ABC

A Handbook of Anti-Biofilm Community

PRODUCERS

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WHU-China
Wuhan University

(IN ALPHABETIC ORDER)

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DUT_China

Project Introduction

Civilization has entered a new stage, and human society is paying more and more attention to sustainable development. Reiterating the impact of Antimicrobial Resistance (AMR) on public health, the United Nations Secretary-General stated in May 2019: “Antimicrobial resistance is a global threat to health, livelihoods and the achievement of the Sustainable Development Goals (SDGs)”, we are also looking for new ways to treat diseases. With multiple breakthroughs in phages engineering resurfacing in public attention, a new form of treatment has given us an alternative approach.

We are looking for a more effective, convenient and low-cost way to help people with phage therapy, now and in the future.

In recent years, yeast artificial

has been becoming the mainstream method for phage engineering. Faced with the difficulty of manipulating phages with large genomes via the classical YAC platform, we improved it to rebuild phage T4. In our research, we explore the assembly of megabase-sized fragments of phage T4 genome and optimize the condition of electroporation of megabase-sized DNA, and we upgrade the classical YAC strategy to make it possible to engineer any phages at many points simultaneously. This will greatly make phage therapy research take a step forward while maintaining the world balanced, beautiful and healthy.

HK_HCY
Tsuen Wan Public Ho Chuen Yiu Memorial College

Team Introduction

As the greenhouse effect around the globe is snowballing, an increasing number of environmentally-friendly products have been invented to mitigate the situation. Biofuel (biodiesel) is one of them. Biofuels are now introduced to many different energy consumption processes, for instance, transport and energy generation. We believe that biofuel would be indispensable in the near future so it is important to enhance the quality of biofuels.

According to our research, biofilms that formed in biofuels have brought detrimental effects to the production and energy efficiency to the fuels. Among the microorganisms forming biofilms in the fuel, Lactobacillus fermentum accounts for the majority. To help reduce the biofilm formation in the fuel, our team targets quorum sensing(QS) in the biofilm with two approaches. The first one is to lower the level of autoinducer-2(AI-2) by degrading the signalling molecules with kinase LsrK. Another approach is to inhibit the activity of LuxS (AI-2 synthase) aligning a putative high-affinity peptide ligand. Our method is to express the recombinants in E. coli, then extract its cell free lysate and insert into the biofuel to interfere with the QS mechanism.

SUSTech_Shenzhen

Project Introduction

Pseudomonas can utilize simple and inexpensive carbon and nitrogen sources as exogenous nutrients to synthesize products for industrial purposes. In addition to the primary metabolites that can be used as important catalysts for the synthesis or degradation of certain compounds, Pseudomonas can also synthesize a variety of secondary products, including six types of complex compounds or polymerizations that have industrialization prospects or have achieved industrialization, such as alginate and vitamin B12.

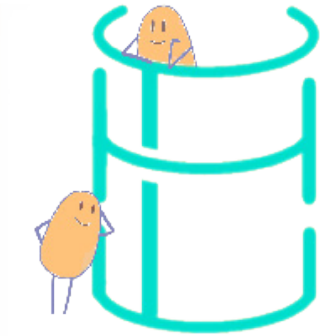
Our team present a chemical biology approach to develop a broad-spectrum molecule that can protect Pseudomonas from phages infection. We attempt to utilize the Target-Directed Screening technique to find a small molecule compound which disrupts the translation of a critical protein during phage infections, and we have successfully found a compound with biological activity recently. In the presence of this small molecule inhibitor, the phages will be disabled to infect Pseudomonas, thus providing a solution to the problem of bacterial infection in large-scale industrial production to a certain extent. Also, we can use the small molecule inhibitor and phage to regulate the biofilm application in sewage treatment.

Tsinghua

Project Introduction

The project of Tsinghua 2020 iGEM group, NO mediated biofilm allayer(NBA), is purposed to utilize engineered E.coli to produce signal molecule nitric oxide to remove the biofilm of P.aeruginosa. when this spy germ senses the QS molecule BHL from P.aeruginosa bioflim, it is expected to be activated and express exogenous gene NOS(Nitric Oxide Synthase), producing NO and leading to the dispersion of biofilm. With the great advantages of synthetic biology techniques, the project NBA aims to provide a feasible solution to P.aeruginosa biofilm contamination in industry and household devices.

Scanning the QR code to read our Popular science cartoon, "kill the biofilm".



UM_MACAU

Project Introduction

Our team visited a famous aquarium near our university, the Chimelong Ocean Kingdom. We learned that the cleaning of biofilm is a hard but important work due to the rapid growth of biofilm. The physical cleaning method of biofilm, which needs divers to dive to the aquarium with brushes to scrub the biofilm away, is the only method using now. However, it is not effective, time-consuming and requires manpower. Moreover, the high pressure in large aquarium gives damage to the divers' body. It is also difficult to clean the corners and crevices in a small tank. Last but not least, there are some fragile creatures, such as the jelly fish and the corals, divers need to pay more attention on them to not hurting them.

Through the visit of the Chimelong Ocean Kingdom, we know more about the importance and cleaning method of biofilm. In order to those problems, we designed a better method: Biofilm Removing Bacteria.

We engineered the BL21 bacteria strain of E.coli. The T7 promoter drives the expression of LuxR, which recognizes the signaling molecule AHL which is secreted by biofilms. Combined with AHL, LuxR would bind to the pLuxR to express the adhesive protein, Ag43, and digestive enzymes, DNase and protease. Our engineered bacteria will bind to the biofilm and degrade it efficiently.

WHU-China

Project Introduction

We aim at rationally engineering probiotics to address the problem of nosocomial infections in respiratory tract, especially ventilator-associated pneumonia (VAP) during COVID-19 pandemic. The chassis of our project is Escherichia coli Nissle 1917, recognized as the most amenable probiotic, and the target pathogen is Pseudomonas aeruginosa, a representative nosocomial pathogen notorious for its quorum sensing-based virulence behaviours. We construct two modules to endow our chassis with the capability as 'the negotiator':

(i) Quenching Module: heterologously overexpressing quorum quenching enzymes to parley with 'criminals' (*P. aeruginosa*) by degrading AHLs;

(ii) Sensing Module: sensing PQS and excreting appropriate amounts of chemokines to recruit 'police squads' (immune cells) for eradication. Notably, we leverage *E. coli* lysate-based cell-free system for rapidly prototyping genetic parts of interest, in order to accelerate the design-build-test-learn cycle of our project as well as to give insights into the elegance of cell-free expression renaissance.

DUT-China

Meet our friends!

I am T4 phage, the phage material most often active in human laboratories. My appearance is as delicate as if I had been designed by a craftsman with a ruler -- a regular icosahedral head filled with DNA, the genetic information that defines my identity; A hollow tubular trunk with tiny fangs at the end, like a syringe, helps me inject genes into bacteria;

And finally six long tails, with which I could cling fast to the surface of the bacteria, and make the poor creature my prey, or new home, as it were. Once my genetic material settles in the bacteria, I can let it produce all the parts I need and assemble another T4 phage. When I—or our team is strong enough, I can leave this depleted shell and look for the next target.

T4

You may think I'm a dangerous guy, but don't worry, because I only prey on E. coli, as for the rest of the cells, I can't even attach to their surfaces. It is precisely because of this specificity that I have the potential to fight against a single strain, and I am gradually exerting a unique value in this field.

M13

I'm an M13 phage, a small but powerful phage. Like my bacteriophage companion T4, I will specifically look for E. coli and infect them. I also have many characteristics: First, I'm much smaller than a T4 phage, and I don't have a big brain to hold the genetic material, so my DNA is much shorter.

Second, I also lacked a long tail, so the appearance became a featureless rod-like shell, and I was classed as a filamentous phage. However, I still have a lot of use: besides infecting E. coli, I can also be modified to transport foreign genes, which is also of great value in the field of genetic engineering.

Lambda

I am lambda phage, a relatively mild and friendly phage. Although I look similar to T4 bacteriophage, I am different from T4 bacteriophage which is keen to destroy host bacteria. The genetic material injected into E. coli by me will not immediately start to guide the production of various components, but will first form a closed DNA circle.

The ring replicates along with the division and reproduction of E. coli itself. Only when the living environment changes drastically will I initiate the synthesis of new phages. This gentle characteristic allows me to bring some exogenous genes to E. coli, so I can often be seen in the laboratory.

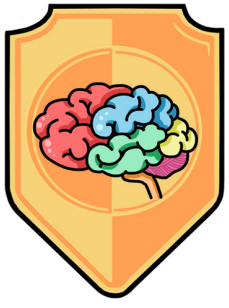
IgG

I'm an IgG antibody, a type of mammalian immunoglobulin, and the "main force" against infection. If a pathogen, be it a bacterium or a virus, invades the body, our figure will be spread all over the serum and various tissues. Second, I also lacked a long tail, so the appearance became a featureless rod-like shell, and I was classed as a filamentous phage. However, I still have a lot of use: besides infecting E. coli, I can also be modified to transport foreign genes, which is also of great value in the field of genetic engineering.

CPISPR

My name is CPISPR, which is an abbreviated name, and my full name is "Clustered short palindrome repeats at regular intervals". In case you still don't understand who I am, let me start with the CRISPR/Cas9 system, which helps people find a specific sequence on a long DNA sequence, cut it down, and replace it with a pre-designed sequence on the other end. This whole process is under people's control, so it is possible to realize the site-specific editing of genes. And the basis of this system is a repeating sequence that evolved by bacteria to resist phage intrusion, which is me, CPISPR. In this way, an "immune system" has become a powerful tool for gene editing and is playing a huge role.

The blood-brain barrier

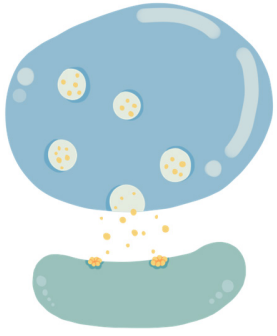


I am the blood-brain barrier, an important wall protecting the brain. Tightly arranged endothelial cells, continuous intact basement membranes, and glial membranes made of astrocytes are the main structures of this city wall. I strictly monitor all the blood flowing to the brain, and any "stowaway", especially pathogens trying to enter the brain tissue, cannot get what they want. They can only be turned away, trapped in the capillaries, waiting for immunity The system clears them.

Nutrients

In this war called natural selection, we are neutrals. We provide all life forms-whether they are high or low, consumers or producers, natural enemies or prey-with the matreials and energy needed for life activities. Although we don't know whether we will be used to build an immune barrier or produce toxins, as long as there is life to maintain, we will lend a helping hand.

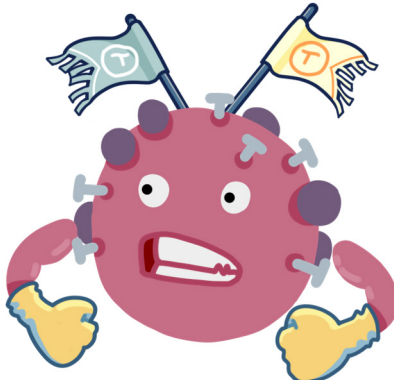
Perforin



I am perforin, a glycoprotein from cytotoxic T lymphocyte and cytoplasma granula of NK cells. I can form a polyperforin tubular channel on the cell membrane and quickly dissolve and destroy the cells!

Cytotoxic T cells

Virus and tumor cells shiver at me. The cytokines I secreted is the right hand of other immune cells! Natural killer cells and I are the guardian angels of the body.



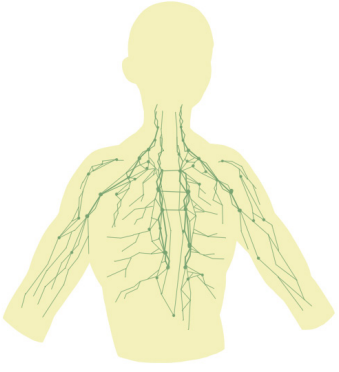
Kanamycin

I am an antibiotic that binds to the 30S ribosome to misread the mRNA interfering with the protei n synthesis with subsequent bacteri al death. Although a lot of bacteria are afraid of me, but there are also lots of them which have immunized me...

Macrophage

I can eat harmful bacteria, foreign bodies and aging cells, and also make some disguised antigenic show their prototype when I reach the lesion region by chemotaxis. Also, I can secrete lysozyme and complement to participate in the defensive reaction of organism at the same time.

Lymphatic circulation



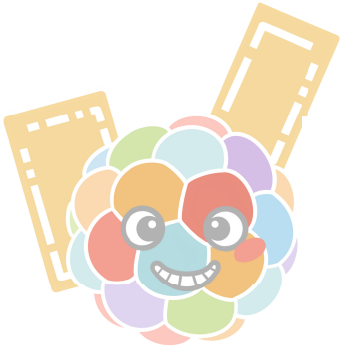
I widely exist in mammals, where tissue fluid enters the lymphatic system via capillary lymphatics to form a lymphatic circulation. I am mainly responsible for recovering protein, transporting fat and other nutrients, regulating the fluid balance between plasma and tissue fluid, clearing red blood cells from tissues, the bacteria invading the body and other particles. I play an important roal in the circulatory system. And I provide the assistance and effective supplement with blood circulation. Organism can not maintain normal life activities without me.

Penicillin

I am the first antibiotic discovered by human beings and one of the most commonly used antibiotics. It could be argued that my emergence marked the dawn of a new era in the treatment with antibiotics.

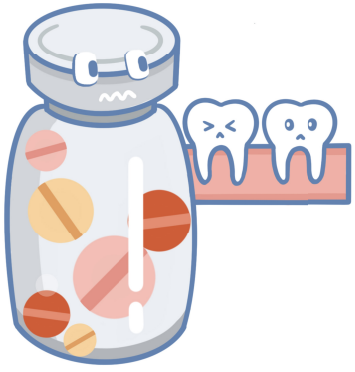
I can inhibit transpeptidases involved in the synthesis of bacterial cell wall mucopeptides and affect the formation of cell walls. So that bacteria lacking cell walls die of expansion deformation.

Growth and development



Growth refers to the growth and morphological changes of various organs and systems of the body, which is the change of quantity while development refers to the differentiation and perfection of cells, tissues and organs and the maturity of functions,
which is a qualitative change. Growth is the material basis of development, and the state of development and maturity is reflected in the change of growth amount. Human growth and development is the maturation process from oosperm to adults.

Tetracycline



I am a broad-spectrum antibiotic that can bind to the mitochondrial 70S subunit and inhibit the synthesis of mitochondrial protein. So bacteria, Rickettsia, chlamydia or mycoplasma are afraid of me. However, due to antibiotics abuse, the resistance of various pathogenic bacteria towards me has become quite prevalent.

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What are biofilms?

Biofilms are formed by one or even more types of microorganisms on surfaces. These organisms include bacteria, fungi and protists. Biofilms contain extracellular polymeric substance(EPS), which allows them to bind on the attached surfaces.

One of the common biofilms formed on humans is the dental plaque. It can be found on the surface of our teeth. In nature, biofilms are also commonly found underwater, underground or even on animal and plant tissues.

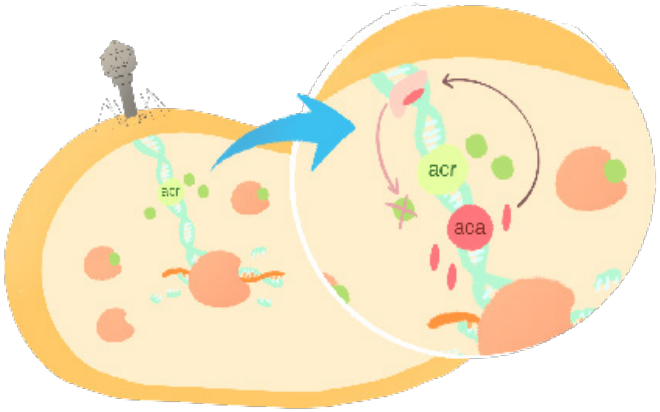
What is quorum sensing?

Quorum sensing(QS) is also called “cell communication”. It is important for bacteria to exchange information among them. This process involves extracellular signalling molecules ,autoinducers, they are then detected by the receptors on another cell existing in its cytoplasm or membranes.

Bacterias are classified into gram-positive and gram-negative, conducting different QS systems. The former uses autoinducing peptides(AIPs) while the latter uses small molecules, autoinducers(AIs). After these substances are detected by the cell, corresponding responses are made.

SUSTech_Shenzhen

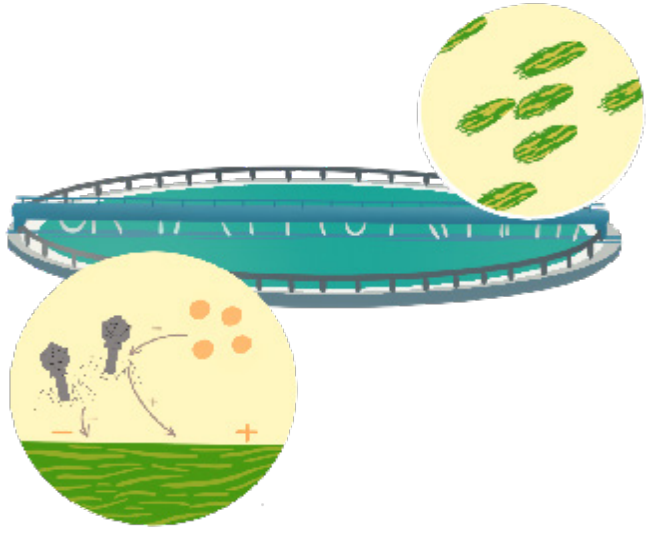
Fight Biofilm with CRISPR-Cas



The CRISPR-Cas system is an adaptive immune system existed in bacteria and archaea, which can resist the invasion of foreign genetic elements, such as bacteriophage. Phages express anti-CRISPR (Acr) proteins to inhibit the CRISPR- Cas system, preventing their own genomes from being destroyed.

Most acr genes are adjacent to anti-CRISPR-associated genes, which encode proteins that bind to DNA. Recent research showed that Aca proteins could act as crucial repressors of the transcription of acr genes. In the absence of Aca proteins, the high transcriptional level of acr genes disrupts the expression of downstream genes, which can result in the death of phages.

Pseudomonas has great potential in bioremediation due to its strong degradability, so they can be used in sewage treatment plants. However, phages in the environment are a major threat to the application of Pseudomonas in sewage treatment.



According to previous studys, we believe the compound we found could be applied to protect Pseudomonas from phages invasion. At the same time, phages and the compound can be used together to control the biodegradation membrane made by Pseudomonas, so that its thickness can be kept within the range of the best degradation effect.

Introduction to Synthetic Biology

At the beginning of 2019, synthetic biology was rated as one of the most anticipated biotechnologies in 2019 by Nature journal, one of the most authoritative and prestigious academic journals in the world. The field of synthetic biology has also "lived up to expectations" and has achieved a series of highly anticipated achievements. So what is the mystery of such mysterious synthetic biology that fascinates scientists?

Israeli researchers have created a new strain of E.coli that uses carbon dioxide as a carbon source instead of conventional organic compounds. Israeli researchers used metabolic redistribution and laboratory evolution to transform E.coli into autotrophs. This strain can also consume carbon dioxide in the air to provide itself with nutrition and energy.

This feat of synthetic biology demonstrated the amazing plasticity of bacterial metabolism, and this achievement also provides a framework for future carbon-neutral bioproduction.

Except the viruses in nature, the genes of every organisms are composed of 4 kinds of bases. The 4 genetic codes of A, G, C, and T that have accompanied us for billions of years constitute the DNA of organisms in the world, but a new study shows that scientists have expanded the genetic code nucleotides from four to eight. These nucleotides are similar in appearance and behavior to natural nucleotides and can even be transcribed into RNA.

The essence of synthetic biology is to use the DNA bases to synthesize the biological gene sequence we want, and this amplified genetic code system can provide new clues for finding more complex molecular structures that can support life. Synthetic biology is not only a pure biological field, but also includes computer, information engineering and physics. In terms of computer digital signals, Ahmad "Mo" Khalil of Boston University and Caleb Bashor of Rice University and others have used synergy to design genetic circuits that can decode frequency-dependent signals and perform dynamic signal filtering. Synergy can be seen as a signal processing function, it can be used as an analog-to-digital converter, which is a device that can convert linearity into switching.

What is CRISPR?

In 1987, Japanese scientists discovered a weird DNA sequence in the genome of the large intestine rod. A certain segment of DNA will be repeated continuously, and there will be an interval of the same length between the repeated segments. Scientists named this regular sequence **CRISPR** (Clustered, Regularly, Interspaced, Short palindromic repeats)

The CRISPR system is a natural ‘immune system’ of prokaryotes, which is present in most bacteria and archaea. When they are invaded by a virus, the virus will inject its own DNA into the bacteria in an attempt to occupy the resources in the bacterial cell to replicate more viruses.

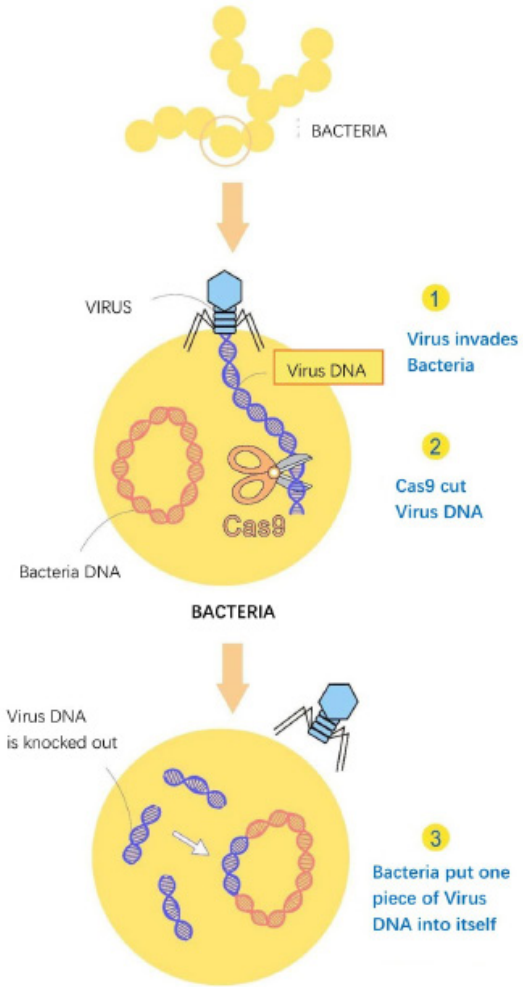


Figure. Virus is invading the bacteria
(Source: Research.yowu)

Bacteria can recognize and destroy viral DNA. After the bacteria resist the virus invasion successfully, they can extract a small piece of viral DNA and store it in a specific area of its own genome. When it encounters a virus invasion again, it can identify the virus according to the DNA fragment and cut the virus DNA to make it invalid with the enzyme Cas9 which can cut DNA. According to the characteristics of CRISIPR and Cas9, scientists have transformed it into the most efficient gene editing tool.

How to Use CRISPR to Edit DNA?

The key to gene editing is to find a molecular-grade scissors that can cut DNA without cutting it randomly. The endonuclease Cas9 can precisely lock DNA fragments with a piece of RNA. After the genes have been cut, how can I attach new genes? Since cells are born to automatically repair damaged DNA, as long as the correct genes are sent to the nucleus, they have the opportunity to be used by the cells to repair the gaps cut by Cas9 and complete gene editing.

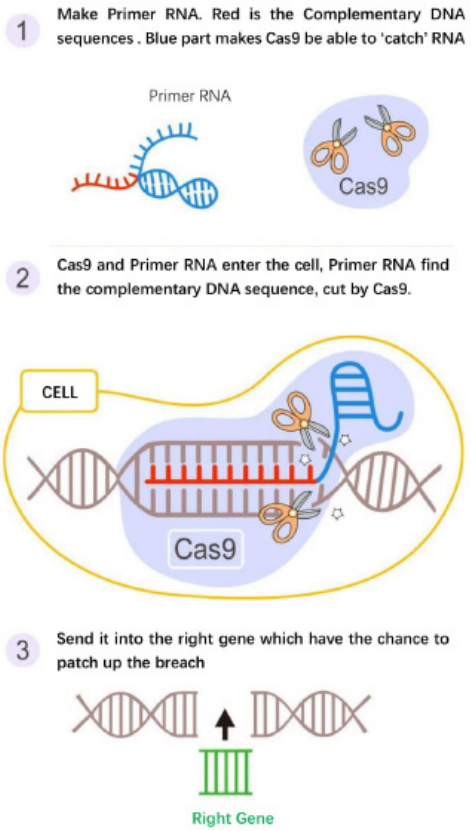


Figure. How CRISPR/Cas9 work
(Source: Research.yowu)

Bottleneck Period of Gene Editing Technology

Unfortunately, the Cas9 scissors still have many technical problems and are not suitable for direct treatment. It is not 100% accurate when applied to human DNA. Therefore, there is no guarantee that Cas9 cuts the right position every time. The wrong position can cause serious consequences. It is safer to take the cells out and edit them in vitro to ensure that it is accurate before putting them back into the body, such as modifying the genes of immune cells to treat immune diseases. CRISPR can also be applied to stem cells to edit genes in vitro, and then put them back into the body to differentiate into various healthy cells. Although CRISPR is a sharp pair of scissors in gene editing, it is still in a state of improvement. For example, sometimes CRISPR/Cas9 enzymes will leave the target cell and act on other normal cells. Sometimes, even if the target is correct, the expression effect may not be the same, and affects gene expression.

Biofilm

What is Biofilm?

Biofilm is a bacterial film formed by a group of bacteria, which never stop growing. Bacteria produce extracellular polysaccharide polymers to make bacterial cells adhere to each other to form a membrane-like structure. This is a self-protective mode of microorganisms. As long as the conditions met the bacterial reproduction, the bacteria will grow and multiply quickly to form a film. Biofilm is commonly found on glass hoses, rocks, driftwood, and even biological surfacess in aquariums.

Conditions for Biofilm Formation

Types of bacteria: There are different bacteria in the fish tank. We cannot distinguish with naked eye. We need to pass professional inspection methods to know which type of bacteria the biofilm belongs to.

Adhesion surface: It is difficult to adhere to a smooth surface, on the contrary, it is very easy to adhere to a rough surface. Such as stones, driftwood, and plastic pipe walls.

pH: pH is an important factor affecting the growth and reproduction of bacteria. There are acidic, neutral, or alkaline corresponding bacteria. Most bacteria prefer growing in a neutral environment, but there are exceptions.

Temperature: Temperature will affect the growth rate. Different bacteria adapt to different temperatures and reproduce at different speeds. Generally, the higher the temperature of the bacterial flora, the faster the reproduction speed, and vice versa, but there are exceptions. For example, nitrifying bacteria are designated to reproduce fastest at 25℃.

Water pressure: Under strong water pressure, the cell structure of bacteria will be destroyed, causing its death. For example, E.coli will become 5-10 times longer under a pressure of 40 MPa, causing changes in the permeability of the cell wall and cell membrane, which will eventually rupture and die.

Biofilm in Daily Life

The following pictures show typical fish tank biofilms. They are white flocs, and composed of polysaccharides and lipids (extracellular polymeric substances/EPS). It is a multi-layer material composed of bacteria, fungi and other eukaryotes. The source of its constituents is the carbon provided by fish feed that converted into fish metabolites. Such carbon is called dissolved organic carbon (DOC) and combines with oxygen on the water surface.

Biofilm is generally formed in new water (new fish tanks). At this time, the water environment is unstable (pH, temperature, oxygen) and is not suitable for the growth of bacteria. Therefore, the bacteria activated their own protective mechanism, which is "grouping." For gram-negative bacteria, their specific inducer for "grouping" is N-acetyl homoserine lactone (AHLS), while for gram-positive bacteria the inducer is an oligopeptide. Furanone exists in both gram-positive and negative bacteria. The bacteria that form biofilm in water are generally *Acinetobacter spp*., *Pseudomonas spp*., *Klebsiella spp*., and *E. coli.* , *Staphylococcus aureus*, *Aeromonas spp* and *Enterococcus spp*.

This biofilm adheres to the soil. The microorganisms in soil also produce EPS. Both the biofilms in water and that exposed to air are in white form. It suggested that they can be formed from similar bacteria. For the aquatic creatures, the biofilm in water does not bring them benefits or even compete for oxygen with them. EPS is also produced when microorganisms form biofilm in soil. It benefits the soil and plants. EPS helps gathering soil particles, maintaining plants humidity and captuing nutrients. The application of EPS in soil agglomeration and the technology of EPS helping plant applications are under development.

If we have not cleaned our water bottle or kettle for a few days, and a small amount of water is left, the inner bottom will indeed form a viscous biofilm. Using a bottle with biofilm may ingest harmful germs. However, the substance formed at the bottom of the water bottle may also be scale. In the process of boiling water, the carbonate, calcium and magnesium ions combine to form calcium carbonate and magnesium carbonate precipitate, then become saturated and form scale. The scale does not harm the human body. Limescale is generally pale yellow solid, and does not have the strong viscous sensation of biofilm. There is also chances that both of them exist at the this time, so we encourage everyone to regularly clean their water bottles, especially the kettle.

Fish Culturing

The common treatment of fish tank with biofilm is to use a water purifying reagent or replace the water every two to three days to keep the water clean. When the water in the fish tank turns yellow or muddy, there are obvious tiny particles, or bacterial biofilm appeares. In addition, adding commercially available water purifying reagent, we choose to replace the water or clean the fish tank with brush usually.

Adding chemicals is very efficient. A good-quality water purifying reagent only needs a few drops adding into the fish tank. There will be flocculent white precipitate appear at the bottom of the fish tank immediately. After waiting for about 20 minutes to let them settle completely, turn on the filter to remove the floc. Then, the water will become clear. It is based on chemical principles. The water purification reagent uses ion complexation to precipitate the organic matter in water, quickly condense the impurities in the fish tank which cannot be processed by the physical filtration system, and form large flocs that can be filtered by the filter cotton, so the water will become clean and clear.

The main components of physical detergents are zeolite powder, activated carbon etc., which are mainly particles that sink to the bottom and have the nature of adsorption. For the chemical agent, it is mainly water solvent. The common ingredients are polyalanine chloride, polyalanine ferric chloride, aluminum sulfate, ferrous sulphate. Among them, aluminum sulphate and ferrous nitrate have a stimulating and harmful effect on the eye epidermal mucosa of fish, while aluminum ions and ferric iron are toxic substances.

Physical agents are not harmful to water quality, but you should pay attention to the cleaning of bottom mat materials (filter sponge, sand). The chemical agents have a certain effect on fish and bacteria. These polymerizes have colloidal properties, so there is a high chance of agglomeration happen in the fish gills, causing serious damage to the fish because of gill blockage.

Most bacteria exist in the form of biofilms. Biofilm is some sticky substances secreted by bacteria, these substances fix the bacteria on a stable surface and the bacteria will use it as a base then multiply on it. With the increasing of bacteria, the thickness of biofilm will also increase, forming a visible gray-white bacterial biofilm. Nitrifying bacteria like other saprophytes, need to form biofilms before they start to reproduce. Therefore, they are mainly attached to the surfaces of objects in the aquarium, and even the surfaces of organisms, such as fish, glass, water pipes, fish tank landscaping, etc.

/There are many reasons for the turbidity of aquarium water, such as overfeeding, insufficient filtration system in the aquarium, insufficient number of nitrifying bacteria etc. However, changes of water or brushing of the tank frequently will destroy the stable fish tank ecosystem and have a certain impact to fish. The important bacteria in the aquarium---nitrifying bacteria. The main function of nitrifying bacteria is to reduce the content of nitrite in water. Nitrite is carcinogenic and harmful to fish. Secondly, nitrifying bacteria can also improve water quality. It can decompose food residues in fish tanks, excrement and clean up other organic matter.

WHU-China

What happens in our lungs during infection?

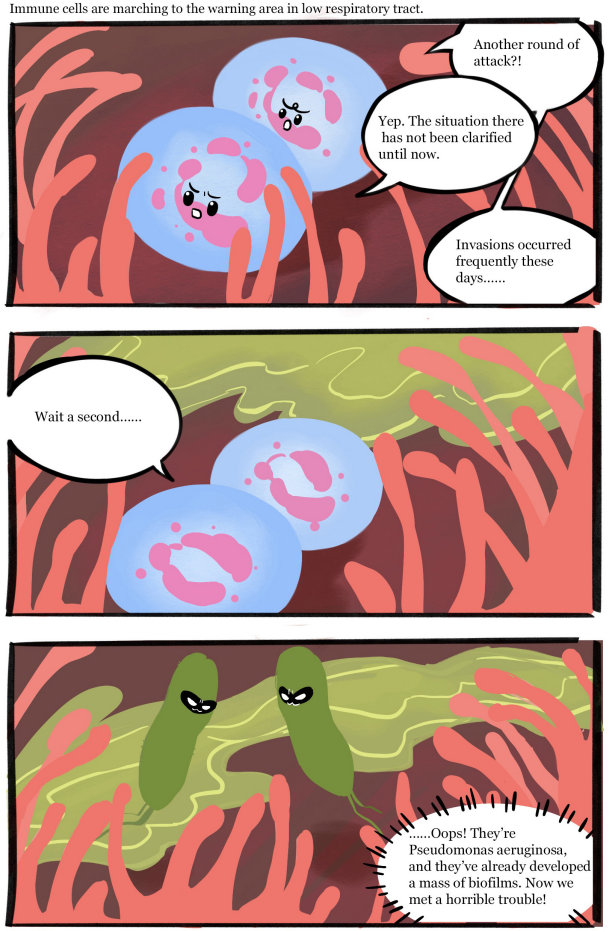
Ventilator-
Associated
Pneumonia

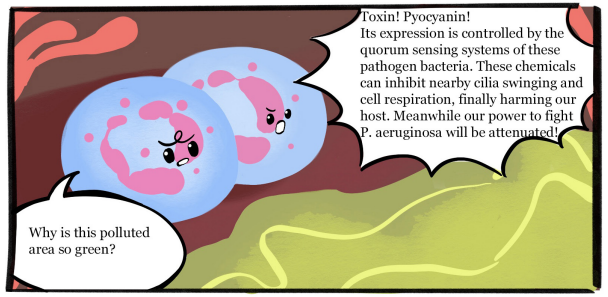
Police squads
-Neutrophils

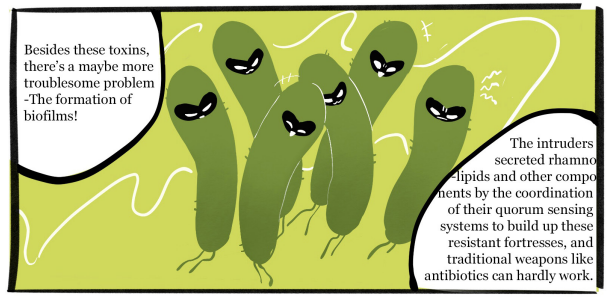
Criminals
-Pseudomonas aeruginosa

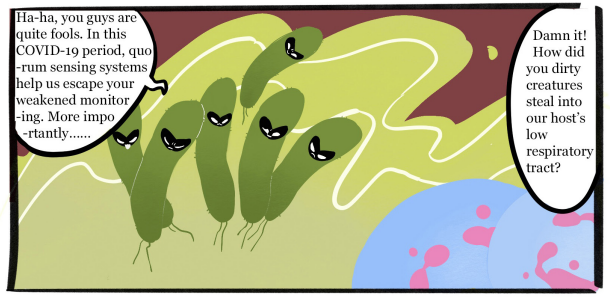
Negotiators
-engineered probiotics

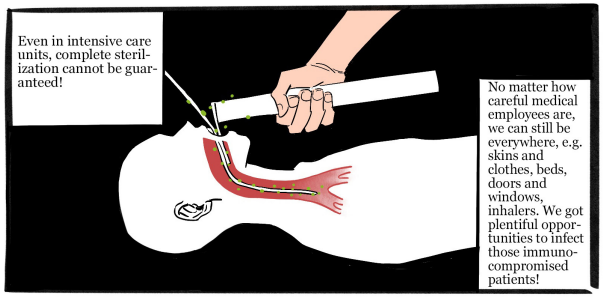
Immune cells are marching to the warning area in low respiratory tract.

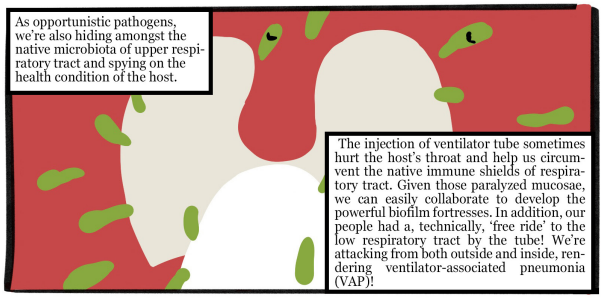


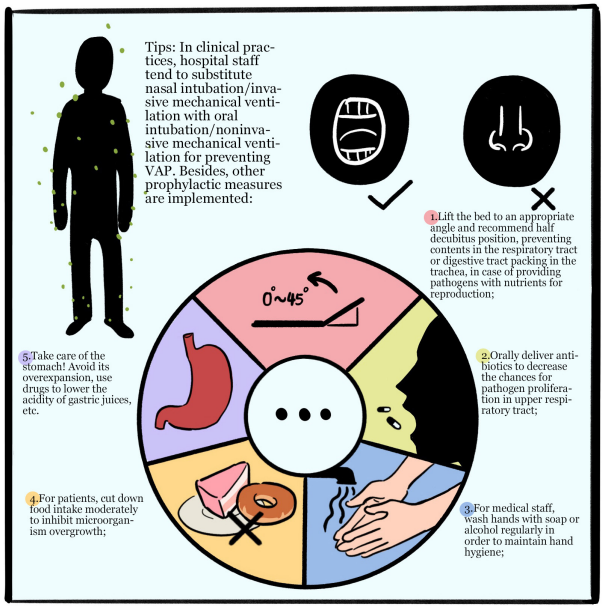

Why is this polluted area so green?
Toxin! Pyocyanin! Its expression is controlled by the quorum sensing systems of these pathogen bacteria. These chemicals can inhibit nearby cilia swinging and cell respiration, finally harming our host. Meanwhile our power to fight P. aeruginosa will be attenuated!


Besides these toxins, there's a maybe more troublesome problem -The formation of biofilms!
The intruders secreted rhamno lipids and other compo nents by the coordination of their quorum sensing systems to build up these resistant fortresses, and traditional weapons like antibiotics can hardly work.


Ha-ha, you guys are quite fools. In this COVID-19 period, quo -rum sensing systems help us escape your weakened monitor -ing. More impo -rtantly.....
Damn it! How did you dirty creatures steal into our host's low respiratory tract?


Even in intensive care units, complete sterilization cannot be guaranteed!
No matter how careful medical employees are, we can still be everywhere, e.g. skins and clothes, beds, doors and windows, inhalers. We got plentiful opportunities to infect those immunocompromised patients!


As opportunistic pathogens, we're also hiding amongst the native microbiota of upper respiratory tract and spying on the health condition of the host.
The injection of ventilator tube sometimes hurt the host's throat and help us circumvent the native immune shields of respiratory tract. Given those paralyzed mucosae, we can easily collaborate to develop the powerful biofilm fortresses. In addition, our people had a, technically, 'free ride' to the low respiratory tract by the tube! We're attacking from both outside and inside, rendering ventilator-associated pneumonia (VAP)!


Tips: In clinical practices, hospital staff tend to substitute nasal intubation/invasive mechanical ventilation with oral intubation/noninvasive mechanical ventilation for preventing VAP. Besides, other prophylactic measures are implemented:
1.Lift the bed to an appropriate angle and recommend half decubitus position, preventing contents in the respiratory tract or digestive tract packing in the trachea, in case of providing pathogens with nutrients for reproduction;
0°~45°
2.Orally deliver antibiotics to decrease the chances for pathogen proliferation in upper respiratory tract;
3.For medical staff, wash hands with soap or alcohol regularly in order to maintain hand hygiene;
4.For patients, cut down food intake moderately to inhibit microorganism overgrowth;
5.Take care of the stomach! Avoid its overexpansion, use drugs to lower the acidity of gastric juices, etc.

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