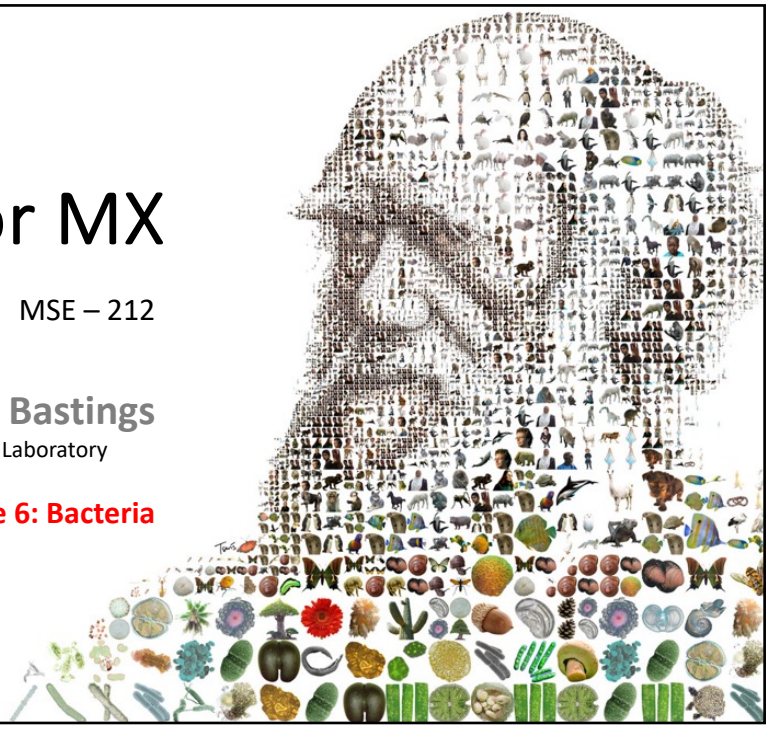
Biology for MX

MSE – 212

Prof. Maartje M.C. Bastings
Programmable Biomaterials Laboratory

Course 6: Bacteria



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Course Content

BLOCK 1: Introduction and engineering with cellular components

Lecture 1.	Intro to biology and cells	(February 21)
Lecture 2.	Proteins and protein based materials	(February 28)
Lecture 3.	DNA and DNA-based materials	(March 6)
Exercise 1.	*Proteins, peptides and DNA*	*(March 13)*

BLOCK 2: Inter- and intracellular action

Lecture 4.	ECM, adhesion and artificial matrices	(March 20)
Lecture 5.	Virus, antibodies and immune engineering	(March 27)
Lecture 6.	**Bacteria**	**(April 10)**
Exercise 2.	*Nanoparticles and Scaffolds*	*(April 17)*

BLOCK 3: Physics of biological processes

Lecture 7.	Receptors and targeting	(April 24)
Lecture 8.	Endocytosis	(May 1)
Lecture 9.	Signaling and communication	(May 8)
Exercise 3.	*Engineering functionality*	*(May 15)*
Lecture 10.	Revision and conclusion	(May 22)
Open office.	*Questions, discussion, exam prep*	*(May 29)*

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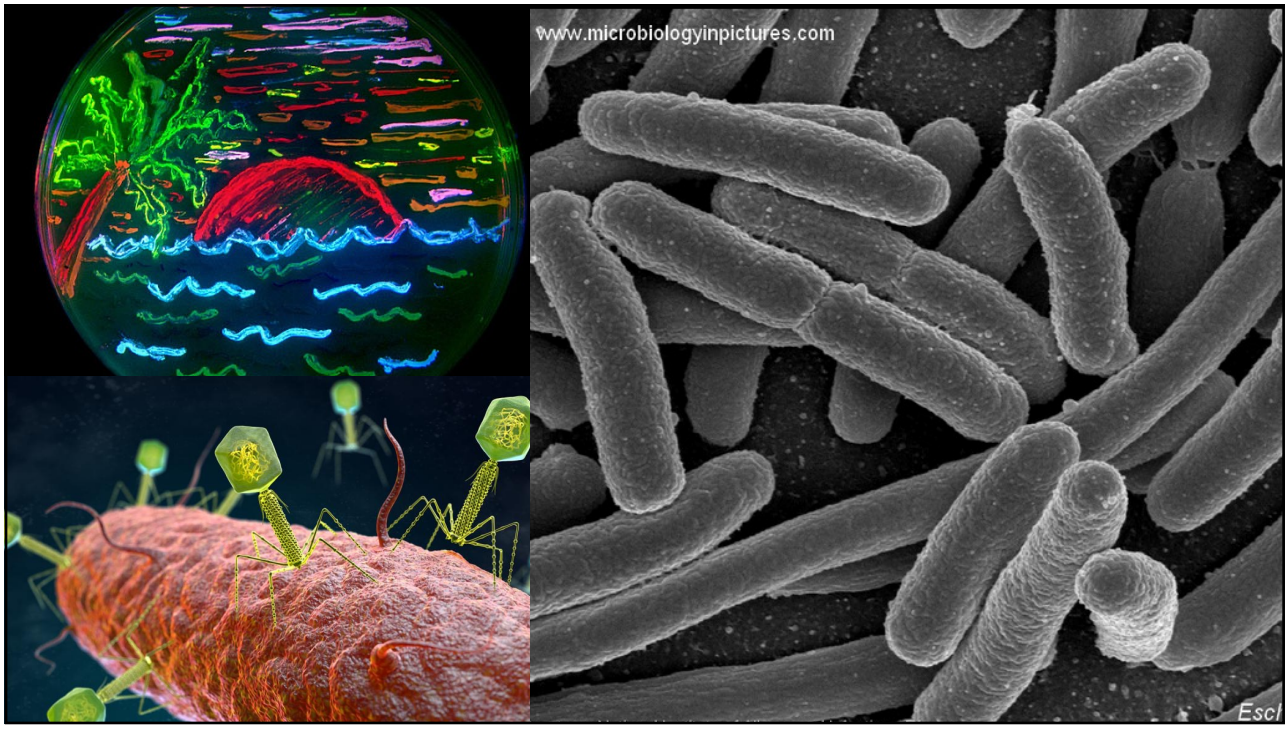

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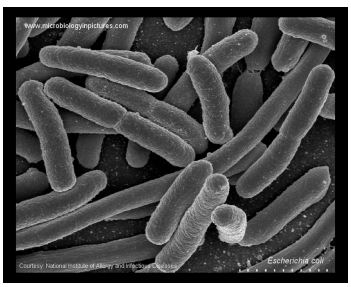
EPFL

On Today's Menu:

Part 1

Bacteria
- Types
- Function
- Importance



Part 2

Engineering with Bacteria
- Proteins
- Biofilms
- Living Materials

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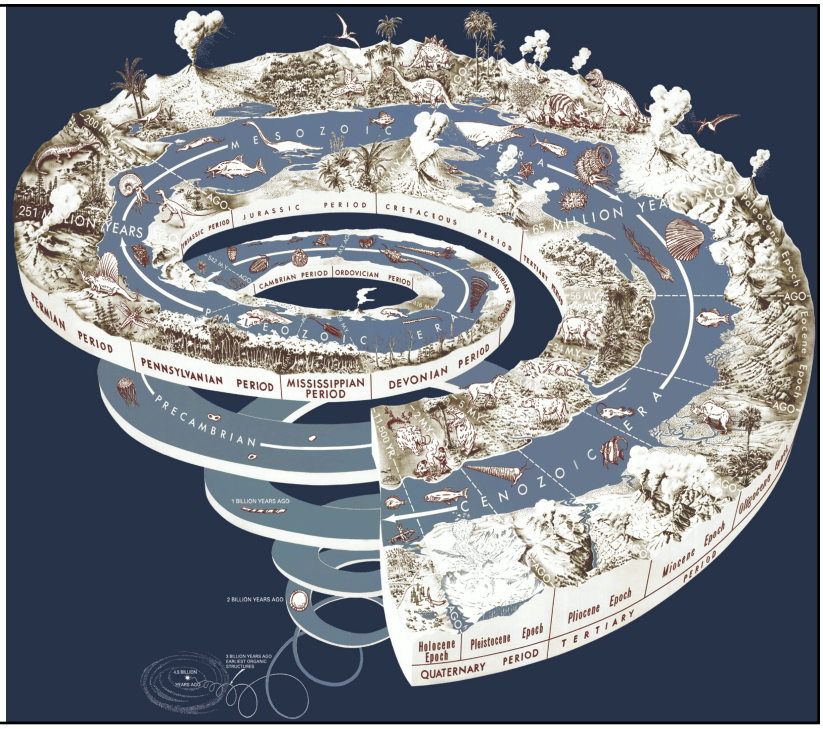
Bacteria

Bacteria are small single-celled organisms. **Bacteria** are found almost everywhere on Earth and are vital to the planet's ecosystems.

The ancestors of modern bacteria were unicellular microorganisms that were the first forms of life to appear on Earth, about 4 billion years ago. **For about 3 billion years, most organisms were microscopic, and bacteria and archaea were the dominant forms of life.**



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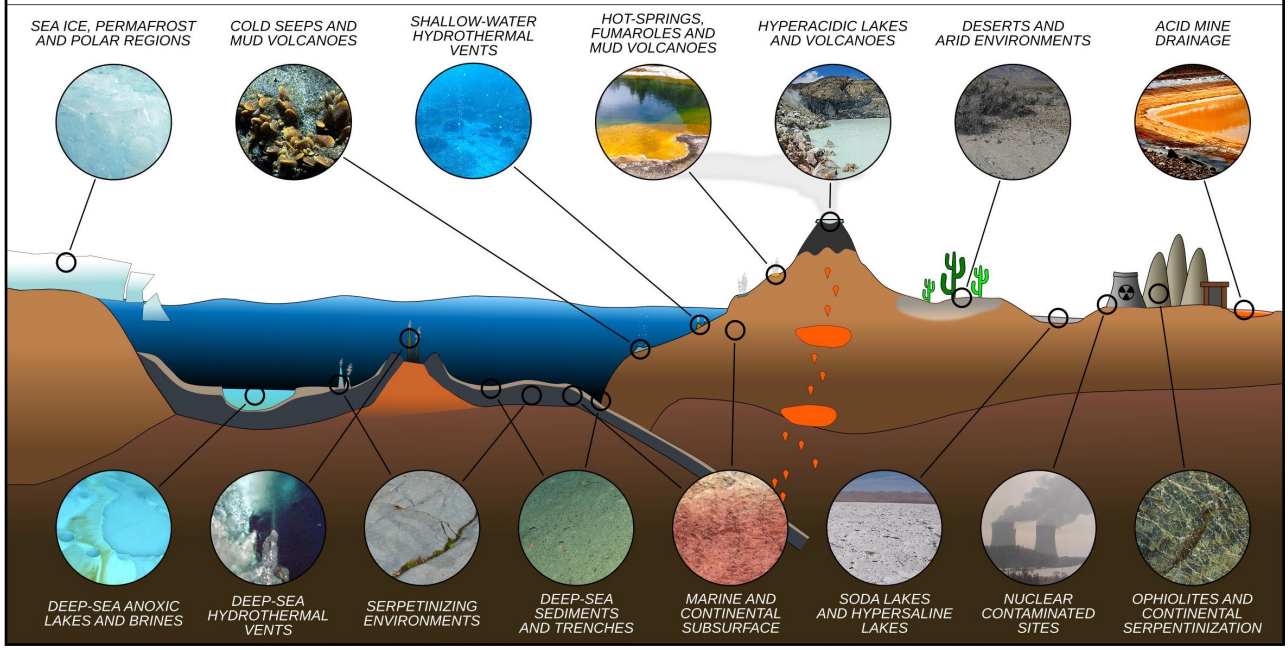
Bacteria - Extreme Conditions

Front. Microbiol., 15 April 2019 | https://doi.org/10.3389/fmicb.2019.0078



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Bacteria - Extreme Conditions

The microorganism Sulfolobus acidocaldarius lives in extreme environments, such as Emerald Hot Spring in Yellowstone National Park. (Image credit: Rennett Stowe / flickr)

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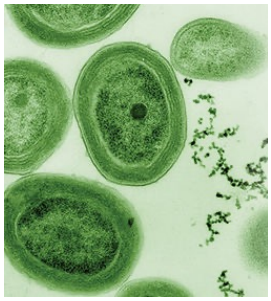
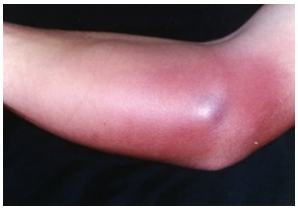
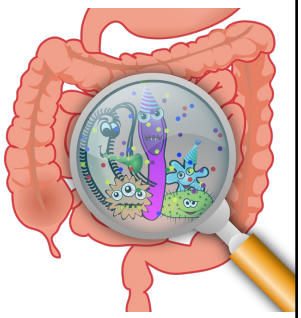
Bacteria - Function

Photosynthesis	(oxygen)
Fermentation	(fuel, wine, cheese)
Pathogenicity	(infection, food poisoning)
Symbiosis	(immune system, digestion)



5% of worlds oxygen





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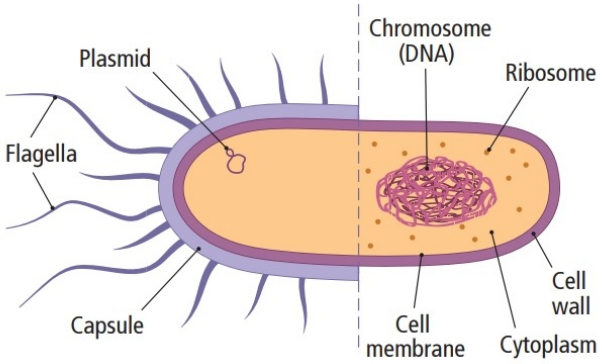
Bacteria - Structure

Bacteria are single celled microbes. The cell structure is simpler than that of other organisms as there is no nucleus or membrane bound organelles. Instead their control centre containing the genetic information is contained in a **single loop of DNA**. Some bacteria have an extra circle of genetic material called a **plasmid**.

The plasmid often contains genes that give the bacterium some advantage over other bacteria. For example it may contain a gene that makes the bacterium resistant to a certain antibiotic.

Left side:

Sometimes present



Right side:

Always present

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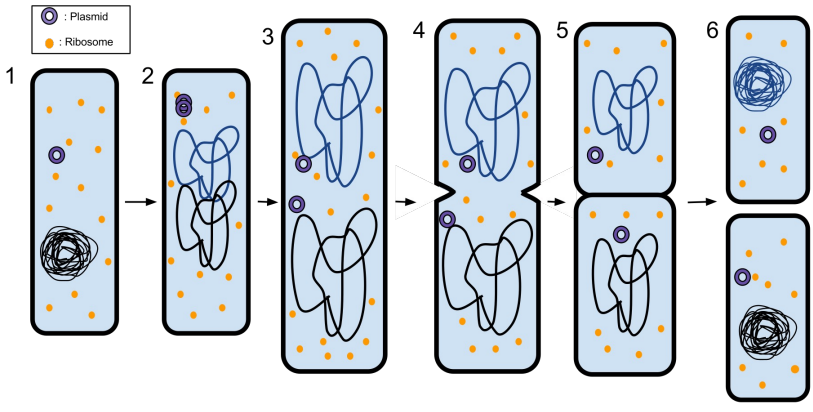
Bacteria - Division (Binary Fission)

Schematic diagram of cellular growth (elongation) and binary fission of bacilli.

The following steps occur:

1) Normal bacterium
2) Uncoiled and duplicated DNA
3) DNA is pulled to the separate poles of the bacterium as it increases the size to prepare for splitting.
4) The growth of a new cell wall begins to separate the bacterium
5) The new cell wall (septum) fully develops, resulting in the complete split of the bacterium.
6) The new daughter cells have tightly coiled DNA rods, ribosomes, and plasmids; these are now brand-new organisms.



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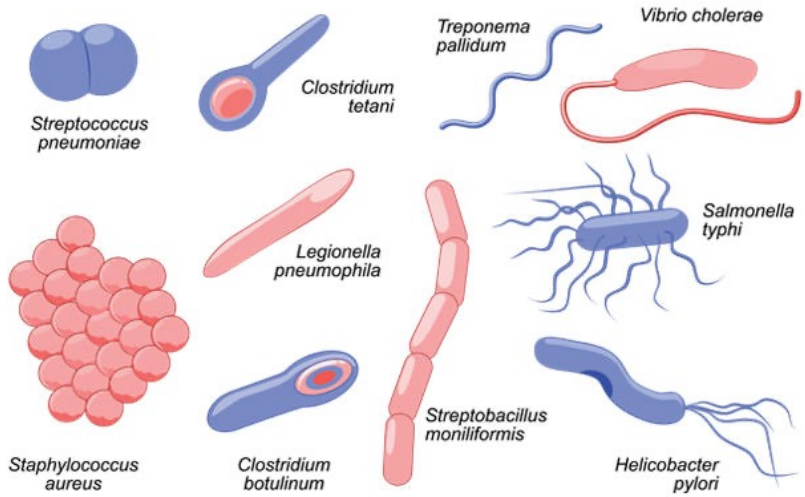
Bacteria - Types



https://microbiologysociety.org/why-microbiology-matters/what-is-microbiology/bacteria.html

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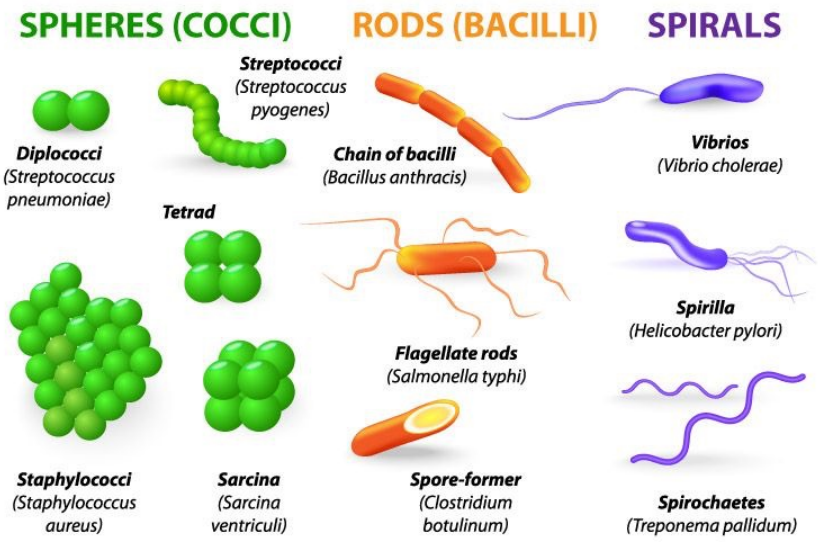
Bacteria - Shapes

Bacteria are classified into five groups according to their basic shapes:

spherical (cocci),
rod (bacilli),
spiral (spirilla),
comma (vibrios),
corkscrew (spirochaetes).

They can exist as single cells, in pairs, chains or clusters.



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Bacteria - The Good and The Bad

Bifidobacterium

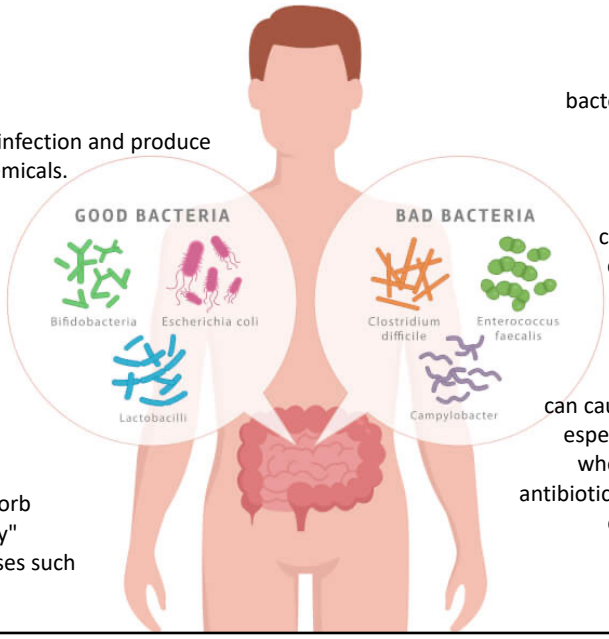
digest dietary **fiber**, help prevent infection and produce **vitamins** and other important chemicals.

E Coli

Most **E**. **coli** strains are harmless and play an essential role in keeping the digestive system healthy, helping to digest food and producing Vitamin K.

Lactobacilli

can help us break down food, absorb **nutrients**, and fight off "unfriendly" organisms that might cause diseases such as **diarrhea**.



Clostridium difficile

bacteria that can infect the bowel and cause diarrhoea.

Campylobacter

can infect humans, sometimes causing campylobacteriosis, a diarrhoeal disease

Enterococcus faecalis

can cause life-threatening infections, especially in hospital environment, where the naturally high levels of antibiotic resistance found in E. faecalis contribute to its pathogenicity

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Bacteria - Food

Nature uses microorganisms to carry out fermentation processes, and for thousands of years mankind has used **yeasts**, **moulds** and **bacteria** to make food products such as **bread, beer, wine, vinegar, yoghurt and cheese**, as well as **fermented fish, meat and vegetables**.

Food Spoilage

There are two types of pathogenic bacteria that target different categories of food. The first type is called **Clostridium botulinum** and targets food such as meat and poultry, and **Bacillus cereus**, which targets milk and cream.

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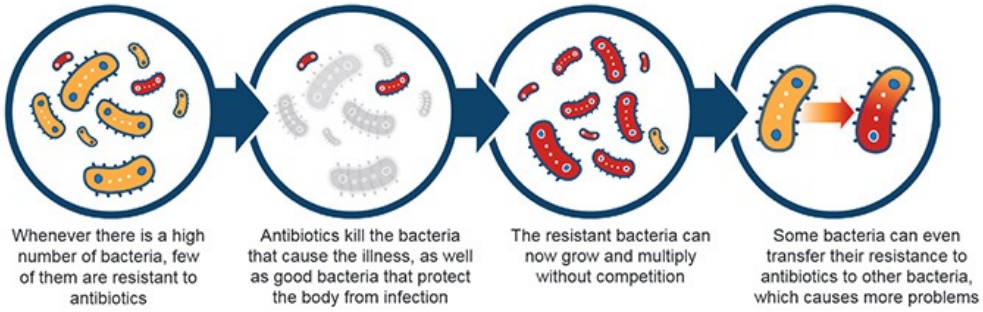
Bacteria - Antibiotic Resistance



Key facts

*Antibiotic resistance is one of the biggest threats to global health, food security, and development today.
*Antibiotic resistance can affect anyone, of any age, in any country.
*Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process.

*A growing number of infections – pneumonia, tuberculosis and salmonellosis – are becoming harder to treat as the antibiotics to treat them become less effective.
*Antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality.

https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

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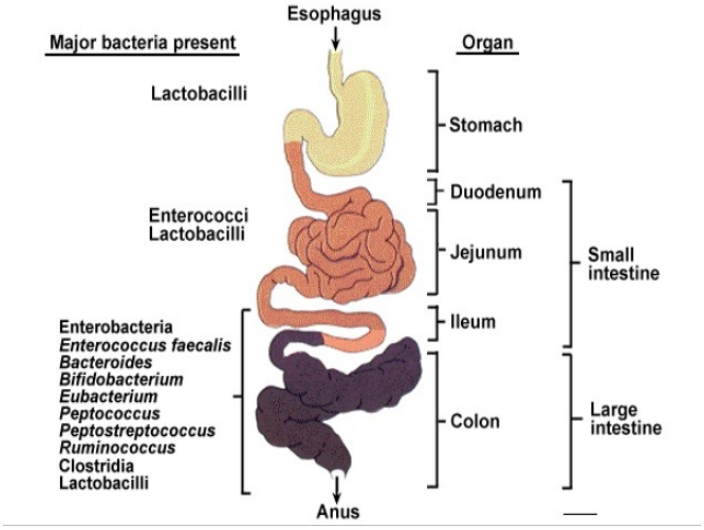
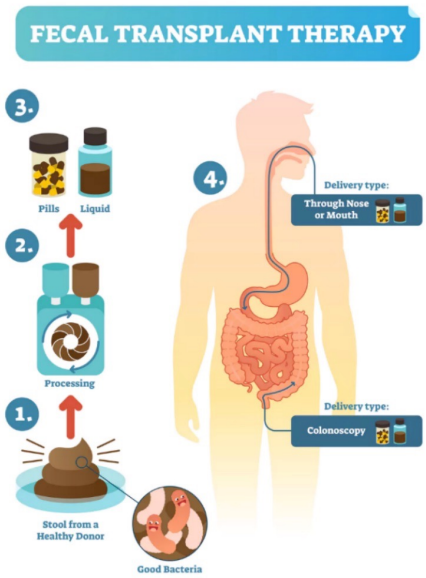
Bacteria - Medicine: Fecal Transplant





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Bacteria - Medicine

C. diff infections typically result from the use of broad spectrum antibiotics that alter the microbiota balance, allowing C. diff to colonize.

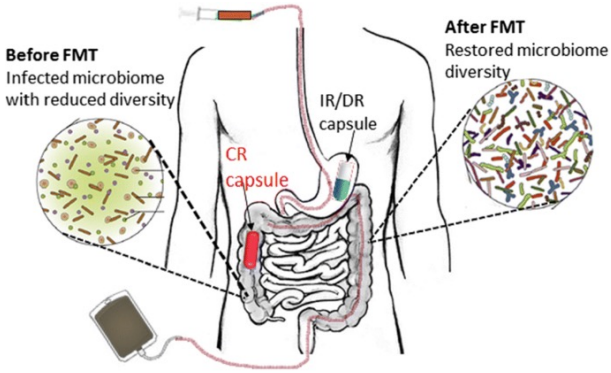
Typical treatment of C. diff with antibiotics, can further disrupt the microbiome of the gut often leading to a cyclical recurrence of C. diff with 35% of patients experiencing recurrence

success rates of treatments against *Clostridium difficile*

vancomycin: 25%

fecal transplant: 90%

the "poop pill"



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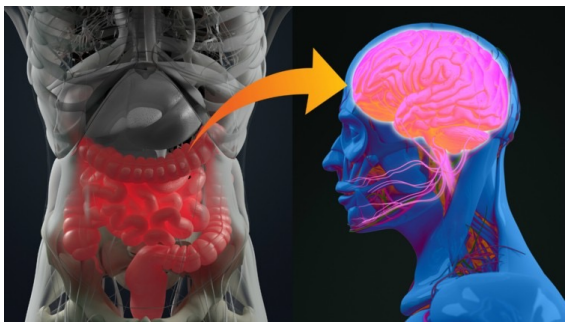
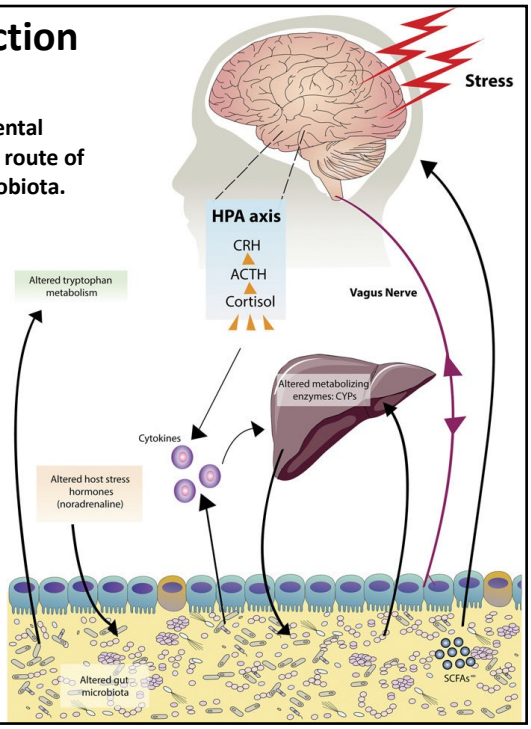
Brain-Gut Connection

Recolonization of gut flora can be used effectively in the **treatment of mental disorders** because of the existence of the gut-brain axis, the **bidirectional route of communication between the brain and the gut, specifically the gut microbiota.**



→ **TED Talk Dr. Giulia Enders (On Moodle)**



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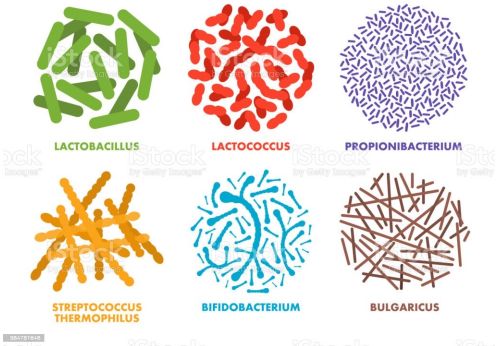
Bacteria - ProBiotics

Probiotics are **living bacteria or fungi** that confer **health benefits**.

They have **3 mechanisms of therapeutic effect**: antimicrobial effects, strengthening lining of the intestines, and immune modulation.

These mechanisms help alter and diversify gut flora to benefit overall health. The antimicrobial effect helps prevent the growth of bacteria that cause illness. Probiotics also help strengthen tight junctions, multiprotein complexes lining the intestines (as well as other organs and regions of the body) to prevent passage of materials.

Leaky gut is the term used when **tight junctions of the intestines** are disrupted and bacteria can exit the intestines. Leaky gut is implicated in many gastrointestinal disorders. Probiotics stimulate production of a protein that maintains the strength of the intestinal barrier and have beneficial effects on local and systemic immune system responses.



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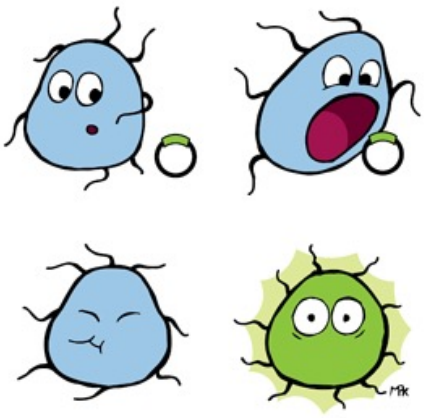
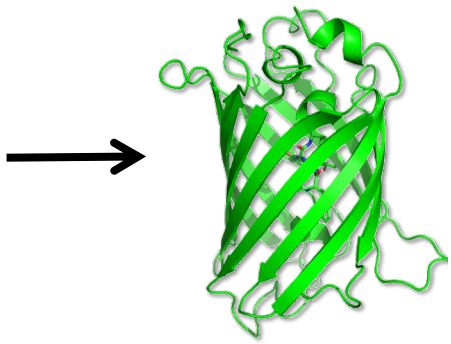
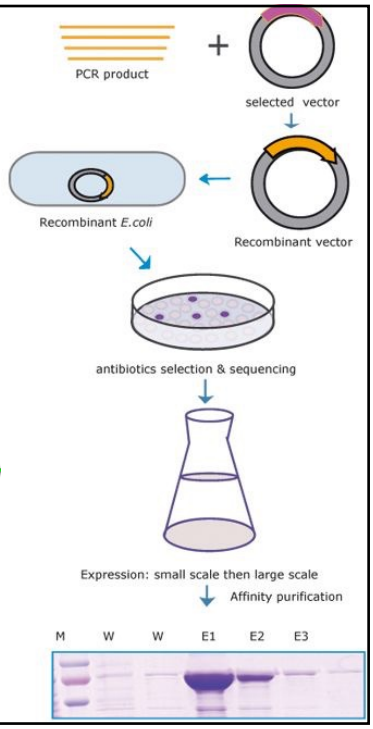
Bacteria – in Engineering

Protein Production







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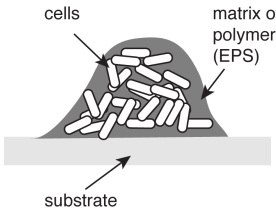
Biofilms

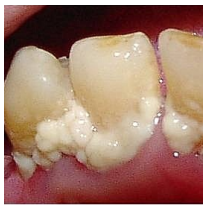
The majority of bacteria in the natural world exist as **biofilms**: organized communities of cells in a network of extracellular matrix (ECM) composed of polysaccharides, proteins, nucleic acids and other biomolecular components.



biofilms are both viscous and elastic

biofilms are porous and permeable

there is no flow within a biofilm



Why do bacteria form biofilms?

protect from predators

protect from antimicrobials

staying put in a nutrient rich environment

protect from invasion (competition)

gene exchange

Courtesy of A. Persat p-lab.science

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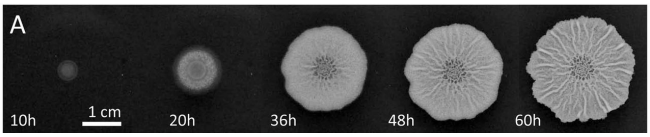
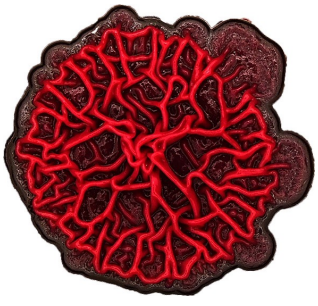
Bacteria and Materials

many biofilms form wrinkles

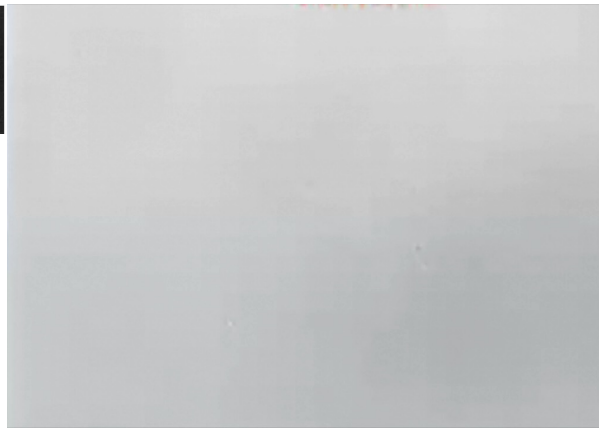


Bacillus subtilis biofilm



Pseudomonas aeruginosa biofilm

formation of a *Vibrio cholerae* biofilm



Courtesy of A. Persat p-lab.science

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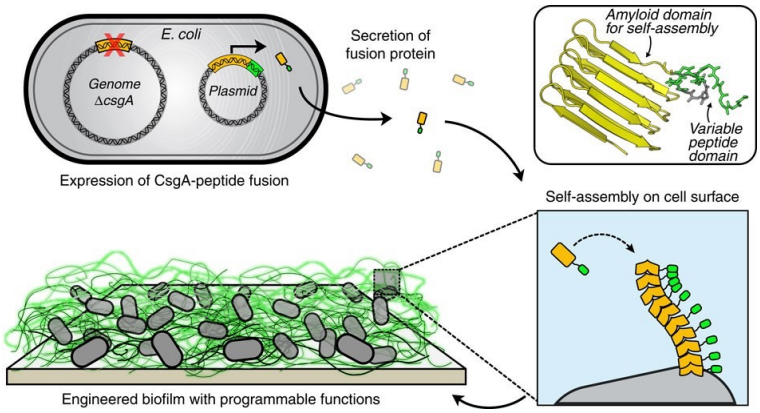
Living Materials

Advances in our understanding of bacterial systems in the past century have expanded the role of the microbe from being regarded solely as a health threat to being exploited as a **genetically programmable factory for the production of biomolecules and chemicals**.

Use the **domestication of biofilms** as a **platform for programmable and modular self-assembling extracellular nanomaterials**, with the bacterium serving as a **living foundry** for the **synthesis of raw building blocks**, their **assembly into higher order structures upon secretion** and the **maintenance of the material** as a whole over time.



Nat Commun **5,** 4945 (2014).
https://doi.org/10.1038/ncomms5945

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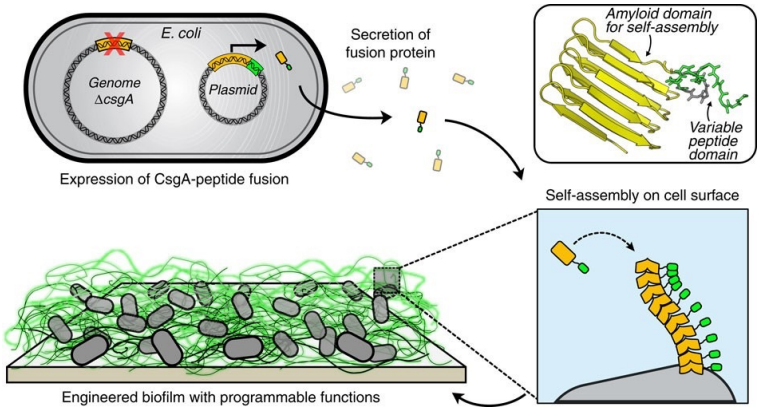
Living Materials

The **curli system** is the primary proteinaceous structural component of *E. coli* biofilms

The curli system exhibits numerous features that make it an ideal platform for the type of materials engineering by way of synthetic biology

Curli are highly robust **functional amyloid nanofibres** with a diameter of approximately **4–7 nm** that exist as **extended tangled networks encapsulating the cells**. Curli are formed from the extracellular self-assembly of CsgA, a small secreted 13-kDa protein. A homologous outer-membrane protein, CsgB, **nucleates** CsgA assembly and also **anchors** the nanofibres to the bacterial surface. Detached curli fibres can also exist as non-cell-associated structural components of the ECM.



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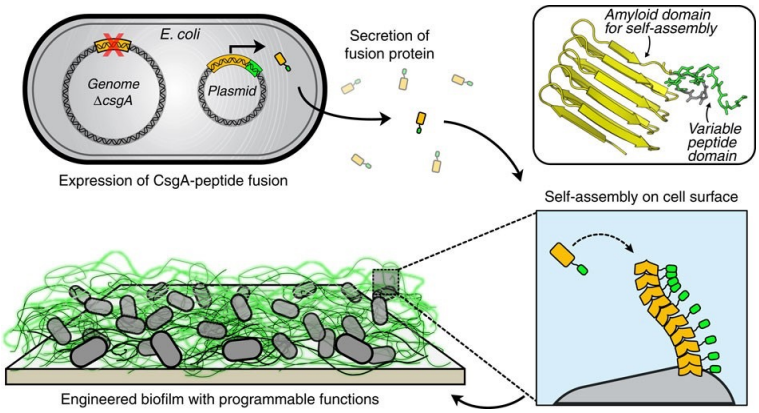
Living Materials

ΔcsgA cells heterologously express and secrete fusion proteins consisting of an **amyloidogenic domain** (CsgA, shown in orange) and a **functional peptide domain** (green). This secreted fusion protein self-assembles into an extracellular network of amyloid nanofibres that are **anchored onto the cell surface**, resulting in a biofilm material with programmed non-natural functions.

Functions that can be engineered into curli nanofibres :

1) inorganic nanoparticle templating,
2) specific abiotic substrate adhesion
3) the site-specific covalent immobilization of an arbitrary functionalized recombinant protein.



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Living Materials

Peptide	Sequence	Length (aa)	Type	Specific function
HIS	HHHHHH	6	Affinity Tag	Affinity Tag
GBP	EPLQLKM	7	Substrate binding	Graphene edge binding
FLAG	DYKDDDDK	8	Affinity Tag	Affinity Tag
CNBP	HSSYWYAFNNKT	12	Substrate binding	Carbon nanotube binding
A3	AYSSGAPPMPPF	12	Substrate binding	Gold surface binding
CLP12	NPYHPTIPQSVH	12	Mineral templating	Hydroxyapatite nucleation
QBP1	PPPWLPYMPPWS	12	Substrate binding	Quartz/glass binding
SpyTag	AHIVMVDAYKPTK	13	Protein display	Covalent capture/display of proteins
CT43	CGPAGDSSGVDSRSVGPC	18	NP templating	ZnS templating
MBD	KCTSDQDEQFIPKGCSKGSGGSG	23	Substrate binding	Binding to stainless steel surfaces
AFP8	DTASDAAAAAALTAANAKAAAELTAANAAAAAAATAR	37	Substrate binding	Ice crystal binding
Mms6	GGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQSDEEVELRDALA	59	NP templating	Magnetite templating

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https://doi.org/10.1038/ncomms5945

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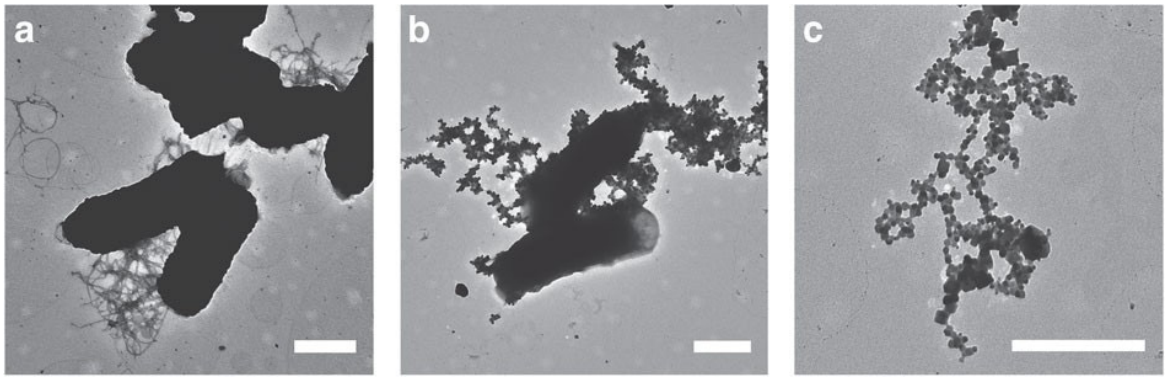
Nanoparticle Templating

Strategy for the large-scale *de novo* production of conductive nanowires.

Silver nanoparticles were templated by A3-BIND biofilms incubated in aqueous AgNO3. Representative TEM micrographs demonstrate that PHL628 ΔcsgA cells producing wild-type CsgA (a) show no nanoparticle templating, whereas A3-BIND (b) templates nanoparticles after incubation in 147 mM AgNO3 for 4 h. (c) A higher magnification of the Ag nanoparticles organized on A3-BIND nanofilaments is shown. All scale bars are 0.5 µm.



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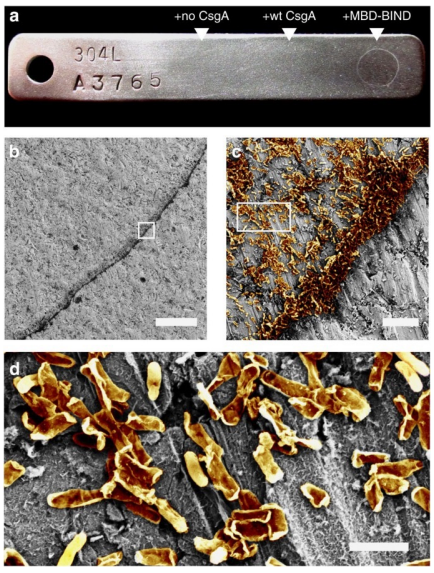
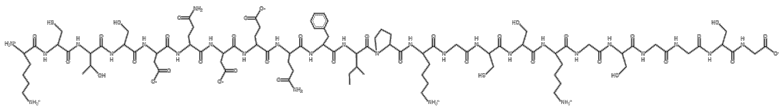
Adhesion to Steel

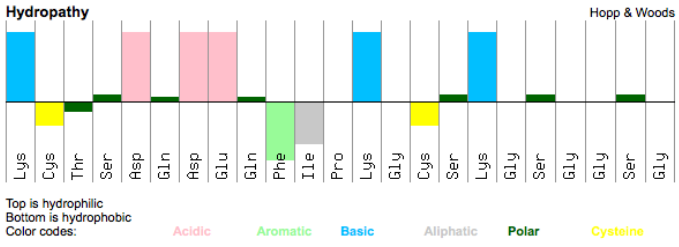
Useful for localized biofilm growth to specific materials in bioreactors

(a) Adhesion of PHL628 ΔcsgA cells producing no CsgA (left), wild-type CsgA (middle) and CsgA-MBD (right) was tested by spotting induced cultures onto a 304L steel coupon and incubating for 48 h at 4 °C.
(b) The MBD-BIND biofilms were analysed by FE-SEM. Scale bar is 250 µm.



Peptide: KCTSDQDEQFIPKGCSKGSGGSG





https://pepcalc.com

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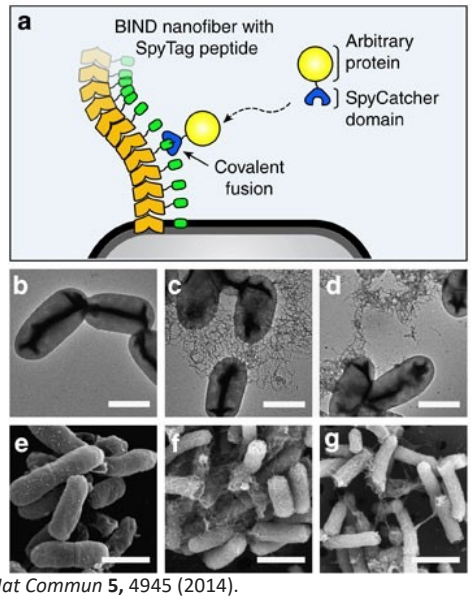
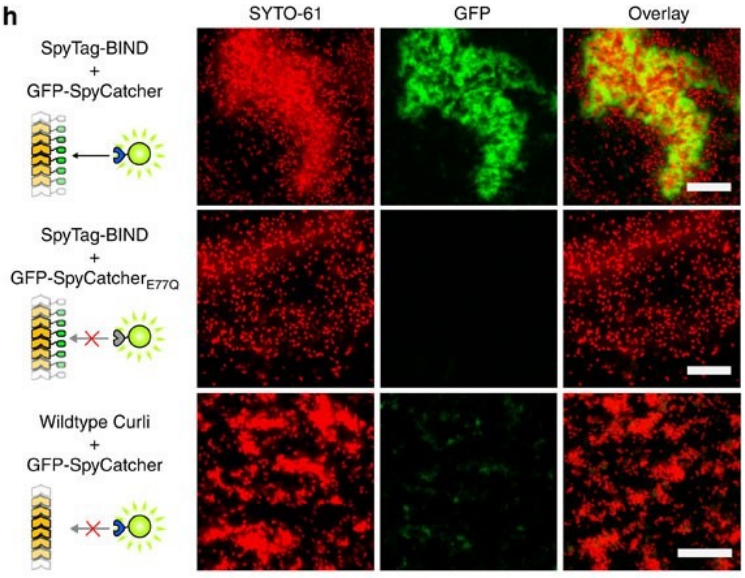
Immobilization of Proteins





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Conclusion

Bacteria are the earliest form of life on earth and have been around for a long time.

They have adapted to live on many different substrates and perform many different functions.

We use many types in our food production, though other types will make our food go bad.

For us human, bacteria in our gut are extremely important for our well-being, and the brain-gut connection is currently being explored as potential treatment solutions for mental health diseases.

Bacteria for engineering are very useful for the production of proteins, as well as functional materials.

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