Mentouri Brothers University – Constantine 1 Faculty of Natural and Life Sciences

Department of Microbiology

Master 1 course: Molecular Biology of Microorganisms

Course material: Interactions of Microorganisms

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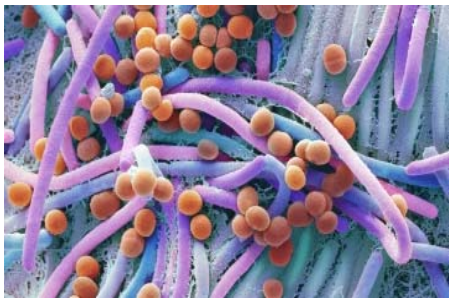
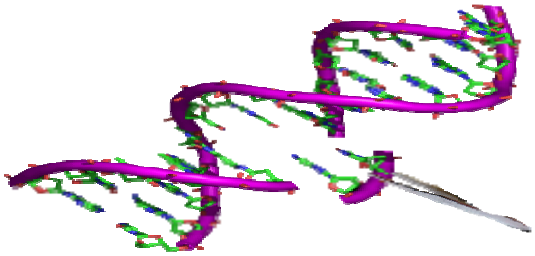
Interactions of Microorganisms





Foreword

Scope and Objective of the Course Document

Microbial interactions refer to the complex relationships and interactions that occur between microorganisms, including bacteria, archaea, viruses, fungi, and protozoa, in various ecosystems. These interactions play a crucial role in shaping the structure of microbial communities, their functioning, and profoundly affect ecosystems, human health, industry, the environment, and sustainability.

Understanding microbial interactions contributes to ecosystem ecology, biotechnology, and drug discovery.

Knowledge of microbial interactions is vital for the conservation of endangered species and ecosystems.

Knowledge of microbial interactions can inform policies and strategies for managing infectious diseases.

Recognizing the importance of microbial interactions can pave the way for innovative solutions to global challenges in health, agriculture, and environmental sustainability.

Our understanding of microbial interactions has expanded dramatically with advances in technology, revealing complex networks of interactions in diverse ecosystems.

Research on the human microbiome has elucidated how microbial interactions affect human health, including the gut microbiota that influences digestion and immune system development.

This course handout, “Interactions of Microorganisms,” is dedicated to unraveling the complex and fascinating world of microbial interactions. Microorganisms are essential to shaping our environment and impacting various aspects of life on Earth. This course handout delves deeper into how microorganisms interact with each other, with other organisms, and with their environment.

This course handout provides a comprehensive and accessible resource for students. Its primary purpose is to serve as an educational tool, providing readers with a clear

understanding of the fundamental principles governing microbial interactions and their ecological importance.

This document is aimed to Master 1 students in the Molecular Biology of Microorganisms specialty. Its main objective is to complete their training in general microbiology already studied in the undergraduate cycle. The program includes three main chapters:

Interactions between Microorganisms and physical environment.

Interactions between Microorganisms.

Interactions between Microorganisms, animal and humans.

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1 Introduction to Microbial Interactions

1.1 Defining Microbial Interactions

Microbial interactions refer to the complex relationships and interactions that occur between microorganisms, including bacteria, archaea, viruses, fungi, and protozoa, in various ecosystems. These interactions are pivotal in shaping microbial communities' structure and functioning and profoundly affect ecosystems, human health, industry, and environmental sustainability**(Kontro and Yaradoddi, 2021).**

Our understanding of microbial interactions has expanded significantly due to technological advancements, revealing intricate networks of interactions in diverse ecosystems. The human microbiome research has elucidated how microbial interactions affect human health, including gut microbiota influencing digestion and immune system development(Figure 1). Here are some interactions:

- **Symbiosis:** Symbiotic interactions encompass mutualism, commensalism, and parasitism. Mutualism benefits interacting organisms (e.g., nitrogen-fixing bacteria and leguminous plants).
- **Antagonism:** Antagonistic interactions include predation, competition, and amensalism. For example, we could cite competition for resources like nutrients or space among bacteria.
- **Syntrophy:** Syntrophic relationships involve cross-feeding between microbes, where one microbe consumes the waste products of another (e.g., methanogenesis in anaerobic digestion) **(Kothamasi & al., (2009).**

1.1.1 Methods for Studying Microbial Interactions

- **Genomics:** Advances in DNA sequencing technologies enable the identification of microbial species and their functional potential.
- **Metabolomics:** Studying the metabolites produced by microorganisms can reveal interactions, such as the exchange of nutrients.
- **Microbiome Analysis:** Analyzing the composition and diversity of microbial communities provides insights into potential interactions **(Figure 1).**

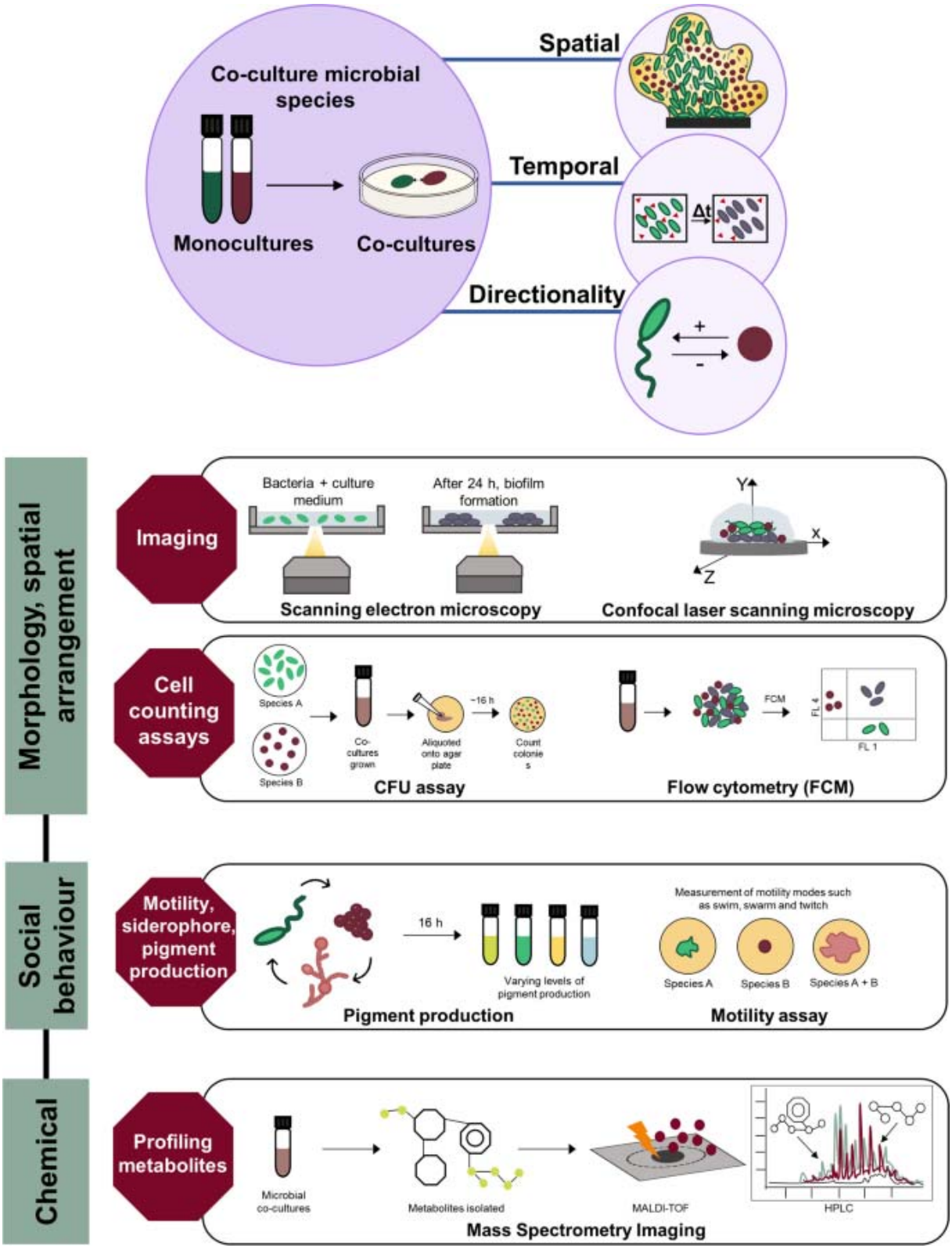

Figure 1: Overview of qualitative methods used to study microbial interactions(Shanchana &al., 2024).

1.1.2 Advantages of Studying Microbial Interactions

❖ **Scientific Research:** Understanding microbial interactions aids ecosystem ecology, biotechnology, and drug discovery.

❖ **Conservation Efforts:** Insights into microbial interactions are vital for conserving endangered species and ecosystems.

❖ **Policy-Making:** Knowledge of microbial interactions can inform environmental policies and strategies for managing infectious diseases.

However, some challenges appear when we study microbial interactions, including at least:

❖ **Complexity:** Microbial communities are incredibly diverse and complex, making it challenging to unravel all interactions.

❖ **Inaccessibility:** Some microbial interactions occur in extreme environments or deep underground, making them difficult to study directly.

❖ **Ethical concerns** may arise when studying human microbiota.

Defining microbial interactions is a multifaceted endeavor with broad implications for science, conservation, and policy-making. While challenges and limitations exist, technological advances and research methods continue to deepen our understanding of these interactions, offering promising avenues for future exploration and application. Recognizing the importance of microbial interactions can pave the way for innovative solutions to global challenges in healthcare, agriculture, and environmental sustainability **(Harcomb& al., (2014).**

1.2 Significance in Various Fields

The concept of significance in various fields holds immense importance across different domains of study, from scientific research to policy-making and conservation efforts. In this context, significance refers to the meaningful impact, relevance, or importance of a particular phenomenon, finding, or factor within a specific field or discipline. Understanding and assessing significance is crucial for decision-making, resource allocation, and the advancement of knowledge.

Significance in various fields is a subjective evaluation that depends on the context and objectives of each discipline. In general, it can be categorized into several aspects:

- **Statistical Significance:** In scientific research, statistical significance is often used to determine whether observed data is likely due to a natural effect or a random occurrence. It is calculated using statistical tests like t-tests or ANOVA. For instance, in medical research, the significance of a new drug's effectiveness is assessed through clinical trials, measuring its impact on patient outcomes.
- **Environmental Significance:** In ecology and conservation biology, the significance of a particular species or habitat lies in its contribution to ecosystem stability, biodiversity, and overall ecological health. For example, the importance of preserving coral reefs is evident as they provide habitats for numerous marine species and protect coastlines from erosion.
- **Policy Significance:** In policy-making, significance pertains to the impact of policies or decisions on society, the economy, and the environment. Policymakers evaluate the effectiveness of various options to make informed choices. An example is assessing the importance of carbon emissions reduction policies in combating climate change.

The assessment of significance has evolved with advancements in data analytics, technology, and interdisciplinary collaboration. Here's a brief overview of its current state in some key fields:

- **Scientific Research:** Significance testing remains a cornerstone of scientific research, but there's a growing recognition of the need for effect size, practical significance, and statistical significance. Researchers increasingly emphasize the importance of replicability and robustness in results.
- **Conservation Efforts:** Conservation biology has shifted towards a more holistic understanding of significance, considering not only individual species but also the interconnectedness of ecosystems. Landscape-scale conservation planning and incorporating traditional ecological knowledge are becoming more significant.
- **Policy-Making:** Policy analysis now employs various tools, such as cost-benefit analysis and environmental impact assessments, to gauge the significance of proposed policies. Data-driven decision-making and evidence-based approaches are on the rise**(Faust, K. (2021),.**

There are many advantages of studying significance in various fields, including, for example:

- **Informed Decision-Making:** Understanding significance helps decision-makers make informed choices by weighing the importance of various factors or options.
- **Resource Allocation:** It aids in efficiently allocating resources and directing efforts towards areas of greater significance.
- **Improved Research:** Scientific research ensures that studies focus on meaningful findings, reducing the likelihood of spurious results.
- **Conservation Prioritization**: In Conservation biology, studying interactions helps prioritize efforts toward preserving critical habitats and species.
- **Policy Effectiveness:** For policymakers, assessing significance ensures that policies have a tangible impact on societal issues**(Braga and Araújo, 2016).**

The concept of significance in various fields plays a pivotal role in shaping the direction of research, conservation efforts, and policy-making. Its evolution reflects the dynamic nature of these domains. While challenges persist, the benefits of understanding and assessing significance are evident, driving progress and informed decision-making across diverse fields of study.

2 Biofilms

2.1 Introduction

Biofilms, a pervasive and tenacious microbial phenomenon, have gained increasing attention due to their remarkable resistance to removal and treatment. This resistance was first highlighted by Characklis in 1973, revealing the biofilm as a complex structure comprising a multitude of microorganisms, including bacteria, fungi, diatoms, and protozoa, adhering to both living and non-living surfaces. In 1978, William J. Costerton introduced the term "biofilm," emphasizing the significance of these sessile, structured communities**(Floyd & al., 2017).**

Biofilms have wide-ranging implications across various sectors, both beneficial and detrimental. They prove indispensable in agriculture, offering plant protection and aiding soil bioremediation. Conversely, biofilms wreak havoc in the medical and pharmaceutical fields, causing diseases in humans and animals, contaminating medical implants, and contributing to a significant portion of hospital infections, often associated with devices like catheters and implants. These infections, primarily caused by pathogens like Staphylococcus epidermidis and *Staphylococcus aureus*, lead to persistent and chronic challenges in treatment (**Chang & al., 2011).**

The formation of biofilms, characterized by microbial aggregation, is governed by environmental conditions and surface properties, including pH, temperature, and nutrient availability **(Figure 2).** The extracellular polymeric substances (EPS) produced by bacteria within biofilms play a pivotal role in their resilience, safeguarding against environmental stressors and therapeutic interventions. These substances create a complex 3D architecture, effectively shielding the microorganisms within. Biofilms also engage in quorum sensing (QS) mechanisms, mediating cell-to-cell communication and gene regulation, further enhancing their adaptability **(Castillo-Juárez *& al.*, 2015)**.

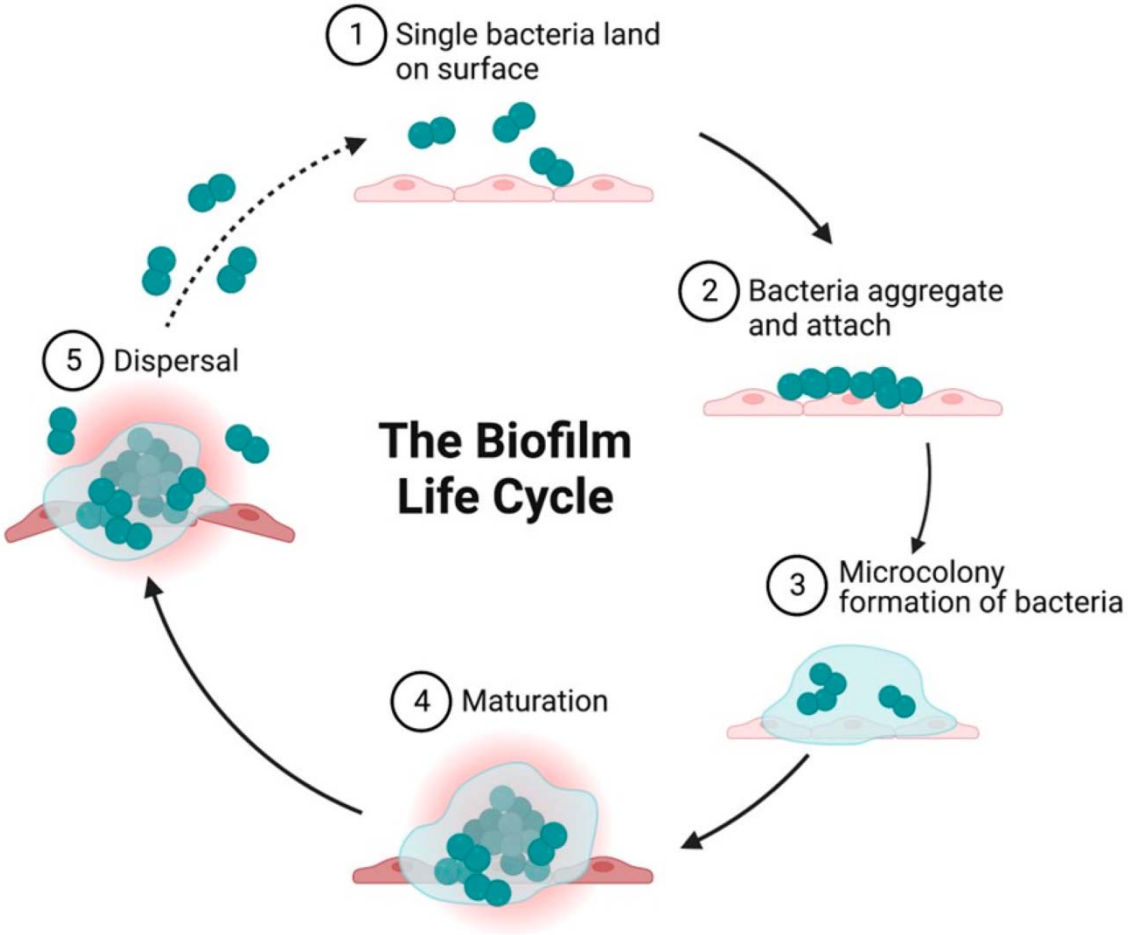

Figure 2 : Diagrammatic illustration showing the growth cycle of a biofilm by a single bacterium species on a solid surface (Satish Sharma & al ., 2024).

The surge in biofilm research over the past decade is evident through numerous publications and growing commercial interest. Advances in molecular biology techniques, including microscopy, spectroscopy, and bioinformatics, have propelled our understanding of biofilms, opening new avenues for research and developing anti-biofilm agents. As biofilms continue to exert their influence across diverse sectors, this review provides a comprehensive perspective on their positive and negative roles and explores the potential of plant derivatives as anti-biofilm agents **(Hobley & al., 2015)**.

2.2 Composition of Biofilms

Biofilms are intricate communities of microorganisms that produce EPS, primarily composed of water, which accounts for up to 97% of the biofilm matrix. This aqueous component is the conduit for nutrient flow within the biofilm structure. Within the biofilm

architecture, two principal elements exist a network of water channels facilitating nutrient transport and densely packed cells devoid of prominent pores.

The microbial cells in biofilms exhibit distinctive physiological and physical properties, rendering them resilient to antibiotics and immune system defenses. Microorganisms capable of biofilm formation activate specific genes that trigger the expression of stress-related genes, leading to the development of resistant phenotypes. Cell density, nutritional conditions, temperature, pH, and osmolarity can induce these changes. In essence, biofilms can be likened to primitive multicellular organisms when considering their water channel systems for circulation **(Arunasri & Mohan., 2019 ;Rather & al., 2021**).

As summarized in **Table 1**, the chemical composition of biofilms underscores their resilience against diverse environmental challenges.

Table1. Biofilm chemical composition **(Rather &al., 2021)**

Components	**Percentageofmatrix**
Microbialcells	2-5%
DNAandRNA	<1-2%
Polysaccharides	1-2%
Proteins	<1-2%(includingenzymes)
Water	Upto97%

2.3 Biofilm Formation and Its Development

Biofilms are a predominant microbial growth form, and understanding their formation is crucial. The process of biofilm development is complex, influenced by various factors such as the matrix, material surface, medium, cell metabolism, and signal molecules.

It can be divided into three main stages: attachment, maturation, and dispersion

(**Figure 3**). Initially, bacteria attach to the biomaterial surface and form microcolonies, but this attachment is reversible and dependent on environmental factors like hydrodynamics, temperature, and pH. Physical interactions promote attachment, including polarity, van der Waals forces, and protein adhesion. Flagella enhances interactions and reduces repulsion between cells and surfaces. Bacterial adhesins, like fimbriae and polysaccharide adhesin, further stabilize attachment **(Kotakonda and Venkata, 2019).**

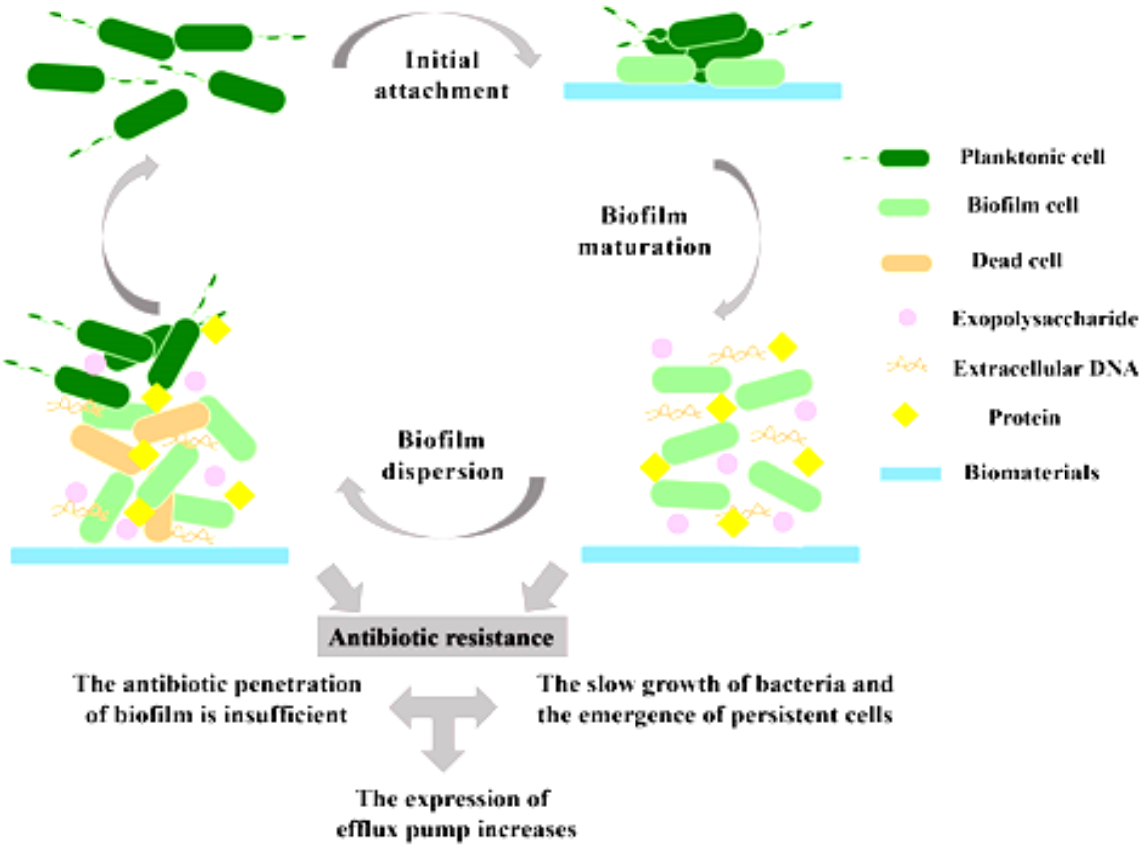

Figure 3: The process of biofilm formation and antibiotic resistance mechanisms. Biofilmformation Is divide in three steps:Initial attachment of the biofilm,maturation,and Biofilm dispersion. The mechanisms of antibiotic resistance in biofilms are also divided into three factors: The antibiotic penetration of the biofilm is insufficient; The bacteria grow slowly, and persistent cells emerge in biofilms; The expression of efflux pumps increases **(Floyd and Hadjifrangiskou, 2017)**.

Adhesion is pivotal for bacteria to form biofilms, leading to persistent infections, such as *Pseudomonas aeruginosa* in cystic fibrosis patients, *Burkholderia pseudomallei* in respiratory infections, and attaching-effacing *E. coli*in intestinal infections. Bacterial adhesion to medical devices also fosters biofilm formation, posing a threat to human health.

Once attached, bacterial cells proliferate, forming microcolonies. When the bacterial density reaches a threshold, quorum sensing (QS) systems are activated, regulating biofilm maturation, motility, and virulence factor expression. Different bacteria secrete specific QS signal molecules, such as AHL, AIP, and AI-2. For instance, PQS molecules in *Pseudomonas aeruginosa* play a vital role in biofilm maturation. EPSs stabilize the biofilm structure, prevent attacks from antibacterial agents and immune cells, and contribute to its three-dimensional architecture **(Rather & al., 2021).**

As the biofilm matures, nutrient depletion and toxin accumulation trigger dispersion, which can be active or passive. Active dispersal involves the dissolution of EPSs, upregulating flagellar proteins, and downregulating attachment genes. Passive dispersion depends on external factors like enzymatic degradation and shear forces. Enzymes like PelA and PslG break down biofilm matrix components, aiding dispersion. Shear forces can also release cells from biofilms (**Floyd and Hadjifrangiskou, 2017).**

Ultrasonic treatment and laser-induced shock waves have been used to disperse biofilms passively. However, once dispersed cells encounter favorable conditions, they can reattach and reform biofilms, perpetuating infection.

The prevailing model of biofilm development, consisting of five sequential steps, has been established *in vitro*, primarily focused on *Pseudomonas aeruginosa* and extrapolated for *Staphylococcus aureus*. It includes attachment, colony formation, maturation, dispersion, and seeding dispersal. Recent studies suggest limitations in this model's applicability *in vivo* and different settings. An alternative model posits that bacteria can switch forms depending on substrate, colonizing bacteria, and micro-environmental conditions **(Reddersen andWiegand, 2022).**

2.4 Mechanisms of Antibiotic-Resistant Biofilms

Mechanisms of antibiotic-resistant biofilms encompass a complex interplay of factors that defy conventional explanations and pose significant challenges in the clinical treatment of biofilm infections. Biofilms, as highly successful bacterial life forms, are prevalent in diverse environments and instrumental in fostering antibiotic resistance. Studies have revealed that antibiotics fail to exert their antibacterial functions within biofilms, often leading to the emergence of superbugs, thereby complicating infection treatment. Biofilm formation is also closely linked to inflammation, exacerbating persistent infections, notably in pulmonary, burn, and medical device infections in cystic fibrosis patients caused by *P. aeruginosa* **(Ciofu & al., 2017).**

One key mechanism behind the resistance exhibited by biofilms is the prevention of antibiotic penetration. The EPS forming the biofilm matrix serves dual roles: structural stability maintenance and acting as a barrier against antimicrobial agents. Negatively charged EPS neutralize the positive charge of aminoglycoside antibiotics, rendering them ineffective. Furthermore, enzymes in the biofilm matrix can deactivate antibiotics, with β-lactamases

particularly adept at this task. Additionally, the biofilm matrix limits antimicrobial agents' permeability, further hampering their effectiveness **(Al-Ansari& al., 2020).**

Another facet of biofilm resistance lies in slow-growing or persistent cells within the biofilm structure. The biofilm matrix impedes the penetration of antimicrobial agents, leading to the development of persistent cells that remain unaffected by antibiotics due to their metabolic inactivity. This phenomenon creates a transient resistance distinct from genetic resistance mutations.

A third mechanism revolves around the increased expression of bacterial efflux pumps in biofilms **(Figure 5**). These membrane proteins help maintain bacterial homeostasis by expelling toxic substances, and their upregulation in biofilms enhances antibiotic resistance. Various families of efflux pumps, including ABC, SMR, MATE, RND, and MFS, play pivotal roles in biofilm antibiotic resistance by actively expelling antibiotics or reducing their cytoplasmic concentrations **(Roy & al., 2018).**

The multifaceted nature of biofilm resistance is further illustrated in **(Figure 4),** which categorizes mechanisms into four classes: active molecule inactivation, altered target sensitivity, reduced drug concentration at the target site, and efflux systems. These mechanisms, which encompass limited diffusion, enzyme-mediated neutralization, heterogeneous functions, slow growth rates, and persistent cells, contribute to the formidable antibiotic resistance observed in biofilms.

Notably, the physical barrier of exopolysaccharides impedes antibiotic diffusion, while the inactivation of antibiotics occurs upon binding to the biofilm matrix. Such complexities challenge our understanding of biofilm resistance, necessitating a comprehensive approach to combat these persistent bacterial communities **(Carrascos & ., 2021).**

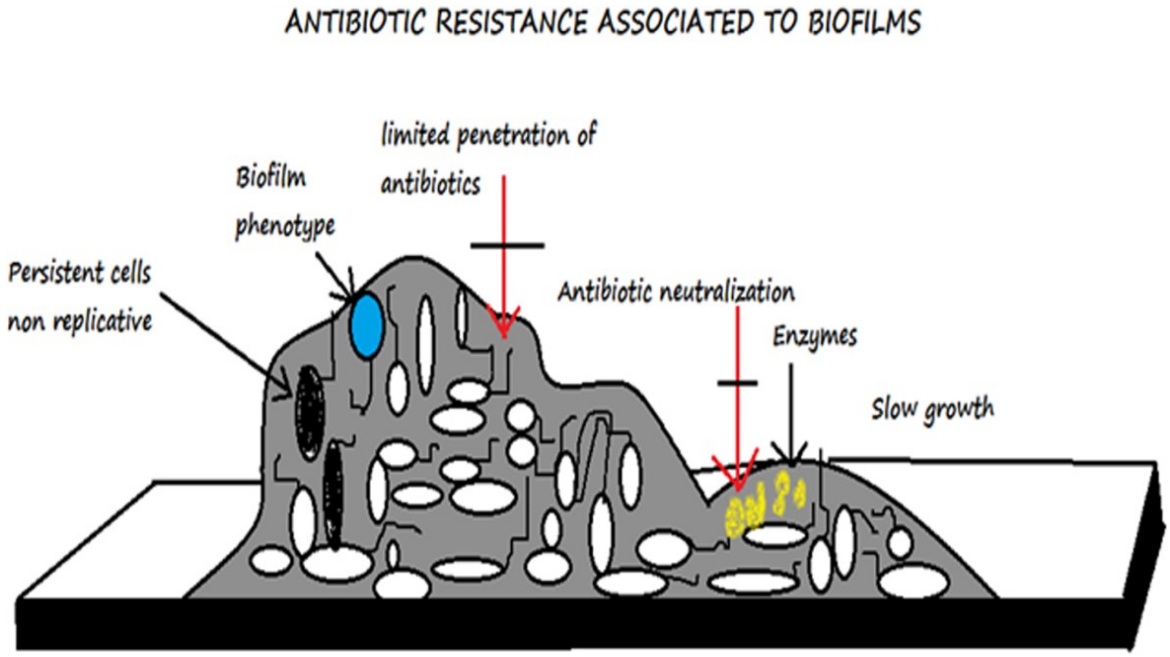

Figure4 : Antibiotic resistance associated with biofilm.Description of the key mechanisms involved in antibiotic resistance,such as enzyme-causing neutralizations,presence of persistent (non-dividing) cells,and biofilm phenotype (**Berlanga & Guerrero, 2016).**

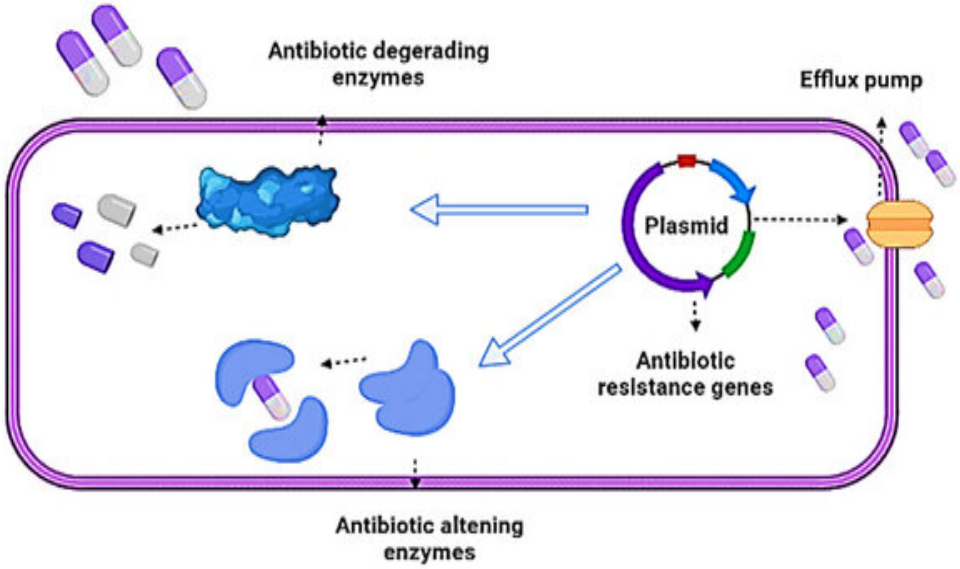

Figure 5: Mechanism of bacterial resistance to antibiotics (https://www.researchgate.net/publication/354258938).

2.5 Biofilms and CRISPR-Cas System

The CRISPR-Cas system, a family of DNA repeats found widely in prokaryotes, is a defense mechanism against plasmids and phages. It consists of CRISPR loci, short sequences repeated numerous times, typically about 250 times, alongside cas genes (CRISPR-associated genes) located adjacent to the CRISPR loci. This system operates through two fundamental mechanisms: first, by acquiring proto-spacers from foreign DNA at the leader end of the CRISPR locus during the adaptation stage, and second, by targeting invasive DNA or RNA during the interference stage **(Zegans & al., 2009).**

Recent studies indicate a connection between the CRISPR-Cas system and biofilm formation in bacteria. Evidence suggests that genes responsible for biofilm formation and the CRISPR-Cas system are intertwined. This system regulates bacterial physiology and influences virulence, pathogenicity, and EPS formation. Moreover, the CRISPR system can be harnessed as a precise and secure approach to combat microbial infections. It involves specific cleavage of the Cas9 complex, a regulator of bacterial virulence **(Xie & al., 2024).**

CRISPR-associated genes and proteins are expressed in various Gram-positive and Gram-negative bacteria associated with humans. Several biofilm-related diseases can be treated by modulating the expression of multiple virulence genes (**Figure 6).** Researchers have explored this system for developing anti-biofilm strategies, such as CRISPR inhibition (CRISPRi). This process enables gene knockdown at numerous levels and targets genes like LuxS, a regulator of quorum signaling **(Smalley & al., 2022)**.

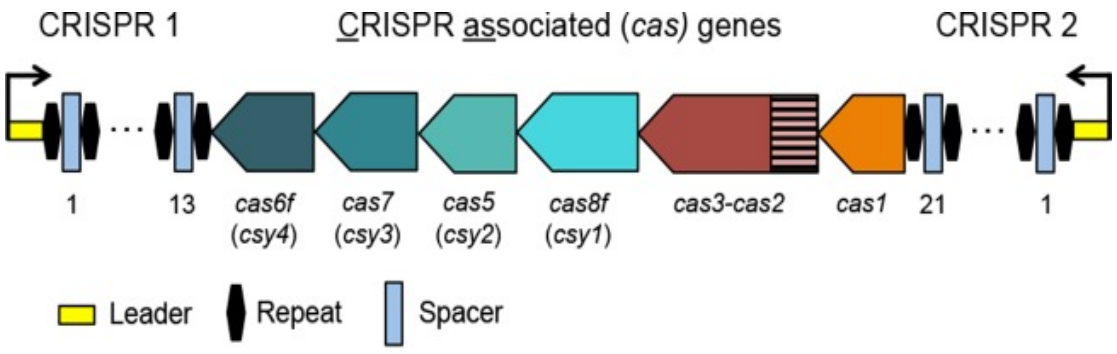

Figure 6 : *Pseudomonas aeruginosa* strain UCBPP-PA14 contains a type I-F CRISPR-Cas system organized such that the canonical type I-F cas genes are flanked by two CRISPR arrays, termed CRISPR1 and CRISPR2, containing 13 and 21 spacers, respectively**(Zegans& al., 2009).**

2.6 Economic Importance of Biofilms

Biofilms play a significant role in various aspects of life, including environmental and health-related scenarios. They are complex communities of microorganisms that adhere to surfaces and form protective matrices. Understanding the economic importance and health implications of biofilms is crucial **(Camara & al., 2022).**

In the realm of food-processing industries, microbial biofilms pose substantial risks. These biofilms can form on equipment and food products, leading to food spoilage and foodborne diseases. Notably, approximately 60% of foodborne outbreaks are attributed to biofilms. These biofilms are composed of various species, making it essential to study their diverse nature's impact on different sectors such as fresh produce, dairy, meat, fish processing, seafood, fermentation, and brewing. Controlling biofilms is vital to ensure food safety at various production stages. Typical food industry pathogens include *Escherichia coli*, *Bacillus cereus*, and *Campylobacter jejuni*. On the other hand, biofilms can play a positive role in microbial fermentation by offering unique benefits such as cell immobilization, resistance to toxic compounds, and long-term cell activity **(Bengtsson-Palme, J. (2020).**

The sector's sustainability in agriculture is critical for feeding the growing global population. Biofilm fertilizers produced by agriculturally important microbes (AIMs) can revolutionize sustainable agriculture. AIMs, including plant growth-promoting rhizobacteria (PGPR), enhance plant development and productivity while also aiding in bioremediation and serving as biocontrol agents. Additionally, using multispecies biofilms in agriculture, particularly those involving bacteria and fungi offers advantages like unique polysaccharide production and improved soil ecology. These biofilms can interact with plants in mutually beneficial, commensal, or pathogenic ways, contributing to soil health and plant growth (**Hassani and Hacquard, 2018).**

Furthermore, biofilms have significant potential in the bioremediation of organic pollutants, offering eco-friendly, cost-effective solutions. Microbes, such as *Pseudomonas, Arthrobacter, Alcanivorax, Bacillus*, and *Rhodococcus* species, can efficiently degrade hydrocarbons found in marine environments. Biofilm-based wastewater treatment is crucial to address the increasing demand for clean water, especially in areas with high wastewater generation. Nitrogen and phosphate removal from wastewater is vital to prevent environmental issues like algal blooms and eutrophication. Bacterial biofilms contribute to

these removal processes, and bioaugmentation with specific bacteria enhances nitrogen and phosphate remova **(Ciofu,& al., 2017).**

Moreover, biofilms are utilized in biogas production through anaerobic digestion, offering a sustainable energy source. Advanced wastewater treatment facilities based on biofilms provide several advantages, including concurrent removal of multiple pollutants, cost-effectiveness, and energy efficiency. Algal biofilms can also grow on wastewater, freeing it from heavy metals and pollutants. These algae can be harvested for biofuel production, potentially aiding crop irrigation during droughts **(Kraft and Ackerly, 2014 ; Herrgård and Nielsen, 2021)**.

2.7 Biofilms in Health

Biofilms pose significant challenges to human health. They are associated with pathogenic bacteria responsible for diseases like dental plaques, cystic fibrosis, infective endocarditis, urinary tract infections, and chronic wounds **(Figure 7).** These bacteria exhibit resistance to treatment methods due to multidrug resistance genes and various mechanisms, leading to growing concerns about antimicrobial resistance.

Conversely, biofilms containing beneficial bacteria, such as *Lactobacillus* and *Bacillus* strains, promote human gut health by aiding tissue growth and boosting the immune system.In healthcare settings, biofilms on medical devices like catheters, breast implants, dental implants, prosthetic joints, contact lenses, and ventilators cause persistent and recurrent infections resistant to antimicrobial treatments. This situation requires urgently exploring techniques like sterile surgical procedures, antibiotic prophylaxis, and antimicrobial coatings to prevent biofilm growth **(Rather & al., 2021**).

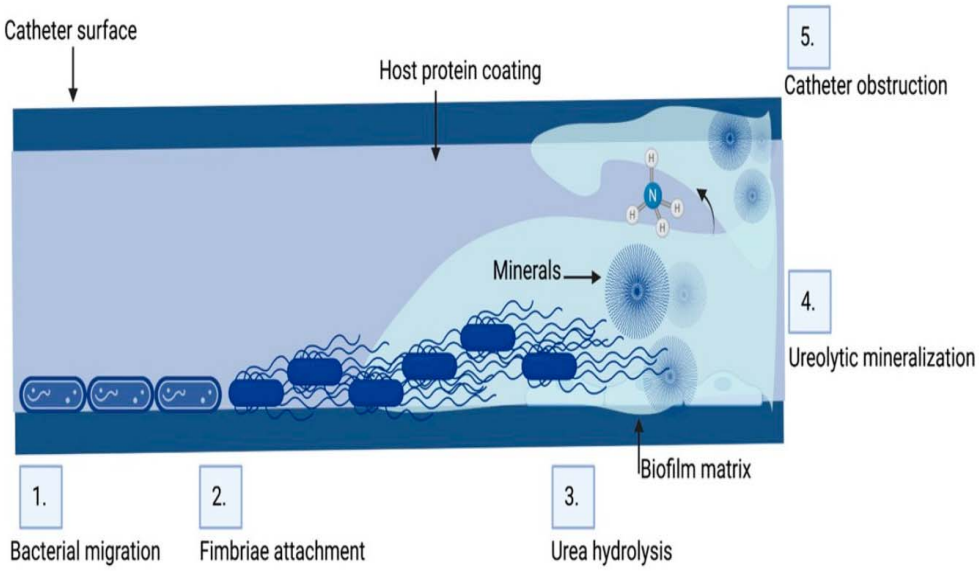

Figure 7: Biofilm formation and pathogenesis mechanism of CAUTI(Satish Sharma & al ., 2023). Created on Biorender.com (31 March 2023).

The environmental conditions created on the catheter surface make it an ideal site for bacterial attachment and formation of biofilm structures. (1) Bacteria migrates through the periurethral area along the catheter surface. (2) Fimbriae attach to the body-fluid-derived catheter surface or directly to the catheter material inducing EPS production and biofilm formation. (3) Some bacteria such as P. mirabilis produce enzymes involved in the hydrolysis of urea in urine into ammonia, increasing the local pH leading to the production of minerals in urine which results in struvite crystals. (4) Struvite formed is incorporated into the developing biofilm—a process called ureolytic mineralization, which is also facilitated by the capsule polysaccharides. (5) Fully developed crystalline biofilm eventually causes catheter obstruction.

Tissue-related biofilm infections occur when microorganisms form biofilms on tissues and implants, causing diseases like dental biofilms, cystic fibrosis-related lung infections, infective endocarditis, chronic wound infections, and periprosthetic joint infections. These biofilms are notorious for their resistance to antibiotics and the immune system.

Biomaterial-associated biofilms resist eradication once formed, often afflicting medical devices like mechanical heart valves, prosthetic joints, endotracheal tubes, and contact lenses. This results in prolonged hospital stays, surgeries, and extensive antibiotic treatments **(Roy & al., 2018).**

2.8 Treatment of Biofilms on the Surface of Biomaterials

Treatment of biofilms on the surface of biomaterials presents ongoing challenges. Current approaches involve high-dose antibiotic administration and, if symptoms persist, surgical replacement of the infected implant (**Figure 8**). However, rising antibiotic resistance necessitates alternative strategies to combat clinical medical device-related biofilm infections (**Shade & al., 2012 ; Arunasri and Mohan, 2019).**

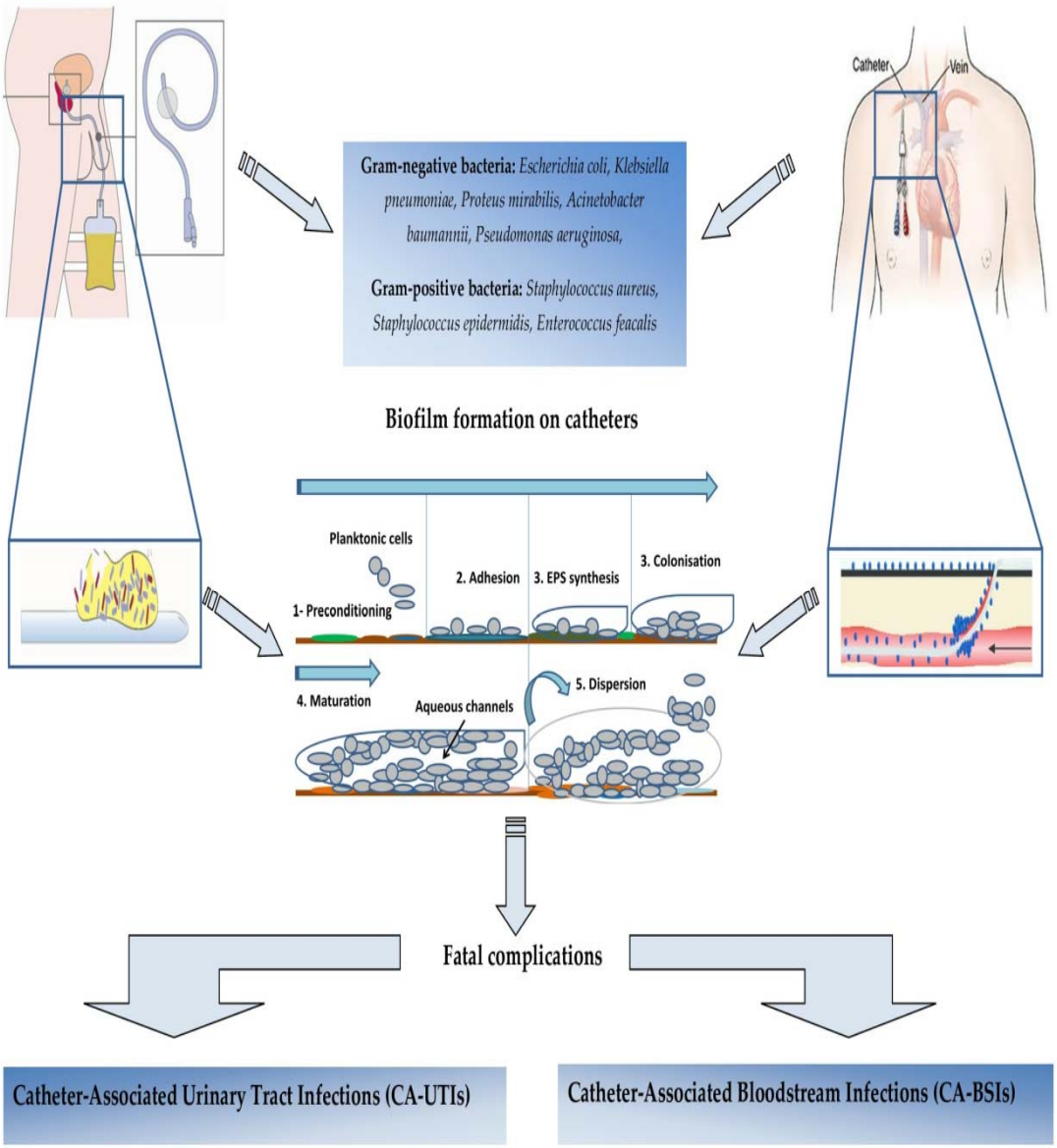

Figure 8: Biofilms formation on catheters (Beloin & al., 2016).

In the biomedical field, antibacterial materials are crucial in treating biofilm infections. Pathogens commonly responsible for medical device-related infections, such as methicillin-resistant *S. aureus* (MRSA) and *E. coli*, often necessitate antibiotic treatment. However, traditional antibiotic therapy can lead to side effects and drug-resistant strains. Antibacterial coatings applied to implant surfaces have emerged as a valuable strategy to address this issue. These coatings can be categorized into two types: active coatings, releasing antimicrobial agents, and passive coatings, which prevent bacterial attachment **(Al-Ansari & al., 2020 ;Bengtsson & al., 2020).**

2.8.1 Active Coatings

Active coatings frequently incorporate antibiotic or silver compounds on implant surfaces to combat medical device-related infections. For example, hydroxyapatite coatings combined with antibiotics show anti-biofilm effects. Nanomaterials, like silver nanoparticles, exhibit antibacterial solid and anti-biofilm properties. Additionally, enzymes and antimicrobial peptides have shown promise in inhibiting biofilm formation **(Al-Ansari & al., 2020).**

2.8.2 Passive Coatings

Passive coatings focus on preventing bacterial attachment. Strategies include using hydrophilic polymers like hyaluronic acid, hydrogel coatings, and heparin coatings. Hydrophilic surfaces reduce bacterial adhesion, offering a non-cytotoxic approach to preventing biofilm formation.

2.9 Modification of Implant Surfaces

The physicochemical properties of implant surfaces influence cell adhesion and subsequent biofilm formation. These characteristics include electrostatic interactions, surface energy, roughness, and chemical group modifications. Surface modification can be achieved through physical or chemical methods.

2.9.1 Physical Modifications

Physical methods, such as surface treatments, can effectively change the wettability and roughness of implant materials. Superhydrophobic surfaces with nano- or microscale

structures naturally repel water and limit bacterial adhesion. Techniques like femtosecond laser-induced surface structures and double etching have shown promise in reducing bacterial colonization and biofilm formation **(Berlanga and Guerrero, 2016).**

2.9.2 Chemical Modifications

Chemical modifications alter the chemical properties of material surfaces to regulate initial microorganism adhesion and biofilm formation. Self-assembled monolayers (SAMs) enable precise control of surface properties. Functional chemical modifications, such as quorum-sensing inhibitors (QSIs), disrupt biofilm formation mechanisms by interfering with bacterial communication **(Castillo-Juárez & al., 2015).**

2.10 Methods of Combating Biofilms

Biofilm formation is closely associated with heightened resistance of cells to antimicrobials. Compounds capable of inhibiting biofilms hold promise for treating infections.

Biofilm-forming bacteria exhibit antibiotic resistance levels up to 1000 times higher than their planktonic counterparts. Biofilms impede antibiotic diffusion due to low membrane permeability and fewer outer surface porins. Various approaches have been proposed to combat biofilm development, including antiseptics, disinfectants, antibiotics, bacteriophages, enzymes, essential oils, surface modifications, and QS inhibitors **(Chang & al., 2011)**.

Phytoextracts derived from the diverse plant life on Earth have shown promise *in vitro* for addressing biofilm-related infections. Plant extracts rich in secondary metabolites and bioactive compounds offer potential treatments for biofilms. These extracts have been tested against bacteria and fungi, which can form biofilms.

Nanoparticles offer a non-conventional approach to targeting biofilm-related microbial infections. Their small size, high sensitivity, and large surface area-to-volume ratio make them suitable for penetrating and destroying biofilms **(Fuqua& al., 2001).**

Antimicrobial peptides (AMPs) are short chains of amino acids with broad-spectrum antibacterial activity. They can disrupt biofilms by interfering with microbial cell membranes, bacterial QS signaling systems, EPS, and bacterial stress response regulation. AMPs have both hydrophobic and hydrophilic properties, enabling them to bind to bacterial cell

membranes, penetrate biofilms, and destroy bacterial cells. Plant-derived AMPs have shown promise against human pathogens and could be used with antibiotics and other bioactive molecules to combat biofilms **(Daniel and Chassaing, 2021)**.

Anti-virulence compounds target factors responsible for infection processes without affecting pathogenic bacteria, making them a promising approach for biofilm control. These compounds can inhibit quorum sensing, preventing gene expression in biofilm production.

Plant-derived substances, such as sulfur-containing compounds, monoterpenes, terpenoids, phenylpropanoids, benzoic acid derivatives, diarylheptanoids, coumarin, flavonoids, and tannins, have demonstrated quorum sensing-inhibiting properties. Ethanolic extracts of certain plants have also shown anti-virulence capabilities against pathogenic bacteria without being toxic to the host.

Phage therapy is another approach to combating biofilms. Bacteriophages, viruses that infect bacteria can penetrate cells and produce exopolysaccharide-degrading enzymes, disrupting biofilms. Challenges in phage therapy include immune system interactions, administration methods, resistance development, and the need for clinical trials (**Figure 9).**

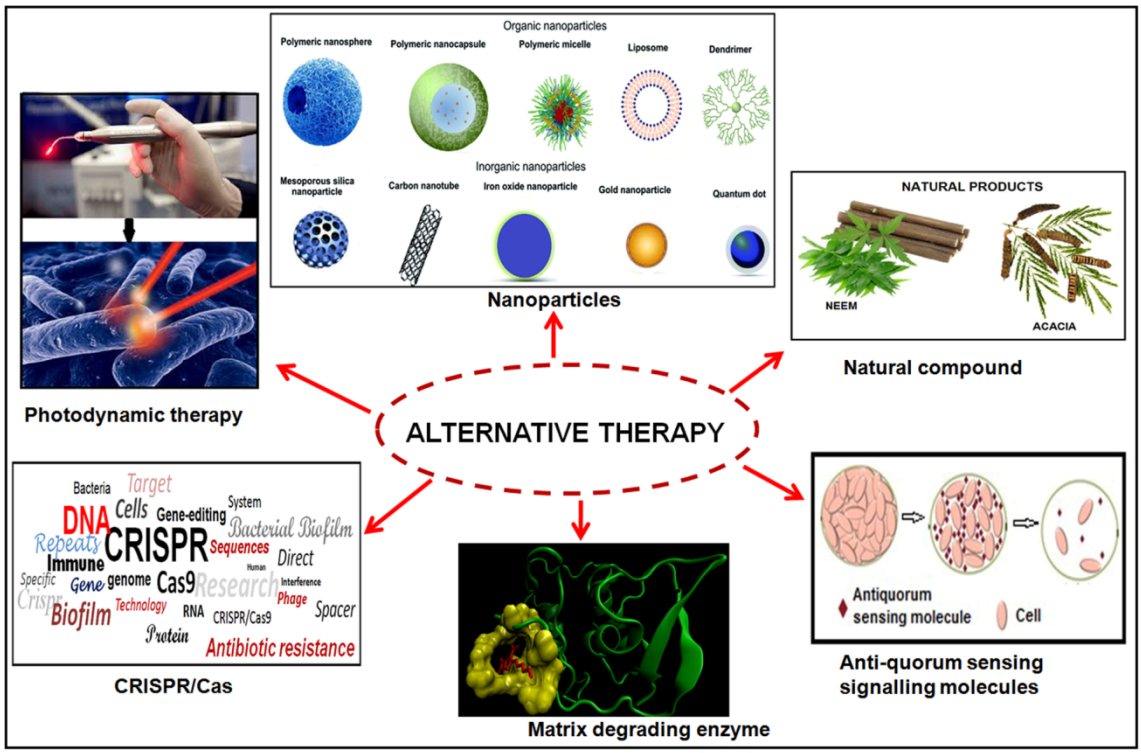

Figure 9: Diagrammatic representation of the alternative approaches against antibiotic resistant biofilms communities (Divakar &al., 2019

3 Quorum sensing

3.1 Introduction

Bacteria, previously believed to be capable only of simple processes and single-celled life, are now recognized for their ability to collaborate in multi-cellular groups. Coordinated behaviors encompass bioluminescence, virulence factor production, secondary metabolite production, competence for DNA uptake, and biofilm formation. These processes are ineffective when undertaken by a single bacterium acting alone. Success requires population-wide coordination of individual cells. Bacteria employ quorum sensing, a cell-to-cell communication process to orchestrate collective behaviors **(Bzdrenga & al., 2016).**

Quorum sensing involves the production, release, accumulation, and group-wide detection of extracellular signaling molecules known as autoinducers. Gram-negative quorum-sensing bacteria use small molecules as autoinducers, detected by cytoplasmic transcription factors or transmembrane two-component histidine sensor kinases. In both cases, autoinducer-receptor complexes regulate the expression of quorum-sensing-dependent target genes.

Gram-positive bacteria, on the other hand, utilize oligopeptides as autoinducers and transmembrane two-component histidine sensor kinases as partner receptors. Often, these complexes activate the expression of the autoinducer synthase gene, elevating the extracellular autoinducer concentration as bacteria enter quorum-sensing mode. This feedforward autoinduction loop synchronizes behaviors across the bacterial population **(Lasarre& al., 2013 ;Bzdrenga & al., 2016).**

Bacteria typically integrate information encoded in multiple quorum-sensing autoinducers into gene expression control, facilitating intra-species, intra-genera, and inter-species communication, including interactions with microbiota. Quorum-sensing circuits frequently incorporate feedback and feedforward regulatory loops to fine-tune responses, adjust input-output ranges, reduce noise, and determine whether cells adopt an individual or group lifestyle program. Quorum-sensing circuits can also intersect with global regulators like the alternative sigma factor RpoN, RNA-binding proteins Hfq and CsrA, and the nucleoid protein Fis to refine quorum-sensing-dependent gene expression further **(Papenfor and Bassler, 2016).**

Our current understanding of quorum-sensing mechanisms primarily involves studying traditional well-mixed pure laboratory cultures. While these studies have yielded fundamental insights into the molecular mechanisms underlying quorum sensing in various bacteria, it's important to note that bacteria often exist in mixed-species and non-ideal conditions with fluctuations.

Furthermore, they form structured surface-bound communities called biofilms. Therefore, recent research efforts have focused on defining how quorum sensing functions in realistic bacterial habitats, including spatially structured and fluctuating conditions that mimic natural bacterial niches such as heterogeneous 3D biofilms, environments with fluid flow, and within eukaryotic hosts where pathogens interact with the host microbiota **(Redderson & al., 2022).**

3.2 QUORUM SENSING BACTERIA

Quorum-sensing bacteria produce and release chemical signal molecules known as autoinducers, and their external concentration increases with rising cellpopulation density. Bacteria detect these autoinducers' minimal threshold stimulatory concentration, leading to gene expression and behavior alterations. Through these signal-response systems, bacteria coordinate specific behaviors on a population-wide scale, effectively functioning as multicellular organisms.

These systems are assumed to share commonalities because bacterial communication is a fundamental process. However, system variations likely stem from optimization for survival within specialized niches unique to each bacterial species. Consequently, the signals, receptors, signal transduction mechanisms, and target outputs of each quorum-sensing system reflect the distinctive biology of the respective bacterial species (**Miller and Bassler, 2001**).

Quorum sensing, a vital communication mechanism among bacteria, plays a pivotal role in the formation and behavior of biofilm communities. Biofilms in diverse environments such as soil, riverbeds, sewage, and even plant and animal tissues consist of bacteria encased in an extracellular matrix composed of polysaccharides, proteins, and extracellular DNA. Unlike well-mixed liquid cultures, biofilms are inherently heterogeneous and subject to dynamic changes, posing questions about nutrient acquisition and diffusion within them. Understanding the dynamics of quorum sensing within these biofilm structures is fundamental to this field of study **(Ciofu & al., 2022).**

Bacteria exhibit varying behaviors in biofilm formation based on their quorum-sensing states. For example, *Pseudomonas aeruginosa* forms biofilms at high cell density (HCD) in response to autoinducer accumulation, while Vibrio cholerae and *Staphylococcus aureus* initiate biofilms at low cell density (LCD), with autoinducer accumulation repressing this process.

However, one common thread is that increased fluid flow necessitates a higher bacterial biomass to initiate quorum sensing. Interestingly, biofilm formation can be enhanced under flow conditions, leading to spatial fate decisions and distinct biological functions within the biofilm structure.

In biofilm communities and other bacterial populations, bacteria often secrete extracellular molecules akin to public goods. Quorum sensing tightly regulates the production of these public goods, and mechanisms such as spatial structure and social policing promote cooperation while deterring cheating within bacterial populations. Understanding these dynamics is paramount for maintaining population fitness in biofilms and various bacterial communities (**Natarajan and Bhatt, 2020).**

Quorum sensing varies between Gram-negative and Gram-positive bacteria, each employing distinct signaling mechanisms. In Gram-negative bacteria like *Vibrio fischeri*, LuxIR-type systems are the hallmark, utilizing acyl-homoserine lactone (AHL) signals. These systems, exemplified by *V. fischeri*, induce behaviors like bioluminescence in response to AHL accumulation, enabling coordinated responses to population density **(Papenfor and Bassler, 2016)**.

LuxIR-type systems are prevalent among Gram-negative proteobacteria, promoting intraspecies communication due to the specificity between LuxR proteins and their cognate AHL signals**(Figure 10).** To prevent premature activation, mechanisms like increased LuxR protein stability upon AHL binding and active export of AHL signals maintain precise coordination of quorum-sensing circuits in these bacteria (**Waters and Bassler , 2017**).

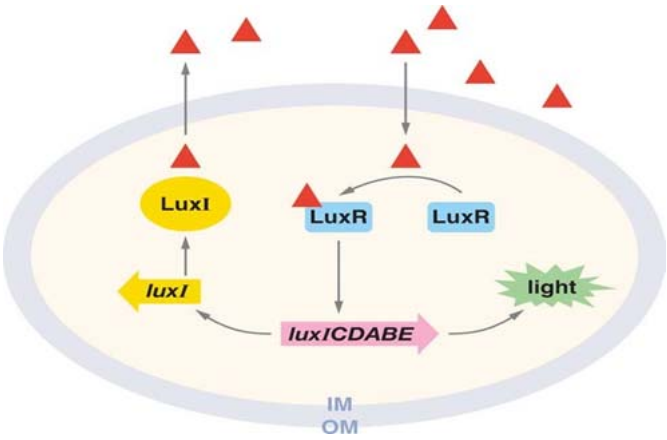

Figure10: Quorum sensing in*Vibrio fischeri*; aLuxIRsignalingcircuit. Red trianglesindicatetheautoinducer that LuxI produces.OM,outermembrane;IM,innermembrane (Shamebo & al., 2016)

In contrast, Gram-positive bacteria employ a quorum-sensing mechanism centered around modified oligopeptides as signaling molecules and membrane-bound sensor histidine kinases as receptors. Signal release in Gram-positive bacteria involves intricate processes, including peptide cleavage from precursor peptides and the addition of chemical groups. *Staphylococcus aureus*is a prime example, where quorum sensing, regulated by the Agr system**(Figure 11)**,governs a gene expression switch between attachment and virulence**(Novick , Geisinger , 2008).**

Intraspecies competition in *S. aureus* strains based on Agr system specificity influences disease outcomes, showcasing the importance of cell-cell communication in niche establishment. This intricate interplay between signal-receptor pairs may contribute to the evolution of new bacterial species **(Henke and Bassler,2004).**

Furthermore, Gram-positive streptomycetes, known for their production of antibiotics, employ γ-butyrolactones as autoinducers to control morphological differentiation and secondary metabolite production. These signals, structurally distinct from AHL autoinducers used by Gram-negative bacteria, do not cross-communicate, highlighting the specificity of quorum-sensing mechanisms within different bacterial groups **(Nepomuceno& al., 2023)**.

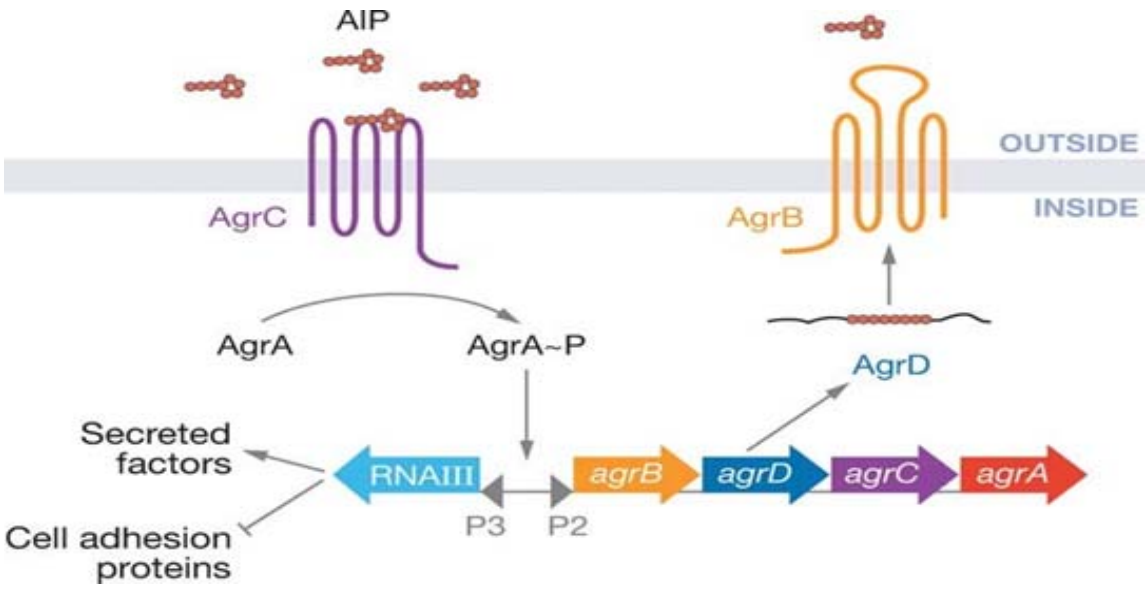

Figure11: Using a two-component response regulatory system,*Staphylococcus aureus* detectsand responds to an extracellular peptide. Small red circles indicate the AIP.P2andP3 designate the promoters for *agr BDCA* and RNAIII, respectively (Henke and Bassler,2004).

3.3 QUORUM-SENSING NETWORK ARCHITECTURE

The study of quorum-sensing networks in bacteria reveals intricate mechanisms of cell-cell communication, shedding light on the various molecular arrangements facilitating this process. This research offers insights into information dissemination, detection, relay, and response within these systems **(Figure 12).**

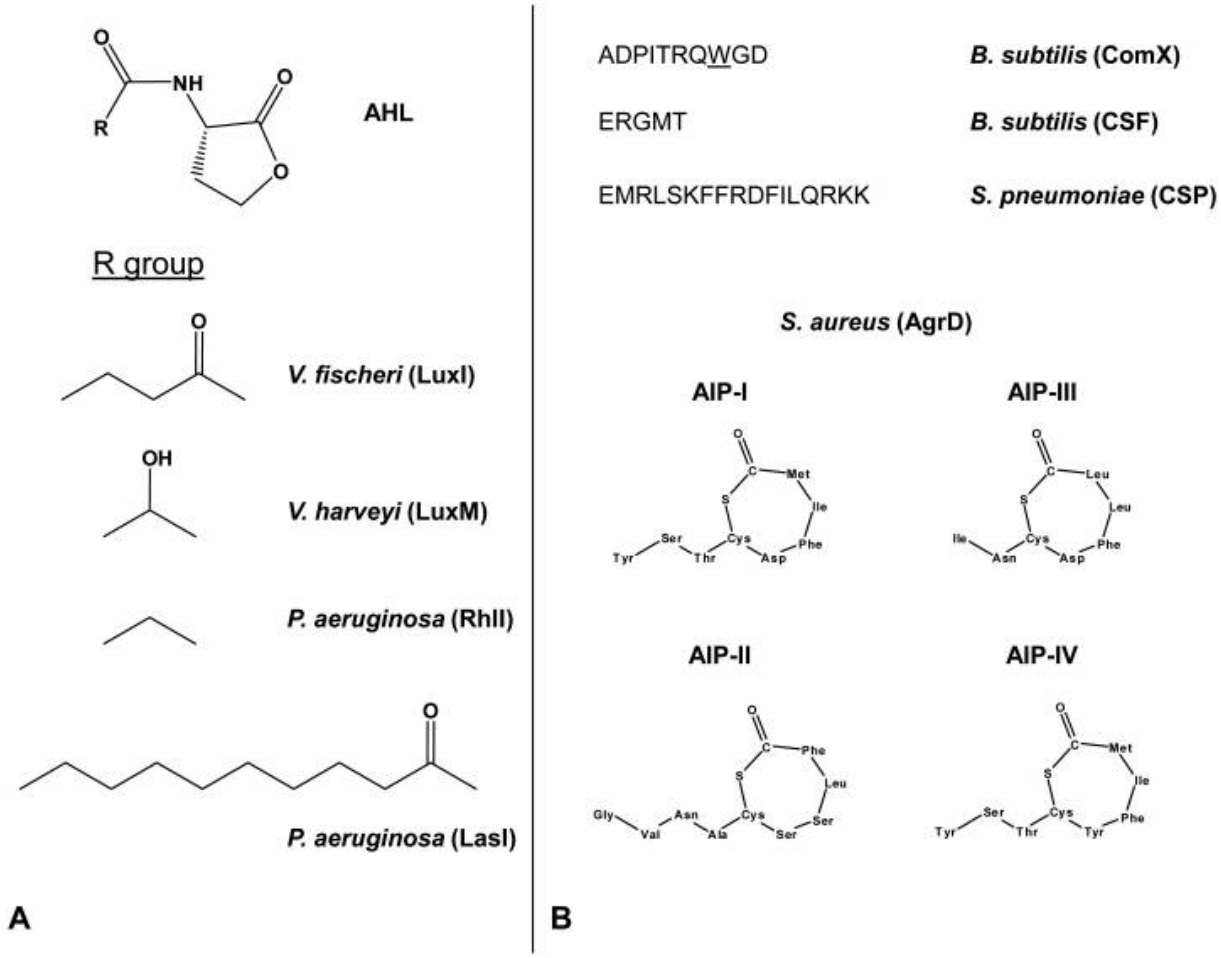

Figure 12 : Structures of bacterial autoinducers **(Fuca&al., 2001).**

(A) Homoserine lactone autoinducers produced by different Gram-negative bacteria. (B) Amino acid sequences of three peptide autoinducers, ComX, CSF, and CSP, produced by Gram-positive bacteria. The underlined tryptophan in *B. subtilis* ComX is isoprenylated. The four different AIPs produced by *S. aureus*. (C) DPD, the precursor to AI-2. In the presence of boron, AI-2 exists as *S*-THMF-borate. In the absence of boron, AI-2 exists as *R*-THMF. (D) Structure of *V. cholerae* CAI-1 and Amino-CAI-1. (E) Structure of the PQS autoinducer of *P. aeruginosa*.

3.4 QUORUM-SENSING REGULONS

The advent of genomic profiling has revealed that quorum sensing in many bacteria exerts global control over gene expression. Two transcription profiling studies in *S. pneumoniae* identified over 150 competence-regulated genes, categorized as early, late, delayed-induction, and repressed **(Shanker and Federle, 2017).**

Early genes are responsible for signal production, export, and detection, while some late genes facilitate DNA internalization. Delayed genes play roles in bacterial stress responses. Gene-disruption experiments showed that only 23 out of 124 quorum-sensing-controlled genes are required for competence. Mutants of *S. pneumoniae* and related streptococci with impaired quorum sensing exhibit defects in various pathways, including biofilm formation, acid tolerance, bacteriocin production, and virulence **(Fuqua & al., 2001).**

These results suggest that quorum sensing in streptococcus initiates a global developmental program, with competence development being just one aspect. Transcriptome analyses of *P. aeruginosa* identified 616 genes as part of the regulon, with 222 genes being repressed upon adding autoinducers**(Figure 13**). Although these experiments were conducted under different conditions, they highlight the role of quorum sensing in gene regulation **(Akanksha & al., 2015).**

Moreover, transcriptional analysis of *V. cholerae* quorum-sensing mutants revealed that quorum sensing represses the entire virulence regulon (>70 genes). These recent whole-genome quorum-sensing studies have two significant implications. First, quorum sensing enables bacteria to switch between distinct genome-wide programs, challenging the notion of bacteria as primitive single-celled organisms.

Bacteria now appear to undergo complex developmental programs similar to eukaryotic organisms. Second, quorum sensing represses large groups of genes, suggesting that its primary function may be to initiate activities beneficial to bacterial group participation and terminate processes more suitable for bacteria living in relative isolation outside a community structure **(Clay Fuqua, 2006).**

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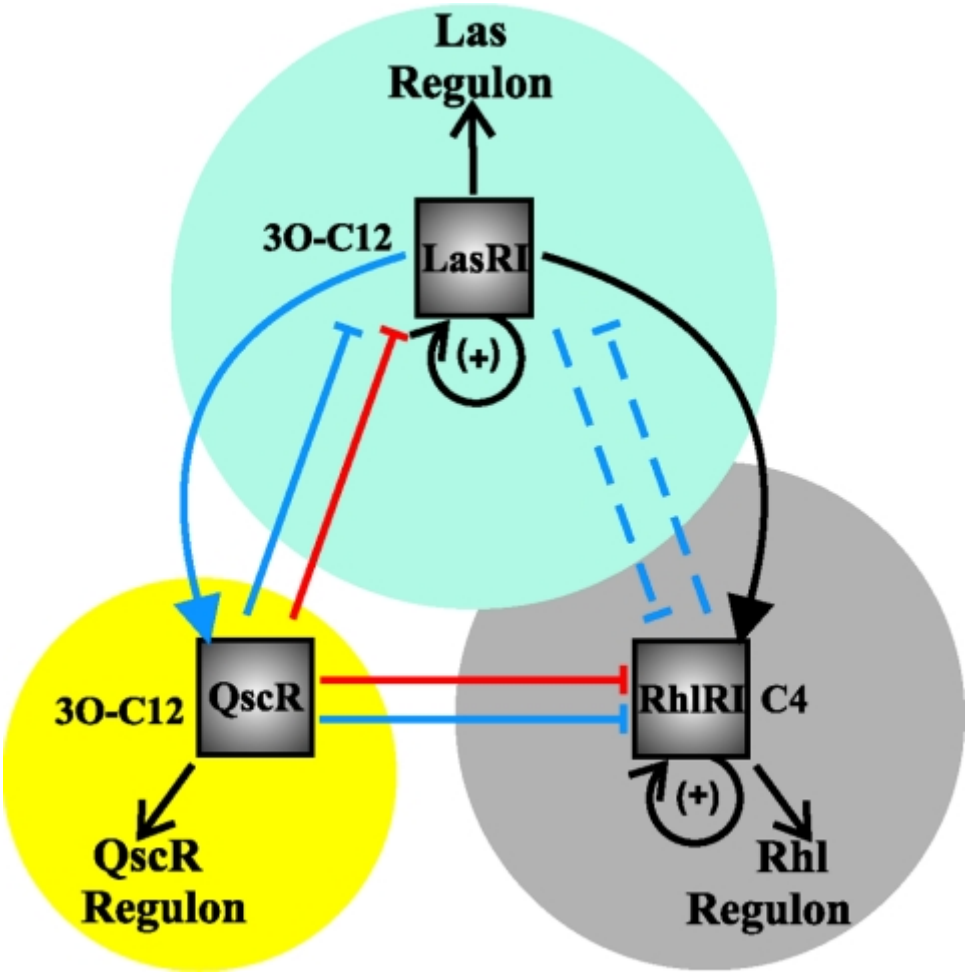

Figure 13: Model of *P. aeruginosa* quorum-sensing network. Black arrows indicate direct transcriptional control (positive/negative or as indicated), blue arrows indicate protein-AHL interactions, and red arrows are protein-protein interactions. Solid arrows are well-supported mechanisms of regulation, while dashed arrows are more tentative. Looped arrows indicate positive feedback on cognate AHL synthesis via LasR and RhlR. Underlying circles represent genes under direct transcriptional control of each LuxR-type protein (Fuqua, 2006).

3.5 INTERSPECIES COMMUNICATION AMONG BACTERIA

Quorum sensing extends beyond global gene expression control, enabling communication among bacteria within and across species **(Figure 14)**. This concept emerged through the discovery of autoinducer AI-2, a signal employed by *V. harveyi* in quorum sensing. LuxS, responsible for AI-2 synthesis, is found in approximately half of the sequenced bacterial genomes, with confirmed AI-2 production in numerous species affecting gene expression.

LuxS participates in the SAM metabolism pathway, producing S-adenosylhomocysteine (SAH) as a byproduct. In bacteria with LuxS, SAH is converted into adenine, homocysteine, and the signaling molecule DPD by Pfs and LuxS enzymes. DPD is a reactive product with various possible reactions, suggesting that different bacterial species may recognize distinct DPD-derived molecules as AI-2 signals**(Castillo-Juárez & al., 2015).**

V. harveyi AI-2 is (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate (S-THMF borate), while S. typhimurium AI-2 is (2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R-THMF). These molecules are linked through DPD's cyclization with two stereochemistries and subsequent hydration and borate addition.

The presence of boron in *V. harveyi* AI-2 is notable, as it is abundant in marine environments but scarce in terrestrial ones. This chemistry can be manipulated by altering boron concentrations. High boron promotes *V. harveyi* AI-2 formation, while low boron favors S. Typhimurium signal production, affecting gene expression responses in these species (**Lasarre 2013**).

These investigations reveal that bacteria use a shared biosynthetic pathway to create signal intermediates influenced by their environment's chemistry. Other DPD derivatives may exist, and some bacteria might possess multiple AI-2 receptors, adjusting behaviors based on different DPD derivatives. LuxS is the sole enzyme required for synthesizing this family of interconverting signal molecules, representing an efficient method for evolving a complex bacterial language.

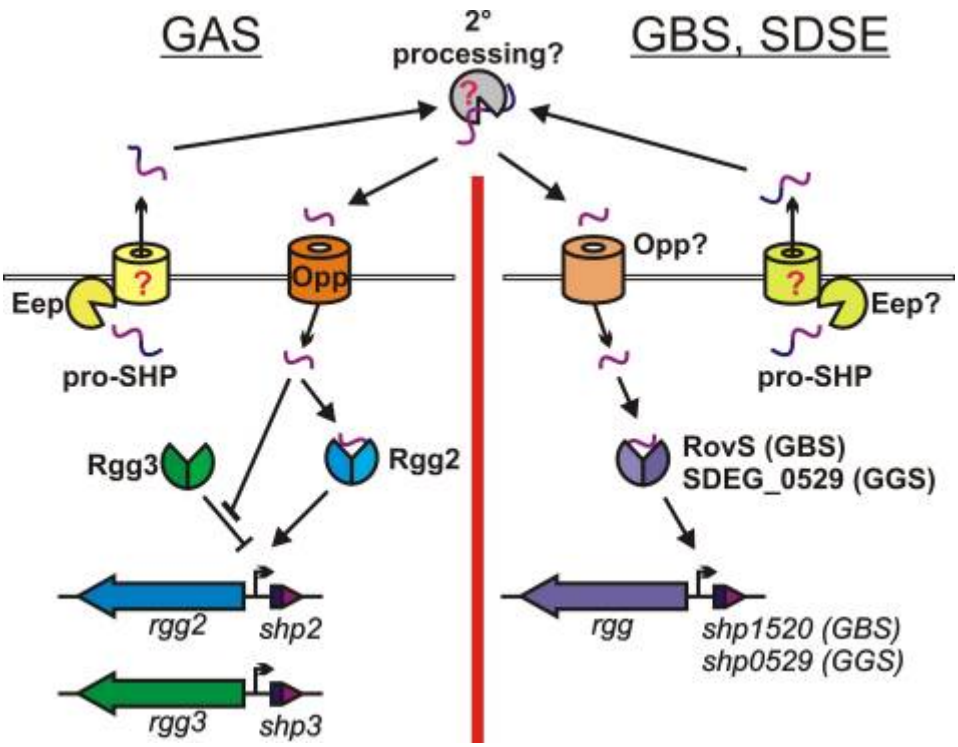

Figure 14: multiple streptococcal species, including at least one member of the pyogenic group, utilize proteins belonging to the Rgg family of transcriptional regulators to mediate QS activity (chang& al., 2011).

3.6 QUORUMQUENCHING

Quorum sensing plays a crucial role in globally controlling the physiology of bacterial populations, often occurring at the interfaces of different bacterial populations or the host-bacterial boundary.

Disrupting quorum sensing can provide a competitive advantage in environments where bacterial populations compete for limited resources. Similarly, a host's ability to interfere with bacterial cell-cell communication prevents colonization by pathogenic bacteria that rely on quorum sensing to coordinate virulence**(Figure 15).** As a result, mechanisms have evolved to interfere with bacterial cell-cell communication in processes known as quorum quenching**(Lasarre & al., 2013 ;Bzdrenga & al., 2016).**

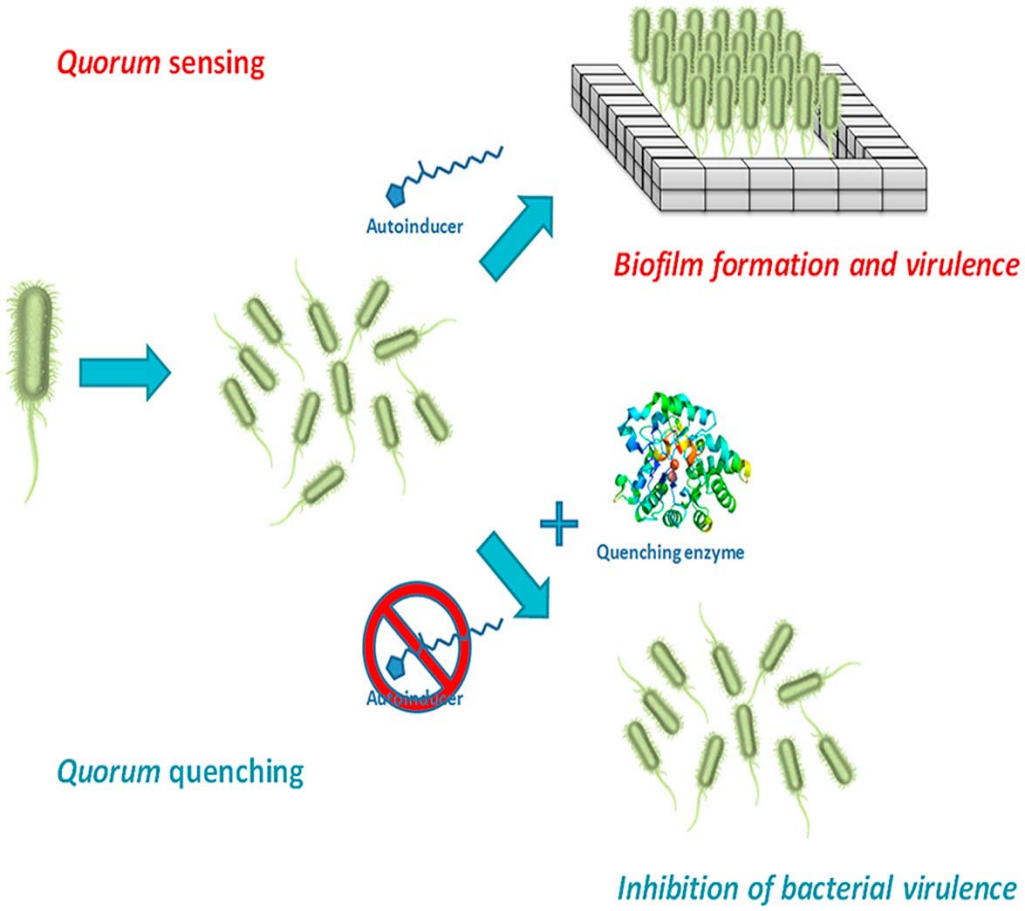

Figure 15: Inhibition of bacterial virulence by Quorum quenching (Janek Bzdrenga &al., 2016)

3.6.1 Biotechnological Applications of Quorum Quenching

Natural quorum-quenching processes are being explored for antimicrobial therapies. Overexpression of aiiA in plants confers resistance to pathogens requiring AHL-controlled virulence factors. Synthetic antagonists of *S. aureus* AIP provide resistance to infection in mice. Halogenated furanone attenuates bacterial virulence in animal models. These approaches offer alternatives to traditional antibiotics, potentially reducing bacterial resistance and may enhance industrial-scale production of bacterial products**(Hansen& al., 2015**).

3.7 EVOLUTION AND MAINTENANCE OF QUORUM SENSING IN BACTERIA

Quorum sensing in bacteria facilitates collective activities like synchronized antibiotic or protease secretion, prompting inquiries about evolution, costs, fidelity, cheating, and

eavesdropping in bacterial communication. Similar behaviors occur in social insects due to kin selection. Microorganisms also sacrifice individuals for group advantages during spore development.

Two examples underscore the importance of maintaining quorum sensing: squid eliminates cheater cells in *Vibrio fischeri*, and *Agrobacterium tumefaciens* optimizes growth while retaining virulence**(Bertani & al., 2007).** Eavesdropping mechanisms exist, exemplified by *Pseudomonas aeruginosa* detecting AI-2 from other bacteria in cystic fibrosis patients and *Salmonella* enterica responding to AHL signals for self-protection in a host **(Figure 16).** The ecological and evolutionary implications of quorum sensing in bacteria remain a subject of ongoing exploration, providing insights into group dynamics and behavior evolution**(Smalley &., 2022).**

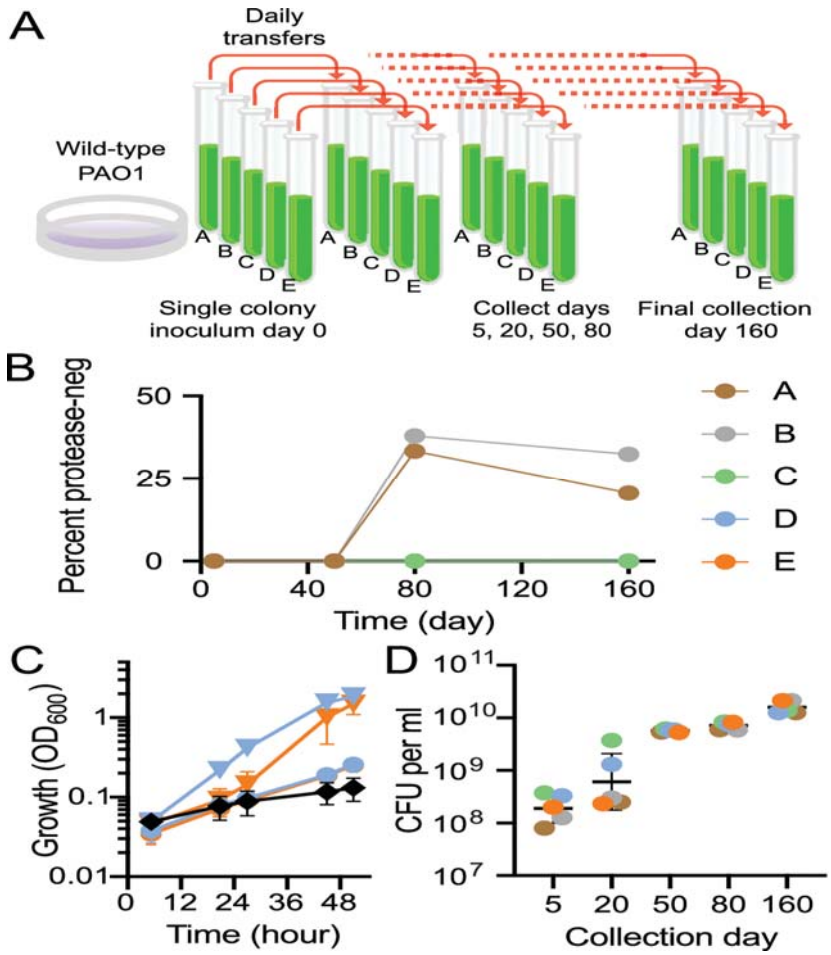

Figure 16: Long-term evolution of *P. aeruginosa* PAO1 serially passaged in a medium (CAB) that requires quorum sensing for growth (Smalley &., 2022).

4 Unculturable bacteria

4.1 Introduction

As our microbial understanding advances, we now recognize the existence of 61 distinct bacterial phyla, with a staggering 31 lacking any cultivable representatives. Similarly, within the enigmatic archaeal phylogenetic tree, 54 species have been cultured to date, representing just a fraction of the hidden diversity, with 49 lineages mostly evading cultivation attempts. This deficiency in cultivation leads to the underestimation of complex bacterial communities and raises the specter of missing out on pivotal organisms vital to ecosystems or pathogens of plants and animals **(Stewart, 2012)**.

Molecular culture-independent techniques have gained prominenceto address this limitation. These methods involve characterizing mixed bacterial populations in environmental biomass and samples from diverse hosts, including humans, through PCR amplification of housekeeping genes, especially the 16S rRNA gene. These molecular methods have uncovered a wealth of novel phylotypes inhabiting a vast spectrum of habitats, from oceans to soils and the intricate microbiota within human health and disease **(Sharm & al., 2005)**.

4.2 Reasons for 'unculturability'

Understanding the existence of unculturable bacteria has been a longstanding challenge in microbiology, with initial clues emerging from microscopy. The vast number of bacterial cells observed under the lens greatly exceeded the colonies that grew on Petri plates, giving rise to "The Great Plate Count Anomaly." This phenomenon varied across environments, initially leading to the hypothesis that non-growing cells were dead. However, subsequent investigations revealed their metabolic activity despite their inability to replicate on standard laboratory media **(Sharma & al., 2011)**.

Molecular tools, particularly DNA sequencing, played a pivotal role in shedding light on the hidden diversity of unculturable bacteria. PCR amplification and sequencing of phylogenetically informative markers like the 16S rRNA gene unveiled a vast ocean of bacterial diversity that had remained invisible to traditional culturing methods. Starting with the 11 bacterial phyla described by Woese in 1987, the number of bacterial divisions has

expanded to at least 85, with the majority lacking cultured representatives. The DNA sequencing from mixed populations consistently revealed members of these missing phyla, underscoring their genuine presence in nature **(Zheng &., 2024).** For instance, the candidate phylum TM7 has been repeatedly detected in various environments, highlighting the vast genetic and biochemical diversity eluding laboratory cultivation **(Figure 17).**

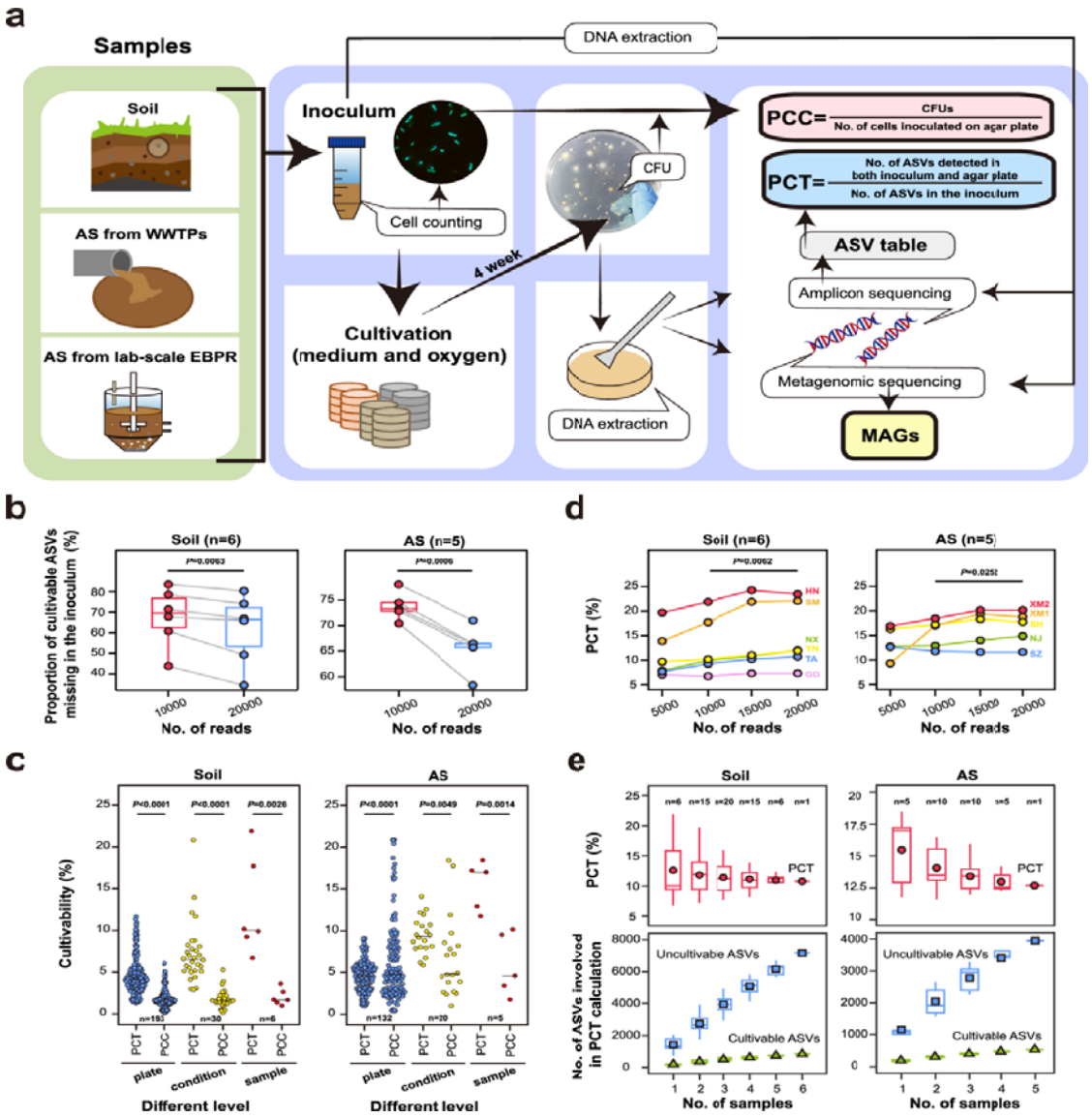

Figure 17 : Determination of proportion of cultivable taxa (PCT) and the proportion of cultivable cells (PCC) in soil and activated sludge (AS) samples(Zheng &., 2024).

a A diagram illustrated the simultaneous measurement of PCT and PCC in this study. **b** Under various sequencing depths, a significant proportion of cultivable amplicon sequence variants (ASVs) could not be detected in the inoculum samples of soil and activated sludge (AS). **c** PCTs and PCCs were compared at levels of plate (single plate), condition (all plates for a specific condition), and sample (all plates of all culture conditions for a given sample). **d** The variation of PCT with increasing sequencing depth was shown. **e** The number of detected cultivable amplicon sequence variants (ASVs) (only those also detected in the corresponding inoculum), uncultivable ASVs, and PCT changed with an increasing number of samples. Comparison analysis was performed using a paired one-tailed *t*-test for **a**–**c**. In **b** and **e**, the boxplot distributions include median, minimum, maximum, 25th and 75th percentiles, and outliers (defined as points more than 1.5 times the interquartile range [IQR]). Source data are provided as a Source Data file.

The reasons behind the unculturability of certain bacterial species are multifaceted. Some may have been overlooked due to low prevalence or slow growth, challenging their isolation via conventional culture methods. Genetically distinct phenotypically identical phylotypes can be erroneously grouped using traditional biochemical identification approaches **(Bodor & al., 2020).**

Conversely, genuine barriers to isolation on artificial media exist. Some bacteria have stringent growth requirements, such as specific nutrients, pH conditions, incubation temperatures, or oxygen levels. Different cultivation approaches yield distinct groups of bacteria from comparable samples, emphasizing the impact of culture conditions on isolating specific bacteria.

Competition for nutrients among mixed organisms, bacteriocin release, or the presence of antibacterial substances can inhibit growth. Furthermore, biofilm formation, a natural consequence of bacterial colonization, involves complex interactions and communication between species, affecting their growth and behavior **(Bodor, 2020)**.

Bacterial growth factors, like the resuscitation-promoting factor (Rpf), have been identified **(Figure 18)**. These factors potentially remodel the peptidoglycan in cell walls, stimulating growth. Moreover, signaling molecules unique to the natural habitat are essential for many bacteria's growth.

Without these beneficial interactions and signals, some bacteria may temporarily enter a state of low metabolic activity, appearing resistant to culture in isolation. This state is a survival strategy in an unfamiliar environment devoid of essential factors rather than an

inherent resistance to culture. Thus, the complex interplay of ecological, physiological, and biochemical factors contributes to the enigma of unculturable bacteria**(Stewart, 2012).**

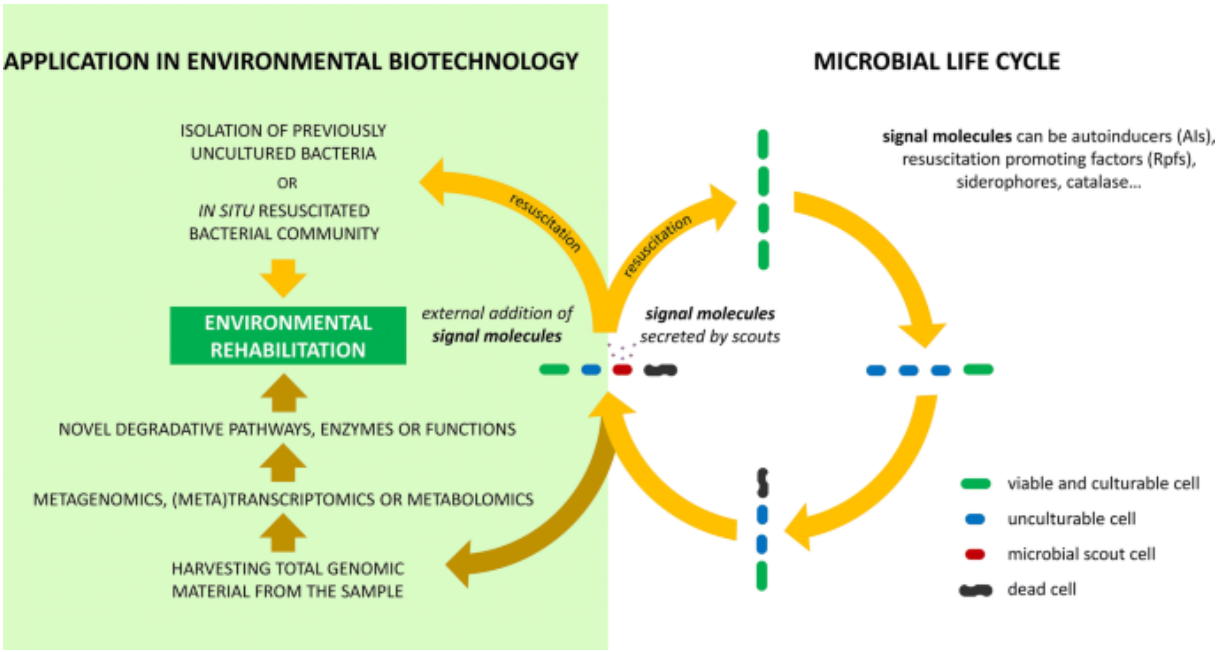

Figure 18: Life cycle of unculturable microorganisms and their environmental potential **(https://rdcu.be/dYsrU)**

4.3 WHAT IS THE SIGNIFICANCE OF THESE UNCULTURABLE BACTERIA?

Measuring biological diversity involves tallying the species described within a specific branch of the tree of life. For instance, it's estimated that between 800,000 and 1.2 million insect species are documented, owing to their accessibility and the attention they've received. Insects, found widely and requiring minimal magnification for observation, also benefit from the immense diversity of niches they occupy.

In contrast, bacterial diversity is anticipated to be even higher, with potential orders of magnitude more species. This is likely because most insect species host unique bacterial endosymbionts besides the various niches that bacteria inhabit in the environment. However, despite this potential diversity, only slightly over 7,000 validly described bacterial species exist. This limited number is attributed to the effort required to characterize new species and

the difficulty in culturing many bacteria, a necessity for species description**(Sharma, & al., 2005)**.

The need for cultivation is unique to microbes, and it arises from the challenge of gathering physiologically relevant data about bacteria without pure cultures**(Figure 19).** Consequently, only a minute fraction of bacterial diversity has been cultured or identified as a species. The missing bacteria, encompassing phyla and smaller phylogenetic subdivisions, likely harbor the most metabolic diversity among bacteria and all life domains**(Phom, 2012)**.

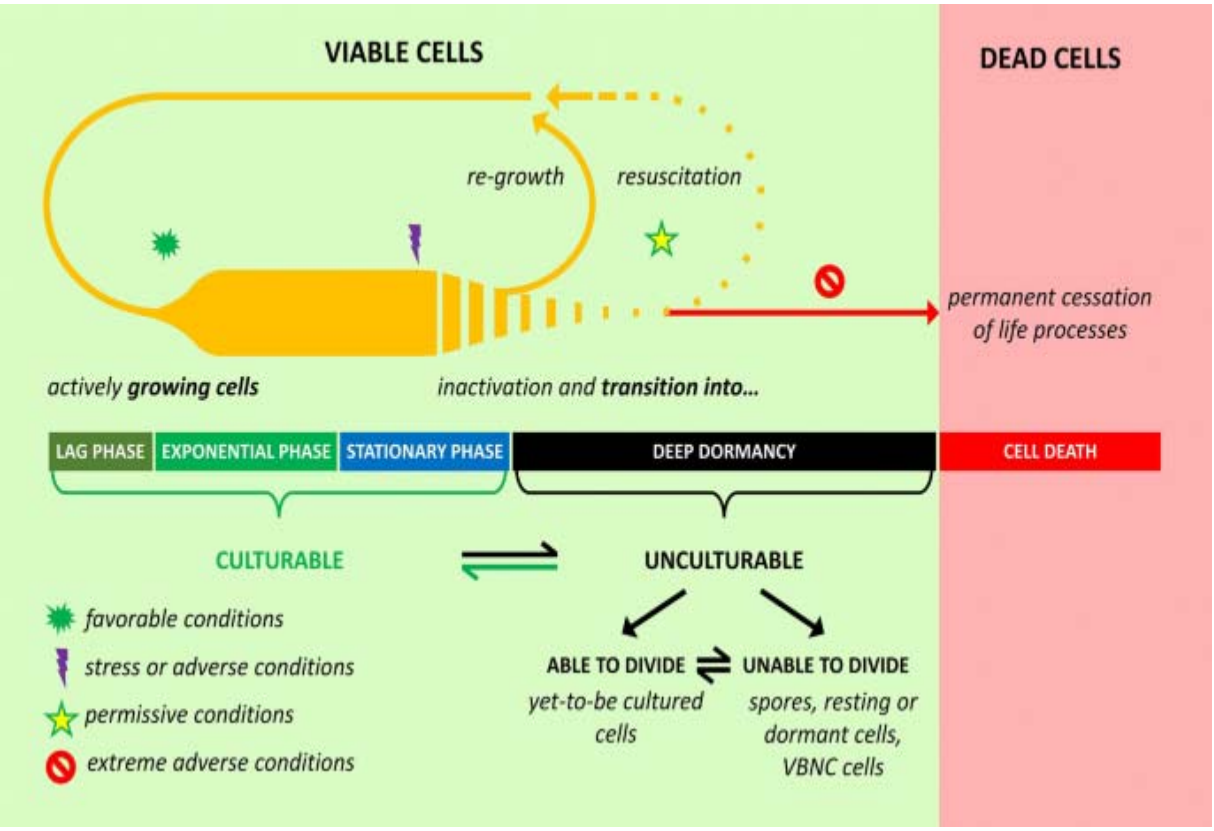

Figure 19 : Schematic diagram of the transition of bacterial cells between different physiological states (**https://rdcu.be/dYsrU**)

4.4 WHY ARE THEY NOT GROWING IN THE LABORATORY?

The challenge of cultivating these bacteria in the laboratory stems from the complexity of replicating their natural environment accurately. Despite dedicated efforts and ingenuity, pinpointing the specific environmental factors that need replication, such as nutrients, pH levels, osmotic conditions, temperature, and more, presents a formidable challenge. Attempting to manipulate all these variables simultaneously results in an

intricate,multidimensional matrix of possibilities that cannot be exhaustively explored within practical timeframes**(Pham and Kim, 2012).**

Traditionally, microbiologists have strived to design synthetic media that mimic the suspected environmental conditions of the target organism. This approach has successfully cultivated thousands of bacteria species now considered culturable. However, recent advancements in the field have introduced two interconnected strategies that have significantly expanded our ability to culture a broader range of bacteria.

Firstly, researchers have begun to harness the environment as a tool for nurturing microbes, capitalizing on its natural intricacies to facilitate growth. Secondly, a promising avenue has emerged in the form of coculturing bacteria with their counterparts from the same environment, enhancing the chances of success in cultivating previously elusive strains. These innovative approaches are driving progress in cultivating bacteria that were once challenging to grow in laboratory settings **(Vartouki, 2010)**.

4.5 Techniques used to culture the 'unculturable'

In recent years, significant strides have been made to cultivate the 'unculturable,' those elusive bacterial species that have resisted traditional culturing methods. Environmental microbiology has been at the forefront of these developments, focusing on diverse habitats such as soil and aquatic environments, both marine and freshwater **(Figure 20).**

Traditionally, culture media have leaned towards being nutrient-rich, favoring the rapid growth of bacteria at the expense of slow-growers that thrive in nutrient-poor conditions. However, dilute nutrient media have emerged as a successful tool in cultivating previously unculturable bacteria from various aquatic and terrestrial environments. Techniques like filtration, density-gradient centrifugation, elutriation, and extinction-dilution have been employed further to narrow down bacterial diversity in mixed samples before cultivation.

Slow-growing bacteria, especially in soil, require extended incubation to outcompete other species effectively. This approach has successfully isolated strains, including those from the SAR11 clade and the TM7 Division. Furthermore, some bacteria have specific nutrient or chemical requirements for growth, and modifying cultivation media with these substrates can aid in isolating previously 'unculturable' organisms **(Stewart, 2012).**

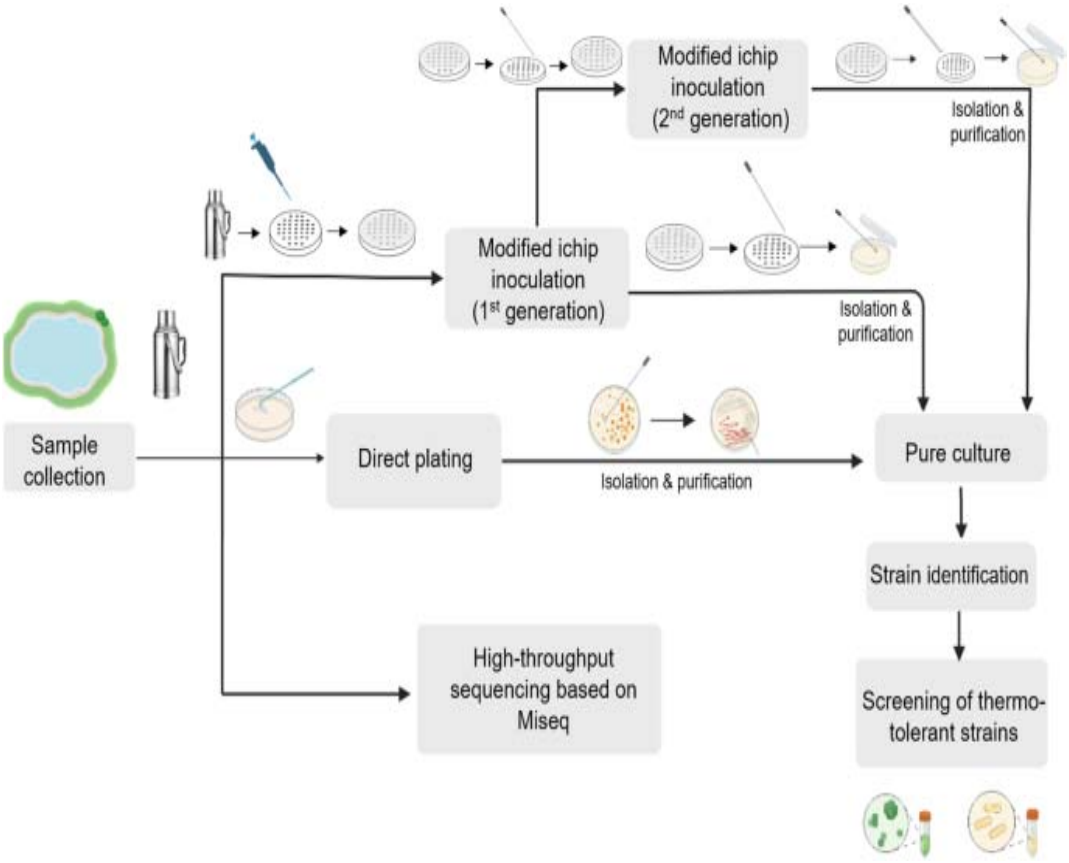

Figure 20:Use of modified ichip for the cultivation of thermo-tolerant microorganisms from the hot spring(https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-023-02803).

Cocultivation with helper strains has proven effective in laboratory cultivation, with factors released from these helper bacteria stimulating growth even in the absence of the helper strain. Signaling molecules like cyclic AMP and acyl homoserine lactones have been employed to enhance cultivation efficiency, though their effects can vary between species. Some bacteria rely on coculture relationships for growth, shedding light on their unique requirements.

Simulating natural environments *in vitro* has also played a role in cultivating previously uncultivated organisms. Techniques such as diffusion chambers, encapsulation in agar spheres, and soil substrate membrane systems have been used to mimic natural conditions, leading to successful cultivation **(Bodor2020)**.

Molecular biology has enabled the detection and sortingof specific target bacteria for selective enrichment or physical isolation. Oligonucleotide probes and advanced techniques

Tike FISH, CARD-FISH, colony hybridization, flow cytometry, and cell sorting have paved the way for isolating and studying previously elusive bacterial strains **(Figure 21).**

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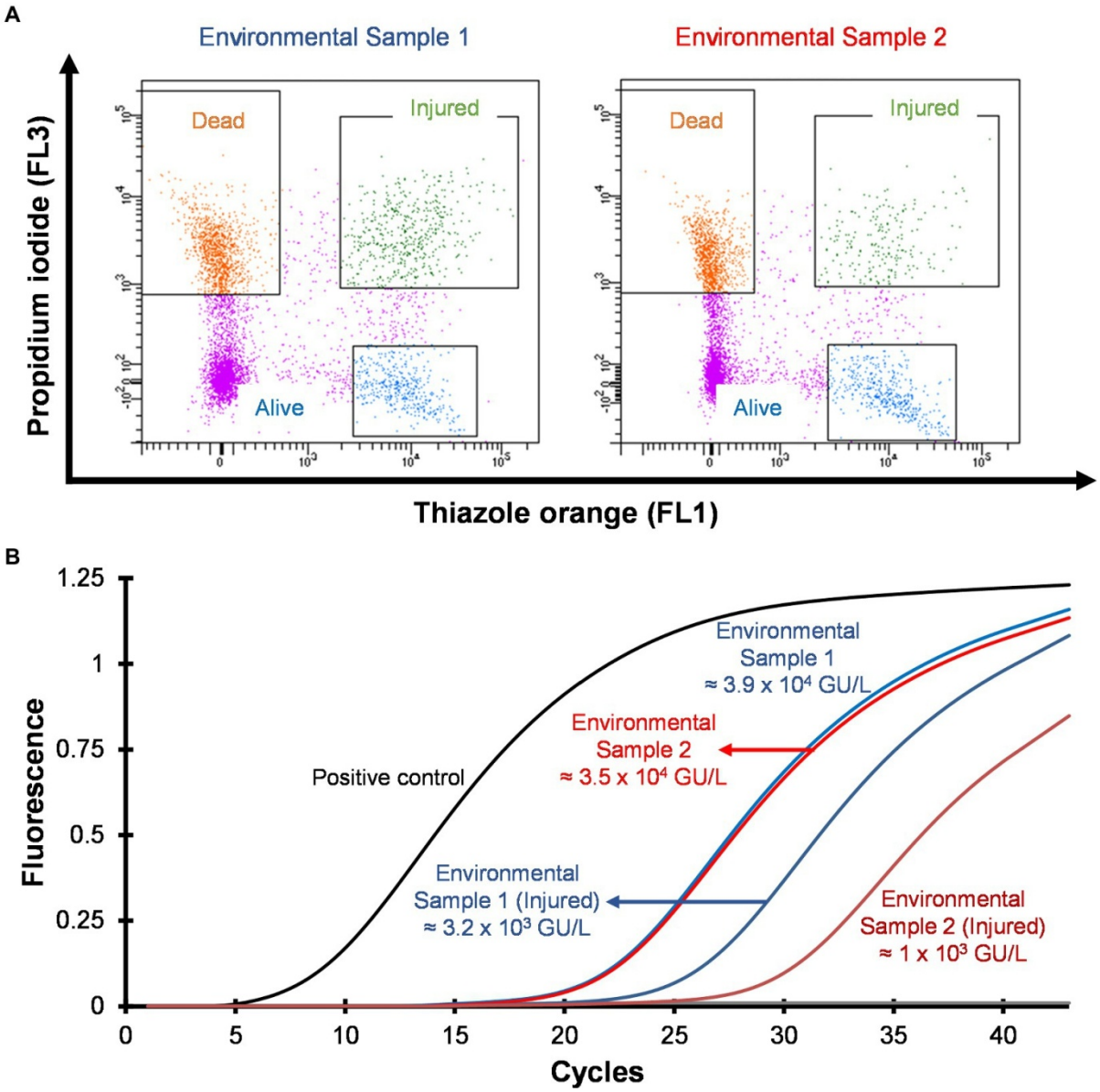

Figure 21 : Detection and quantification of VBNC *L. pneumophila* from potable water samples. **(A)** Cytograms of culture negative potable water samples. **(B)** qPCR of whole water sample and VBNC *Legionella* and estimation of GU/L after viability cell sorting using flow cytometry shown in **(A)**. This image clearly demonstrates the difference in the concentration of *Legionella*, indicating presence of dead *Legionella* of its DNA in the water sample.

4.6 Methods used for detection of VBNC bacteria

4.6.1 Bright Field Microscopy with Nalidixic Acid

Nalidixic acid (20–40 mg/L) is used to stop cell division. After exposure to nalidixic acid, viable cells continue to grow and will appear elongated, whereas the nonviable metabolically inactive cells will retain their original shape and size. The cells are then observed under a microscope. Viable cells will be seen as elongated, whereas VBNC/dormant cells will be seen as oval and large **(Josephson& al., 1993).**

4.6.2 Fluorescent Microscopy

Various fluorescent staining procedures can be used to determine VBNC organisms. Frequently used stains are acridine orange, 4,6-diamino-2-phenyl indole (DAPI), fluorescein isothiocyanate (FITC), indophenyl-nitrophenyl-phenyltetrazolium chloride (INT), and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) The mode of action of these dyes and the reactions observed are summarised in **Table 2.**

Table 2 : Fluorescent dyes used for detection of VBNC bacteria (Fakruddin &al., 2013)

Dye	Mechanism	Reaction
Acridine orange	The staining response depends on the ratio of DNA to protein in the cells	Actively reproducing cells appear green but slow-growing or nonreproducing cells at time of staining appear orange
Di-amino-phenyl-indole (DAPI)	Differential staining	Living cells look green under fluorescent microscope
Indophenyl-nitrophenyl-phenyl tetrazolium chloride (INT)	INT reacts with dehydrogenase enzyme to produce formazone and red color, thus living cells appear red	Living cells appear red.
Fluorescein isothiocyanate (FITC)	Enzyme activity in living cell	FITC stains living cells violet or blue

Molecular Techniques

Hybridization probes are nucleic acids (DNA/RNA) which have been chemically or radioactively labeled and are used to detect complementary target DNA/RNA. Specific amplification of DNA targets in bulk DNA extracts from environmental and clinical samples permits detection of specific organisms or groups of related organisms without the need to cultivate them, provided the appropriate unique primers are used.

These procedures do not discriminate between culturable and nonculturable forms of the target organisms. Due to the failure of distinguishing between dead or live cells by DNA-

based methods, RNA-based methods are a more valuable estimate of gene expression and/or cell viability under different conditions **(Alaa Eldin and Mansour, 2024).**

This technique is more able to discriminate between culturable and nonculturable forms of an organism. Furthermore, reverse transcriptase PCR (RT-PCR) can distinguish between live and dead cells **(Figure 22)**. This is possible because it is an mRNA-based method and mRNA is short-lived (half-life less than 1 minute). Messenger RNA is only present in metabolically active cells and not found in nature after cell death. RT PCR can detect nonculturable but active or live cells**(Yaron and Matthews, 2002** ; **Tyson & al ., 2005)**.

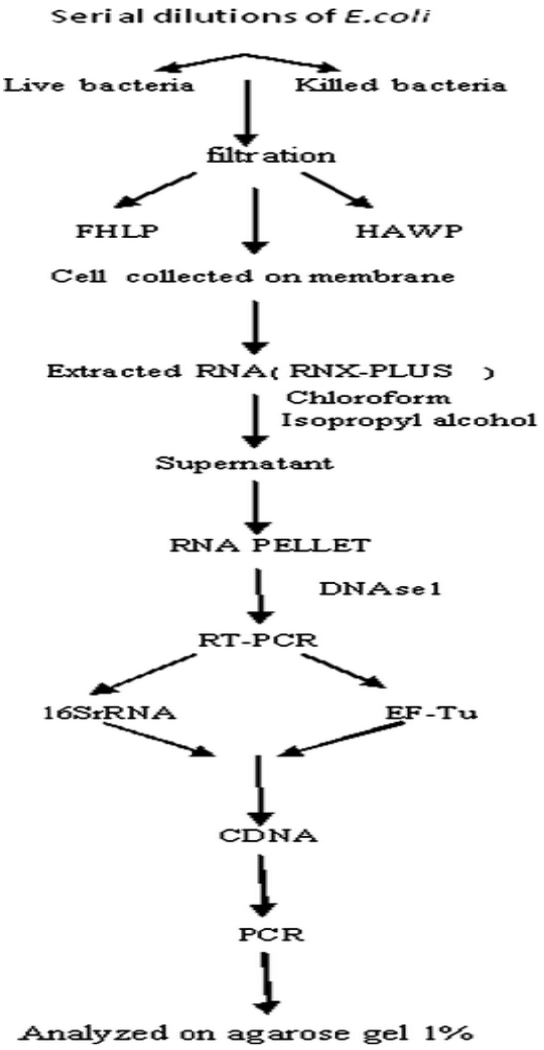

Figure 22 : Flow chart illustrates the RT-PCR procedure for detecting *16S rRNA* and *EF-Tu* mRNA of *E.coli*(Molaee& al., 2015)**.**

5 Interactions and Dynamics of Microbial Populations

5.1 Introduction

The microbiome of Earth constitutes a substantial proportion of the planet's biomass, exerting influence on ecosystem processes such as global element cycling, mainly carbon, and playing a role in the well-being of plants and animals. Recent advancements in DNA sequencing have provided valuable insights into microbial biogeography, explicitly focusing on bacteria and fungi. These advancements have shed light on the significant impact of environmental conditions on the variety of microbial communities. The inquiry persists: Is there a consistent pattern in which abiotic influences shape distinct microbial communities throughout the many environments found on Earth**? (Bharti 2014).**.

Microorganisms, including viruses, bacteria, archaea, and protists, establish intricate ecological networks characterized by various interactions. Identifying these interactions is a significant challenge, although it is of utmost importance in comprehending microbial ecosystems. Multiple tactics provide light on the dynamics of communities and the functioning of ecosystems.

Niche theory, a fundamental concept in ecology, also applies to microorganisms. Current scientific investigations are focused on examining microbial habitats, including arbuscular mycorrhizal fungi, and elucidating their associations with other environmental parameters, such as temperature and soil p^H **(Vartoukian & al., 2010).**

The success of organisms is influenced by energy limits resulting from severe temperatures. However, there is a need for further study on biotic interactions to understand this phenomenon fully.Contemporary technologies are used to investigate the identification and function of intricate microbial communities, therefore uncovering their crucial biogeochemical significance and interaction processes **(Brusseau, 2019)**.

Ecological niches include the fundamental aspects of species' functions and needs within ecosystems, including the environmental conditions they rely upon and their effects on other species. Comprehending these facets is essential for attaining a complete understanding of ecology **(Figure 23).**

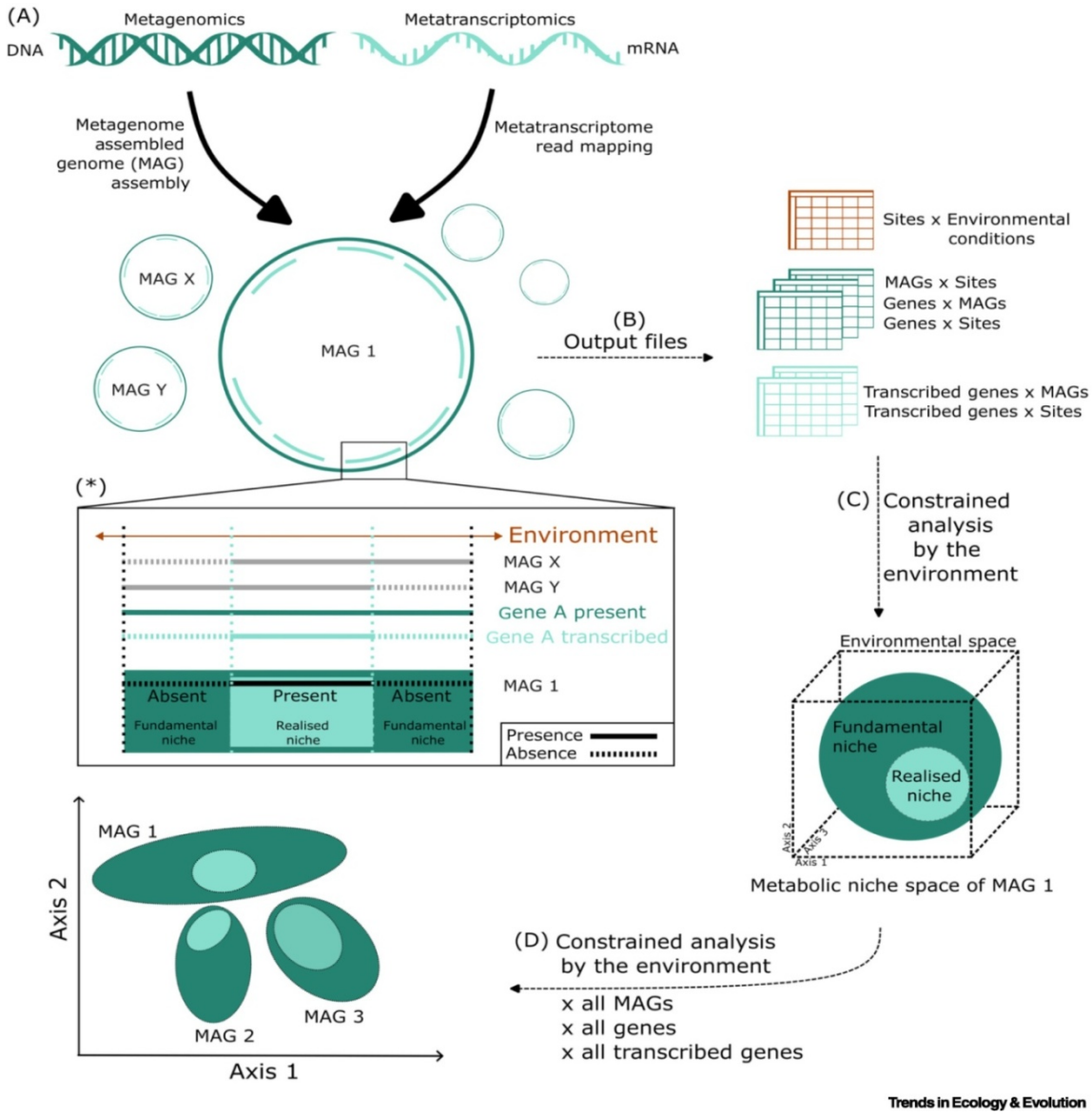

Figure 23: The metabolic niche framework (Malar and Gusam, 2023).

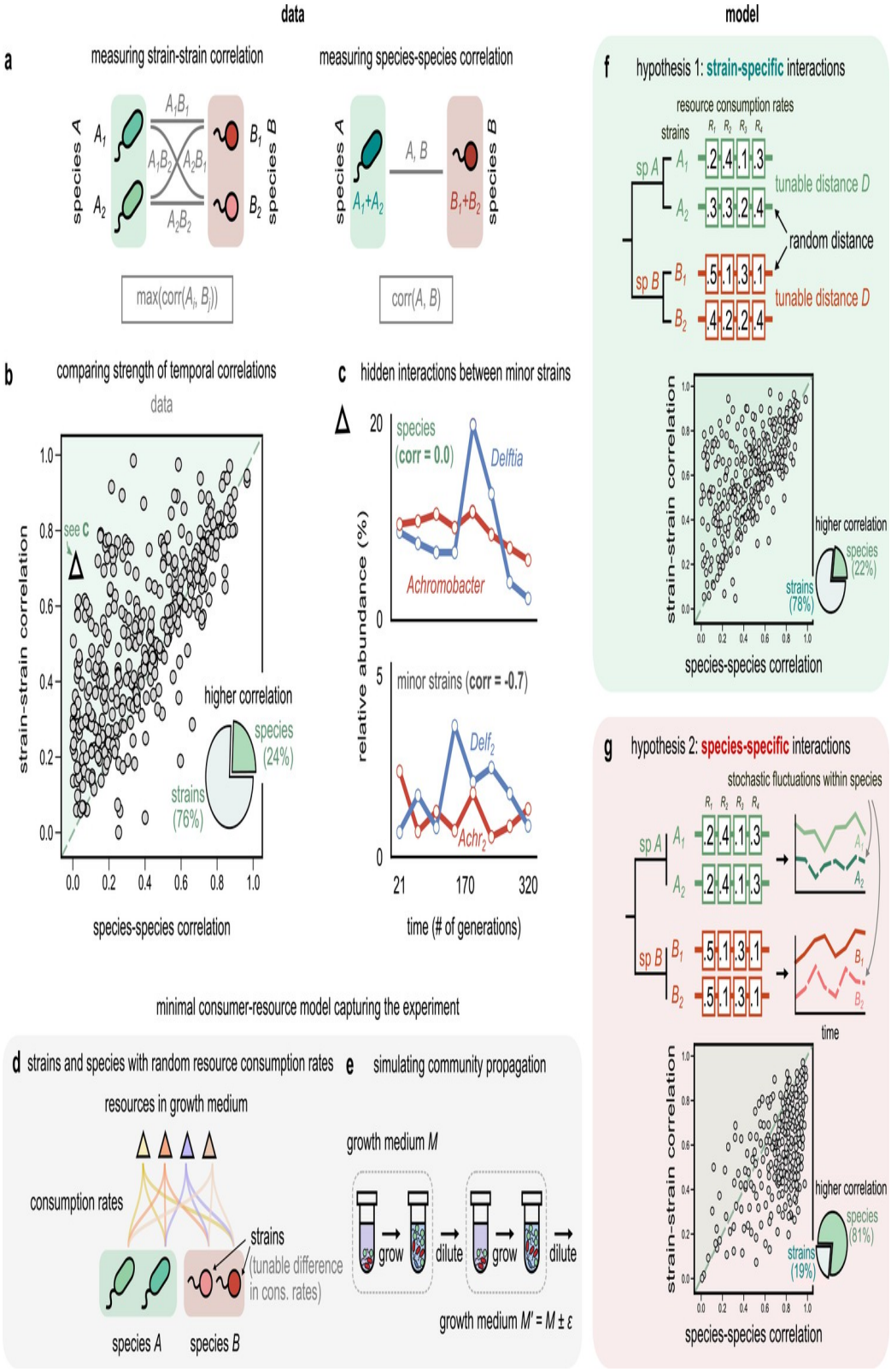

Figure 24 :(a) Schematic showing how we measured dynamical correlations at the strain and species level for a species pair *A* and *B*. **(b)** Scatter plot of the dynamical correlation between species

in a community and the highest correlation between their corresponding strain pairs. shown in (**c**). Top: relative abundance plots of two uncorrelated species measured over the experiment. Bottom: relative abundances of the minor strains for the same species, which are strongly negatively correlated. (**d, e**) Schematics of our models showing how species are split into strains. (**f, g**) Scatter plots of the expected dynamical correlations using our models, (**f**) where strains are ecologically distinct (hypothesis 1) and (**g**) identical (hypothesis 2), similar to (**b**). Schematics of the consumption rate matrices for both models (hypotheses) are also shown **(Goyal& al., 2022)**.

5.2 What is microbial community ecology?

5.2.1 What is a microbial community?

Community ecology emerged from plant and animal ecology, defining communities as multi-species assemblages where organisms coexist in the same environment and interact. This discipline aims to analyze the structure of biological groups, their functional interactions, and how community composition changes over time and space **(Kontro, and Yaradoddi, 2021).**

Recent developments in community ecology emphasize the interdependence between biological assemblages and their abiotic environments. Understanding the tight relationship between microbes and their microscale physical and chemical surroundings is crucial for defining microbial communities.

Microbial communities are crucial in ecosystem dynamics, influencing productivity and resilience. To comprehensively analyze these communities, we must delve into several essential elements **(Biton 2010)**.

5.2.1.1 Analysis of Community Functional Pathways

Microbial communities facilitate biogeochemical reactions, converting organic elements into inorganic forms for primary producers. These reactions sustain organisms and impact geological processes. Analyzing material and energy flow through microbial metabolic networks could offer predictive insights into ecosystems, though this requires advanced methods.

5.2.1.2 Interactions Among Organisms

Understanding microbe-microbe and microbe-metazoan interactions is vital for grasping ecosystem dynamics and individual organism evolution. While we catalog potential interactions, mechanistic understanding lags. Although interspecies competition is challenging to analyze naturally, laboratory studies provide insights. Interactions encompass various activities, from membrane-bound transporters to enzyme production, extending beyond resource competition **(Feichtmayer, 2017)**.

Table 3: Summary of qualitative methods available to study microbial interactions with a description of the characterized microbial interactions/behavior(Shanchana &al., 2024)

Phenotype	**Method**	**Microbial interaction**
Morphology		
Physical co-adherence	Fluorescence-based co-aggregation assay using two-chamber assay and PET membranes	Oral biofilms: *Candida albicans* co-localizes with *Fusobacterium nucleatum*
Colony morphology	Time-lapse imaging using MicrObial CHAmber (MOCHA) with double decker agar plates	Pellicle formation and colony morphology changes after release of extracellular DNA (eDNA) *Bacillus subtilis.* Novel colony morphology observed in *Bacillus amyloliquefaciens*
Mixed species biofilm structures	Scanning electron microscopy (SEM), transmission electron microscopy (TEM), confocal and fluorescence microscopy (CLSM)	Mixed biofilms of etiologic strains of *Aspergillus fumigatus* and *Staphylococcus aureus* isolated from infectious keratitis
Morphogenesis	IncuCyte time-lapse imaging and Neutrotrack (NT) analysis	Co-incubation of *Pseudomonas aeruginosa* with *Aspergillus fumigatus*: Siderophores pyoverdine and pyocyanin suppressed mycelial expansion of *A. fumigatus* in concentration dependent

Phenotype	Method	Microbial interaction
Morphology		
		manner
Spatial arrangement		
Host microbial habitat	In vitro *Hydra* models on R2A agar plates to determine colony forming units (CFU) per individual	Microbiome of freshwater polyp *Hydra*: *Curvibacter* sp. strain AEP1.3 and *Duganella* sp. strain C1.2
Increased fitness and productivity	Biofilms cultured in three-channel flow chamber and visualized using time-lapse confocal microscopy	*Pseudomonas putida* and *Acinetobacter* sp.
Chemical compounds released		
Volatile compounds	Microbes cultured in nutrient limited agar followed by exposure to volatile compounds to assess difference in transcriptional response	Soil bacterium *Pseudomonas fluorescens* exposed to volatiles produced by soil co-inhabitants *Collimonas pratensis*, *Serratia plymuthica*, *Paenibacillus* sp., and *Pedobacter* sp.
Quorum sensing signals	Liquid chromatography-mass spectrometry–based metabolomic analysis	Metabolites produced by bacterial and fungal endophytes associated with brown algae (*Ascophyllum nodosum*, *Pelvetia canaliculata*, *Laminaria digitata*, and *Saccharina latissimi*) interferes with bacterial autoinducer-2 (quorum quenching)

5.3 Biodiversity at multiple trophic levels

Global change is leading to species loss across various trophic groups, potentially affecting the ecosystem services crucial for human well-being. Predicting the functional consequences of declining biodiversity across these groups is challenging because their effects can complement or oppose each other. While the diversity of plants and microbes works together to enhance nutrient cycling, plant and herbivore diversity have contrasting effects on biomass stocks **(Figure 25**).

It has been revealed that changes in multitrophic richness and abundance were better predictors of ecosystem service effects than changes in any individual trophic group. Multitrophic richness exhibited solid and positive relationships with provisioning, regulating, and cultural services, surpassing the effects of plant richness alone. High species richness in multiple trophic groups proved essential for maintaining ecosystem functioning, particularly for regulating and cultural services.

Multitrophic abundance also influenced ecosystem functioning, albeit with generally weaker effects than individual trophic groups. The simultaneous presence of certain trophic groups could dampen the impact of others. For instance, abundant predators partially offset large herbivores' positive effects on supporting services.

Overall, the critical role of species richness in driving ecosystem functioning has been highlighted and underscores the importance of considering total biomass abundance, especially for supporting and provisioning services. The findings emphasize that maintaining high levels of species richness across various trophic groups is crucial for keeping multiple ecosystem services. The results are consistent across different multifunctionality scenarios and reveal low functional redundancy among trophic groups **(Hassani & al., 2018; Brusseau & al., 2019).**

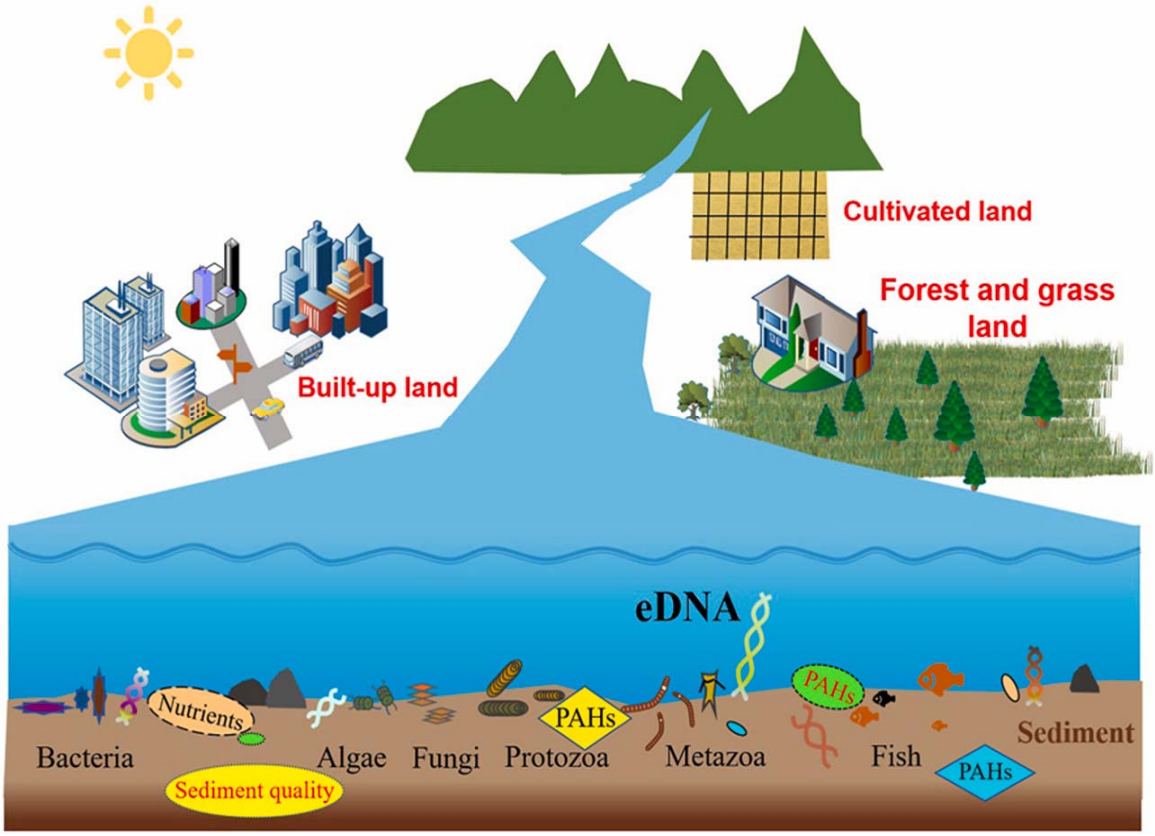

Figure 25: multi-trophic communities were assessed using DNA metabarcoding in a relatively stable riverine sediment compartment to investigate the biodiversity dynamics (https://doi.org/10.1016/j.envpol.2024.124884).

5.4 Microorganisms as Soil Quality Indicators

Microorganisms serve as valuable indicators of soil quality, offering insights that physical and chemical analyses or studying higher organisms may not provide. They are pivotal in essential nutrient cycling, soil structure maintenance, pollutant degradation, and various ecosystem services. Microbes respond rapidly to natural perturbations and environmental stress due to their short generation times and close interactions with their surroundings, driven by their high surface-to-volume ratio. This ability allows microbial analyses to effectively assess soil quality status, making shifts in microbial population and activity indicative of changes in soil quality **(Basio& al., 2020)**.

Microbial indicators encompass properties or impacts extending beyond the information they represent. Various levels of microbial study exist, including individuals, populations,

functional groups (such as autotrophic nitrification, arbuscular mycorrhiza, and specific soil enzymes), **(Figure 26)** the entire microbial community (evaluated through genetic or physiological diversity, quantitative methods, and parameters like microbial biomass, basal respiration rate, nitrogen mineralization, denitrification, and general soil enzymes), and the ecosystem level, which consolidates data from the other levels. The selection of specific indicators is crucial, considering their discriminating potential and relevance to soil quality in agricultural systems, considering the sensitivity to disturbances **(Sharma &al., 2011; Shade & al., 2012).**.

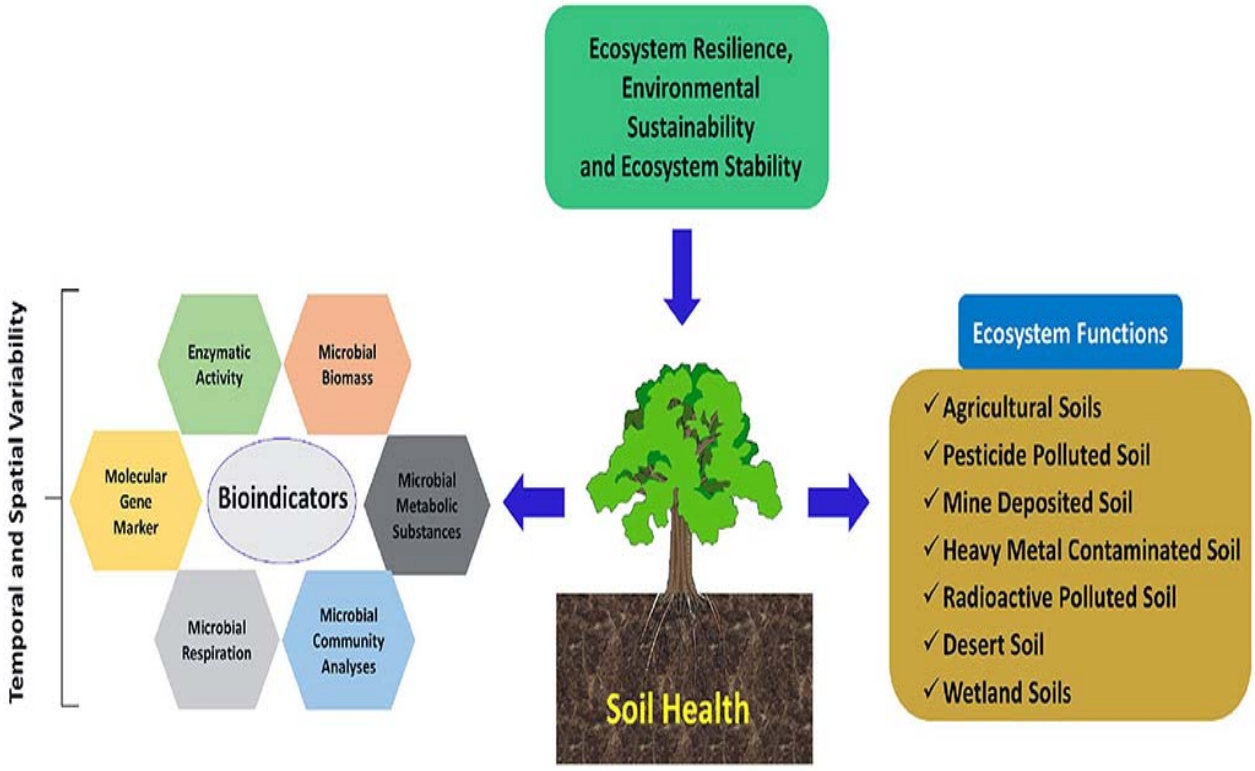

Figure 26: Schematic representation of soil health as an indicator of ecosystem resilience and stability.(https://doi.org/10.3389/fmicb.2022.938481)

6 Associational effects in the microbial neighborhood

6.1 Away for wardin spatial microbial ecology

In a world beyond well-mixed culture flasks, microorganisms exist within multispecies communities characterized by unique spatial organization. This spatial structure plays a pivotal role in shaping the composition and function of microbial communities, yet it often goes overlooked in field and laboratory studies **(Figure 27).** Microbes engage in communication and interactions with their neighbors through physical, chemical, and biological mechanisms, and the spatial arrangement of these communities enhances and consolidates these interactions.

Doing so holds the promise of expanding our understanding beyond communities with simple bipartite interactions in well-mixed environments to explore intricate, spatially structured communities interconnected by dense interaction networks **(Faust & al., 2021)**.

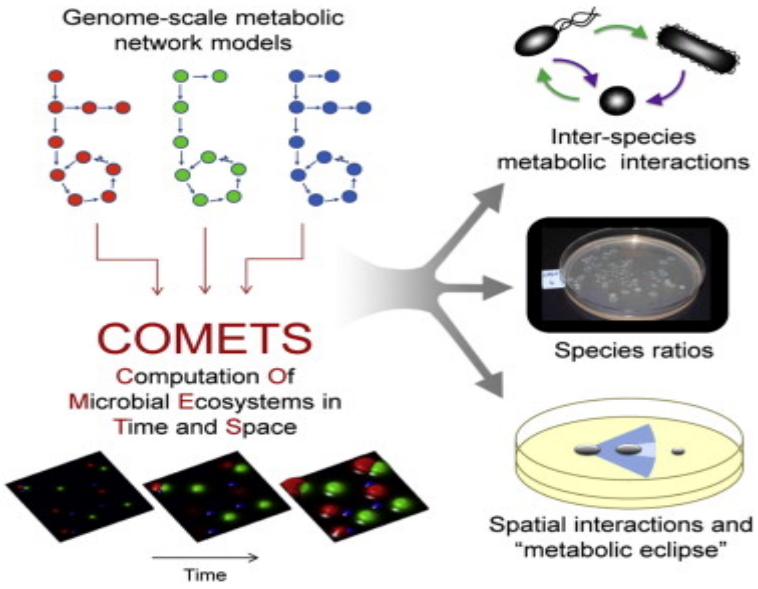

Figure 27: Metabolic Resource Allocation in Individual Microbes Determines Ecosystem Interactions and Spatial Dynamics(Harcombe & al., 2014**)**

6.2 Associational Effects

Consumer-resource interactions have been a focal point of research in plant ecology, particularly emphasizing the relationships between plants, pests, and pathogens due to their significant economic and societal implications. These investigations have led to various ecological concepts, including Associational Effects (AE). AE encompasses interactions involving plants and herbivores, predators and prey, and plants and pollinators.

Unlike other fundamental ecological interactions like competition and mutualism, AE represents an indirect interaction wherein a modulating species, often called a plant neighbor, influences the interaction between a focal plant and an effecting herbivore species**(Figure 28)**. This modulation can take shape through interaction chains or higher-order interactions (HOI), depending on whether the indirect effect emerges from changes in herbivore density (interaction chain) or shifts in per capita competitive effects between herbivore and focal species (HOI)**(Santoyo&al., 2021).**

What sets AE apart is its versatility, as the outcome for the focal plant species can either be positive, known as Associational Susceptibility (AS), or negative, termed Associational Resistance (AR). Moreover, AE considerations account for species' identity in the focal species' direct vicinity at specific spatial scales. This distinguishes AE from other indirect interactions, such as "apparent competition," which describe purely negative indirect interactions between species through shared natural enemies without considering spatial aspects.

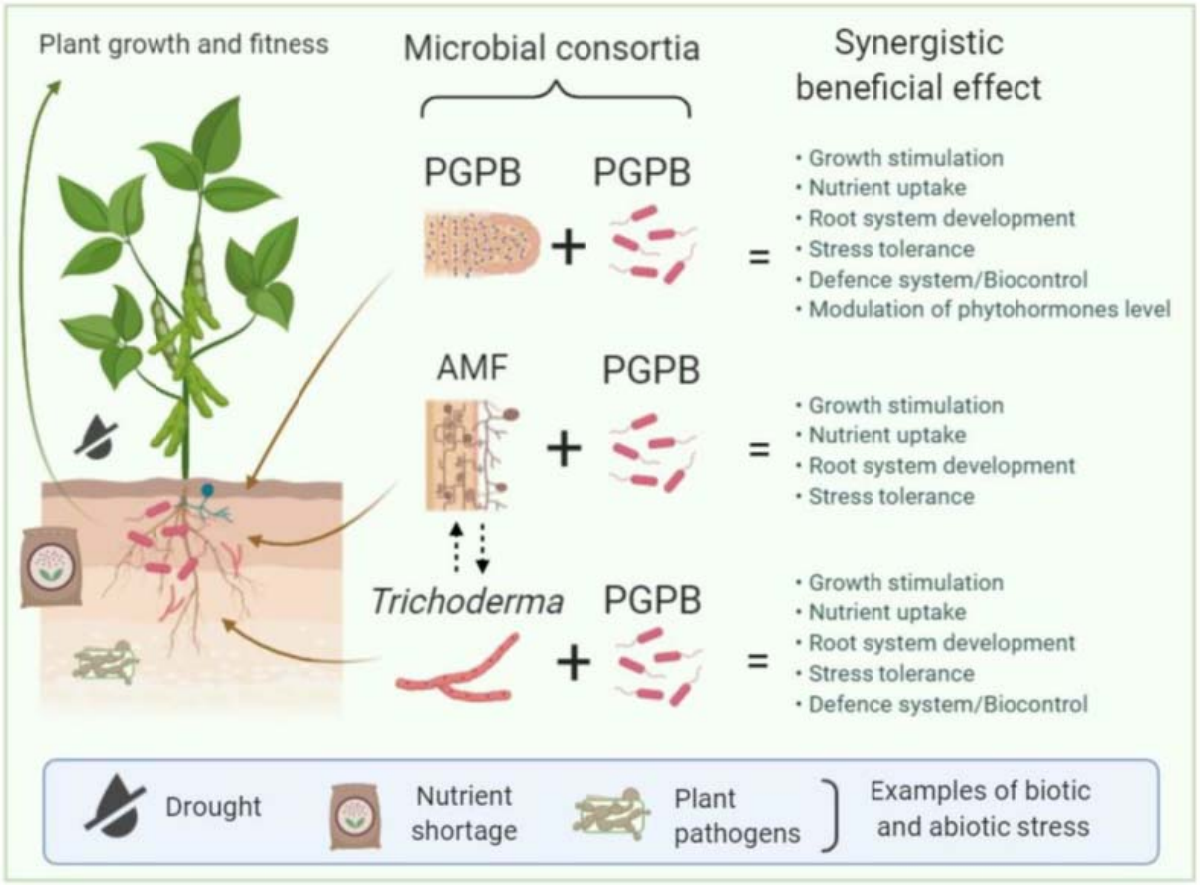

Figure 28 :Microbial consortia. Rhizosphere microorganisms like plant-growth-promoting bacteria (PGPB), arbuscular mycorrhizal fungi (AMF), and fungi from the genus *Trichoderma spp*. can establish beneficial interactions with plants, promoting plant growth and development, increasing the plant defense system against pathogens, promoting nutrient uptake, and enhancing tolerance to different environmental stresses. Rhizosphere microorganisms can influence one another, and the resulting consortia of PGPB + PGPB (e.g., a nitrogen-fixing bacterium such as *Rhizobium spp*. and *Pseudomonas fluorescens*), AMF + PGPB, and Trichoderma + PGPB may have synergetic effects on plant growth and fitness, providing the plant with enhanced benefits to overcome biotic and abiotic stress. Dashed arrows indicate beneficial interactions between AMF and Trichoderma (Santoyo & al., 2021)

Understanding these AE linkages within plant-herbivore interactions has provided ecologically informed agricultural practices to safeguard crops against herbivore attacks. This protection is achieved by strategically placing adjacent plants within crop plots or introducing non-crop patches near focal crop plots.In microbial ecology, the focus extends to various interactions within diverse species communities. Due to the complexity of these dense interaction networks, researchers often resort to reductionist approaches, breaking down the community into more manageable subsets. Nevertheless, consensus is growing that predicting

the dynamic behavior of a community as a whole solely from observations of interactions among species subsets is a challenging endeavor **(Basio& al., 2020)**.

To address this challenge, holistic approaches emphasize the role of diversity in determining community stability and productivity. However, it is essential to delve into diverse relationships to uncover the specific ecological mechanisms driving emergent properties within communities. Herein lies the attempt to apply the AE conceptual framework to microbial ecology, bridging the gap between holistic and reductionist perspectives. This adaptation considers the number of modulating species and the interactions between focal and effecting species, integrating the influence of biotic (modulating species) and abiotic (spatial structure) factors.

This adaptation allows for a better understanding of species behavior within complex microbial communities and the impact of spatial organization on interaction patterns. In the context of microbial ecology, AE is defined as the influence of one species (effector) on another (focal), mediated by other community members (modulators). The outcome of this modulation can be either positive (Associational Benefit, AB) or negative (Associational Detriment).

6.3 Mechanisms underlying AE in microbial communities

Antagonistic interactions in microbial communities can be attributed to physical, chemical, and biological mechanisms. In a study involving a predatory bacterium, a diverse prey community was found to reduce predation. This phenomenon is believed to occur because predation-resistant bacteria create protective barriers around vulnerable counterparts, effectively shielding them from predators **(Figure 29).**

Biofilm formation is another mechanism that alters the physical environment of bacteria, providing collective protection against phage or protist attacks. Resource competition among community members can induce bacterial cell aggregation due to changes in surface properties, offering physical protection against predation as predators cannot access bacteria within the inner part of these aggregates **(Rather & al., 2021).**

Bacteria not only affect the physical but also the chemical environment. Metabolic byproducts like lactic acid or antibiotics can influence the growth of other microbes and potentially impact predators. Volatile organic compounds (VOCs) emitted by bacteria serve

as weapons in microbial warfare, effectively countering various antagonists, including fungi, protists, and other bacteria. In unsaturated soils, VOCs act over greater spatial scales than soluble compounds, possibly safeguarding VOC-insensitive bacteria near the producing population.

Predators often focus on abundant prey types, and this frequency-dependent selection can either enhance or diminish predation. For instance, in a study on phage epidemics in a bacterial population, immunity strongly depended on the relative frequency of resistant and susceptible individuals and spatial population structure. While bacteria and phage could coexist in a structured habitat, they collapsed in a well-mixed environment due to the low frequency of resistant individuals **(Daniel& al ., 2021)**.

Figure 29: Starving colony of *Myxococcus xanthus* forms fruiting bodies(Natarajan and Bhatt, 2020).

While the preceding section highlights the prevalence of antagonistic interactions in microbial ecology literature, fewer studies delve into the potential adverse effects of modulators on cooperating species. (**Pande et al., 2022)** demonstrated that non-cooperating

bacteria can exploit public goods produced by a cross-feeding consortium, leading to reduced productivity in the latter. The spatial structure plays a crucial role in this modulation outcome, as spatial segregation on agar surfaces protects cross-feeders from exploitation by non-cooperators.

6.4 Expansion of the concept to abiotic stressors

Expansion of AE initially emerged to elucidate changes in plant vulnerability to herbivores. More recently, this concept has broadened its scope to encompass herbivore-herbivore associations and their susceptibility to various factors, including predators, pathogens, parasites, or parasitoids.

Conceptually, the interaction can be viewed as amensalism, where the stressor inflicts harm upon the focal species without suffering any consequences. Modulator species come into play by shielding the focal species, diminishing stress levels (e.g., through the degradation of toxic compounds), or altering the physiology of the focal species. The outcome is contingent upon the specific mechanism at play, with spatial organization either amplifying or attenuating the impact of the stressor.

This expanded perspective enhances our understanding of the significance of compositional and spatial complexity in microbial communities' stability. It was observed that antibiotic-resistant strains confer resistance to nearby sensitive cells, indicating the presence of Antibiotic Buffering (AB) within bacterial colonies. Neighboring cells protect focal species, reducing their vulnerability to abiotic stress. Another noteworthy mechanism was observed in *Streptococcus mutans* populations, exhibiting greater tolerance to antibacterial agents when coexisting in mixed biofilms with *Veillonella parvula*, as opposed to mono-species biofilms.

Transcription analysis unveiled that *V. parvula* induces gene expression changes within *S. mutans*, enhancing antibiotic resistance. Besides spatial shielding and trait modifications, AB can also arise from alterations in resource availability. It has been proposed that antibiotic-resistant cells can exploit substrates released from lysed-sensitive strains to offset the fitness costs associated with maintaining resistance **(Bengtsson-Palme, 2020).**

In addition to negative effector chemicals like antibiotics, this expansion also encompasses chemicals that positively impact the focal species, such as signaling molecules.

The degradation of quorum-sensing signals by other bacteria can disrupt communication among populations and is thus being explored as a potential therapeutic approach for bacterial diseases **(Figure 30).**

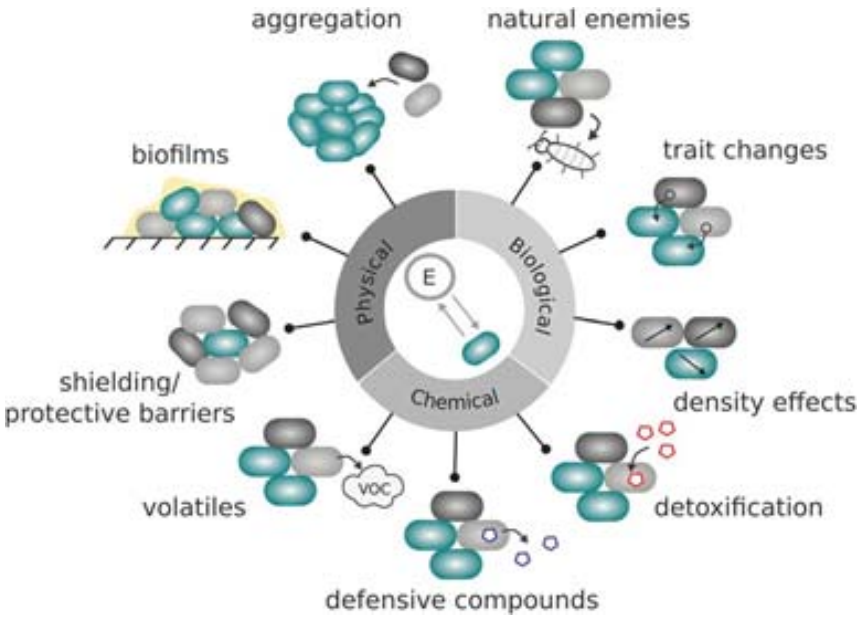

Figure 30: Physical, chemical, and biological mechanisms causing AE inbacterialcommunities (Worrich& al., 2019).

6.5 Implications of AE in Microbial Ecology

Bacterial multispecies assemblies play crucial roles in both engineered and natural systems. However, there is a significant lack of understanding regarding the principles governing their community, structural integrity, and functional stability. This knowledge gap dramatically hampers our ability to engineer and manage microbial consortia to enable, restore, or enhance desired functions.

While synthetic microbial ecology offers the opportunity to design communities with specific properties, these designs have predominantly been implemented in well-mixed culture systems. Yet, research has demonstrated that spatial organization can profoundly influence the interactions and behaviors of bacterial species and impact their resilience to stress. Introducing the concept of Auto-Ecology into microbial ecology represents a crucial step in advancing our comprehension of fundamental aspects of microbial interactions within complex and structured communities.

It also allows us to develop consortia that maintain long-term stability in dynamic environments characterized by fluctuating conditions, competitive pressures, and potential predation **(Worrich & al., 2019)**.

7 Microbialinteractions

7.1 Introduction

Microorganisms are rarely encountered as single species populations in the environment, since studies in different habitats has shown that an enormous richness and abundance variation are usually detected in a small sample, suggesting that microbial interactions are inherent to the establishment of populations in the environment, which includes soil, sediment, animal, and plants, including also fungi and protozoa cells. The many years of coevolution of the different species lead to adaptation and specialization and resulted in a large variety of relationships **(Figure 31)** that can facilitate cohabitation, such as mutualistic and endosymbiotic relationships, or competitive, antagonistic, pathogenic, and parasitic relationships **(Faust, 2012).**

These interactions involve all ecological aspects, including physiochemical changes, metabolite exchange, metabolite conversion, signaling, chemotaxis and genetic exchange resulting in genotype selection. In addition, the establishment in the environment depends on the species diversity, since high functional redundancy in the microbial community increases the competitive ability of the community, decreasing the possibility of an invader to establish in this environment **(Grosskopf and Soyer, 2014)**. Therefore, these associations are the result of a co-evolution process that leads to the adaptation and specialization, allowing the occupation of different niches, by reducing biotic and abiotic stress or exchanging growth factors and signaling (Figure 33). Microbial interactions occur by the transference of molecular and genetic information, and many mechanisms can be involved in this exchange, such as secondary metabolites, siderophores, quorum sensing system, biofilm formation, and cellular transduction signaling, among others **(Braga & al., 2016)**.

7.2 Microbial interactions types

Microorganisms live in close contact with each other and to multicellular hosts, usually including many species. Additionally, microbes are exposed to variations in the environment, which in turn affect the interactions **(Figure 31).** Microbial interactions are thus highly complex, and many mechanisms and molecules are involved **(Braga& al., 2016)** . Studies on microbial interactions led to significant findings in microbiology, botany, zoology, and ecology. Research on microbial interactions also enabled discoveries for clinical, industrial,

and biotechnological applications, e.g., antimicrobial drug development based on natural products like QS interfering compounds.

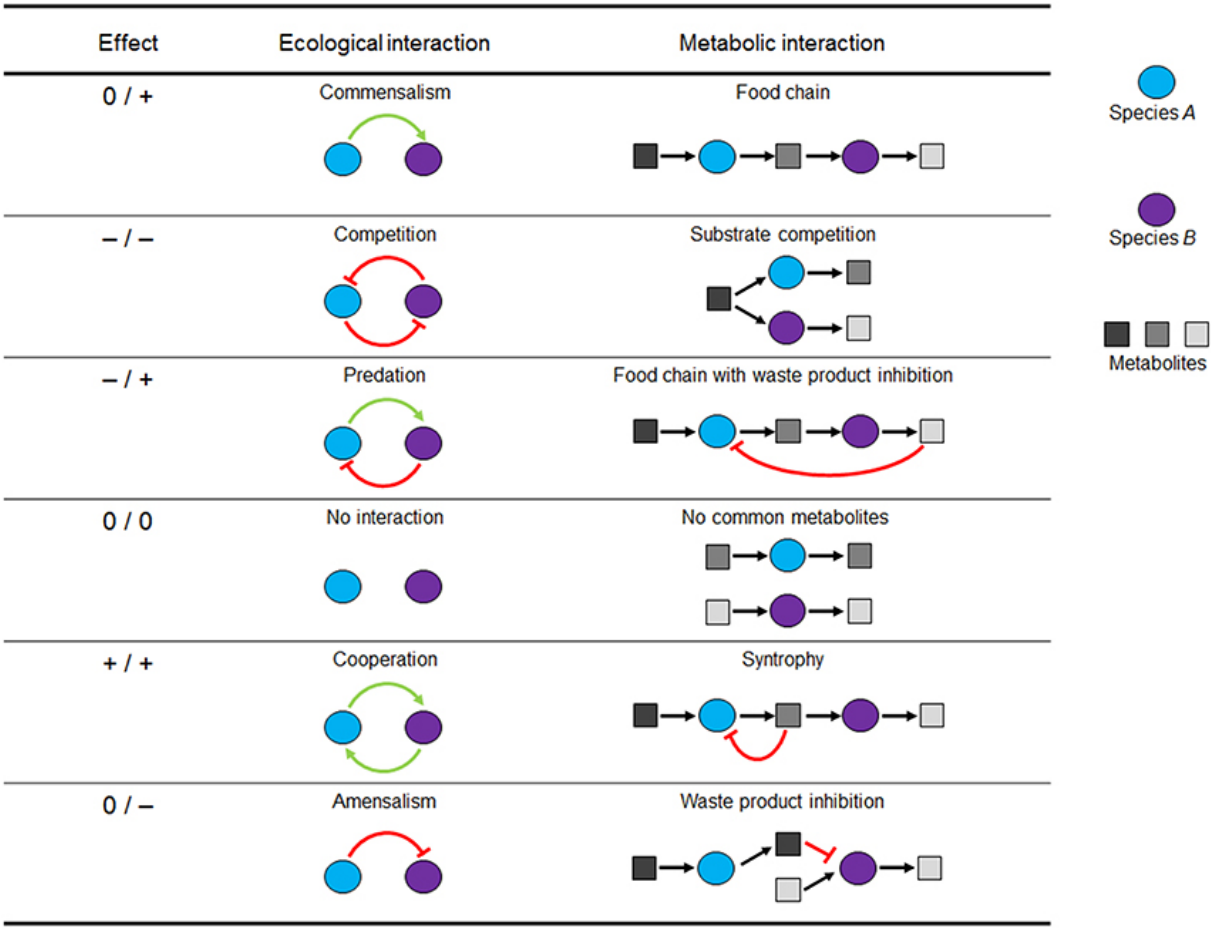

Figure 31 : Pairwise microbial interactions in environmental processes. For each interaction partner, there are three possible outcomes: positive (+), negative (–), or neutral (0). Metabolic but not ecological interactions can be modeled using metabolic networks. Figure adapted from (Großkopf and Soyer, 2014).

7.2.1 **Symbiosis :** A relationship in which two dissimilar organisms (symbionts) live in close association with one another.

7.2.2 **Commensalism :** A relationship between two species in which one is benefited and the other is not affected, neither negatively nor positively.

7.2.3 **Mutualism :** Mutually beneficial relationship between two species.

7.2.4 **Parasitism :** A relationship between two species in which one benefits (parasite) from the other (host); it usually involves some detriment to the host **(Kubicek and Druzhinina, 2007)**.

Some exemples about microbiol interactions are summarized in **Table 4** :

Table 4 : Types of microbial interactions adapted from **(Barton and Northup, 2011)**.

Interaction	Characteristic	Species A	Species B	Example
Mutualism	Symbiosis needed for survival in a specific habitat	Benefits	Benefits	Root nodules
Synergism	Another improves the growth of one partner	Benefits	Benefits	Crossfeeding of acetate between bacteria
Commensalism	One partner benefits and the other is not harmed nor improved	Benefits	Not affected	trification with *Nitrosomonas* and *Nitrobacter*
Parasitism	Host is compromised	Benefits	Harmed	*Bdellovibrio sp.* and BALO require Gram-negative bacterium for growth
Competition	Rivalry for space and nutrients	Harmed	Harmed	Soil bacteria compete with fungi for nutrients
Antagonism	Product(s) of one partner impact another	Not affected or benefits	Harmed	Production of antibiotics

With the expansion of research into host-associated communities, ecological approaches are poised to intersect with clinical studies. Strain-level networks for healthy human gut communities have already been established, distinguishing Bacteroidetes and Firmicutes into separate clusters.

These networks have also been used to identify members of alternative gut communities known as enterotypes. Recently, a comprehensive microbial association network encompassing 18 human body sites has been constructed, revealing relationships within and between body sites using a combination of similarity measures and sparse regression.

Cross-body-site associations are predominantly observed among microorganisms in related body sites, aligning with the concept that different body sites represent distinct microbial niches **(Figure 32).**

The network indirectly captures alternative community configurations, such as those observed in dental plaque formation, the gut, and the vagina. Additionally, comparing phylogenetic and functional similarity among associated microorganisms highlights that closely related pairs tend to co-occur in related habitats, while exclusive relationships are more common in distantly associated pairs **(Rayen & al., 2019).**

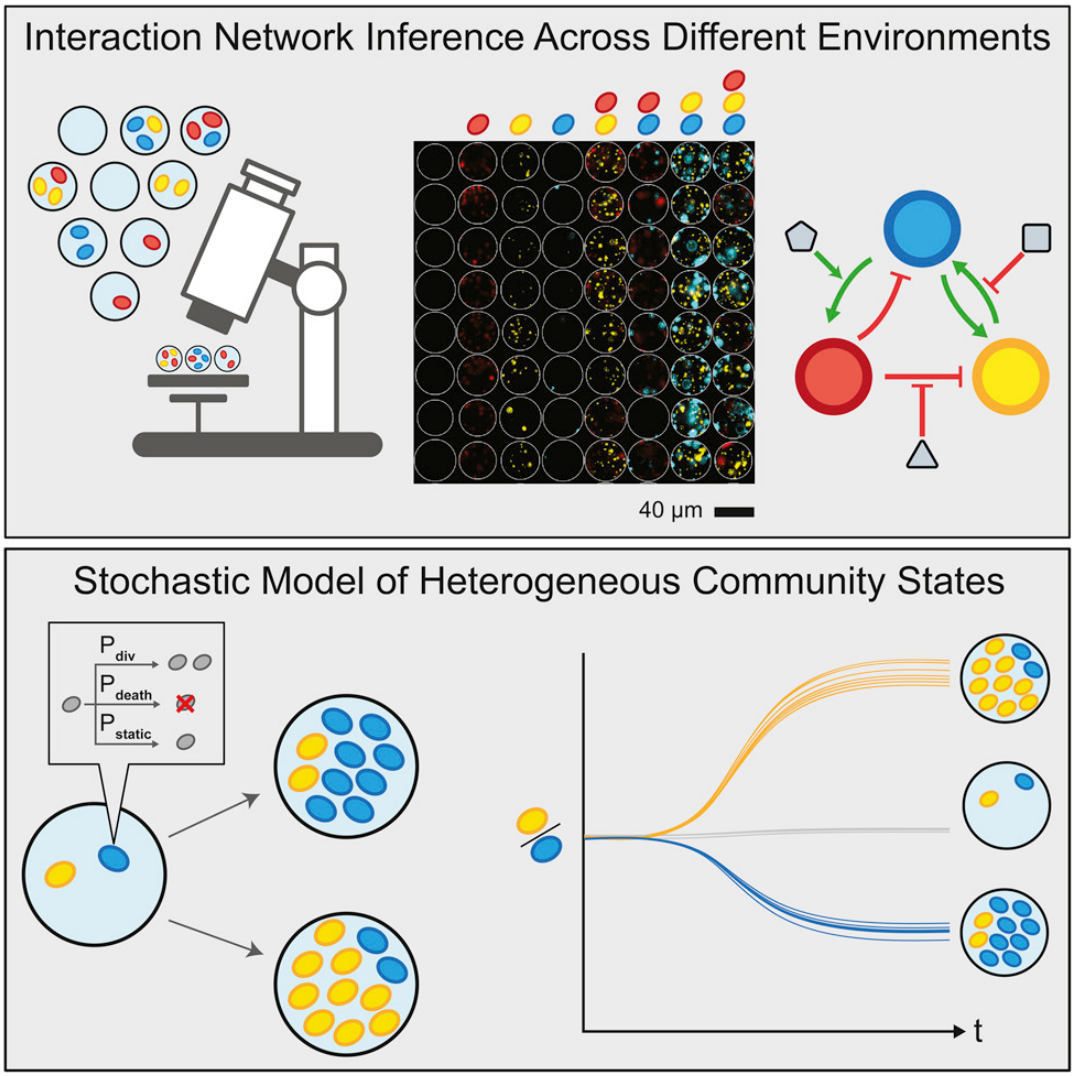

Figure 32 : Interactions Network inference across different Environment (Ryan & al., 2019)

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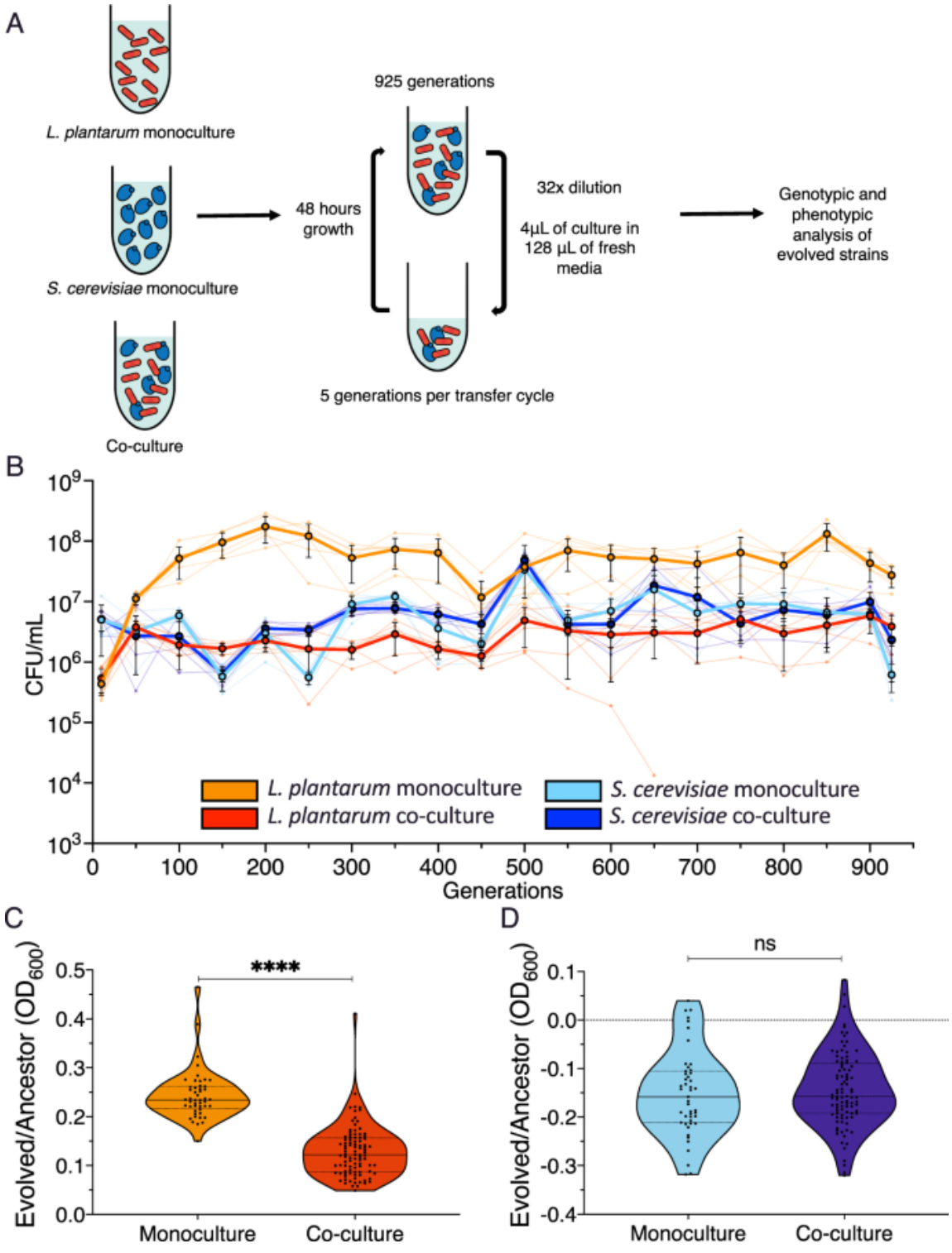

Figure 33 : *L. plantarum* adaptation is limited by co-culture with *S. cerevisiae*(Barber & al., 2022).**A** Replicate populations of *L. plantarum* (red) and *S. cerevisiae* (blue) were propagated in either monoculture or co-culture conditions for 925 generations. **B** Population carrying capacity estimated by colony-forming units (CFU) per mL of *L. plantarum* and *S. cerevisiae* in monoculture and co-culture over 925 generations. Measurements were taken at 50 generation intervals. Each point shows the average of six independent evolution experiments. Growth assays of (**C**) *L. plantarum* and

(**D**) *S. cerevisiae* after 925 generations of evolution, measured as the relative difference in optical density compared with the ancestral strain. Error bars show the S.E.M.

7.3 Dynamic modeling of microbial interactions

Dynamic modeling of microbial interactions involves moving beyond static representations of microbial communities, offering a deeper understanding of their behavior over time. While inferred networks provide a snapshot of community status, dynamic models are essential for studying microbial populations' stability, perturbation, and succession **(Sharma & al., 2011 ; Srinivasan, & al ., 2024)**.

❖ Community Stability

Microorganisms' rapid growth and short generation times are ideal for studying community stability and response to perturbations. Mathematical models can systematically assess the impact of species removal to identify keystone species, crucial thresholds, or chaotic behavior. This is particularly relevant in ecosystems like the gut microbiome, where the effects of invading species or microbiota modulation are of great interest.

❖ Alternative Stable States

Recent discoveries have revealed alternative microbial communities in ecosystems like the vagina and gut. Identifying the nature and drivers of these alternatives is crucial. While challenging, dynamic models can help verify alternative stable states by assessing their stability, response to perturbation, and occurrence in the same environment. Repeated experiments can validate the existence of multiple steady states.

❖ Microbial Succession

Microbial succession occurs in various environments, such as dental plaque biofilms, plankton communities, and infant guts. Network inference captures mutual exclusions but cannot distinguish succession from other relationships. Mathematical models can simulate succession dynamics, exploring factors like dispersal, random processes, and the impact of initial conditions. Evolutionary changes in microorganisms also play a role in succession dynamics.

❖ **Challenges in Dynamic Microbial Community Modeling:**

✓ Handling Multiple Species: Models must accommodate many species and consider unmonitored community members, like bacteriophages.

✓ Accounting for Random Processes: Realistic models should incorporate random processes and acknowledge that interaction strengths between species can evolve rapidly.

✓ Choosing the Appropriate Level of Modeling: Model granularity, whether at the species or pathway level, depends on the research questions and system characteristics.

✓ Game Theoretical Approaches: In systems where microorganisms cooperate in biofilms, exploring game theoretical modeling approaches can be beneficial.

The most significant challenge lies in integrating metabolic data into dynamic models. While static metabolic models using flux balance analysis (FBA) exist, they assume stable, steady states and require high-quality metabolic reconstructions. Dynamic extensions of FBA have been attempted, but exploring alternative modeling techniques that demand less detail may be worthwhile due to the inherent complexities of microbial communities **(Herrgård and Nielsen, 2021).**

8 Genetically Modified Microorganisms

8.1 Introduction

In recent years, the landscape of biotechnology has witnessed remarkable advancements, particularly in recombinant DNA technology. This innovation has opened up new avenues for exploring and harnessing the metabolic potentials of microorganisms, giving rise to genetically modified microorganisms (GMMs). These GMMshave entered various sectors, impacting human health, agriculture, bioremediation, and multiple industries, including food, paper, and textiles. The allure of genetic engineering lies in its ability to enhance molecular diversity and chemical selectivity, ensuring safer handling of otherwise hazardous agents while significantly reducing production costs **(Aguilera & al., 2013).**

These genetic modifications serve various purposes, from generating new proteins and food ingredients to improving the production of existing ones and tailoring the characteristics of proteins for novel applications. GMMsrefers explicitly to microorganisms, such as bacteria and fungi, including yeasts, that have been deliberately modified using modern biotechnology techniques. While other methods exist for altering the genetic makeup of microorganisms, they don't always fall under the regulatory definitions of genetic engineering or genetic modification **(Figure 34).**

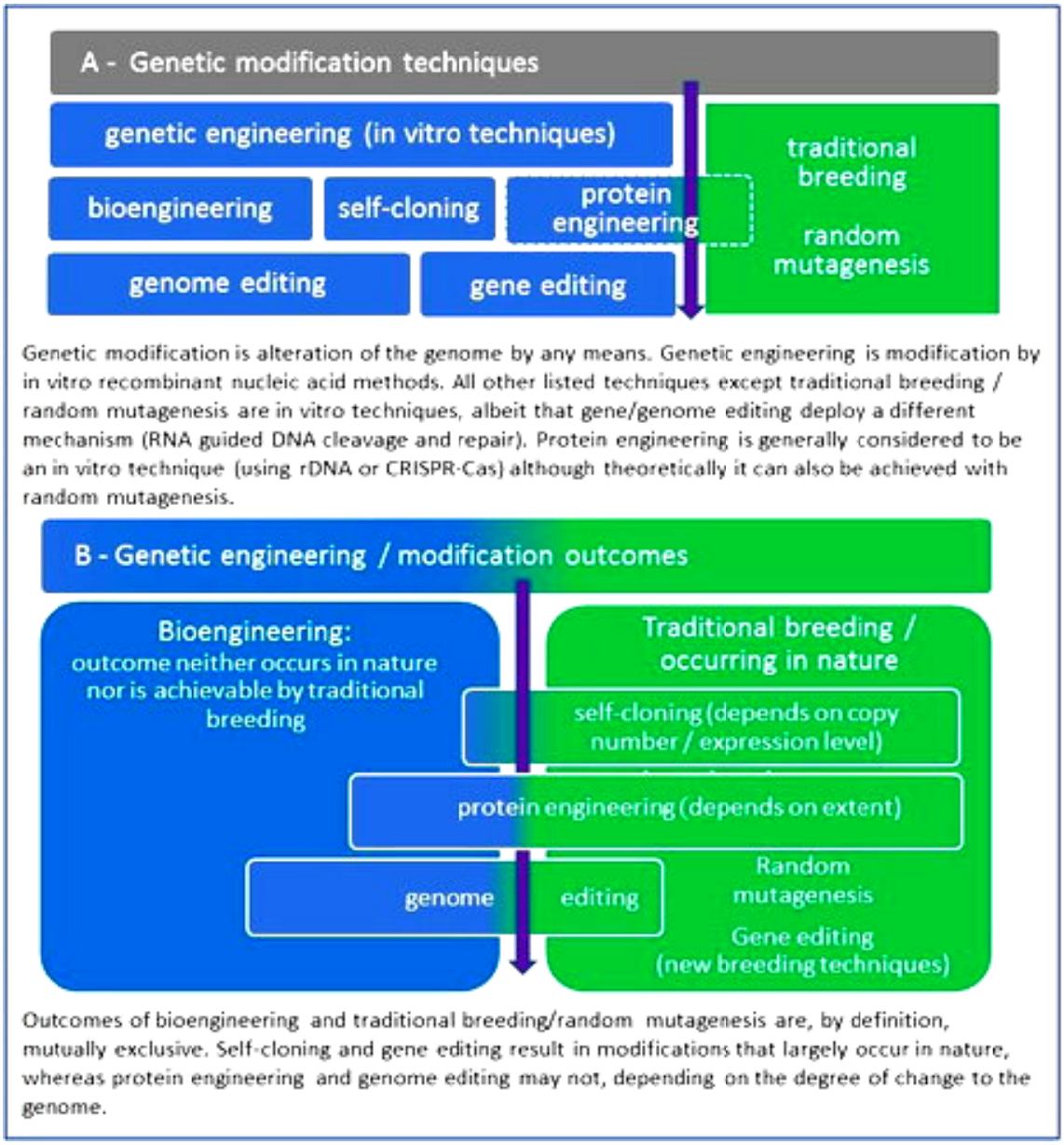

Figure 34 :Differentiation among genetic modification techniques (A) and modification/engineering outcomes (B) (EFSA. 2011a) .

8.2 Definition of GMO

A genetically modified organism (GMO) is any organism whose genetic material has been altered using genetic engineering techniques. The exact definition of a genetically modified organism and what constitutes genetic engineering varies, with the most common being an organism altered in a way that "does not occur naturally by mating and/or

natural recombination". A wide variety of organisms have been genetically modified (GM), including animals, plants, and microorganisms (**Chilton, 2016 ; Blakemore, 2019**).

8.3 Production of GMO

Genetic modification can include the introduction of new genes or enhancing, altering, or knocking out endogenous genes. In some genetic modifications, genes are transferred within the same species, across species (creating transgenic organisms), and even across kingdoms.

Creating a genetically modified organism is a multi-step process. Genetic engineers must isolate the gene they wish to insert into the host organism and combine it with other genetic elements, including a promoter and terminator region and often a selectable marker.

A number of techniques are available for inserting the isolated gene into the host genome. Recent advancements using genome editing techniques, notably CRISPR, have made the production of GMOs much simpler. Herbert Boyer and Stanley Cohen made the first genetically modified organism in 1973, a bacterium resistant to the antibiotic kanamycin.

The first genetically modified animal, a mouse, was created in 1974 by Rudolf Jaenisch, and the first plant was produced in 1983. In 1994, the Flavr Savr tomato was released, the first commercialized genetically modified food. The first genetically modified animal to be commercialized was the GloFish (2003) and the first genetically modified animal to be approved for food use was the AquAdvantage salmon in 2015 **(Russo, 2003 ; Zhang & al., 2016)**.

research, food production, industrial protein purification (including drugs), agriculture, and art. There is potential to use them for environmental purposes or as medicine. Fungi have been engineered with much the same goals. Viruses play an important role as vectors for inserting genetic information into other organisms. This use is especially relevant to human gene therapy.

There are proposals to remove the virulent genes from viruses to create vaccines. Plants have been engineered for scientific research, to create new colors in plants, deliver vaccines, and to create enhanced crops. Genetically modified crops are publicly the most controversial GMOs, in spite of having the most human health and environmental benefits **(Esvelt and Wang , 2013)**

8.4 Molecular Tools for GMMs

In the realm of GMMs, a set of essential molecular tools facilitates the manipulation of these organisms to express desired traits. These tools encompass gene transfer methods, cloning vectors, promoters for gene expression control, and selectable marker genes for identifying recombinant microorganisms **(Figure 35), (EFSA, 2011a).**

8.4.1 Gene Transfer Methods

Gene transfer methods are pivotal in delivering selected genes into the desired host microorganisms. One prevalent approach is transformation, where plasmid DNA is assimilated by recipient microorganisms during their competence stage, typically occurring at a specific growth phase. For instance, *Escherichia coli*, commonly used for cloning and therapeutic protein production, achieves plasmid DNA uptake through calcium chloride or rubidium chloride treatment **(Stemke, 2004).**

In more complex cases, such as with antibiotic-producing Streptomyces, transformation necessitates the preparation of protoplasts using lysozyme to remove most of the cell wall. Protoplasts are combined with plasmid DNA and polyethylene glycol to enhance DNA uptake. Various variables, like growth conditions and polyethylene glycol parameters, must be explored for optimal protoplast formation.

Electroporation provides an alternative method for DNA transformation, involving brief high-voltage pulses to make recipient cells electrocompetent. This method is preferred when protoplast transformation efficiency falls short. Electroporation finds utility in industrially significant microorganisms like *Streptomyces*, *Corynebacterium*, *Bacillus*, and lactic acid bacteria. Additionally, methods have emerged to transfer DNA directly from DNA-harboring cells to recipients without DNA isolation.

Conjugation represents another method for introducing plasmid DNA into microorganisms, involving a donor strain containing the gene of interest and transfer functions on the chromosome. This method offers advantages, such as bypassing the need for protoplast formation and cell wall regeneration and facilitating the transfer of single-stranded plasmid DNA **(Hanlon and Sewalt, 2021)**.

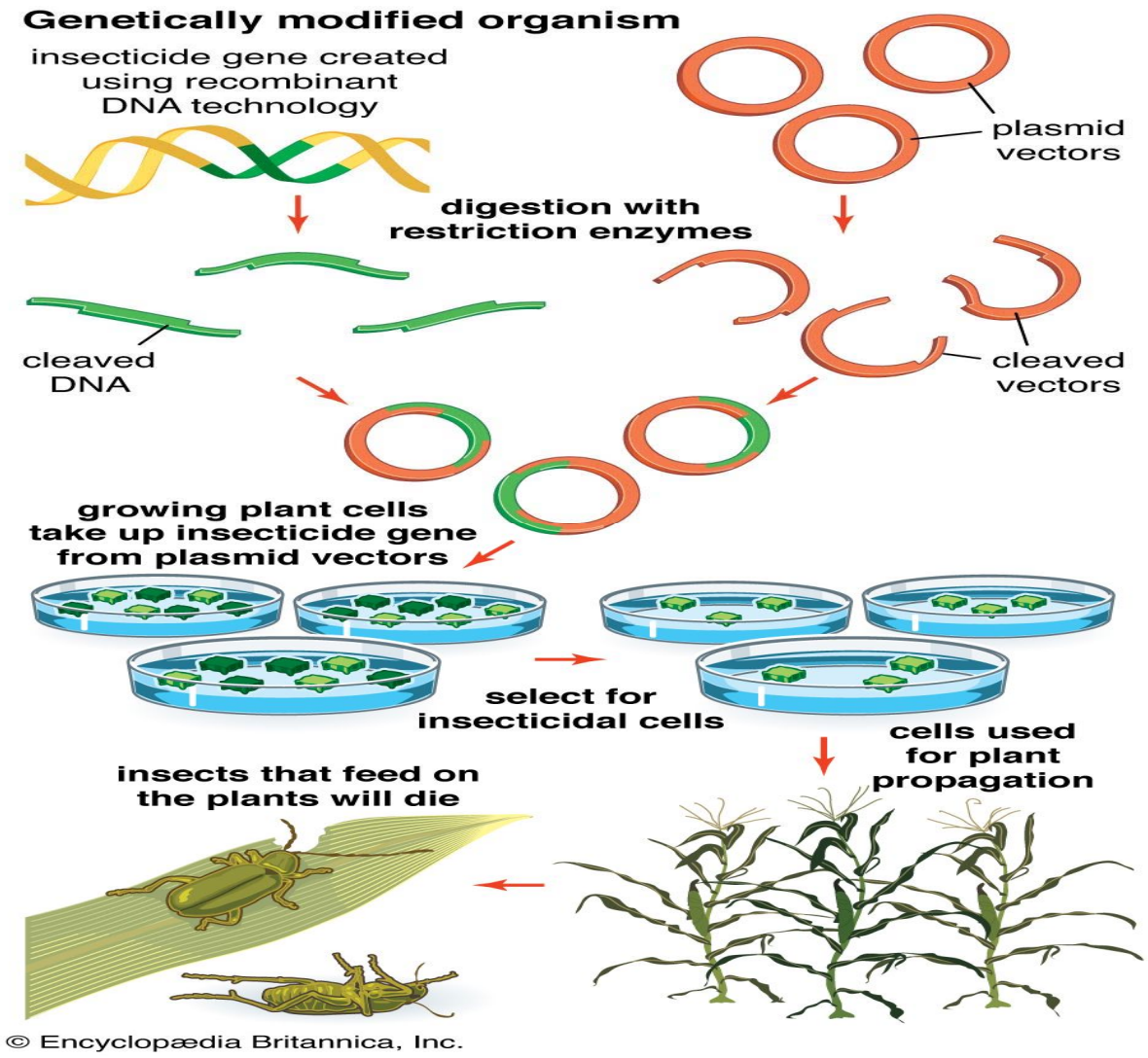

Figure 35: Scientific methods that include recombinant DNA technology are used to produce genetically modified organisms.Encyclopædia Britannica, Inchttps://www.britannica.com/science/genetically-modified-organism#/media/1/897705/122433

8.4.2 Vectors

Selecting an appropriate cloning vector depends on the gene transfer method, desired outcomes, and microorganism application. Various vector classes serve different purposes, from replicating vectors expressing desired genes to cosmid and bacterial artificial chromosome vectors capable of carrying large DNA fragments. Conjugal vectors transfer genes from easily manipulated organisms like *E. coli*to more challenging hosts. Gene replacement vectors enable stable gene integration, while food-grade vectors omit antibiotic resistance marker genes **(Udriste and Badulescu, 2017).**

8.4.3 Promoters

Promoters, segments of DNA regulating gene expression, come in two primary types: constitutive and inducible. Constitutive promoters remain active continuously, while inducible promoters activate only under specific conditions **(Figure 35).**

Choosing the right promoter is crucial for optimizing target gene expression. Examples include the lac promoter in *E. coli*, induced by IPTG, and alternative promoters like the arabinose and cold-shock promoters, which offer cost-effective alternatives for large-scale production. Promoters responsive to pH, dissolved oxygen, or osmolarity are also potential choices for industrial applications **(Faust, 2021)**.

8.4.4 Selectable Marker Genes

Selectable marker genes, often encoding antibiotic resistance, play a vital role in identifying transformed cells. They apply selection pressure, inhibiting nontransformed cell growth. Based on organism-specific properties, alternative selection systems have been developed for microorganisms unsuitable for antibiotic resistance marker genes. For instance, lactic acid bacteria use systems tied to lactose metabolism, proteolytic activity, DNA synthesis, and bacteriocin resistance. In yeast, selection systems based on yeast genes, like the YAP1 gene, resist specific inhibitors and effectively reduce nontransformed cells **(Udriste and Badulescu, 2017).**

8.5 Strategies for GMMs

Several strategies have been developed to create GMMs with desired traits. These strategies include:

8.5.1 Disruption of Undesirable Gene Functions

Disrupting a gene function can be achieved by cloning a DNA fragment internal to the target gene into a suitable vector. Upon introducing the recombinant plasmid into the host organism, the internal fragment of the gene, along with the vector, is integrated into the host chromosome via single-crossover recombination. This integration results in the formation of two incomplete copies of the same gene separated by the inserted vector sequence, thereby

disrupting the target gene's function. However, such integration is unstable due to identical DNA sequences on either side of the vector.

Another approach to disrupting gene functions relies on antisense technology, which uses antisense ribonucleic acid (RNA) or DNA sequences complementary to the target genes' messenger RNAs (mRNAs). The binding of an antisense molecule to its complementary mRNA results in the formation of a duplex RNA structure that inhibits the activity of the target gene through various mechanisms **(Aguilera & al., 2013).**

8.6 Applications of GMM-Derived Products

❖ Human Health

Several therapeutic proteins, including insulin, interferons (IFNs), and interleukins, are now produced by GMMs for medical use. Traditionally, these proteins were obtained from human, cow, or pig sources, posing challenges like limited supply, immunological responses, and contamination risks. GMMs offer a solution by producing these proteins efficiently and safely.

The first recombinant therapeutic protein, human insulin, was FDA-approved in 1982. It was produced using genetically engineered *E. coli*containing human insulin genes. Similarly, human growth hormone and IFN y were also made through GMMs. This method ensures these therapeutic proteins' stable supply, cost-effectiveness, and purity **(EFSA, 2011a)**.

❖ Recombinant Vaccine

The Hepatitis B vaccine, vital for preventing severe liver disease, was initially prepared from blood samples of infected individuals. This method was unsafe and costly. Recombinant technology changed the game by expressing the gene responsible for hepatitis B surface antigen in Saccharomyces cerevisiae (baker's yeast). The recombinant vaccine, Engerix®-B, is identical to the previous vaccine but safer, more consistent, and economical (**Aguilera & al., . 2013)**.

❖ Animal Health

Recombinant proteins produced by GMMs also benefit animal health. Bovine somatotropin (bST), a natural cattle hormone regulating growth and milk production, was

traditionally extracted from pituitary glands. The bST gene was expressed in *E. coli*to meet commercial demand, resulting in FDA-approved recombinant bST, marketed as Posilac™. Studies showed increased milk production in cows treated with it.Another example is phytase, an enzyme vital for phosphate absorption in nonruminant animals**(Forabosco & al., 2013)**.

❖ **Recombinant Vaccine to Eradicate Rabies**

Rabies, a deadly viral disease, affects both humans and wildlife. The traditional method involves vaccines derived from attenuated rabies virus strains and is impractical for eradicating the disease in wild animals. A cost-effective solution emerged with a recombinant vaccinia virus expressing the glycoprotein G of the rabies virus. This vaccine is used in eradication programs in Europe and North America **(Concha & al., 2017).**

❖ **Textile Industry**

Microbial enzymes enhance production processes and product quality in the textile industry. Genetic engineering has been applied to increase enzyme production in heterologous hosts. For instance, a-amylase and cellulase, used to remove starch sizes and maintain fabric quality, were produced in higher quantities by recombinant microorganisms.

❖ **Food Industry**

GMMs have been used in the food industry for over 15 years. Notable examples include chymosin for cheese making and pectinases for fruit and beverage processing. Chymosin, traditionally sourced from calf stomachs, is now produced in Kluyveromyces lactis, a safe yeast strain. Pectinases, used for complete pectin degradation in fruit processing, are produced in *A. oryzae* for efficiency and purity (**Panesar & al., 2010 ; Blair & al., 2015 Kärenlampi and Wright , 2016).**

Diagnostic Tools

Diagnostic tests for diseases like AIDS and Alzheimer's disease have been improved using GMMs. Cloning relevant antigenic coat protein genes into *E. coli*enabled large-scale production of diagnostic antigens, overcoming safety and reliability issues.

❖ **Biodegradable Plastics**

Polyhydroxyalkanoates (PHAs) offer an environmentally friendly alternative to petroleum-based plastics. While naturally produced by microorganisms, their slow growth and low yields are impractical for commercial use. *E. coli*transformed with PHA pathway genes efficiently accumulates PHAs, making it a viable choice for large-scale production, offering a sustainable plastic solution**(Sharma & al., 2018)**.

8.6.1 Labeling of GMM-produced food substances as “GMO”

In addition to establishing safety criteria for GMM-produced food substances, regulatory agencies determine whether these substances or food products containing them require labeling as "GMO" (or "Bioengineered" in the United States). Understanding these labeling requirements can be challenging, especially if one is only familiar with regulations applied to agricultural products. However, GMM-produced food substances are typically not classified as "GMO" under certain conditions:

1. **Food substances or food "produced with" a GMM do not need to be labeled GMO:** Regulations differentiate between substances "derived from" or "produced from" genetically engineered sources and those "produced with" a GMM.
2. **Food substances with no detectable DNA need not be labeled GMO:** Several countries require differentiation between GMO-labeled and non-GMO-labeled foods based on the detection of GMO DNA. If analytical methods cannot detect GMO DNA in the final food substance, it is not labeled GMO.
3. **Food substances produced with specific genetic modification techniques do not need to be labeled GMO:** Different jurisdictions define genetic engineering techniques differently. For instance, CRISPR is considered outside the scope of genetic engineering in the United States and Japan if the modification could occur naturally or through traditional breeding.
4. **Other GMO labeling considerations:** Most regulatory frameworks define criteria for labeling foods containing GMOs but not for labeling foods as "non-GMO." This has led to the development of independent, voluntary, non-regulatory GMO labeling frameworks that may deviate slightly from regulatory criteria.

8.7 GMMs in Agriculture

8.7.1 Biological Control of Frost Injury in Plants

Frost damage presents a significant challenge to agriculture, impacting annual crops, deciduous fruit trees, and subtropical plants. In the United States alone, plant frost injury costs exceed $1 billion. Current mechanical methods to mitigate frost damage prove both costly and ineffective.

The primary culprits behind frost damage are ice-nucleating bacteria, including Pseudomonas, Xanthomonas, and Erwinia, which reside on plant surfaces. These bacteria possess a membrane protein that initiates ice crystal formation, leading to plant cell damage. Nonice-nucleating strains can be applied through seeds or foliage to combat these ice-nucleating bacteria. These strains are obtained by treating ice-nucleating bacteria with chemical mutagens. However, chemically induced mutants may suffer multiple mutations, impacting genetic stability and ecological fitness **(Stemke, 2004).**

An alternative approach involves genetically engineering mutants of *Pseudomonas syringae*, lacking ice nucleation genes. As demonstrated in field tests, these engineered strains effectively outcompete ice-nucleating P. syringae on plant leaf surfaces, resulting in significantly reduced frost damage **(Hokanson& al., 2014)**.

8.7.2 Biological Control of Insect Pests

Bacillus thuringiensis (Bt), a naturally occurring soil-borne bacterium, produces larvicidal proteins without harming mammals, birds, or fish. These proteins target specific receptors in the intestinal lining of susceptible insects, causing cell rupture. Bt-based products have been a safe alternative to chemical pesticides for decades, but they have limitations, including environmental instability, a narrow host range, and challenges in reaching larvae within crops.

One solution is to use plant-associated bacteria as delivery vehicles for Bt toxins. Bt toxin genes have been successfully integrated into several plant-associated bacteria, such as *Clavibacter xyli subsp cynodontis* and *Ancylobacter aquaticus*. These genetically modified strains have demonstrated effective pest control, making them promising candidates for insect management **(Zhao & al., 2016)**.

8.7.3 Biological Control of Plant Disease

Traditionally, plant diseases are combated with chemical agents, an expensive and sometimes ineffective approach. An alternative method involves modifying microorganisms to deliver desired chemicals. For instance, *Agrobacterium tumefaciens* causes crown gall disease in plants by transferring T-DNA from a Ti plasmid into plant cells, leading to the overproduction of plant growth hormones and opines. Agrobacterium *radiobacter* K84 produces agrocin 84, a bacteriocin that targets pathogenic *A. tumefaciens* strains **(Zimmer& al., 2018).**

8.7.4 Soil Improvements

Genetic modifications have also been applied to enhance soil fertility. Medicago sativa (alfalfa) grown in nitrogen-rich soils showed improved root nodulation when exposed to genetically modified *Sinorhizobiummeliloti* expressing the *Klebsiella pneumoniae nifA* gene compared to wild-type *S. meliloti*. Additionally, the recombinant S. meliloti significantly increased plant biomass compared to the wild-type strain.

8.7.5 GMMs in Bioremediation

Bioremediation utilizes biological systems to detoxify environments contaminated with heavy metals, organic compounds, radionuclides, and other substances, including explosives, pesticides, and plastics **(Figure 36).**

The pioneering use of MMs in bioremediation involved *Pseudomonas fluorescens* HK44, engineered for naphthalene degradation. Derived from *P. fluorescens* isolated from a heavily contaminated site, HK44 carries a plasmid capable of naphthalene catabolism and a bioluminescence-producing reporter gene (lux) linked to the naphthalene catabolic gene promoter.

Consequently, naphthalene induces gene expression, resulting in naphthalene degradation and luminescence emission, allowing real-time monitoring **(Sharma & al., 2018)**.

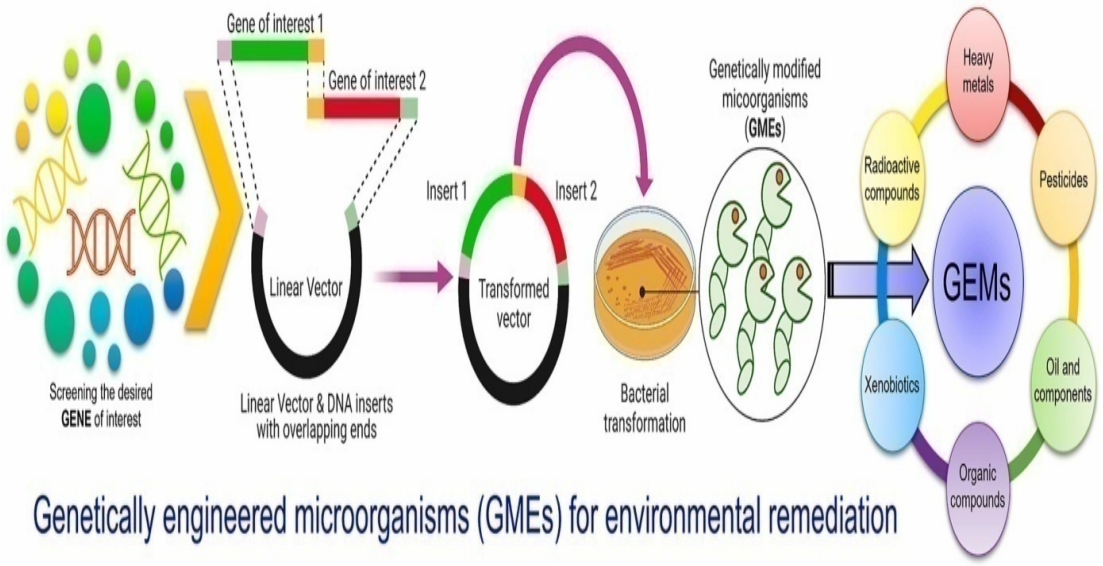

Figure 36: GMMs in Bioremediation (https://doi.org/10.1016/j.chemosphere.2022.136751)

9 MO- gastrointestinal

9.1 Introduction

The human body **(Figure 37)** is a vast reservoir for microorganisms, colonizing diverse regions such as the skin, mouth, reproductive organs, and the gastrointestinal (GI) tract. This intricate microbial community, primarily composed of bacteria, maintains a symbiotic relationship with the human host, which is pivotal in preserving overall health. Within the GI tract, these commensal microbes undertake critical tasks, including providing essential nutrients, fostering the development of the immune system, and thwarting the colonization of harmful pathogens. The precise configuration of the GI microbiota is indispensable for effective gut functioning, hinging upon intricate cell-to-cell communication and interaction **(Vijay and Valdes, 2022).**

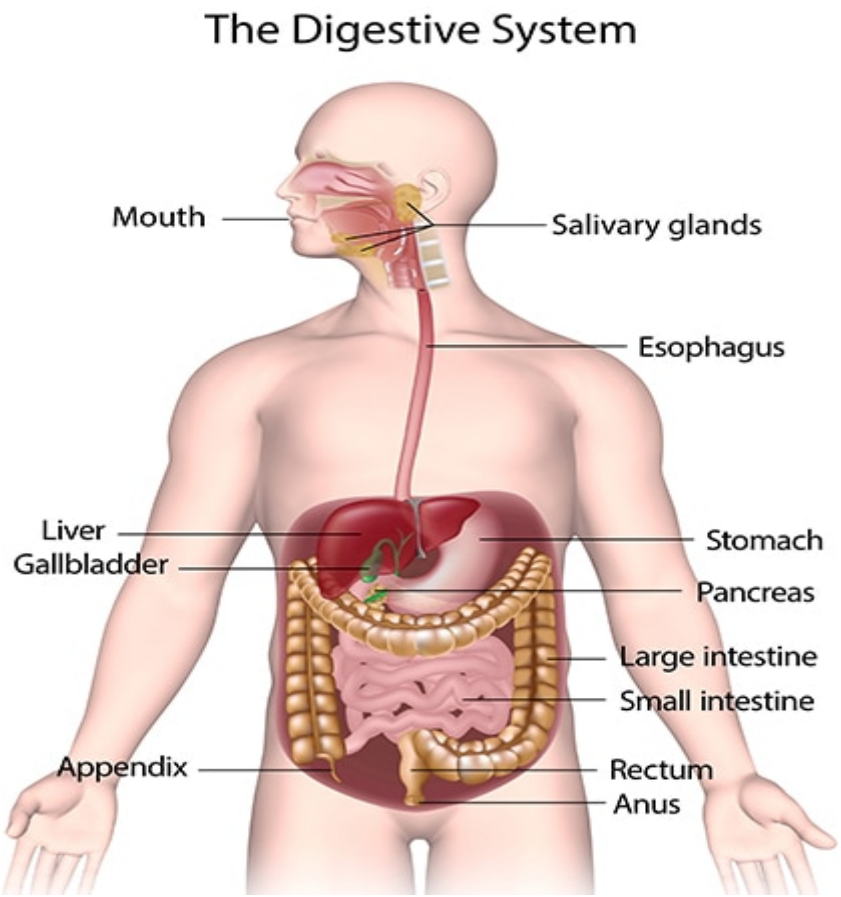

Figure 37: The digestive system (Your **Digestive System** & How it Works - NIDDK

National Institute of Diabetes and Digestive and Kidney Diseases › Health Information › Digestive Diseases. 2017.

Quorum sensing systems have been unearthed in various bacteria inhabiting the human body, encompassing pathogenic strains like *Fusobacterial nucleaum*, *Helicobacter pylori*, *Escherichia coli*, *Salmonellaenterica*, and even probiotics such as *Lactobacillus*. Extensive research has scrutinized the QS signaling systems in these individual bacteria, revealing their involvement in diverse processes related to bacterial colonization, virulence expression, and biofilm formation.

Nonetheless, unraveling the intricate interplay of these systems to construct an effective bacterial communication network and sustain bacterial community equilibrium in multispecies consortiums like the human gut microflora remains a formidable challenge. Infection models have provided invaluable insights into the QS-regulated social behaviors of bacteria within the context of multispecies communities **(Bzdrenga & al., 2016).**

Beyond the confines of the human body, bacteria exist ubiquitously in the environment, pervading air, soil, and water and establishing extensive colonies, especially within the gastrointestinal tract. Their forms exhibit remarkable diversity, manifesting as cocci, rods, spirilla, or budding shapes.

Classification of bacteria hinges on factors like shape, cell membrane characteristics, and energy utilization, with categories such as heterotrophic, phototrophic, or lithotrophic organisms. Thriving under optimal conditions, bacteria can replicate every 20 minutes, rapidly adapting to shifting environmental circumstances.

Traditionally, attention has been directed primarily toward pathogenic bacteria that afflict humans, including Streptococcus pyogenes, Bordetella pertussis, Corynebacterium diphtheriae, Clostridium tetani, *Salmonella t*yphimurium, *Vibrio cholera*, and numerous others.

However, many bacteria coexist harmoniously in close association with humans, the majority of which are not harmful but play crucial roles in maintaining host health. These microbiota wield significant influence over nutrient degradation and absorption, bolster defenses against pathogens, stimulate the immune system, and shape gut health. Remarkable variation in microbiota composition exists among individuals, influenced by genetics, age, personal hygiene, infections, medications, and dietary choices **(Ruan & al., 2020).**

9.2 Microbiota in the Early Years of Life

The neonate's first encounter with microbes occurs during the birthing process, resulting in the progressive establishment of bacterial populations, especially in the opening months of infancy. Following birth, bacteria establish colonies on the oral and nasopharyngeal membranes and the infant's skin and gastrointestinal tract. Surprisingly, there is a lack of notable microbial differences seen across the many habitats of newborns, including the skin, mouth cavity, nasopharyngeal mucosa, and gastrointestinal tract **(Figure 38).** The observation above starkly contrasts the heterogeneous microbial colonization reported in different anatomical regions of maternal individuals**(Galland, L. (2014)**.

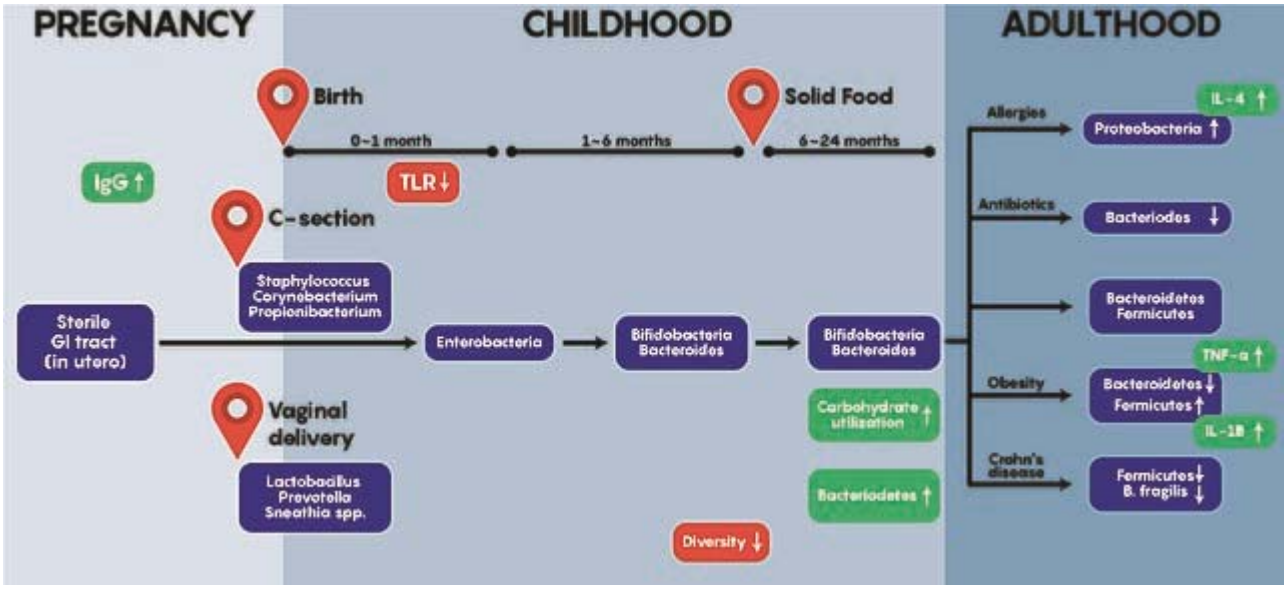

Figure 38: An illustration of the typical developmental colonization of the gut by bacteria. The initial colonies of bacteria that settle depend on the delivery method. In the first week of life, TLR is reduced, which may allow for the formation of stable bacterial colonies in the gut. During the first 6 months, as children are subjected to solid foods, the diversity of microbiota increases. The immune system is able to differentiate the difference between pathogenic and helpful bacteria. Disease appears to correlate with bacteria concentration (http://neuroscience.openetext.utoronto.ca/chapter/chapter-1-the-gut-microbiome-and-its-impact-on-the-brain/).

The manner of administration significantly influences the first bacterial colonisation. Infants born via the vaginal canal tend to receive microbiota that closely resemble the microbial composition of their mother's vaginal flora. *Lactobacillus*, *Prevotella*, and *Sneathia*

often dominate this microbial community. In contrast, neonates delivered by Caesarean section (C-section) often have bacteria primarily present on the skin, including *Staphylococcus, Corynebacterium*, and *Propionibacterium* species. These exhibit significant differences when compared to the microbiota found on maternal skin. There is considerable variation in the underlying microbial pattern seen among infants. During the first stages of life, the composition of the gut microbiota undergoes a progressive transformation. However, it is noteworthy that certain gut microbes maintain unique characteristics, indicating a potential competitive edge for those who colonize the gut early on **(Cogen & al., 2008).**

The establishment of early colonization and the presence of diverse bacterial species play a vital role in facilitating a harmonious interaction between the microbiota and the host organism. After the first 2-3 years, the microbiota attains stability and exhibits a bacterial composition that nearly approximates the mature gastrointestinal tract. Research findings suggest that neonates born by cesarean section (C-section) are more susceptible to wheezing and allergy sensitization during the first two years of their lives **(Ruan & al., 2020).**

9.3 Microbiota Along and Across the Gut

The concentration of microbiota varies significantly along the gastrointestinal tract, demonstrating a remarkable diversity of microbial communities within the gut**(Figure 39).** Starting in the stomach, where harsh acidic conditions prevail, bacterial populations are sparse, with just 101 bacteria per gram of content. However, the numbers steadily rise as we move down the digestive tract. Approximately 10^3 bacteria per gram are in the duodenum, followed by 10^4 in the jejunum and 10^7 in the ileum. However, the real microbial metropolis lies in the colon, where the concentration skyrockets to an astounding 10^{12} bacteria per gram. These microbes primarily belong to the *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phyla, with minor representation from *Actinobacteria, Verrumicrobia, Acidobacteria*, or *Fusobacteria* **(Quigley, 2013)**.

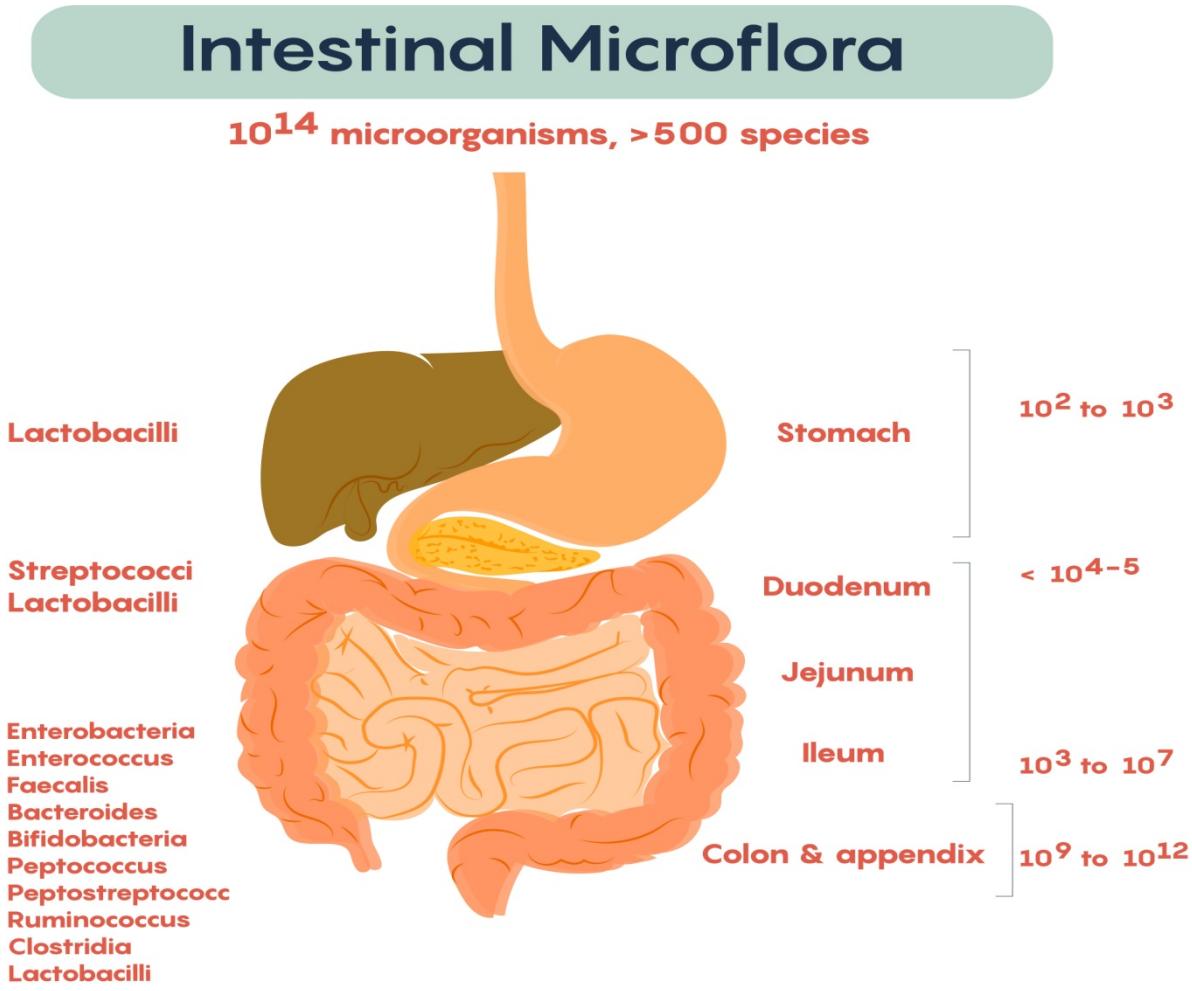

Figure 39: Microbial density in the gut (Quigley, 2013).

But it's not just the sheer numbers that distinguish the gut microbiota. Close examination reveals nuances even within the same phyla. For instance, mucosa-associated bacteria in the distal small intestine and the colon predominantly belong to *Bacteroidetes* and *Firmicutes*, albeit in varying ratios. Proximal gut regions, such as the duodenum, jejunum, and ileum, boast a different microbial lineup, featuring Bacilli, *Streptococcaceae*, *Actinomycinaeae*, and *Corynebacteriaceae* in greater abundance. As we delve into the colon, we encounter increased proportions of *Lachnospiraceae* and *Bacteroidetes*.

This longitudinal variation is not the only intriguing aspect of gut microbiota. There's also a fundamental difference in microbial populations between the gut epithelium and the gut lumen. Goblet cells play a pivotal role by producing glycosylated proteins known as mucins, which form a protective mucus layer that acts as a barrier against most bacteria. Only specialized bacteria, such as *Clostridium, Lactobacillus,* or *Enterococcus*, can adhere to this mucus, utilize it as a nutrient source, or access epithelial cells. In contrast, the fecal

environment harbors a diverse array of bacteria, including *Bacteroides, Bifidobacterium, Streptococcus, Enterobacteriaceae, Enterococcus, Clostridium, Lactobacillus*, and *Ruminococcus* **(Holscher, H.D. (2017).**

In parallel with these microbial intricacies, the gut employs a fascinating communication network based on autoinducers (AIs). Quorum sensing (QS) regulates various bacterial functions, from sporulation to virulence secretion and even interspecies cooperation or competition **(Figure 40).**

Gram-negative bacteria utilize acyl-homoserine lactone (AHL) as their AI, while Gram-positive bacteria rely on autoinducing peptides (AIPs). A universal language, autoinducer-2 (AI-2), transcends these boundaries, fostering inter-species and inter-kingdom communication within the human GI tract.

Another dimension of communication emerges through AIPs, small peptides primarily used by Gram-positive bacteria for QS signaling. AIP signaling can be species-specific and even inhibit QS in other strains, demonstrating its competitive nature within the microbiota. This phenomenon has been observed in commensal Staphylococcus simulans blocking QS in methicillin-resistant *Staphylococcus aureus* **(Castillo-Juárez , 2015)**

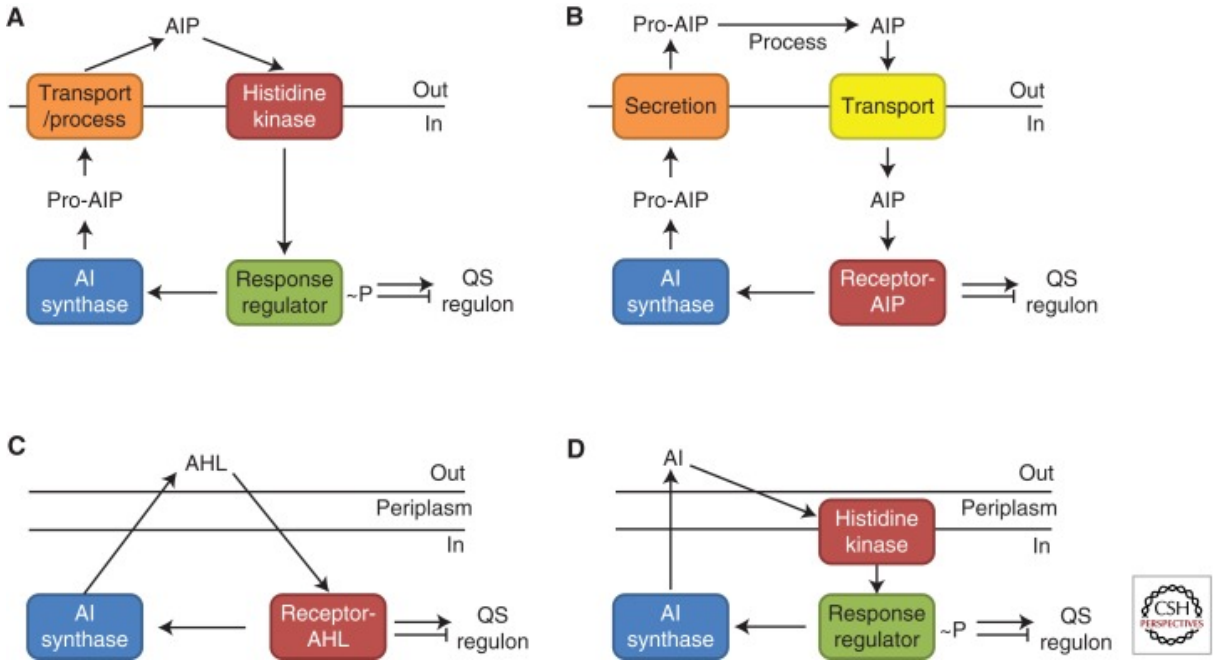

Figure 40: Canonical bacterial quorum-sensing (QS) circuits.(Rutherford and Bassler, 2012).

9.4 Viruses and Fungi

The human intestinal microbiota is a complex ecosystem comprising various microorganisms, including bacteria, archaea, bacteriophages, viruses, unicellular eukaryotes, and fungi. While bacterial components have traditionally received the most attention in microbiota research, the presence of these other microorganisms should not be overlooked.

Recent studies have highlighted abnormal viral patterns in inflammatory bowel diseases, shedding light on the potential significance of viruses in maintaining a healthy microbial community. Bacteriophages, in particular, play a pivotal role in shaping their bacterial hosts' survival, reproduction, composition, and functionality. Notably, 23 distinct bacteriophages common in over 50% of individuals have been identified, and their reduced occurrence has been observed in patients with gastrointestinal complaints **(Barko &al ., 2018)**.

9.5 Host—Microbiota Interaction

The gastrointestinal tract serves as the vital interface between the host and its environment, particularly emphasizing the small intestine due to its substantial surface area dedicated to digestion and nutrient absorption. Given that the intestine harbors the highest bacterial concentration, the host has evolved a dual strategy: tolerance towards beneficial and benign microorganisms while maintaining an efficient defense mechanism against pathogens and bacterial overgrowth.

One pivotal component of this defense is the mucus barrier, which lines the intestine. The dense and highly efficient inner layer serves as the primary defense mechanism. Its high density prevents the penetration of most bacteria, effectively isolating the epithelium from the abundant luminal microbiota. Human mucus is rich in glycosylation, with over 100 different mono-, di-, or trisialylated oligosaccharides described **(Lin and Medeiros, 2023)**.

Only a select few microorganisms can adhere to mucus, primarily to the outer layer, contingent on the presence of lectins. Mucus-binding proteins have been isolated from beneficial bacteria, such as *Lactobacillusreuteri*, *Lactobacillusplantarum*, and *Lactobacillusrhamnosus*. Conversely, pathogenic microorganisms like *Helicobacter pylori, Clostridium jejuni*, and noroviruses have also demonstrated an ability to adhere to mucus,

likely utilizing human histo-blood group antigens on mucins as receptors **(Van Tassell and Miller, 2011).**

The intestinal epithelial barrier, which separates the gut lumen from the lamina propria, comprises absorptive enterocytes, goblet cells, Paneth cells, and endoenterocrine cells. These cells collectively form a physical barrier that restricts the paracellular transport of molecules

(Figure 41).

Emerging evidence suggests a bidirectional influence between human gut microbiota and the brain, forming the gut-brain axis. Gut microbiota influences the central nervous system through neuroendocrine, neuronal, and immune-mediated mechanisms. In turn, the brain impacts gut microbiota via the autonomic nervous system **(William & al., 2014).**

Gut microbiota plays a pivotal role in producing metabolites, including short-chain fatty acids, which affect the intestinal barrier function, mucosal neurotransmitter release (e.g., serotonin), the sympathetic nervous system, and modulation of neurotransmitters (e.g., GABA, serotonin, acetylcholine, histamine, melatonin), as well as brain-derived neurotrophic factor. Additionally, gut microbiota modulates afferent sensory nerves by inhibiting calcium-dependent potassium channels and regulating mucosal immune function **(Barko & al., 2018)**.

Several preclinical studies suggest a connection between gut microbiota and the central nervous system

Dysregulation of the gut-brain axis is implicated in the pathogenesis of various psychiatric and depressive disorders, including autism spectrum disorders (ASD) and affective disorders , as well as neurological disorders like multiple sclerosis (MS), Parkinson's disease, fibromyalgia, and chronic pain syndrome. Even conditions like irritable bowel syndrome and obesity are believed to involve an altered gut-brain axis **(Vijay and Valdes, 2022).**

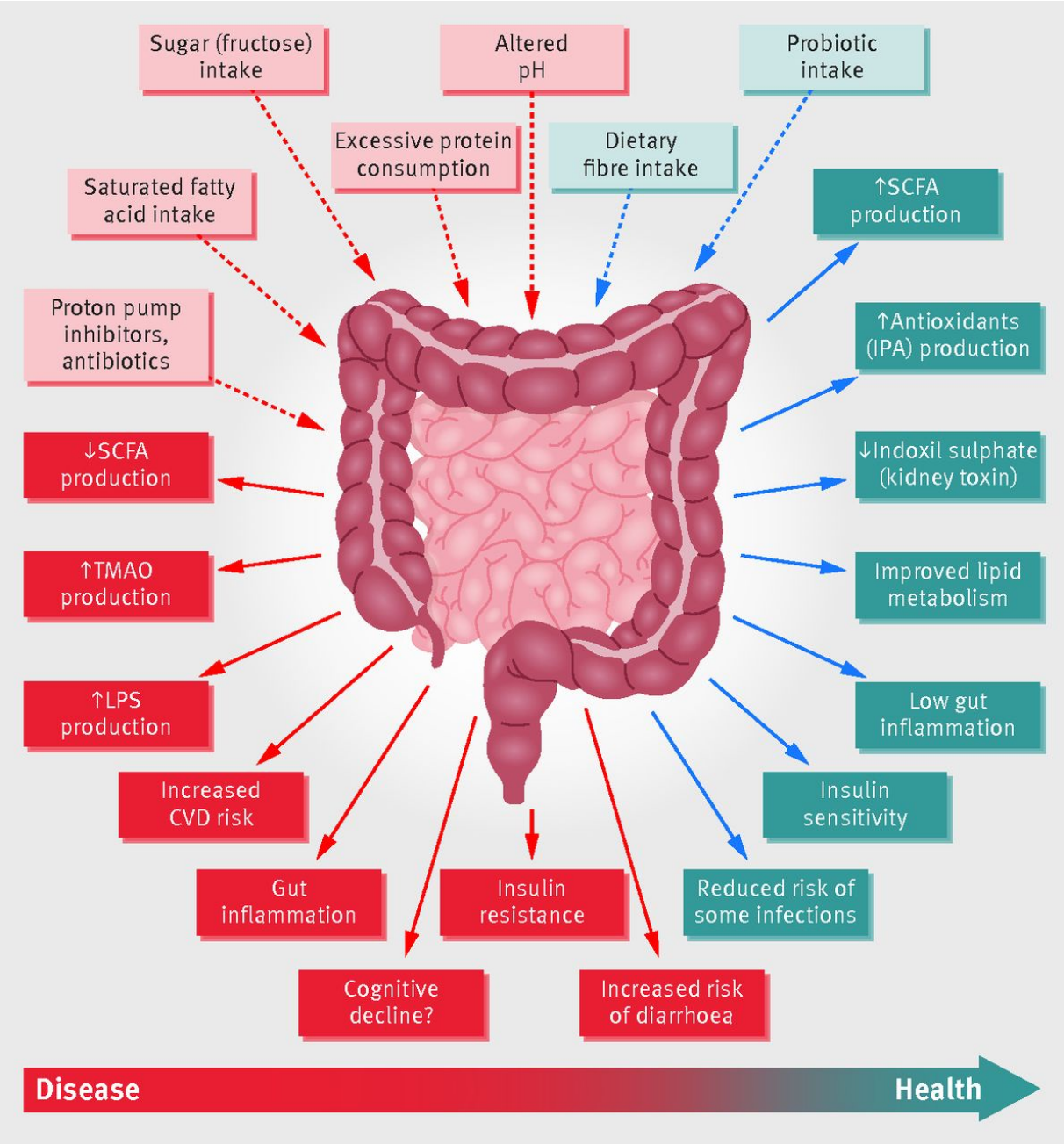

Figure 41 : Schematic representation of the role of the gut microbiota in health and disease giving some examples of inputs and outputs. CVD=cardiovascular disease; IPA=indolepropionic acid; LPS=lipopolysaccharide; SCFA=short chain fatty acids; TMAO=trimethylamine N-oxide (*BMJ* 2018; 361 doi: https://doi.org/10.1136/bmj.k2179).

Although dysbiosis is evident in many neurological disorders, the causal role of gut microbiota in their pathogenesis remains unclear. Nonetheless, normalization of dysbiosis through probiotic supplementation has demonstrated promising effects on neurological and mental symptoms. Animal studies have indicated a reduction in various neurological symptoms, including anxiety, depression, or stress, following treatment with

*Bifidobacterium*and *Lactobacillus* and modulation of neurotransmitter concentrations in the brain. Human studies have also shown some beneficial effects of probiotics on anxiety, depression-related behavior, and stress **(Strandwitz , 2018).**

9.6 Probiotics and Prebiotics

Efforts have been made to identify beneficial bacteria due to the protective role of a well-balanced microbial pattern against disease. Probiotics and prebiotics have garnered significant attention in this context.

Probiotics, defined as living organisms with health benefits, are commonly found in probiotic products containing high concentrations of *Lactobacillus* or *Bifidobacteriumspp*. These organisms support the host's immune system, enhance defense mechanisms through increased anti-microbial defensin production, regulate gut permeability, and serve as primary producers of metabolites, including vitamins (**Figure 42).** Recent meta-analysis results suggest a positive impact of probiotics in children with inflammatory bowel disease, particularly combinations thereof. While probiotics have shown promise in adults with ulcerative colitis, their effects in adult Crohn's disease patients are minimal **(Barko & al., 2018).**

Combining probiotics and prebiotics can facilitate probiotic settlement and multiplication, reducing pathogen concentrations and undesirable metabolites.

Prebiotics are fermentable oligosaccharides like inulin, oligofructose, or fructo-oligosaccharides, which promote the growth of beneficial bacteria, especially *Bifidobacterium*. Some prebiotics, such as fructans and arabinoxylan-oligosaccharides, significantly boost butyrate production, a valuable energy source for enterocytes that contributes to intestinal barrier maintenance. Nevertheless, reducing these oligosaccharides results in lower Bifidobacteriacea levels, despite *Bifidobacteriumspp.*being considered valuable probiotics in gastrointestinal disorders **(Holscher, H.D. (2017).**

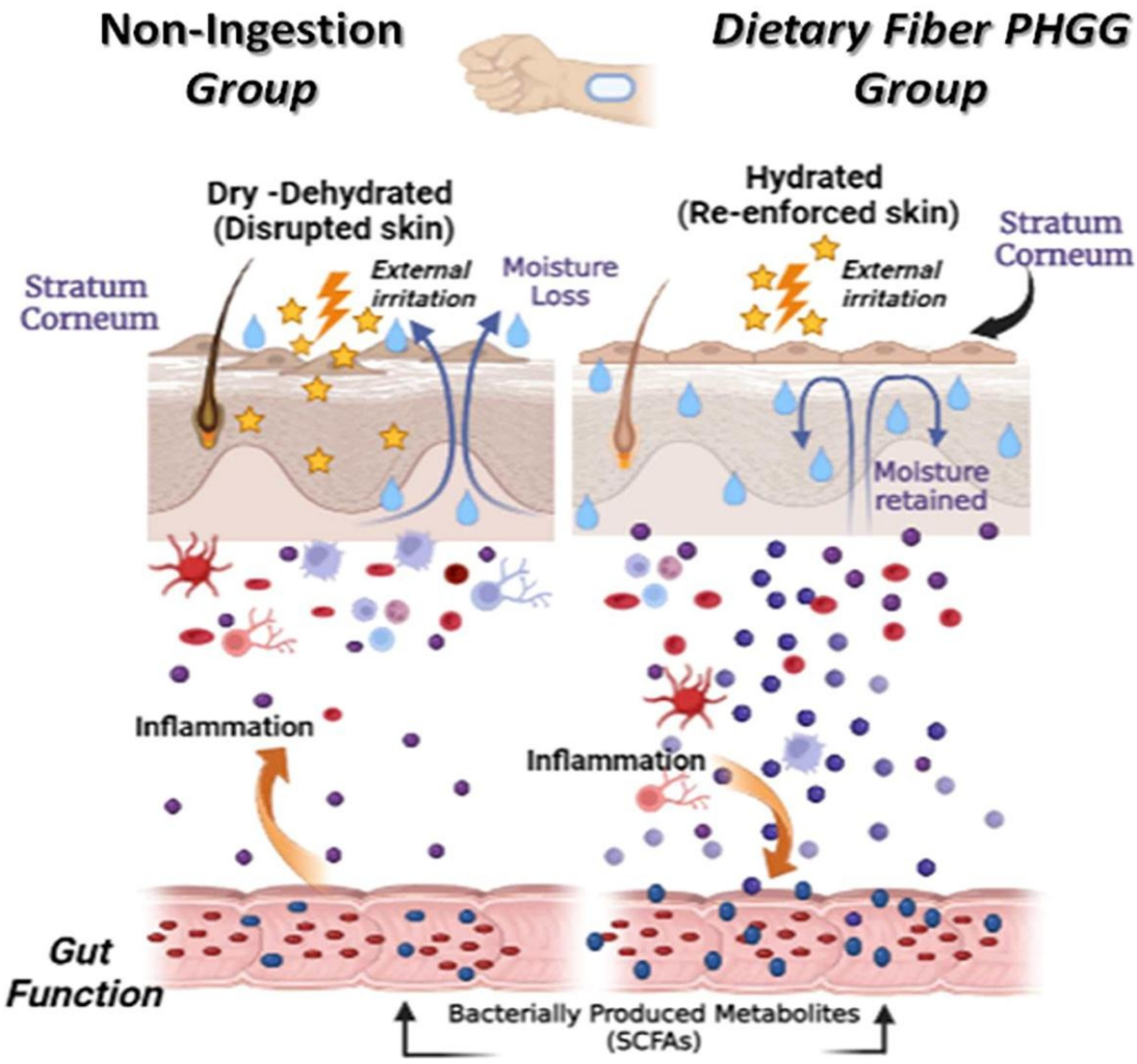

Figure 42: The effects of prebiotic partially hydrolyzed guar gum on skin hydration. (https://doi.org/10.1016/j.jff.2023.105494).

10 Notions of reservoirs (soil, water and plants)

Definition of the reservoir

The reservoir is the place in which biological agents accumulate and proliferate. The latter can grow anywhere, the reservoirs can be found in the environment (**Figure 43):**

- Soil, - fresh or marine waters, - plants, but also on or in a human being or an animal:

- Skin, - respiratory, - saliva, - blood, - wool...

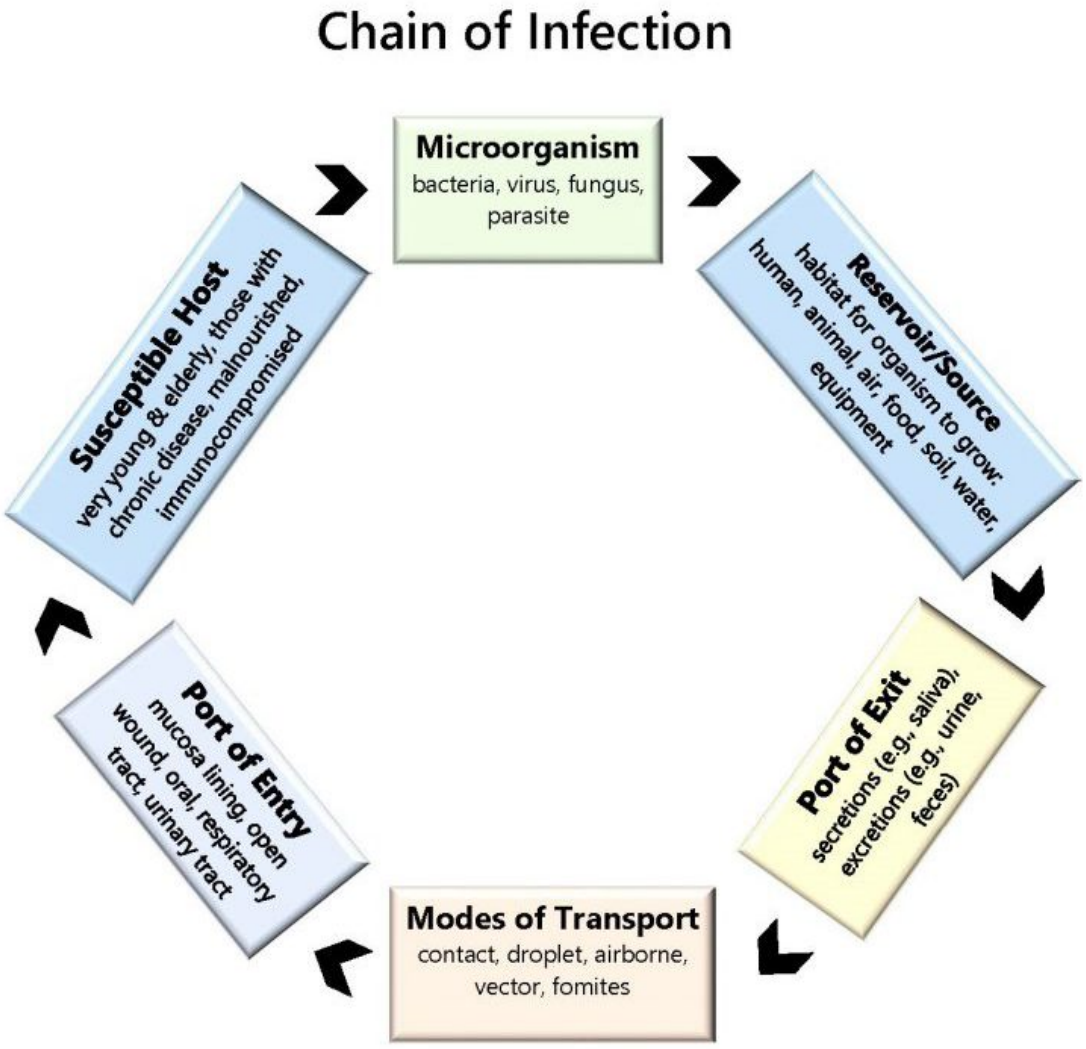

Figure 43 *:* Chain of infection diagram (www.cdc.gov › niosh › learning › safetyculturehc › module-2)

Environmental reservoirs include living and non-living reservoirs that harbor infectious pathogens outside the bodies of animals (Figure 43). These reservoirs may exist on land (plants and soil), in water, or the air. Pathogens in these reservoirs are sometimes free-living. The bacteria *Legionella pneumophila*, a facultative intracellular parasite which causes Legionnaires' disease, and Vibrio cholerae, which causes cholera, can both exist as free-living parasites in certain water sources as well as in invertebrate animal hosts**(Haydon &al., 2002).**

10.1 Soil

The soil is certainly the largest reservoir of microorganisms on our planet. We know that since plants colonized the soil from the marine environment, they have given rise to numerous symbioses with lower bacteria and fungi. These symbioses promote plant growth by facilitating the acquisition of nutrients. In these associations, the colonization of the plant root by soil microorganisms requires a complex molecular dialogue between the two partners leading to specific morphogenesis of the plant as is the case for the associations allowing the symbiotic fixation of the nitrogen (**Figure 44**).

100 g of soil contains on average: 2.1011 bacteria;1 km of mycelial filaments;200,000 insects, worms and protozoa, which represents :1.5 t/ha of bacteria;3.5 t/ha of mycelial filaments.

There is therefore an enormous biodiversity of microorganisms in the soil organized in ecosystems whose role in the growth and health of plants is increasingly highlighted. This microscopic life is subject to enormous pressures which must be taken into account (erosion, compaction, salinization, impermeability, etc.) **(Baize & al., 2013)** .

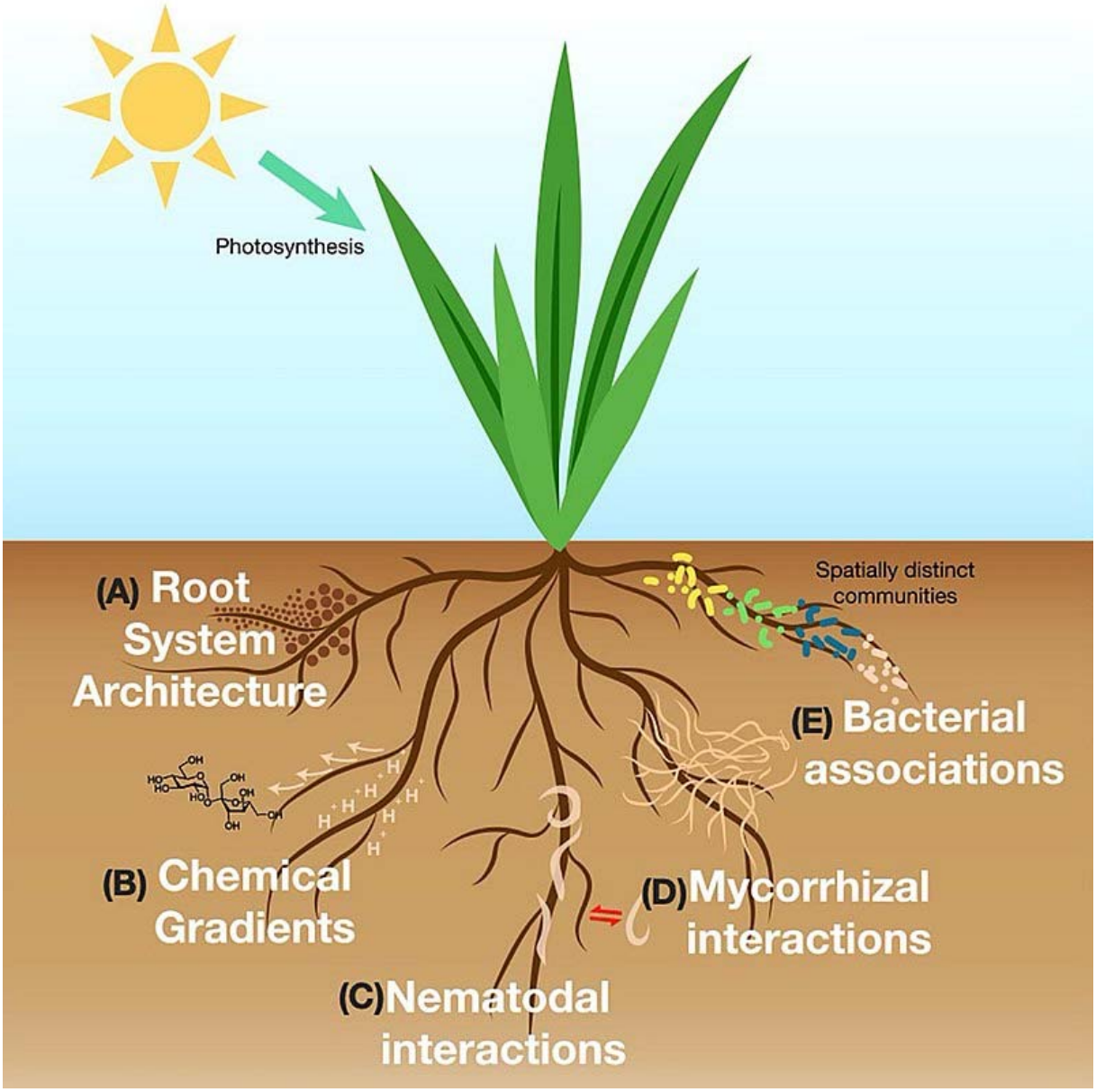

Figure 44 : Some rhizosphere processes in the soil (A) Root system architecture is concerned with structural features of the root and responds to with environmental stimuli. (B) The rhizosphere produces photosynthetically fixed carbon that exudes into the soil and influences soil physicochemical gradients. (C) Free-living or parasitic nematodes interact with the rhizosphere via signaling interactions. (D) Mycorrhizal fungi create intimate relationships with the roots and engage in nutrient exchange. (E) Bacterial composition is distinct upon different parts, age, type of the roots (Yee &al., 2021).

10.2 Plants

Plants evolve in an environment rich in microorganisms that come from multiple sources (e.g. soil, air, water, insects) and which can come into contact with plant tissues. Each plant therefore interacts with an environment composed of a great microbial diversity (several thousand species) belonging to bacteria, archaea, fungi and protists. Furthermore, viruses (including bacteriophages), after infection of living cells, can also be part of these microbial communities. Among this diversity of microorganisms surrounding plants, some can interact directly with them, and even colonize them on the surface or inside their tissues, to form what is called the plant microbiota.

The composition of the plant microbiota varies greatly depending on the genotype of the plant (depending on the plant species and the variety cultivated), the properties of its growing location (soil type, climate and other environmental factors), and the capacity genetics of surrounding microorganisms to interact with the plant **(Brink , 2016).**

At the root level, there is a gradient of biomass and diversity decreasing from the outside to the inside of these organs. In other words, the total number of species and the diversity of these species are greater in the rhizosphere than inside the roots**(Figure 45).**

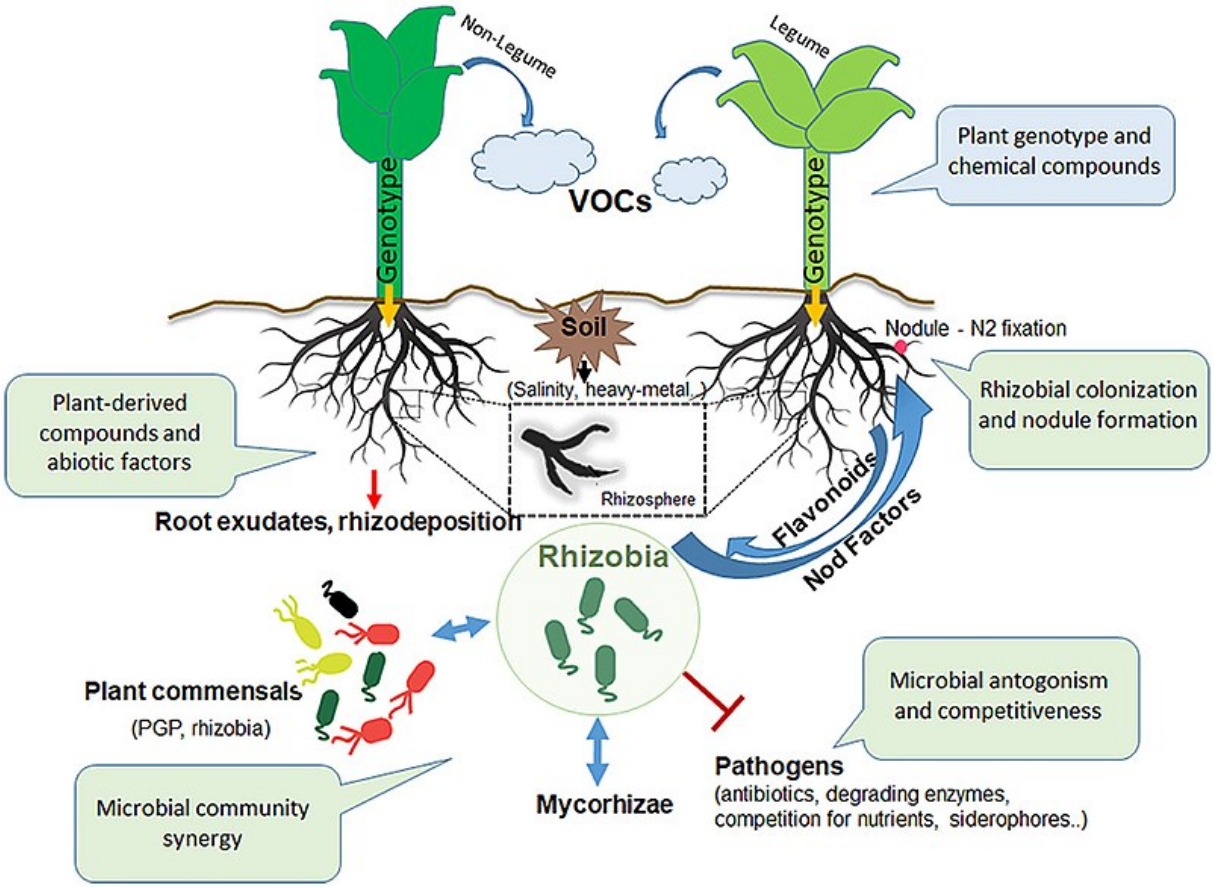

Figure 45: Communication in the rhizosphere Actors and interactions in the rhizosphere: Inter-kingdom and intra-kingdom communication involving plants and microbes in the rhizosphere, including the consistent role of rhizobia. VOCs = volatile organic compounds; PGP = plant growth promoting; AMF = arbuscular mycorrhizal fungi (Checcucci &al., 2020)

10.3 Water

Whatever its origin, marine or continental, water serves both as a resource (food supply, leisure, industrial and agricultural uses, etc.) but also as an environmental or heritage framework (habitat) and supports a certain number of uses and of human activities. In these environments, the microbial component is diverse; it is represented by viruses, bacteria, protozoa, unicellular algae or even microscopic fungi which, by interacting with each other and with their environment, play a major role in the functioning of these ecosystems(Figure 46). However, these ecosystems may contain micro-organisms which are likely to cause more or less serious diseases in humans **(table 5).**

In aquatic environments, pathogenic microorganisms, whether specific or opportunistic, can be an integral part of the natural microbial community. This is the case, for example, of *legionella*, *vibrios* and *amoebae*, which are germs indigenous to natural environments. In other cases, they can be transmitted to natural environments via the discharge of fecal matter from an infested host or from water polluted by fecal matter of human or animal origin (pathogen allochthonous to natural environments) **(Nelson & al., 2009)**.

Among the routes of entry into the human body, the digestive route constitutes by far the most important route, whether through the ingestion of contaminated water or through the consumption of an animal or fruits or vegetables, eaten raw and contaminated by polluted surrounding water. Water contamination is caused, in the majority of cases, by fecal matter which contains pathogenic micro-organisms of enteric origin **(Fenwick, 2006)**.

Waterborne pathogens can occur naturally in aquatic environments. This is the case of bacteria belonging to the group of cyanobacteria, the genera *Legionella*, *Aeromona*s, or the species *Pseudomonas aeruginosa, Burkholderaia pseudomallei*, which are present in fresh water, lakes and rivers, while the species of the genus Vibrio are more dependent on marine and estuarine environments. Other pathogens initially present in fecal matter of human or animal origin are transferred to surface waters via different sources of pollution**(Wilkes &al., 2009).**

Table 5 : Pathogenic microorganisms present in sewage sludge and in household waste **(Déportes I &al.1998).**

Virus	**Bactéries**	**Champignons**	**Protozoaires**	**Helminthes**
Adenovirus	*Arizona hinshawii*	*Aspergillus fumigatus*	*Acanthamoeba*	*Ancylostoma duodenale*
Astrovirus	*Aeromonas spp.*	*Candida albicans*	*Balantidium coli*	*Ascaris lumbricoides*
Calicivirus	*Bacillus anthracis*	*C. guiillermondii*	*Blastocystis hominis*	*Diphyllobothium latum*
Coronavirus	*Bacillus cereus*	*C. krusei*	*Cryptosporidium parvum*	*Echinococcus granulosus*
Coxsachivirus	*Brucella sp.*	*C. tropicalis*	*Dientamoeba fragilis*	*Echonococcus multilocularis*
Echovirus	*Campylobacter perfringens*	*Cryptococcus neoformans*	*Entamoeba histolityca*	*Enterobius vermicularis*
Enterovirus	*Campylobacter jejuni*	*Epidermophyton sp.*	*Giardia intestinalis*	*Hymenolepis nana*
Mixovirus	*Citrobacter sp.*	*Geotrichum candidum*	*Isospora belli*	*Necator americanus*
Parvovirus	*Clostridium botulinum*	*Microsporum sp.*	*Naegleria fowleri*	*Strongyloides stercoralis*
Poliovirus	*Escherichia coli (souches pathogènes)*	*Phialophora richardsii*	*Sarcocystis spp.*	*Taenia solium*
Reovirus	*Klebsiella spp.*	*Trichosporon cutaneum*	*Toxoplasma gondii*	*Taenia saginata*
Rotavirus	*Leptospira interrogans*	*Tricophyton sp.*		*Toxocara cati*
Virus hépatite A	*Listeria monocytogenes*			*Toxocara canis*

Virus	Bactéries	Champignons	Protozoaires	Helminthes
Virus hépatite E	*Mycobacterium pseudotuberculosis*			*Trichuris trichura*
Virus influenza	*Pasteurella pseudotuberculosis*			
Virus de Norwalk	*Proteus sp.*			
	Providencia sp.			
	Pseudomonas aeruginosa			
	Salmonella spp.			
	Serratia sp.			
	Shigella spp.			
	Staphylococcus aureus			
	Streptococcus spp.			
	Vibrio parahaemolyticus			
	Vibrio cholerae			
	Yersinia enterocolica			

10.3.1 Living conditions in the environment

The formation of a reservoir requires certain parameters favorable to the growth of microorganisms.

- The presence of a certain degree of humidity is the first essential condition for the development of biological agents in the environment(**Figure 46).**

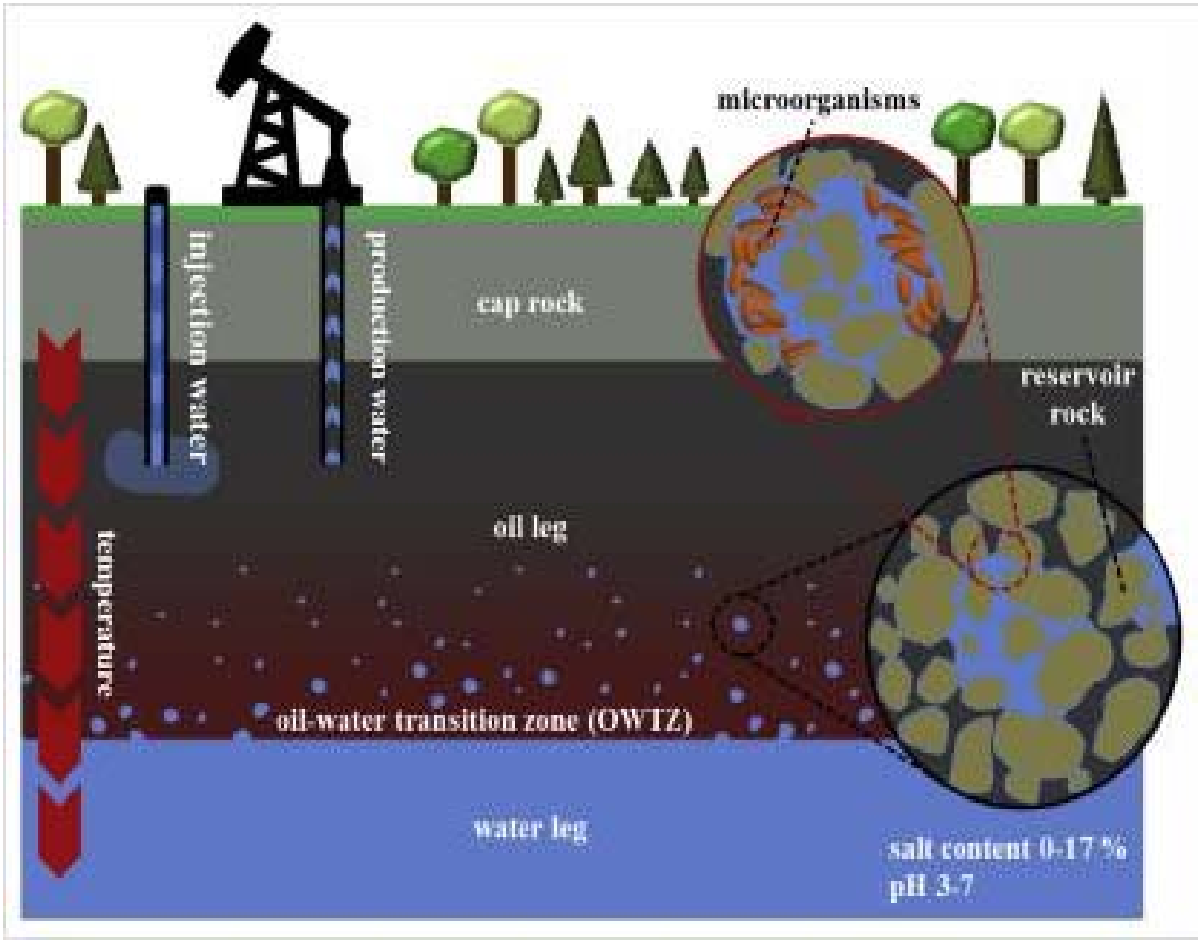

Figure 46 : Schematic scheme of a deep subsurface oil reservoir (oil leg) with underlying brine water (water leg). Most of the biological oil degradation takes place at the oil-water transition zone (OWTZ) and in dispensed water droplets nearby. Microorganisms live attached to rock particles in a thin water film or in dispensed water droplets amidst the oil phase (pannekens& al., 2019).

- Then, depending on how they feed, biological agents require the presence of organic matter or mineral matter.

- In addition, depending on their resistance to desiccation, biological agents multiply at higher or lower temperatures.

- Finally, depending on their metabolism, certain microorganisms grow in the presence of oxygen (aerobic), while others grow in the absence of oxygen (anaerobic).

Each species develops optimally in a defined environment, but can tolerate some variability. Certain micro-organisms can be present in extreme environments:

- from –10°C to +110°C;- pH from 0.5 to 11.5;

- environments saturated with salt such as the Dead Sea;

- high pressure located at –10,500 m depth;

- environment where radiation is 1000 times higher than the lethal dose for humans.

Thus, it is possible to find microorganisms practically everywhere, in nature, but also in the work environment:

- composting platforms,- treatment plants,- grain silos,- offices,- archives,- humidifiers,- laundry, etc.

Microorganisms that can grow in the environment are bacteria, fungi and protozoa. The vast majority of these micro-organisms which surround humans are not pathogenic for the **(Bertrand &al., 2015).**

10.3.2 Conditions of life on or in an organism

Biological agents can also grow on or in a host organism. There they find the necessary nutrients and are adapted to body temperature.

Some symbiotic microorganisms participate in the life of their host: development, regulation of immunity, digestion of food.

They colonize the surfaces of the body in contact with the outside (the skin, the respiratory tract, the digestive tract, the vagina) and thus create a barrier preventing the colonization of pathogenic microorganisms. These symbiotic microorganisms are essentially bacteria, or even fungi.

Other pathogenic biological agents colonize the body causing damage. They divert nutrients for their own benefit, kill cells and cause different symptoms depending on their location. These agents can be viruses, bacteria, fungi, protozoa, or parasitic worms.

Depending on the humidity of the environment and their degree of resistance to desiccation, these body micro-organisms will survive a more or less short time in the external environment **(Helga and Sergiu, 2012).**

10.4 Community life

In the environment or the body, microorganisms live in communities.The colonization of a surface by this community forms what is called a biofilm. The cells are organized in superimposed layers, the deepest of which clings to the surface.Biofilm cells secrete numerous substances forming a thick matrix that covers them.Inside this biofilm, microorganisms exchange molecules between the same species or even between species from different families.

These exchanges are beneficial (the molecules produced by some nourish others) or sometimes aggressive (the molecules produced by some kill others).

It is by studying this last effect that toxins and antibiotic molecules were identified that kill certain bacteria and certain fungi **(Gupta & al., 2013).**

In response to these attacks, only microorganisms that have resistance genes to toxins or antibiotics survive.

The biofilm also includes predators, such as protozoa, which feed on bacteria accessible on the surface of the biofilm.

10.5 Resistance mechanisms

To resist unfavorable environmental conditions, microorganisms have developed various resistance mechanisms.

The first is linked to the appearance of resistance genes to molecules secreted by other cells or biocidal molecules from the chemical industry.

Indeed, given that microorganisms multiply rapidly, there is a high probability of favorable mutations such as resistance genes appearing.

These genes are then passed on to descendants and give rise to resistant strains.

Inside a biofilm, where cells interact closely, resistance genes can also pass from one species to another, or even from one family to another.

When they live in a biofilm, microorganisms protect themselves from mechanical attacks (water or air pressure) and chemical attacks (presence of a biocide), thanks to the matrix

which covers the cells, but also thanks to the stacking of cell layers which protects the deepest microorganisms**(Hobley &al., 2015)**.

Some bacteria, particularly those living in the soil, have the ability to thicken their wall and form a spore that will allow them to resist in the environment.

In spore form, the bacteria cannot multiply, but can survive for a very long time, as shown by the "cursed fields" which contained spores of Bacillus anthracis transmitting, for decades, anthrax to the herds that grazed there. .

Some protozoan cells are naturally resistant to biocides and have the ability to transform into cysts with a thick wall and a slowed metabolism.

This encystment occurs when environmental conditions become unfavorable, or at the time of cell division, or even during the cycle to give the infective form of the parasite.

When protozoa, like amoebae, feed on bacteria, they are trapped and then degraded in digestion vacuoles. However, certain bacteria, such as legionella, can not only resist degradation, but also multiply in the vacuole and escape the amoeba by bursting it.

If environmental conditions become unfavorable, the amoeba encysts and also protects the bacteria it harbors.This is how legionella can protect itself from thermal or chlorine shocks in water pipes, then recolonize the environmen **(Miller & al., 2000)**.

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