Biological drugs, genetically engineered drugs are identified as strategic industries
2023 Outline for Building a Powerful Country with Intellectual Property Rights and Plan for Implementing and Promoting the 14th Five-Year Plan	2023	State Intellectual Property Office	Financial institutions are encouraged to develop financing and insurance products adapted to the characteristics of the intellectual property service industry.
Catalogue for Guidance on Industrial Restructuring (2024 edition)	2023	National Development and Reform Commission	Breakthroughs and applications of pharmaceutical core technologies,
Early trials of living biotherapeutic products: information on chemistry, manufacturing and control	2016	FDA	To advise IND sponsors on early stage clinical trials using live biologics in the United States.
Policy regarding Quantitative labeling of dietary supplements containing Active Microorganisms: an Industry guide	2018	FDA	The CFU index is required to be added to the quantitative label of dietary supplements containing active microorganisms
Decentralized clinical trials of drugs, biologics, and devices	2023	FDA	Recommendations for clinical trials of biological products are provided for manufacturers and other stakeholders to learn from
National Biotechnology and Biofuturing Program	2022	The White House	Increase domestic biofabrication capacity, (i.e., synthetic biology capacity)
U.S. Innovation and Competition Act	2021	Congress	Funding for basic and advanced technology research, including synthetic biology.
A framework for coordination and cooperation in biotechnology	2017	The White House	Contains biotechnology products and products and regulations under the jurisdiction of relevant departments, providing a good process and legal reference for product manufacturers

Note: Although China and the United States have a certain legislative basis for synthetic biology, the current laws on bacterial therapy are not perfect and perfect. However, it can be seen that synthetic biology and biomedicine are the joint investment fields of the two countries.

11. Overview of key companies in EcN industry market

market

1. Synlogic Therapeutics

To use EcN as a chassis for engineered cancer treatment

Introduction: Founded in Boston in 2013, it was listed on NASDAQ through a reverse merger in 2017. It uses the technology of synthetic biology to transform microorganisms and repair metabolic disorders in patients by synthesizing microorganisms carrying special DNA strands (gene circuits) to achieve the effect of treating related diseases.

Product Pipeline



Figure1. Synlogic Therapeutics product pipeline

2. Ardeypharm

Live bacteria tablets

About: Arderpharm, based in Herdeke, Germany, is a leading pharmaceutical specialist in the field of probiotic drug development and production. mutaflor is sold directly with EcN as the main ingredient.

Mutaflor®-with the unique probiotic active ingredient Escherichia coli strain Nissle 1917 for human gut health



Figure 2. EcN is engineered to secrete related proteins

3. Angelo (Shenzhen) Biotechnology Co., LTD

Medicinal protein

Brief introduction: Angro (Shenzhen) Biotechnology Co., Ltd. was established on April 10, 2023. In the field of SOD development and application, it is one of the few synthetic biology enterprises in the world with molecular level acquisition, collision and gene transcription technology, and mass production with scale industrialization. The company provides green biotechnology solutions and the supply of key raw materials for applications in the fields of medicine, agriculture, and food.



Figure 3. Angelo Product Display

12. Forecast of EcN development prospects in China

A wide range of indications:

EcN is originally suitable for the treatment of inflammatory bowel disease (IBD), etc. After modification, its potential application field is further broadened. This includes, but is not limited to, the treatment of cancer, autoimmune diseases, etc., as well as monitoring the condition as a biosensor, which brings a significant market share potential for EcN.

Policy support:

Governments around the world are gradually recognizing the importance of EcN and other biotherapeutic technologies, and have introduced relevant policies to support it. For example, in the 11th Five-Year Plan, the Chinese government increased the investment in scientific research in the field of biomedicine, which provided a good policy environment for the development of EcN.

Meeting clinical needs:

EcN has the advantages of good oral compliance, lasting efficacy, and precision of treatment, which are urgently needed to be met in current clinical treatment. Especially for patients who need long-term medication, EcN provides a more convenient treatment option.

Capital market concerns:

Although there are not many capital market data on EcN at present, with the overall development of the biomedical industry, more and more capital has begun to pay attention to and invest in ECN-related enterprises and R&D projects. From the perspective of the probiotics market, China's probiotics market reached a scale of 109.38 billion yuan in 2022, growing from 64.77 billion yuan in 2018 to 2022, with an average annual compound growth rate of 14%. On the investment and financing situation, probiotics industry investment and financing activity is higher in 2019, nine financing event happened, financing amount is more than 840 million yuan. However, since the outbreak of COVID-19 in 2020, the financing activity has decreased, and the amount of financing has dropped to 33 million RMB in 2022. On industry competition pattern, probiotics industry as a whole market concentration is very high, CR4 in 2022 was 95.7%, the shape of 99.8% on the regional competition, north China and Yangtze river delta region are the probiotic industry concentrated area, capacity of most representative enterprises in north China, including branch billiton biological, blue, etc. Among them, the micro probiotics is located in the first tier, annual production capacity of over 500 tons; The second echelon includes Azure Biology, Ketuo biology, etc., with an annual production capacity between 100 tons and 500 tons. It is expected that the size of China's probiotics market will exceed 190 billion yuan by 2028, with an estimated annual compound growth rate of 10%.

Applications in the field of environmental protection and secretion:

EcN is not only promising in the field of disease treatment, but also has shown potential in the fields of environmental protection and secretion regulation. For example, the application of EcN in bioremediation can help to remove environmental pollution.

Public awareness raising:

As public awareness of probiotics and biotherapy increases, the acceptance and demand for EcN (*E. coli* Nissle 1917) is expected to increase. Probiotics are a group of active microorganisms that can promote the ecological balance of the intestinal microbial flora of the host, and have a positive effect on health. EcN as a kind of probiotics, has a variety of biological functions, including antibacterial, anti-inflammatory, and regulating intestinal flora, etc., which makes it become the ideal candidate of disease treatment and diagnosis.

Market Data:

According to a report by Fortune Business Insights, the global oncology drugs market is expected to grow from \$150 billion in 2021 to \$250 billion in 2028. The growth trend reflects the sustained demand growth and technological progress in the field of tumor treatment. The market potential of EcN (*Escherichia coli* Nissle 1917) as a potential cancer therapy cannot be ignored.

EcN is a probiotic with a variety of biological functions, including antibacterial, anti-inflammatory, and regulation of gut microbiota, which makes it an ideal candidate for disease treatment and diagnosis. Through gene editing techniques, EcN can be engineered to accurate carrier of drug delivery to tumor site, reduce drug damage to the normal cells, improve the effect of treatment.

The growth in the size of the global oncology drugs market is driven in part by the development of targeted therapies and immunotherapy. Targeted therapies dominated the global oncology market, accounting for 61.3% of the market share in 2022. The transformation and optimization of EcN make it expected to become a new member of targeted therapy, providing new possibilities for cancer treatment.

In addition, the demand for oncology drugs is expected to continue to grow as the global population ages and medical technology advances. As a new type of treatment, EcN has great market potential and is expected to occupy a place in the future cancer treatment market.

However, despite the huge market potential, the clinical application of EcN still faces some challenges, including genetic stability, safety and other issues. Widely used in clinic, in order to achieve the EcN, need further research and development, in order to solve these technology and safety problems.

To sum up, with the continuous growth of the global oncology market, EcN as a potential treatment, its market prospect. Through continuous research and technological innovation, EcN is expected to play an important role in tumor therapy field in the future.

Technological advances:

With the rapid development of biotechnology, especially the continuous progress of gene editing technology, the modification and optimization of engineered bacteria such as *Escherichia coli* Nissle 1917 (EcN) have become possible and have shown great potential in clinical application. EcN as a kind of probiotics, has a variety of biological functions, including antibacterial, anti-inflammatory, and regulating intestinal flora, etc., which makes it become the ideal candidate of disease treatment and diagnosis.

In clinical applications, EcN engineering alteration are mainly concentrated in two aspects: surface modification and genetic engineering. through

These transformation, EcN can be used as a drug delivery system, used in the treatment of diseases such as cancer, inflammatory bowel disease. For example, surface modification can enhance the adhesion of EcN to the target, improve its tolerance in the gastrointestinal environment, improve its biocompatibility, and thus improve the therapeutic effect.

Genetic engineering involves the mutation of endogenous genes of EcN or the introduction of foreign genes to achieve specific therapeutic purposes. For example, by means of genetic engineering, EcN can be engineered to express specific drug molecule bacteria, or as a biosensor monitoring condition. These engineered ECNs have shown promising therapeutic effects in animal models, but problems such as genetic stability and safety need to be addressed before they can be widely used in clinical practice.

In addition, ECNs are being used to build smart drug delivery systems that are able to respond to specific biological signals, such as temperature, oxygen concentration, or metabolite levels, to precisely release drugs at target sites. For example, engineering ECNs to locally release the cytokine GM-CSF and blocking nanoantibodies against PD-L1 and CTLA-4 at the tumor site has been shown to significantly reduce the adenoma burden in a mouse model.

Although EcN modification and optimization show great potential in clinical application, but it is still facing some challenges. For example, the genetic instability of bacterial plasmids may lead to uncertain therapeutic effects, and the transfer of antibiotic genes may also pose safety concerns. In addition, the stability and safety of recombinant strains for mass industrial production is also need to focus on problem.

In general, the modification and optimization of EcN is an active research area, and with the continuous progress of technology, it is expected to be more widely used in clinical practice in the future.

13: Side effects and biosafety prospects of EcN related therapies

The safety and genetic plasticity of EcN make it an engineered bacterial vector for the treatment of a variety of diseases. These characteristics of EcN make it potential for a wide range of medical applications, including as a drug delivery system, anti-tumor therapy, and the treatment of specific metabolic diseases. However, there are still some side effects and risks of EcN in the treatment of diseases.

1. Immune response

The immune response activated by bacterial treatment may sometimes lead to side effects. For example, excessive immune response may trigger a systemic inflammatory response complex, and then lead to severe complications such as shock and multiple organ failure. In addition, the activation of the immune system can result in autoimmune diseases, attack normal tissues. one

1. The question of the immunogenicity of engineered EcN in participatory therapy mainly concerns the immune response it may elicit in vivo as a living drug. Although EcN itself has high safety as a probiotic, the immunogenicity of engineered EcN can be affected by many factors. Engineering EcN may express exogenous protein or drugs, these foreign proteins or drugs may cause the host's immune response. For example, EcN is currently used for CRISPR/Cas9 targeted delivery for gene therapy of cancer and other diseases. The Cas9 protein carried by EcN is derived from *Streptococcus pyogenes* and *Staphylococcus aureus*, two bacteria that frequently infect humans. , according to a study at Stanford university were detected in 78% of the donor for SaCas9 antibodies, while 58% of donor in detect antibodies against SpCas9. This indicates that there is a pre-existing adaptive immune response in the human body, and the possible immune response caused by this is considered in clinical applications.²

2. Targeting

In therapies related to the colonization, detection, and treatment of tumor cells, *E. coli* Nissle 1917 (EcN) forms a long-term niche in the gut only in the presence of adenoma tissue in a gene-based engineering mouse model. Although EcN colonized selectively and significantly in tumors in the absence of antibiotic pretreatment in the mouse model compared with adjacent tissues and organs, its presence in adjacent healthy tissues and peripheral organs should not be ignored.⁴⁵

3. Gene transfer risk

The antibiotic resistance genes of general bacteria are relatively stable if they belong to the nucleosomal mutation; On the contrary, if a resistance gene is located in the mobile genetic elements, will probably be horizontal gene transfer, become a library of antibiotic resistance of pathogens in the gastrointestinal tract can express exogenous protein² in disease treatment or medication of the antibiotic gene engineering EcN usually located in the import of foreign plasmid, The transfer of plasmid DNA between bacteria can be achieved by conjugative pili. The transfer of antibiotic genes may confer resistance to pathogenic bacteria in the environment, leading to the development of drug resistance, and further increasing the difficulty of treating bacterial infectious diseases caused by the antibiotic genes.

4. infinite proliferation risks in the body

EcN is currently on the market as a pharmaceutical ingredient and is involved in the treatment of IBD. The rational application of probiotics will benefit patients. However, if ECN is used in IBD patients with severely impaired intestinal mucosal barrier or severe acute IBD, probiotics may transfer from the gut to the blood, leading to probiotic bacteremia and mycosis. Therefore, for intestinal mucosal barrier severely damaged, or severe acute IBD patients need to be vigilant when application of probiotics bacteremia. Vital signs and infection indicators should be monitored. If abnormal, probiotics should be stopped in time and blood culture should be done to identify the pathogenic bacteria. Meanwhile, anti-infection and symptomatic and supportive treatment should be given.²

5. Genotoxicity

Although EcN has probiotic properties, its pks gene island encodes the synthesized genotoxin colibactin, which is genotoxic and can cause DNA double-strand breaks in host cells, cause cell cycle arrest, and eventually lead to cell death, and may be a carcinogenic compound. Current research on EcN engineering renovation, through specific inactivated ClbP peptide enzyme, realize the EcN antagonists and gene activity uncoupling, so as to provide security guarantee for the use of EcN.³

Biosafety prospects of 6. EcN in the therapeutic field

In general, as an engineered bacterium, EcN has a promising prospect in the application of disease treatment and diagnostic strategies. With the continuous progress of synthetic biology and genetic engineering, transformation and optimization of EcN will continue to improve its safety and therapeutic potential, for patients with more effective and safer treatment options.

References:

- [1]史恩宇,王宁,赵溪悦,陈博,曹鸣芯,李长义.细菌介导的肿瘤治疗研究进展[J].中国生物工程杂志,2024(Z1):112-123.DOI:10.13523/j.cb.2307016.
- [2]杨荟平, 王志青, 刘乐, 等.炎症性肠病中应用益生菌的临床疗效与潜在风险[J]. 中华炎性肠病杂志, 2022, 06(2) :155-160 .
- [3]Clémence M, Priscilla B, et al. Deciphering the interplay between the genotoxic and probiotic activities of *Escherichia coli* Nissle 1917. PLoS Pathog. 2019 Sep; 15(9): e1008029.
- [4]Gurbatri CR, Radford GA, Vrbanac L, Im J, et al. Engineering tumor-colonizing *E. coli* Nissle 1917 for detection and treatment of colorectal neoplasia. Nat Commun. 2024 Jan 20;15(1):646. PMID: 38245513; PMCID: PMC10799955.
- [5]Luke JJ, Piha-Paul SA, Medina T, et al. Phase I Study of SYNB1891, an Engineered *E. coli* Nissle Strain Expressing STING Agonist, with and without Atezolizumab in Advanced Malignancies. Clin Cancer Res. 2023 Jul 5;29(13):2435-2444. PMID: 37227176; PMCID: PMC11225568.
- [6]Charlesworth, C. T. et al. Nat. Med. 25, 249–254 (2019).

14. Introduction of teams that useEcNprojects

PekingHSC

curmino: Bacterial platform for cancer therapy based on the VIII secretion system of E.coli Nissle 1917

It is generally believed that Gram-negative bacteria are not capable of secretion. However, we found that E. coli possesses T8SS, which forms Curli fiber on the surface of E. coli. The corresponding gene sequence, called Curli specific gene (Csg), contains seven proteins, csgABCDEFG. Among them, CsgG is the channel that mediates the secretion of CsgA and CsgB. Curli fiber is composed of csgA and CsgB, CsgA is the main component of Curli fiber, and CsgB acts as a condensation nucleus.ⁱ

iGEMers from PKUHSC-China brainstorm a variety of ways to transform T8SS.

1 CsgA - mediated intratumoral protein drug delivery system: CsgA is a kind of amyloid protein, with 5 repeat, there is evidence that csgG only mediated amyloid protein secretion.ⁱⁱ Based on this, we linked CsgA or truncated CsgA to the protein of interest (POI) by using MMP cleavable linker. Using EcN for the natural target of the tumor, the EcN within tumors secrete CsgA - fused POI, using tumor microenvironment high expression of MMPS enzyme, cut off between CsgA and POI would, so as to realize the tumor in situ sustained release POI, achieve high specific tumor destruction.

2 Applying Curli in E.coli Nissle 1917 as ectopic nucleation display system(ENDS) for immune boosting: CsgB acts like a condensation nucleus in the formation of Curli fiber, and makes Curli fiber grow on the surface of bacteria by interacting with CsgF on the surface of bacteria. We targeted the protein on the surface of tumor cells by knockout the CsgF gene of EcN and fusion at the CsgB end. At the end of CsgA neoantigen fusion, which carry a large number of neoantigen of Curli fiber in the tumor cell surface growth, realize the ectopic nucleation for antigen display, Immune cells are recruited by antigens displayed on the tumor surface to achieve immune activation, thereby achieving highly specific tumor killing.

3 ncAA incorporated application of csgA for prodrug click-to-release: We used codon expansion and site-directed modification of unnatural amino acids to site-directed insertion of an unnatural amino acid Tet2.0 with a tetrazine group into csgA. Taking advantage of the natural targeting property of microbacterium to tumors, we can achieve pre-targeting to tumors. Then, TCO caged prodrug was added, and TCO and tetrazine occurred click-release reaction to release active drug molecules, so as to achieve high tumor-specific drug release and killing.

TJUSX

Spidey Microbe: A Parkinson's drug delivery platform leveraging the adhesion and interaction network of probiotics

Probiotics have great potential in the treatment of gastrointestinal tract and gut-brain axis related diseases, but their weak intestinal colonization ability and complex microflora interactions limit the application of probiotics in the treatment field. To overcome these limitations, we designed and constructed the superhero Spidey Microbe, which includes *Escherichia coli* Nissle 1917 and *Lactococcus lactis* F44 flora. Through the intestinal adhesion system based on biotin-avidin binding and the interaction system based on quorum sensing, probiotics can "weave a web" to colonize the gut just like Spider-Man. We expect design genetic circuit control *Escherichia coli* Nissle mucous membrane combined protein expression in 1917 and probiotics mutual adhesion Spidey "silk" of Microbe controllable engraftment of intestinal tract. In addition, we plan to build in the EcN butyrate express module, in *Lactococcus lactis* F44 factory building cell expression in serotonin and l-dopa to give spider-man bacteria more abilities against Parkinson's disease. Our modification of EcN can be summarized into the following parts.

1 Gut colonization Capacity: We designed a self-assembly system based on streptavidin and biotin binding to label the surface of EcN cells using N-hydroxysuccinimide biotin, a reagent that can achieve conjugation by primary amines such as the N-terminus and side chains of lysine residues in proteins and peptides. We constructed an inducible streptavidin-gut mucin-binding protein fusion protein, "spider silk", in *Escherichia coli* Nissle1917 and used a signal peptide to localize the fusion protein to the extracellular space. Under the condition of the induction, can realize controllable engraftment of intestinal tract.

2 Butyric acid production element: Butyric acid, as a short-chain fatty acid, can reduce inflammatory response by improving intestinal mucosal permeability and down-regulating the expression of inflammatory factors. We express our acetyl-coa through acetyltransferase (ACAT), strengthen the metabolism of acetyl auxiliary enzymes to butyric acid A carbon flow. Thus, the butyric acid synthesis of EcN was enhanced.

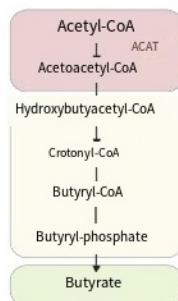


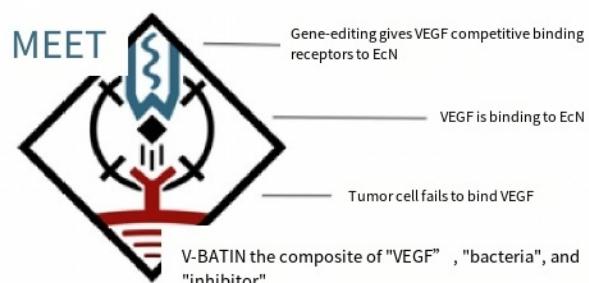
Figure1. Metabolic Pathway for Butyrate Production (from iGEM TJUSX).

USTC

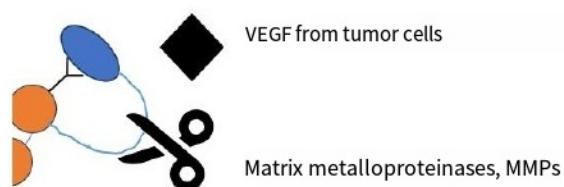
V-batin to construct an engineered anti-tumor strain that competes with vascular endothelial growth factor

A key reason why solid tumors are difficult to treat is that the tumor microenvironment around them inhibits the killing of the immune system, and how to relieve this inhibition is a challenge. At present, the strategy of targeting the tumor microenvironment to inhibit or treat solid tumors has attracted wide attention. However, most of the existing therapies still face challenges such as off-target.

Our group noted that the innocuous *E. coli* Nissle 1917 strain autonomically colonized the tumor microenvironment and showed excellent targeting ability. Therefore, we planned to exploit the angiogenic mechanism of vascular endothelial growth factor (VEGF) in humans to enable EcN to express VEGF-competitive binding receptors under specific conditions in the tumor microenvironment and present them on the bacterial surface. By absorbing and consuming VEGF in the tumor microenvironment and reducing its concentration, angiogenesis in the tumor area can be effectively inhibited to achieve the therapeutic purpose. This strategy provides a new idea for the treatment of solid tumors and demonstrates the potential of engineered bacteria in the treatment of solid tumors.



First, we screened several different VEGF receptors for presentation on *E. coli* using a surface-display system; Secondly, we also designed a series of intrinsic environmental sensing switches, such as hypoxia switches, which can be turned on and expressed only when the bacteria are located in the tumor microenvironment. Thirdly, different from the off-target effects faced by traditional systemic delivery methods, we designed a masking system in order not to affect the VEGF in the normal blood circulation system. VEGFR displayed on the surface of *E. coli* is linked with a masking peptide that is similar to VEGF. The linked structure can be cleaved under the presence of tumor-specific high expression of MMP-14, thus the masking structure can be removed specifically in the tumor microenvironment.



GXU-China

This project uses the combination of engineered bacteria and nanoparticles to achieve precise localization and attack on tumor cells, and provides a feasible scheme for the treatment of triple-negative breast cancer.

EcN was used as the chassis strain of engineered bacteria in this project. The modification of ECN was mainly divided into two parts: genetic engineering and chemical modification of nanoparticles.

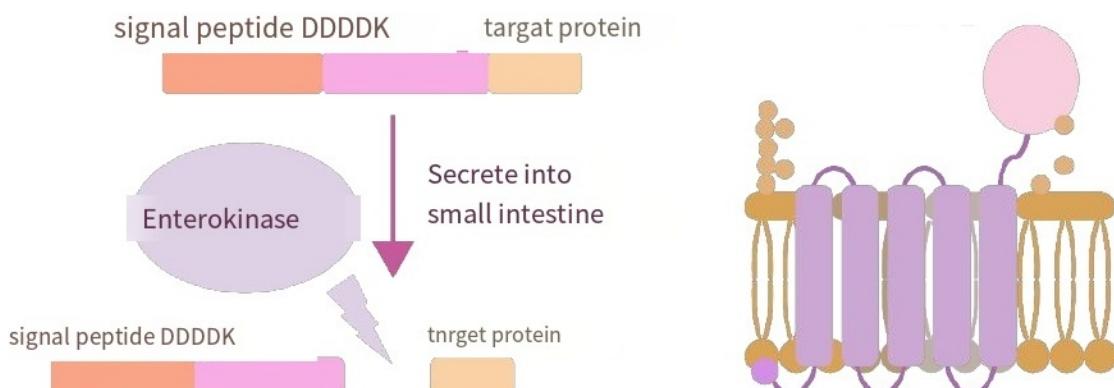
In this project, CD47-targeted SIRPa gene was used to genetically engineering EcN to achieve the purpose of identifying TNBC. SIRPa was transferred into a plasmid vector pET-28a+ using the Golden Gate cloning system. SpyTag/SpyCatcher construct was also used. This year, the team also focused on surface display of EN targeting tumor SIRPa. To properly display a tumor-targeting protein on the cell surface, the target protein (guest protein) needs to be genetically fused to a carrier protein that can facilitate export across the cell envelope and anchor the guest protein to the bacterial cell surface with appropriate surface exposure. SpyCatcher/SpyTag Surface Display system (S3D) was utilized in the project, in which the membrane anchor and passenger are expressed separately and assembled by post-translational coupling. AIDA-I adhesin was selected as a tumor-targeting SIRPa vector. It contains a C-terminal autotransporter structure that forms a porin-like β -barrel channel at the outer membrane to transport the N-terminal guest domain to the cell surface.

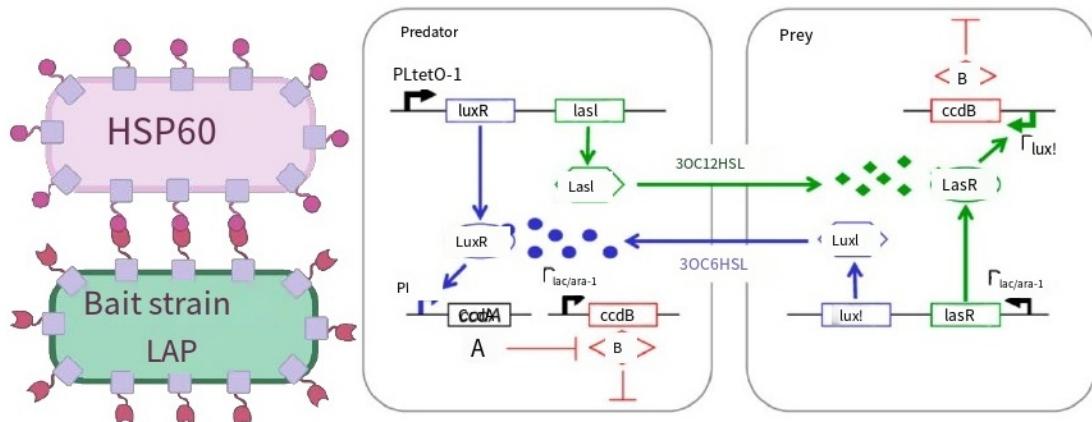
EcN was further chemically modified with peroxidase-like MNo2 nanoparticles to alleviate the hypoxia problem in the tumor microenvironment by reacting with endogenous hydrogen peroxide and generating oxygen in situ. The engineered bacteria were surface-sulfurized and bonded to gold nanoparticles (aunPs) deposited on the surface of MNO2 NPs. These nanoparticles can react with GSH in tumor cells to produce GSSH. The engineered bacteria can survive on lactic acid and compete with tumor cells for glucose availability, prevent lactic acidosis, and ultimately promote the transformation of tumor-associated macrophages (Tams) from a non-inflammatory to an inflammatory phenotype.

Intestide

Short peptides have many superior properties, such as higher stability and lower immunogenicity, making them beneficial for human intestinal stability and safe absorption. However, existing methods for delivering short peptides to the gut still have many limitations. To establish a safe and efficient delivery system for short peptides, we engineered two different strains: peptide producer and peptide regulator. Our design consists of three modules - secretion module, adhesion module and QS (quorum sensing) module. The secretion module, present only in the short peptide producer, fuses the recombinant transmembrane protein Lpp'ompA to the short peptide with an enterokinase cleavage site in between. The engineered bacteria colonize the gut and express the fusion protein. The fusion protein will be localized to the outer membrane of the engineered bacteria and cleaved by enterokinase in the gut to release the short peptide and complete the delivery. The producers and regulators express Hsp60 and LAP adducins, respectively. Naturally occurring Hsp60 protein is present on the surface of the gut. The engineered bacteria expressing LAP adhere to the gut, and the engineered bacteria expressing Hsp60 adhere to the engineered bacteria expressing LAP, and so on, forming a bacterial layer that stably adheres to the gut. QS safety modules exist in the producers and regulators of short peptides. Based on the principle of group regulation, the number of groups of producers and regulators is adjusted to obtain the optimal proportion of short peptide production.

To establish a safe and efficient delivery system for short peptides in the intestine, we selected E.coli Nissel 1917 as our chassis strain. It is a well-recognized and effective probiotic for the treatment and prevention of various intestinal diseases or intestinal inflammation. In order to successfully achieve the secretion, adhesion and population regulation functions, we introduced our designed plasmid into EcN, and carried out a series of verification experiments such as Western Blot and immunofluorescence.





The above diagram shows the fusion protein of secretion module Lpp'OMP_A fused with short peptide

Bottom left diagram of
adhesion module [1] bottom
right diagram of QS module [2]

References:

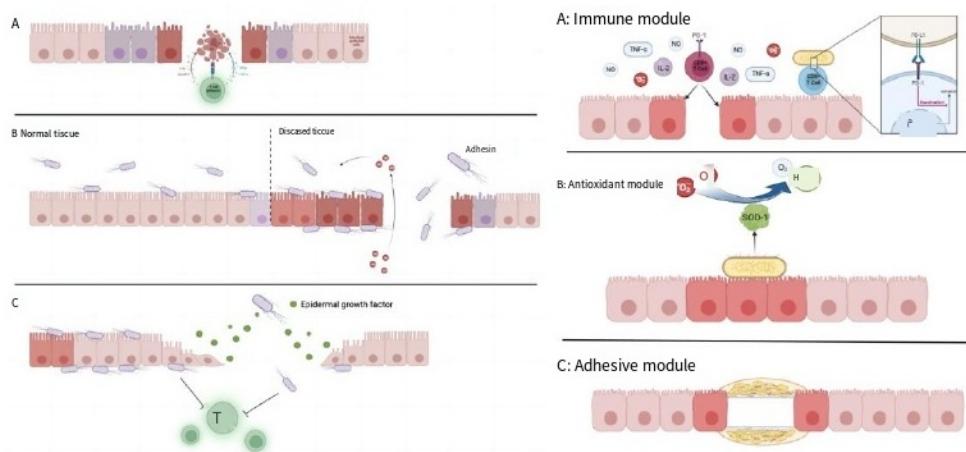
- [1] Timmis K, Timmis JK, Brüssow H, Fernández LÁ. (2019) Synthetic consortia of nanobody-coupled and formatted bacteria for prophylaxis and therapy interventions targeting microbiome dysbiosis-associated diseases and comorbidities. *Microb Biotechnol*. 2019 Jan;12(1):58-65.
- [2] Balagaddé FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. (2008) A synthetic Escherichia coli predator-prey ecosystem. *Mol Syst Biol*. 2008;4:187. doi: 10.1038/msb.2008.24. Epub 2008 Apr 15.

NJTech-China-A

Today, the prevalence of diabetes is increasing year by year. How to treat the growing diabetic population has become a worldwide problem. D-tagatose, as a functional sugar, is a potential therapeutic molecule for type II diabetes. However, there are few reports on the natural 4-tagatose - epimerase that catalyzes the synthesis of tagatose from fructose. At present, the production of tagatose from fructose still has some problems, such as few enzyme sources and low conversion rate. Therefore, the aim of this project is to screen new enzymes with potential tagatose 4-epimerase activity by gene mining. This project combines the techniques of protein engineering, molecular docking and molecular simulation. Through iterative mutagenesis, the conversion rate of tagatose 4-epimerase was further increased to meet the needs of industrial production. In this project, the maximum conversion rate of the mined new tagatose 4-epimerase was increased to 37%, and the maximum yield reached 24 g/(L·h). At the same time, three strategies were proposed to solve the inclusion body problem during the expression process of the novel enzyme: fusion tag, molecular chaperone and fermentation optimization. In addition, a high-throughput screening method based on the tagatose specific green fluorescence biosensor was proposed to screen the best performing mutants to improve the screening efficiency.

NJTech-China

Inflammatory bowel disease (IBD) is a chronic non-specific intestinal inflammation with unknown etiology and no cure. The pathogenesis of IBD is complex, the effect of traditional treatment is not ideal, and patients often face problems such as inflammation recurrence and increased economic burden. In this project, the probiotic *Escherichia coli* Nissle 1917 (EcN) was used as the chassis cell, and five functional modules were designed based on the thinking of synthetic biology. Among them, the sensing module SoxR/SoxS responsive promoter will regulate the opening of the whole gene circuit. By sensing the excess NO in the IBD intestinal environment, we ensure that our gene circuit is only opened in the IBD environment, which ensures certain biological safety and system specificity. The main therapeutic modules include programmed cell death ligand 1 (PD-L1) in the immune module, mussel foot protein (Mfp) in the adhesion module, and superoxide dismutase (SOD) in the antioxidant module, which are used to inhibit immune overreaction, repair damaged intestinal sites, and eliminate reactive oxygen species. The last safe lysis module, PhiX174E, is the ultimate guarantee of biosafety. When the number of bacteria reaches a certain level, it can automatically lyse bacteria. This will not only ensure that our engineered bacteria do not overproliferate in the gut, but also better release our therapeutic modules into the gut environment to achieve better therapeutic effects.



HUBU-4-CHN

CRISPR-Cas9 system, as the third generation gene editing technology, has been widely used in the clinical field. The 2020 Nobel Prize in Chemistry was awarded to two scientists who invented CRISPR-Cas9 technology, which marks the importance and impact of the technology. Subsequently, the research boom of CRISPR-Cas9 technology has mushroomed, and remarkable progress has been made in the fields of biomedicine and molecular diagnostics. However, the rapid, efficient, and accurate delivery of CRISPR-Cas9 systems into cells or organisms remains a challenge. In recent years, researchers have developed a variety of vectors for the delivery of CRISPR-Cas9 system, including viral vectors, liposome nanocarries, and exosomes. However, the selection of vectors with high safety, good targeting, and high economic efficiency is still a key issue.

Bacterial outer membrane vesicles (OMVs) released by Gram-negative bacteria are a promising drug delivery system. Escherichia coli Nissle 1917 (EcN) has been widely studied and used as a non-pathogenic and non-immunotoxic probiotic. In this study, we constructed a safe, efficient and adaptable CRISPR-Cas9 RNP delivery system using EcN as a chassis cell.

First, we genetically engineered wild-type EcN chassis cells by knocking in T7 RNA polymerase to achieve efficient expression of target proteins using the T7 promoter. The Cas9 protein was then co-transformed into EcN with the sgRNA co-expression plasmid and the plasmid containing the surface display element. The engineered EcN bacteria were prepared into protoplasts, extruded by a liposome extruder, and filtered through molecular sieves with different pore sizes to obtain appropriate sizes of OMVs.

To adapt the OMVs to different application scenarios, two strategies were adopted to display the targeting elements on the membrane surface. One is to express the fusion protein of ice nuclear protein InaK and Im7, and take advantage of the high affinity between Im7 and colitin E7 (CE7) to combine the engineered variant of CE7 with Im7 to display different targeting elements on the surface of OMVs. Another strategy is to fuse the Z domain on the extracellular membrane and use its binding property to the Fc region of the antibody to display the commercial monoclonal antibody. The OMVs generated by the above engineering engineering can be used for targeted delivery to different organs, tissues, and cells.

Finally, we will use the constructed Cas9 RNP OVs delivery system to perform targeted gene editing experiments in human cells to evaluate the safety of the vector and the efficiency of gene editing, and lay the foundation for the clinical application of this gene editing delivery platform.

10. Author list

CAI Kailing	Li Lin	Liu Heyu	Zhou Yufan	Sun Yuhao	Chiho Gu
Ho Mi Hwan	Zhu Hongyu	Yan Xujie	Lu Chenxi	Xing Yunqi	Lei Murong
Zhao Yanzong	Su Zhaoyin	Li Qicheng	Yu Shengan	Sun Gu Zihan	Shen Jiawen
Zhou Xinquan	Yang Xinyi	Chen Xinjie	Zhou Suhui	Yang Hongdan	Zhu Chunling
Wang Tao	Chu Huixin	Li Shushu	Li Qike	Hua Qihui	Yu Yingying
Ma Jiacan	Zhou Dekun	Gao Jingyuan	Chen Xinnan	Gao Jiaxin	Chen Jingyan
Hu Chenhao	Chen Yuyan Yan	Zhang Wenrui	Yan Yifei	Huang Furong	Zhu Luping



(In no particular order)

16: Legal statement

The copyright of this report belongs to all iGEM teams involved in the preparation. Without written permission, no institution or individual may reprint, reproduce, publish or cite it in any form. If it is cited and published with the consent of all iGEM teams involved in the preparation, it must be used within the allowed scope and indicate the source. The report shall not be quoted, abridged or modified in any way contrary to the original intention.

The author of this report has professional research ability, and guarantees that the data in the report are from legal and compliance channels. The opinion output and data analysis are based on the analyst's objective understanding of the industry, and this report is not inspired or influenced by any third party.

The views and information presented in this report are for reference only and do not constitute any investment or academic advice. This report is issued only as permitted by the relevant law and is provided for informational purposes only.

The information in this report is based on publicly available information. The iGEM team involved in the preparation of this report has the final right to interpret the accuracy, completeness and reliability of the information. The information, opinions and speculation contained in this report only reflect the judgment of the author and the report on the date of publication. Descriptions in previous reports should not be relied upon as a basis for future performance. The Alliance does not guarantee that the information contained in this report is up to date. At different times, all iGEM teams involved in the preparation may issue reports and articles that are inconsistent with the information, opinions and conjectures contained in this report. The information contained in this report is subject to change without notice by all iGEM teams involved in the preparation of this report, and the reader should be aware of any updates or modifications. Any institution or individual is responsible for and liable for all consequences of any activity carried out using the entire contents of this report.
