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Department of Biological Sciences



Course handout:

Interaction of Microorganisms

Intended for Master 2 students

Specialty: Microbiology and Quality Control

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Preface

Most ecosystems are populated by a large number of diversified microorganisms, which interact with one another and form complex interaction networks.

The microorganism-microorganism or microorganism-host interactions are the key strategy to colonize and establish in a variety of different environments. These interactions encompass multiple ecological processes, including physicochemical modifications, metabolite exchange and transformation, cellular signaling, chemotactic responses, and genetic exchange, ultimately leading to genotype selection.

Interactions within ecological webs may exert positive, negative, or neutral effects on the species involved. The possible combinations of beneficial, detrimental, and neutral outcomes between two interacting partners provide a basis for classifying different types of ecological interactions.

This handout, “Interactions of Microorganisms,” provides students with an in-depth understanding of the diverse interactions that occur among microorganisms, as well as between microorganisms and their biotic and abiotic environments. Through the integration of recent scientific literature, students will explore how microbial interactions shape community structure, drive evolutionary processes, and influence ecosystem functions.

Students enrolling in this course are expected to have a solid foundation in general microbiology, including knowledge of microbial structure, metabolism, and genetics. Familiarity with molecular biology techniques, such as PCR, DNA sequencing, and microbial culturing methods, is essential. A basic understanding of immunology, biochemistry, and cell biology will also support a deeper comprehension of the interactions between microorganisms and their environments, including hosts.

This course handout is intended for Master 2 students in Microbiology and Quality Control. Its primary goal is to reinforce and expand upon the general microbiology concepts acquired during undergraduate studies. The program includes three main chapters:

- Interactions between Microorganisms and physical environment.
- Interactions between Microorganisms.
- Interactions between Microorganisms, plants, human and animals.

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1. Interactions between Microorganisms and physical environment

1.1. Microbial ecology in simple or complex ecosystems « Soil »

1.1.1. Introduction

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.

100 g of soil contains on average: $2 \cdot 10^{11}$ bacteria; 1 km of mycelial filaments; 200,000 insects, worms and protozoa.

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.).

1.1.2. Definition

The definition of the term "soil" has evolved considerably over time, depending largely on how soil has been perceived and utilized.

Soil is defined as a natural body composed of organic and mineral constituents, differentiated into horizons of variable thickness, which differ from the underlying material in terms of morphology, physical constitution, chemical properties, and biological composition.

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1.1.3. Soil Forming Processes

Soil formation is a long term process. It takes several million years to form a thin layer of soil. As soil is a complex mixture of various components, its formation is also more complex.

The Formation of a particular type of soil depends upon the physico-chemical properties of the parent rock, intensity and duration of weathering, climatic and other parameters.

Pedogenesis is the process by which soil is formed. Soils are formed by the physical and chemical weathering of rock.

➤ **Physical weathering** may be one of two types. First, there is disintegration caused primarily by wetting and drying or by freezing and thawing in cracks in the rock. Second, there is erosion caused by the action of glaciers, water, or even wind.

These processes produce a range of particles of varying sizes which are still composed of the same material as the parent rock. Sand and silt particles produced by physical weathering generally consist of single rock minerals, rather than combinations of these, as is the case in their parent rock or in gravel-sized material.

It is important to recognize that no matter how fine the particle size of the material produced by physical weathering may be, it can never have the properties of clay because the chemical conversion needed to form true clay particles is not present.

➤ **Chemical weathering** processes are much more complex and involve chemical changes to the mineral content of the parent rock caused by the action of percolating water, oxygen, and carbon dioxide.

The minerals of which rock is composed are converted into a very different group of materials known as clay minerals. Well-known members of this group are kaolinite, illite

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and montmorillonite, but less well known clay minerals of considerable importance in volcanic areas are halloysite and allophane.

Clay mineral particles are generally crystalline in form and are of colloidal size, that is, they are less than 0.002 mm. These minerals give soil the properties of cohesion and plasticity, which are the distinctive characteristics of clay.

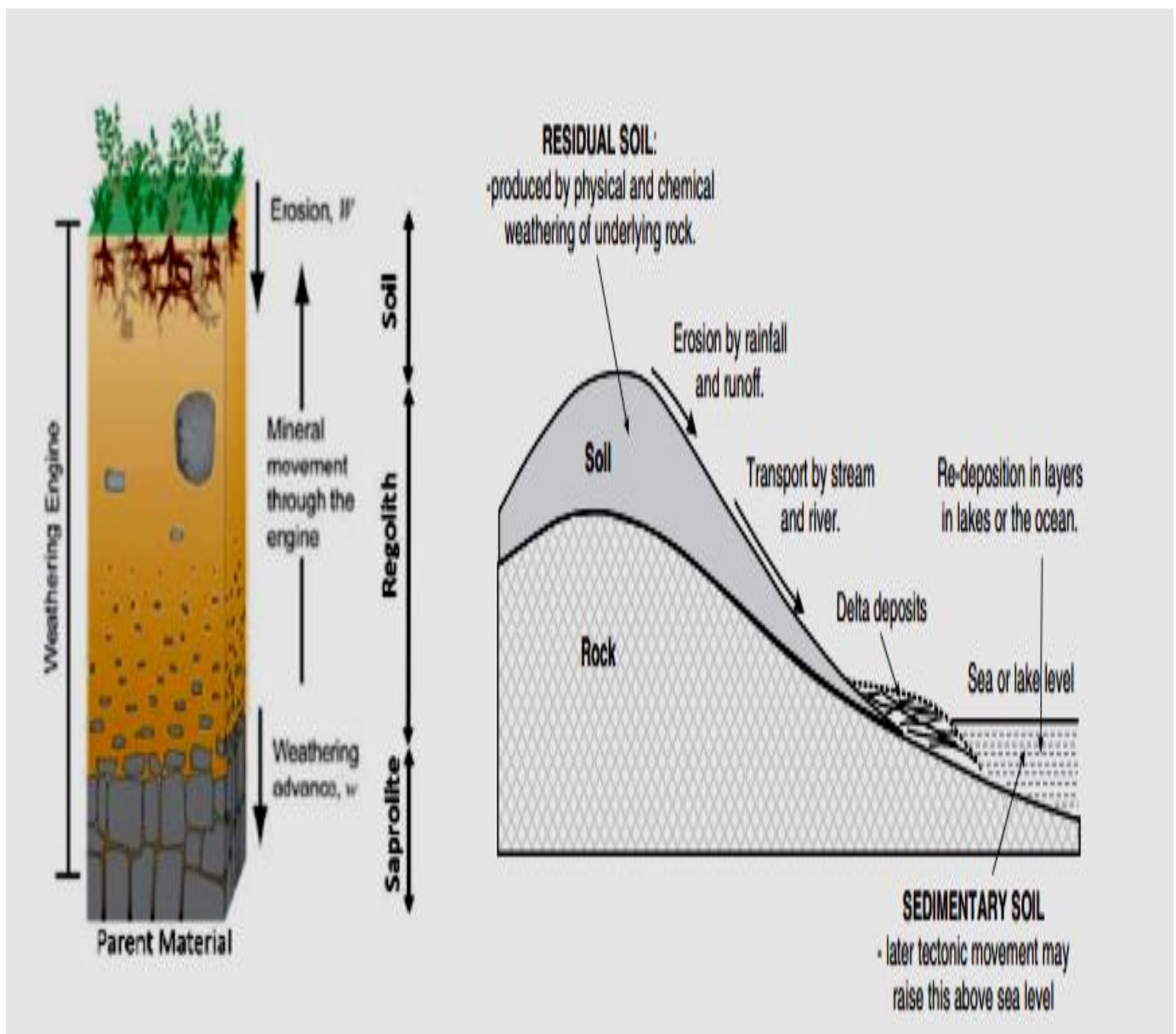


Figure 1. Soil formation processes.

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1.1.4. Soil microorganisms

Soil is one of the most essential natural resources for humans, providing the foundation for agriculture and sustaining ecosystems. Microorganisms play a vital role in the health and productivity of soil, making them crucial to the well-being of the planet. Microorganisms are tiny organisms, including bacteria, fungi, protozoa, and viruses, that are present in soil in large numbers.

Microorganisms constitute $< 0.5\%$ (w/w) of the soil mass, yet they have a major impact on soil properties and processes. About 60-80 % of the total soil metabolism is due to the microflora.

Microbes support many soil processes and functions essential to agroecosystems sustainability. The main soil functions with microbial intervention which support agricultural production are carbon dynamics (decomposition and synthesis of organic matter), nutrient cycling (decomposition, transformation, nitrogen fixation and plant nutrient uptake), soil structure and maintenance (particle aggregation and transport) and biological population regulation (pest and disease control).

Through these functions, microbes also contribute to vital ecosystem services such as climate regulation and the water cycle.

Soil microbes are mainly considered ‘chemical engineers’ because they intervene in soil functions, they decompose organic matter, consume and emit trace gases (methane, nitrous oxide, carbon dioxide), regulate soil acidity (pH), nutrient cycling and availability (nitrogen, phosphorus, iron, sulphur) and regulate organic matter dynamics.

However, microbes can also act as ‘biological regulators’ by providing protection against pathogens, predated on each other and controlling pests and disease, as well as ‘ecosystem engineers’ contributing (though in a limited way) to soil structure and formation.

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1.1.5 Biogeochemical cycles

Natural systems exist in a balance; carbon and all the other elements that make up living things are subject to repeated *recycling*, so that they are available to different organisms in different forms.

Microorganisms are responsible for most of these reactions, oxidising and reducing the elements according to their metabolic needs. The continuation of life on Earth is dependent on the cycling of finite resources in this way.

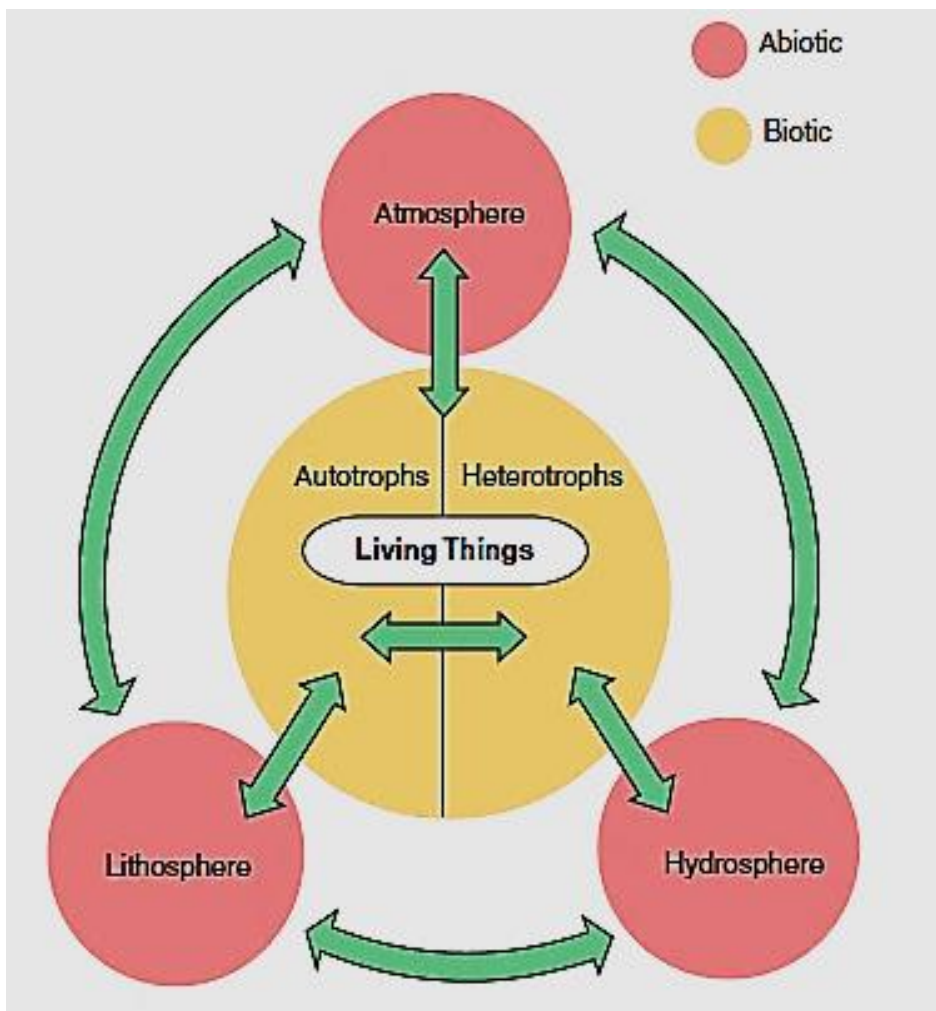


Figure 2. General pattern of biogeochemical cycles.

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1.1.5.1. The carbon cycle

Because carbon is the fundamental atom in all biomolecules and accounts for at least one-half of the dry weight of protoplasm, the carbon cycle is more intimately associated with the energy transfers and trophic patterns in the biosphere than are other elements.

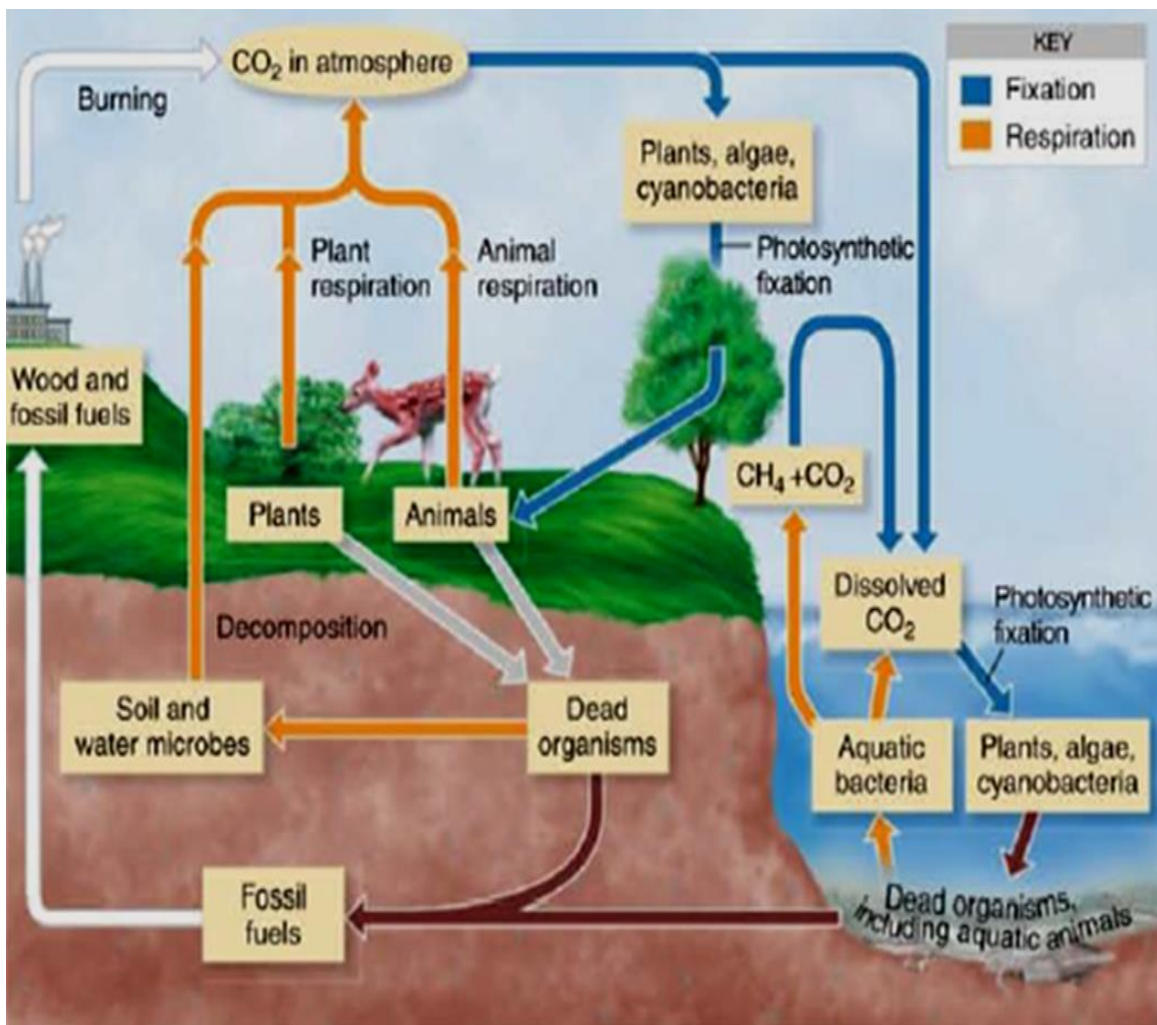


Figure 3. The Carbon cycle.

Atmospheric CO₂ is fixed into organic compounds by plants, together with phototrophic and chemoautotrophic microorganisms. The organic compounds thus synthesised undergo cellular respiration and CO₂ is returned to the atmosphere. The carbon may have been passed along a food chain to consumers before this occurs.

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Carbon dioxide is also produced by the decomposition of dead plant, animal and microbial material by heterotrophic bacteria and fungi.

Methanogenic bacteria produce methane from organic carbon or CO_2 . This in turn is oxidised by methanotrophic bacteria; carbon may be incorporated into organic material or lost as CO_2 .

1.1.5.2. The nitrogen cycle

Nitrogen is an essential element for all living organisms, as it is a fundamental component of proteins and nucleic acids. Although molecular nitrogen (N_2) constitutes approximately three-quarters of the Earth's atmosphere, only a limited number of organisms are capable of utilizing it directly for metabolic processes. These organisms, known as nitrogen-fixing bacteria, convert atmospheric nitrogen into ammonia, thereby making nitrogen biologically available.

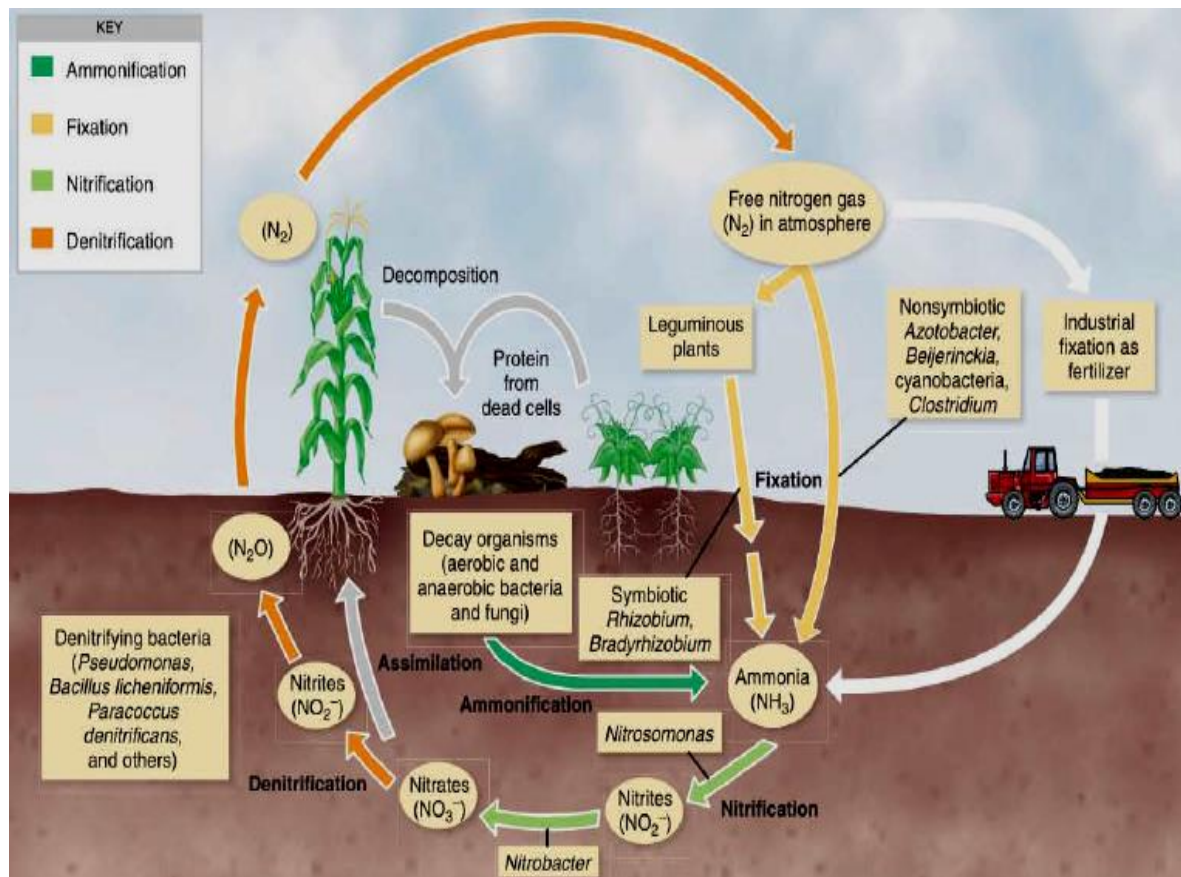


Figure 4. The Nitrogen cycle.

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The nitrogen cycle is relatively more intricate than other cycles because it involves such a diversity of specialized microbes to maintain the flow of the cycle.

The cycle includes four basic types of reactions: nitrogen fixation, ammonification, nitrification, and denitrification.

➤ **Nitrogen fixation** is brought about primarily by nitrogen-fixing bacteria in soil and water, though a small amount is formed through nonliving processes such as lightning. The nitrogen fixers have developed a unique enzyme system capable of breaking the triple bonds of the N_2 molecule and reducing the N atoms, an anaerobic process that requires the expenditure of considerable ATP. The primary product of nitrogen fixation is the ammonium ion, NH_4^+ .

Nitrogen-fixing bacteria live free or in a symbiotic relationship with plants. Among the common free living nitrogen fixers are the aerobic *Azotobacter* and *Azospirillum* and certain members of the anaerobic genus *Clostridium*. Other free-living nitrogen fixers are the cyanobacteria *Anabaena* and *Nostoc*.

➤ **Ammonification**, almost all the nitrogen in the soil exists in organic molecules, primarily in proteins. When an organism dies, the process of microbial decomposition results in the hydrolytic breakdown of proteins into amino acids. In a process called deamination, the amino groups of amino acids are removed and converted into ammonia (NH_3). This release of ammonia is called ammonification.

➤ **Nitrification**, the next sequence of reactions in the nitrogen cycle involves the oxidation of the nitrogen in the ammonium ion to produce nitrate, a process called nitrification. Living in the soil are autotrophic nitrifying bacteria, such as those of the genera *Nitrosomonas* and *Nitrobacter*.

➤ **Denitrification**, the form of nitrogen resulting from nitrification is fully oxidized and no longer contains any biologically usable energy. However, it can be used as an electron acceptor by microbes metabolizing other organic energy sources in the absence of

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atmospheric oxygen. This process, called denitrification, can lead to a loss of nitrogen to the atmosphere, especially as nitrogen gas

1.2. Biofilm

1.2.1. Introduction

Biofilms are networks of aggregated living microorganisms that are formed either on biotic or abiotic moist surfaces. They are well-known predominating micro-ecosystems which are not only distributed widely in most natural environments but can be adapted to survive in extreme environmental conditions, such as UV radiation, high salinity, poor nutrients, high temperature, high pH and antibiotics.

Because of their power of adaptation in extreme conditions, they are assumed to have developed self-protective mechanisms, creating an environment for their growth and survival, in addition to enhancing the communications among the microcolonies within the biofilms.

1.2.2. Composition

Three-dimensional structure of biofilms is established by the layer which is formed by the aggregation and composition of the secretory insoluble substances produced by the associated microorganisms.

The layer made of extracellular substances is collectively known as the extracellular polymeric substance (EPS), which has an indispensable role in the formation of biofilms. EPS also supports the transformation of biofilms from reversible to irreversible and establishes the biofilm on a suitable surface.

Thus, the slimy EPS not only helps organisms adhere to each other but also protects biofilms from any adverse synthetic reactions. Biofilms are diverse, depending on organism types and environmental factors. Most of the biofilms are aggregations of similar or different bacterial species, but they can also be composed of other organisms, such as fungi, algae, or protozoa.

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1.2.3. Formation

Formation of biofilm starts after some free moving microorganisms come into contact with a suitable surface and establish the communication roots to gather sources of nutrients and the production of a slimy material known as EPS.

Overall, biofilm formation has five steps:

- Initial reversible attachment between the bacteria and the friendly surface;

- Irreversible attachment that leads to the formation of monolayer surface of extracellular matrix (ECM) comprising proteins, cellular debris, nucleic acids and polysaccharides;

- Maturation stage I, exhibiting biofilm growth and quorum sensing (QS);

- Maturation stage II, leading to formation of EPS to encase the biofilm and helping with cell-cell and cell-matrix interactions;

- Dispersion stage, separating from parent biofilms and repeating the cycle in another place.

Bacterial attachment on the abiotic or biotic surface starts the biofilm formation. Initial reversible attachment is converted to irreversible attachment by binding of bacteria to the surface by the polymeric matrix, which is made of various exoproteins and substances released by the bacteria themselves.

For example, exopolysaccharide substances (EPSs), proteins, nucleic acids and other substances help bacterial adherence on the surfaces. In addition, certain bacterial surface proteins, such as Aap, sortase and SasG, also play vital roles in irreversible attachment.

After irreversible attachment, bacteria start division with the release of various extracellular matrix proteins (ECMs) for biofilm growth. Proteins, carbohydrates, DNA

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and EPS are some major components of ECMs that support bacterial growth during biofilm extension.

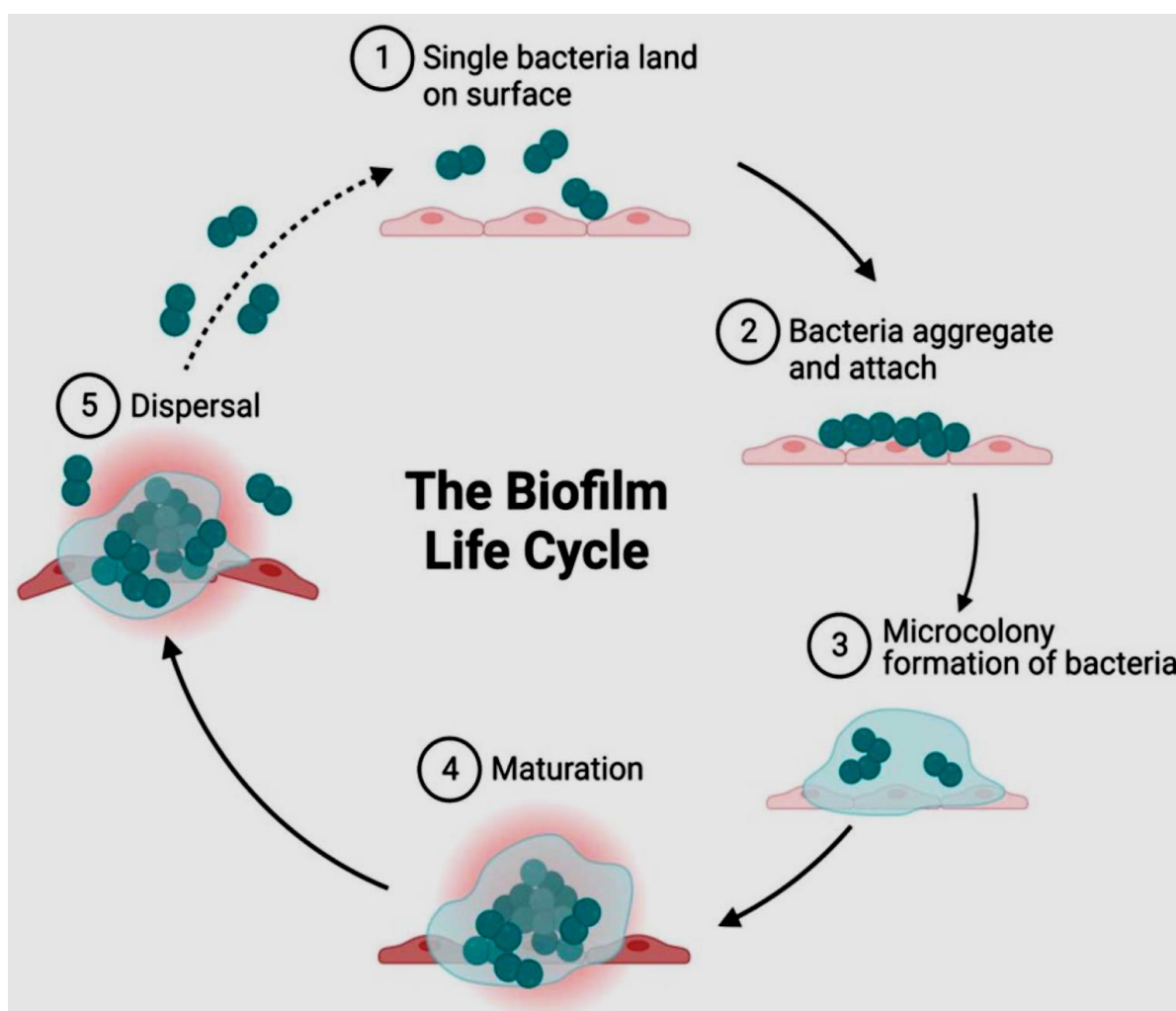


Figure 5. Diagrammatic illustration showing the growth cycle of a biofilm by a single bacterium species on a solid surface.

1.2.4. Importance of biofilms

The sustainability of the agricultural sector is critical for meeting the demands of a growing global population. Biofilm-based fertilizers derived from agriculturally important microbes (AIMs) hold significant potential to transform sustainable agriculture. AIMs, including plant growth-promoting rhizobacteria (PGPR), enhance plant development and productivity, while also contributing to bioremediation and serving as biocontrol agents.

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Moreover, the application of multispecies biofilms particularly those comprising both bacteria and fungi offer additional benefits, such as the production of unique polysaccharides and the improvement of soil ecology. These biofilms can interact with plants in mutualistic, commensal, or pathogenic ways, thereby supporting soil health and promoting plant growth.

Furthermore, biofilms hold considerable promise for the bioremediation of organic pollutants, providing environmentally friendly and cost-effective solutions. Microbial genera such as *Pseudomonas*, *Arthrobacter*, *Alcanivorax*, *Bacillus*, and *Rhodococcus* are capable of efficiently degrading hydrocarbons in marine environments. Biofilm-based wastewater treatment plays a critical role in addressing the growing demand for clean water, particularly in regions with high wastewater production. The removal of nitrogen and phosphate from wastewater is essential to prevent environmental problems such as algal blooms and eutrophication. Bacterial biofilms contribute significantly to these nutrient removal processes, and bioaugmentation with targeted microbial strains can further enhance nitrogen and phosphate elimination.

Moreover, biofilms are employed in biogas production via anaerobic digestion, providing a renewable and sustainable energy source. Advanced biofilm-based wastewater treatment systems offer multiple advantages, including the simultaneous removal of diverse pollutants, cost-effectiveness, and improved energy efficiency. Algal biofilms can also develop on wastewater, facilitating the removal of heavy metals and other contaminants. The harvested algae can subsequently be used for biofuel production, with potential applications in supporting crop irrigation during periods of drought.

1.3. Unculturable bacteria

1.3.1. Introduction

With advances in microbial ecology, we now recognize 61 distinct bacterial phyla, of which 31 have no cultivable representatives. Similarly, within the archaeal domain, only 54 species have been successfully cultured, representing a small fraction of the vast hidden diversity, while 49 lineages largely remain uncultivated. This limitation in cultivation leads to an underestimation of the complexity of microbial communities and raises the possibility of

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overlooking key organisms that are essential for ecosystem functioning or that may act as pathogens of plants and animals.

Despite the abundance of bacterial species in soil, more than 99% of these species cannot be cultured by traditional techniques. In addition, the less than 1% of bacteria that can be cultured are not representative of the total phylogenetic diversity. Limited microbial availability by cultivation has created an emerging need to learn more about the missing species and their functions.

1.3.2. Definition

Uncultivable bacterium, viable but nonculturable bacteria (VBNC) indicates that current laboratory culturing techniques are unable to grow a given bacterium in the laboratory.

Unculturable does not mean can never be cultured but, rather, signifies that we lack critical information on their biology.

1.3.3. VBNC state in bacteria

Like other living organisms, microorganisms are able to interact with changing environmental conditions by triggering specific stress responses and survival mechanisms if the environmental parameters become suboptimal. Bacteria can survive in extreme environments by forming spores or entering a non-sporulating dormant state.

A VBNC state is an adaptive strategy that serves long-term bacterial subsistence under adverse conditions.

Although VBNC cells fail to grow or form colonies on laboratory media, they are very different from dead cells. VBNC cells preserve their cellular integrity, their membranes are not injured and they retain genomic or plasmid DNA. In contrast to dead cells, VBNC cells exhibit metabolic and respiratory activities, have high ATP levels, and may perform transcription and gene expression.

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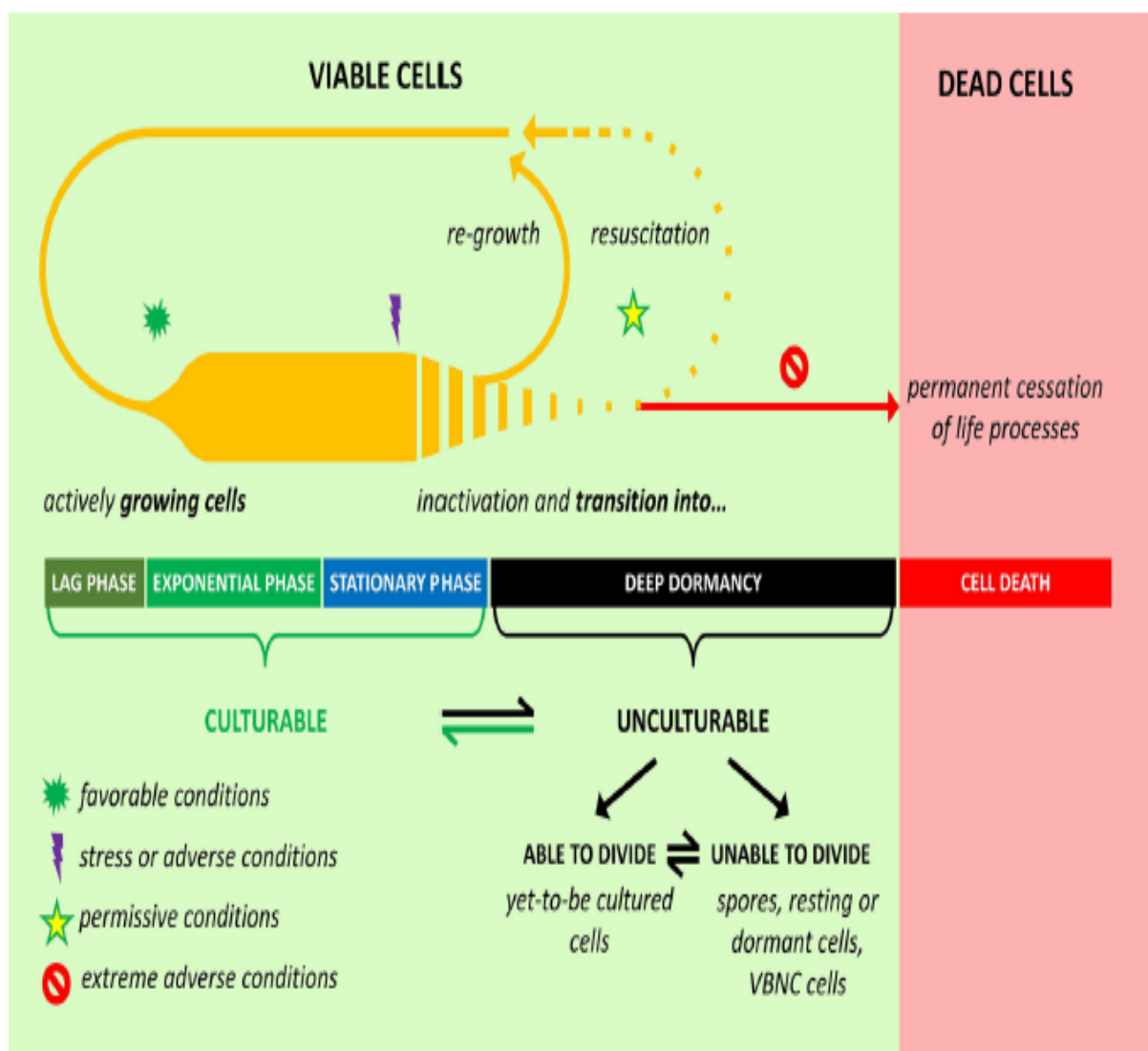


Figure 6. Schematic diagram of the transition of bacterial cells between different physiological states.

1.3.4. Reasons for unculturability

The existence of unculturable bacteria has long posed a major challenge in microbiology, with the first indications arising from microscopy. Observations revealed that the vast number of bacterial cells detected under the microscope far exceeded the number of colonies that developed on Petri dishes, a discrepancy now known as "The Great Plate Count Anomaly." This phenomenon exhibited variation across different environments and initially led to the assumption that non-growing cells were non-viable.

1. Interactions between Microorganisms and physical environment

Subsequent studies, however, demonstrated that many of these cells remain metabolically active despite their inability to proliferate on standard laboratory media.

The factors underlying the unculturability of certain bacterial species are multifaceted. Some taxa may have been overlooked due to low abundance or slow growth rates, which hinder their isolation using conventional culture techniques. Additionally, genetically distinct but phenotypically similar phylotypes can be misclassified when relying on traditional biochemical identification methods.

Conversely, there are intrinsic barriers that prevent the cultivation of certain bacteria on artificial media. Some species exhibit highly specific growth requirements, including particular nutrients, pH levels, incubation temperatures, or oxygen conditions. Indeed, different cultivation strategies often recover distinct subsets of bacteria from comparable samples, highlighting the critical influence of culture conditions on the successful isolation of specific taxa.

The difficulty of cultivating these bacteria in the laboratory arises from the complexity of accurately reproducing their natural environments. Despite considerable effort and methodological ingenuity, identifying the precise environmental parameters that must be replicated such as nutrient composition, pH, osmotic conditions, temperature, and other factors remains a formidable challenge. Attempts to manipulate all of these variables simultaneously generate a highly complex, multidimensional matrix of conditions that cannot be exhaustively tested within practical time constraints.

Traditionally, microbiologists have sought to design synthetic media that approximate the presumed environmental conditions of target organisms. This approach has enabled the cultivation of thousands of bacterial species now classified as culturable. More recently, however, advances in the field have led to the development of two interrelated strategies that have markedly enhanced our capacity to culture a wider diversity of bacteria.

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1.3.5. Detection of uncultivable bacteria

Despite the fact that they are alive, VBNC cells are no longer able to grow either on liquid or semi-solid standard bacteriological media; thus, a lack of colony formation by certainly living cells can be confirmed by using simple plating methods.

Kogure's procedure, which applied acridin orange staining to elongated living cells after exposure to a cell division inhibitor, nalidixic acid, allow the detection of normal-shaped VBNC or dormant cells under a microscope. Once unculturability is confirmed, a wide range of fluorescent stains is available for fluorescent microscopy, which are mostly based on detecting the membrane integrity or enzymatic activity of tested cells.

Molecular methods that exclusively target DNA, such as normal PCR or qPCR, are not capable of differentiating viable cells from dead ones. Combining qPCR with the microbial dye propidium monoazide (PMA), which penetrates only damaged membranes and inhibits PCR amplification by binding to the DNA of dead cells or extracellular DNA, enables the detection of nucleic acids exclusively in living cells. Although there are no specific methods available for the direct detection of VBNC bacteria, a combination of certain techniques may reveal the viability of VBNC bacteria (Table 1).

Table 1. Methods used for detection of VBNC bacteria.

	Purpose of reaction	Mechanism
Fluorescence microscop methods		
Acridine orange staining after the addition of nalidixic acid	Distinguishing actively growing cells from VBNC or dormant cells	Actively growing cells become elongated and fluoresce reddish orange, while VBNC or dormant cells remain normal shaped and have greenish white Fluorescence

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Counterstaining with 5-cyano-2,3-ditolyltetrazolium chloride (CTC) and 40,6-diamidino-2-phenylindole (DAPI)	Differential staining of VBNC and dead cells, once unculturability is confirmed	Viable cells have red fluorescence, while dead cells fluoresce blue
Combination of SYTO_9 and propidium iodide (PI) e.g. LIVE/ DEAD BacLight kit	Differential staining of VBNC and dead cells, once unculturability is confirmed	Viable cells have green fluorescence, while dead cells fluoresce red

Molecular methods

qPCR combined with propidium monoazide (PMA) treatment	Detection of nucleic acids exclusively from living cells, once unculturability is confirmed	PMA penetrates only damaged membranes and inhibits PCR amplification by binding to DNA of dead cells or extracellular DNA.
Monitoring stress-related genes with RT-PCR	Detection of viability, once unculturability is confirmed	Bacterial mRNA has short life-time and is mainly detectable in metabolically active cells

Molecular biology has enabled the detection and sorting of specific target bacteria for selective enrichment or physical isolation. Oligonucleotide probes and advanced techniques like FISH, CARD-FISH, colony hybridization, flow cytometry, and cell sorting have paved the way for isolating and studying previously elusive bacterial strains.

1. Interactions between Microorganisms and physical environment

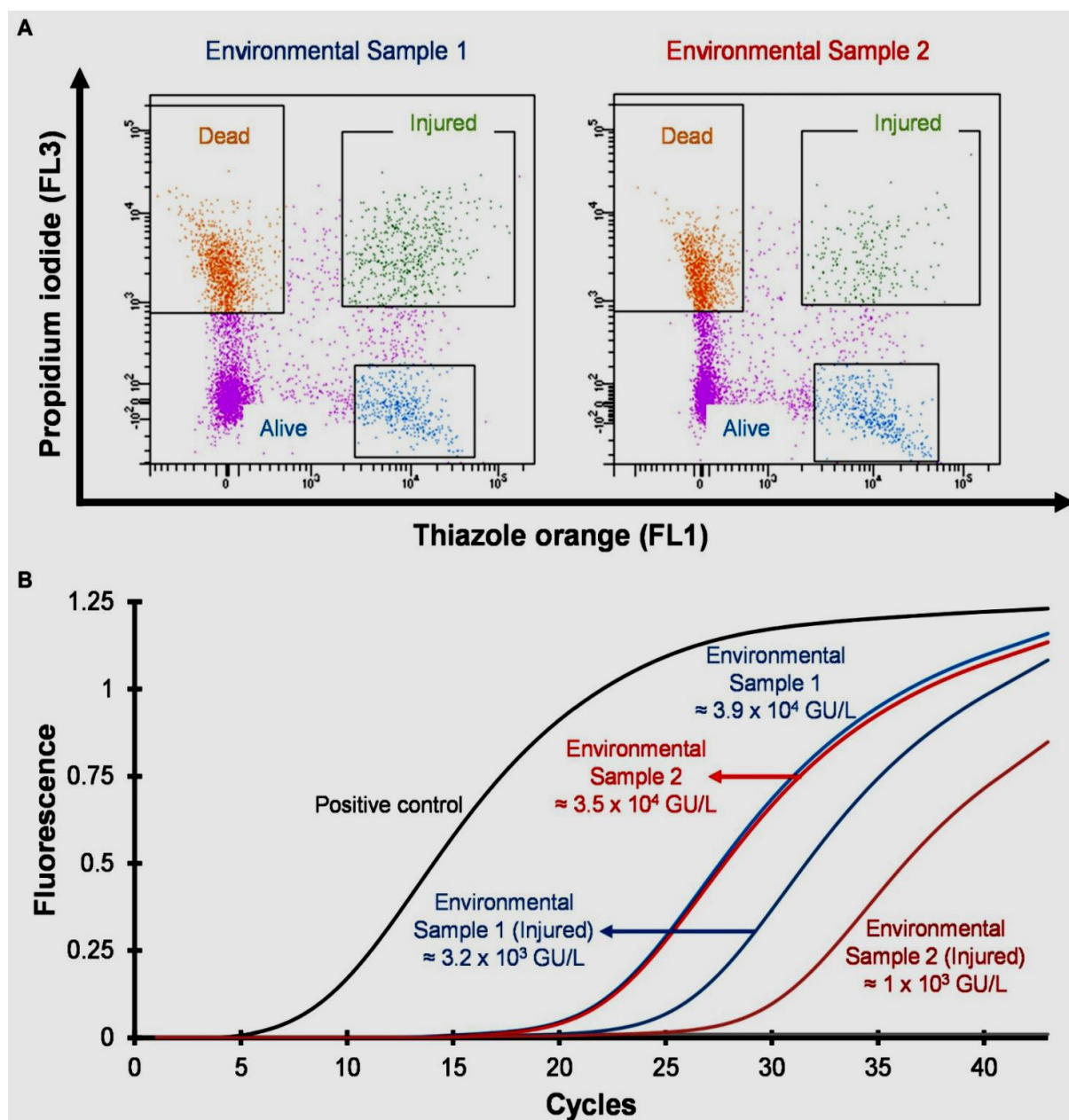


Figure 7. Detection and quantification of VBNC *Legionella pneumophila* in potable water samples.

(A) Cytograms of potable water samples that were culture-negative. (B) Quantification by qPCR of the total water sample and the VBNC *Legionella*, with genome units per liter (GU/L) estimated following viability-based cell sorting using flow cytometry as shown in (A). This figure clearly illustrates the difference in *Legionella* concentration, highlighting the presence of dead cells or free *Legionella* DNA in the water samples.

2. Interactions between microorganisms

2.1. Microbial interactions types

2.1.1. Introduction

Microbial interactions are crucial for successfully establishing and maintaining a microbial population in various environments and on various hosts.

Microorganisms rarely occur as single species populations and are encountered in many hosts environments, thus there is a large variety of types of microbial interactions concerning the organisms involved: Bacteria-bacteria, fungus-fungus, bacteria-fungus, fungus-plant/animal, bacteria-plant/animal and bacteria-fungus-plant/animal interactions.

2.1.2. Beneficial Interactions

2.1.2.1. Mutualism

Mutualism defines the relationship in which some reciprocal benefit accrues to both partners. This is an obligatory relationship in which the mutualist and the host are dependent on each other. When separated, in many cases, the individual organisms will not survive.

2.1.2.2. Commensalism

Commensalism is a relationship in which one symbiont, the commensal, benefits while the other (sometimes called the host) is neither harmed nor helped. One good example is nitrification, the oxidation of ammonium ion to nitrite by microorganisms such as *Nitrosomonas*, and the subsequent oxidation of the nitrite to nitrate by *Nitrobacter* and similar bacteria. *Nitrobacter* benefits from its association with *Nitrosomonas* because it uses nitrite to obtain energy for growth.

2.1.2.3. Cooperation

Cooperation is a mutually beneficial, it benefits both organisms. A cooperative relationship is not obligatory and, for most microbial ecologists, this nonobligatory aspect differentiates cooperation from mutualism.

2. Interactions between microorganisms

Two examples of a cooperative relationship include the association between *Desulfovibrio* and *Chromatium* in which the carbon and sulfur cycles are linked, and the interaction of a nitrogen-fixing microorganism with a cellulolytic organism such as *Cellulomonas*.

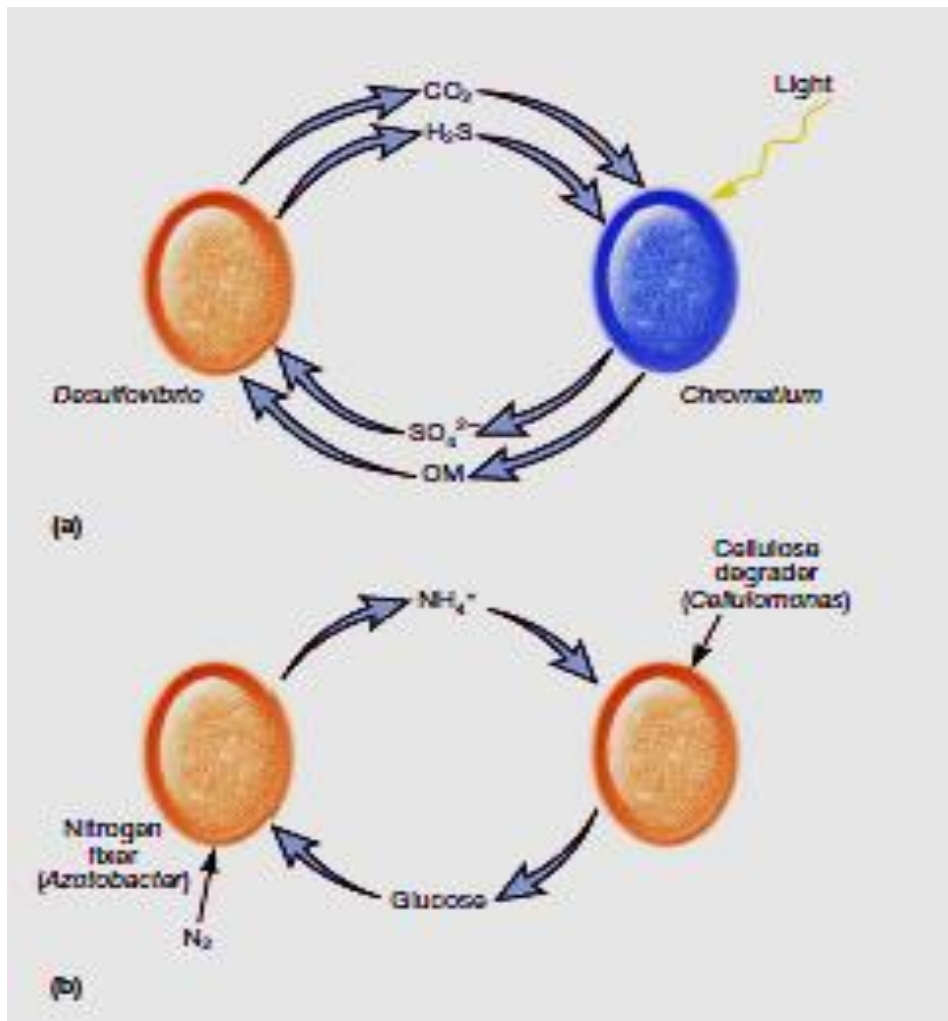


Figure 8. Examples of Cooperative Symbiotic Processes.

2.1.3. Conflictual Interactions

1.3.1. Predation

As is the case with larger organisms, predation among microbes involves a predator species that attacks and usually kills its prey. Over the last several decades, microbiologists have discovered a number of fascinating bacteria that survive by their ability to prey upon other microbes. Several of the best examples are *Bdellovibrio*, *Vampirococcus*, and *Daptobacter*.

2. Interactions between microorganisms

Bdellovibrio is an active hunter that is vigorously motile, swimming about looking for susceptible gram-negative bacterial prey. Upon sensing such a cell, *Bdellovibrio* swims faster until it collides with the prey cell.

2.1.3.2. Parasitism

Parasitism is one of the most complex microbial interactions; the line between parasitism and predation is difficult to. This is a relationship between two organisms in which one benefits from the other, and the host is usually harmed.

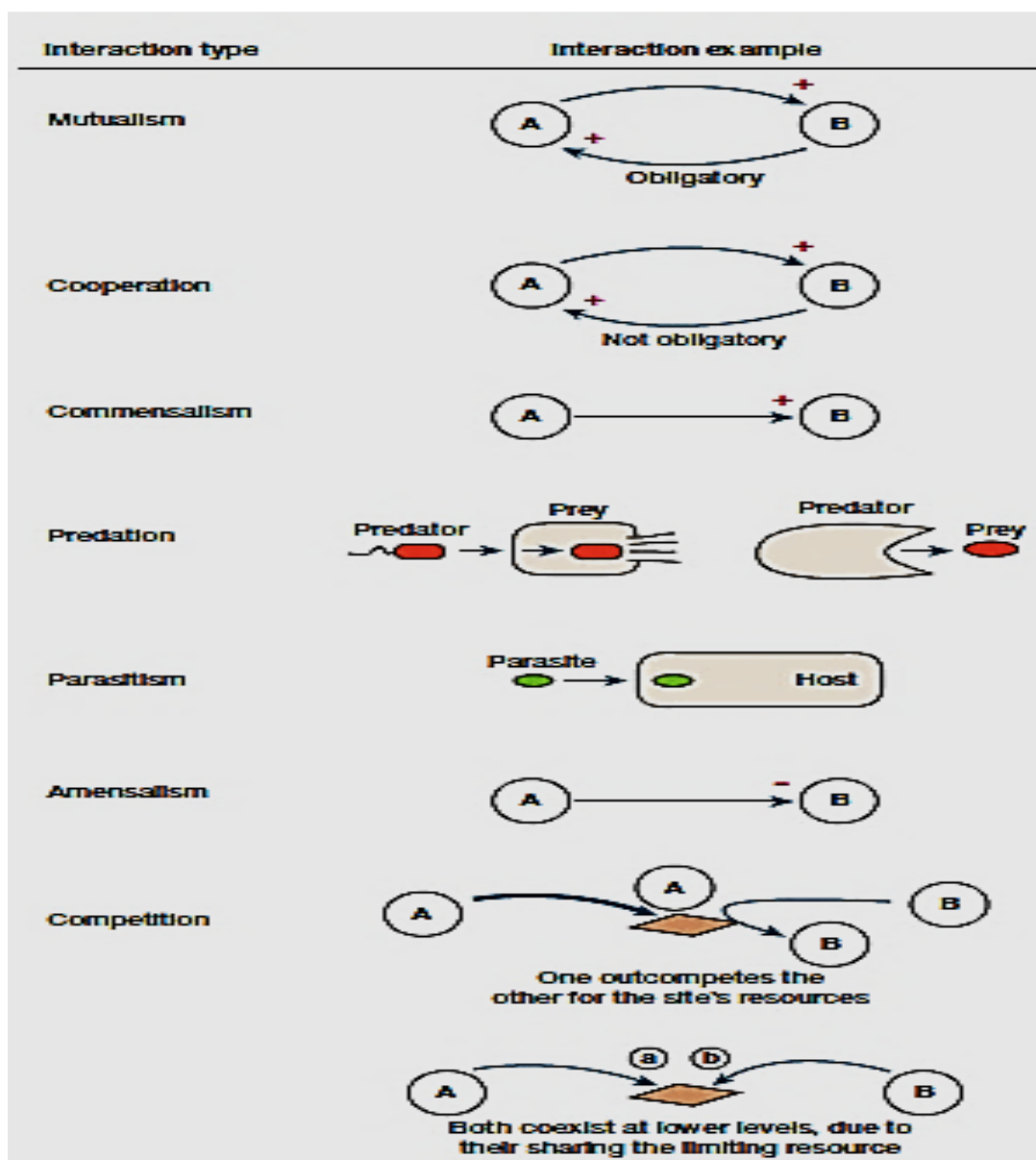


Figure 9. Microbial interactions.

2. Interactions between microorganisms

2.2. Quorum Sensing

Many bacteria use a cell-cell communication system called Quorum Sensing (QS) to coordinate population density-dependent behaviors. This process involves the production and detection of low molecular weight molecules, so-called autoinducers (AI), which either diffuse over the cytoplasmic membrane or are actively transported and detected explicitly by a specific receptor.

The first QS system described was in the 1980s in *Vibrio fischeri* (formerly known as *Photobacterium fischeri*) bacterium. In the sea, it is in a low population density and does not luminesce. Therefore, when it is in a symbiotic association with fishes and squids it luminesces. After the autoinducer molecule reaches a threshold luminescence genes are activated. This light matches the moonlight, making the squid invisible to the predators below. QS systems were found in both Gram-negative and Gram-positive bacteria.

Gram-negative bacteria communicate via acylated homoserine lactones (AHLs), in *V. fischeri* this system is called LuxR/LuxI (as illustrated in Figure 10). The signaling molecule or autoinducer (AHL) ligates to the transcriptional regulator LuxR, this ligation is very specific, used for interspecies communication.

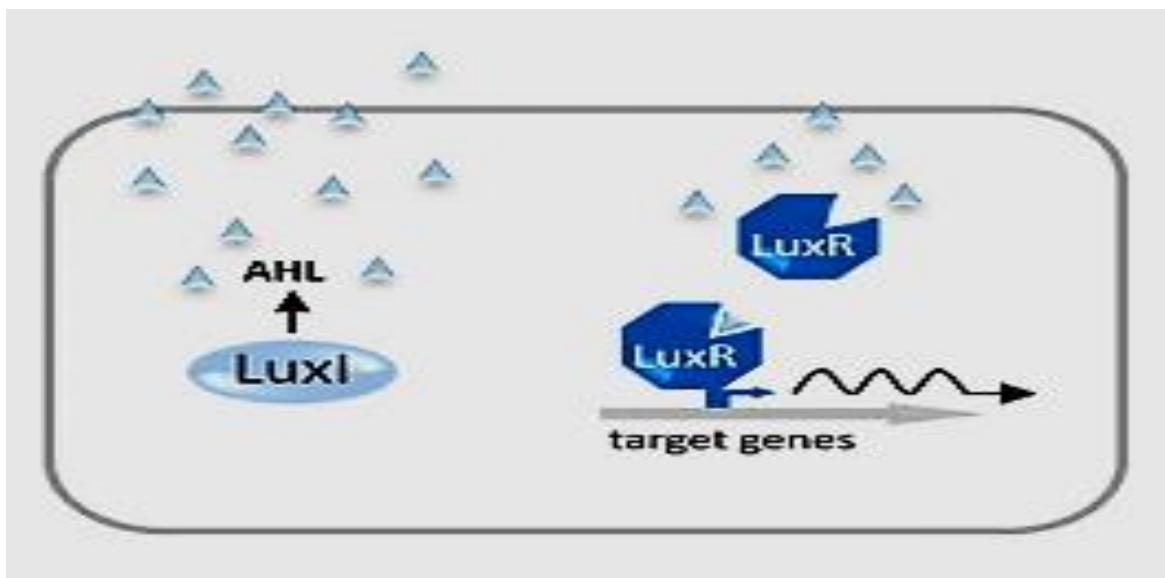


Figure 10. Quorum sensing system-Gram-negative bacteria.

2. Interactions between microorganisms

Gram-positive bacteria communicate using modified oligopeptides and two-component regulatory systems (as illustrated in Figure 11). The signaling molecules are either unmodified or posttranslationally modified small peptides secreted via ABC exporter proteins. Phosphorylation of the receptor kinase due to peptide binding activates the regulatory protein, which acts as a QS target gene transcription factor.

Virulence factor production in *Bacillus cereus* and *Staphylococcus aureus*, the competence of *B. subtilis*, and the biofilm formation of *Streptococcus pneumoniae* are examples of QS-dependent gene regulation in Gram-positive bacteria.

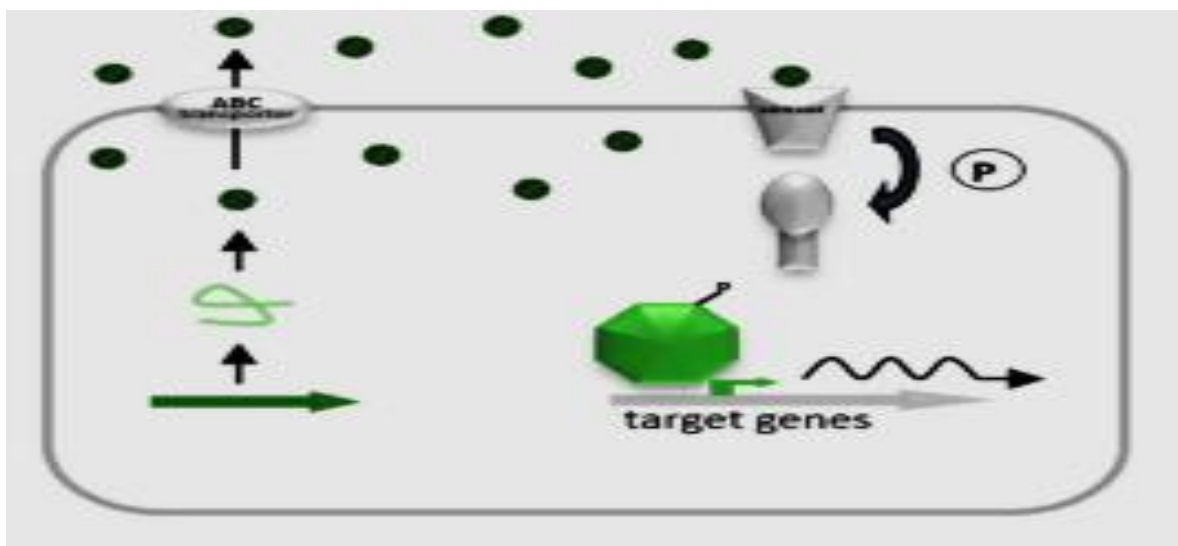


Figure 11. Quorum sensing (QS) system-Gram-positive bacteria.

The emergence of genomic profiling has demonstrated that quorum sensing in numerous bacterial species governs global regulatory networks and broadly modulates gene expression. In *Streptococcus pneumoniae*, transcriptome-wide analyses have identified more than 150 competence-regulated genes, which are classified into early, late, delayed-induction, and repressed categories.

Early competence genes primarily mediate the production, export, and sensing of the quorum-sensing signal, whereas several late genes are involved in DNA uptake and internalization. Delayed-response genes contribute to various bacterial stress-adaptation processes.

2. Interactions between microorganisms

Gene-disruption studies have revealed that only 23 of the 124 quorum-sensing regulated genes are essential for competence development. Mutants of *S. pneumoniae* and related streptococcal species with defective quorum-sensing systems display impaired functionality across multiple pathways, including biofilm formation, acid tolerance, bacteriocin synthesis, and virulence.

In *Pseudomonas aeruginosa*, transcriptomic analyses have identified 616 genes within the quorum-sensing regulon, of which 222 are repressed upon the addition of autoinducers (Figure 12).

Although these studies were performed under different experimental conditions, they collectively underscore the central role of quorum sensing in global gene regulation.

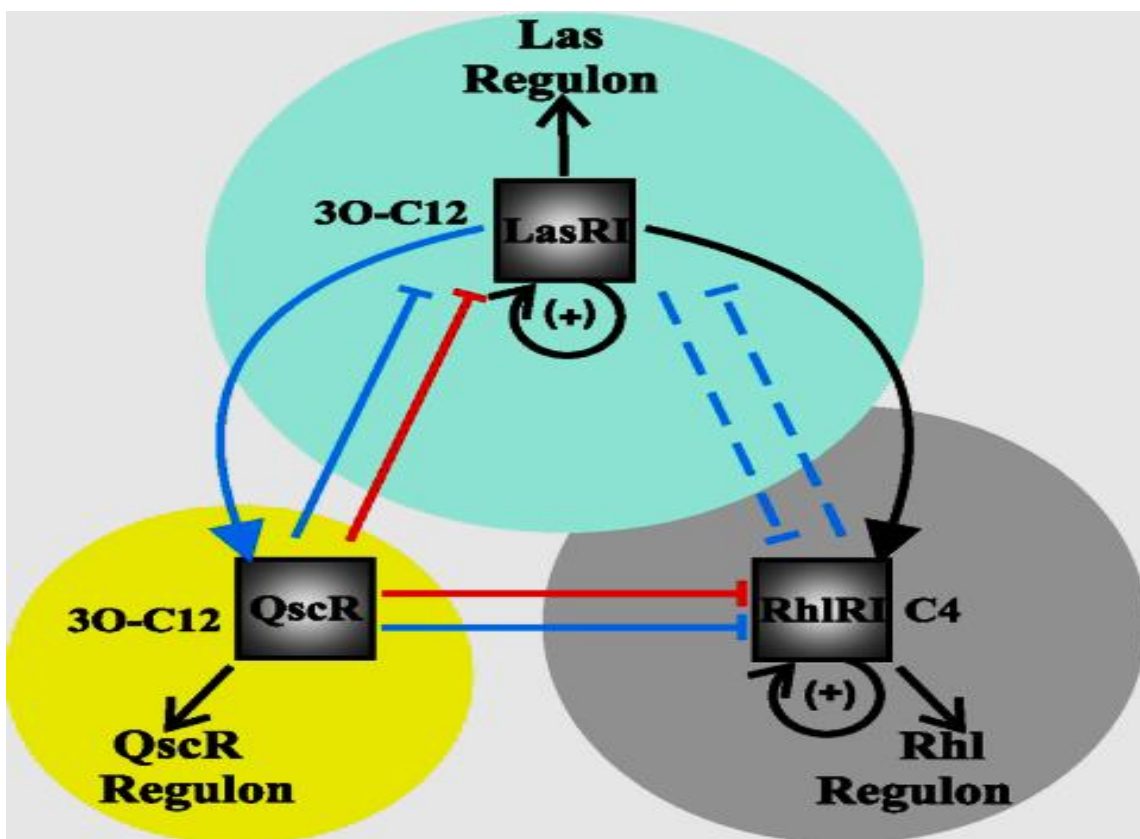


Figure 12: Model of the *P. aeruginosa* quorum-sensing network.

Black arrows represent direct transcriptional regulation either activation or repression, as indicated. **Blue** arrows denote interactions between regulatory proteins and their corresponding AHL signal molecules, **Red** arrows depict protein-protein interactions. **Solid** arrows correspond to well-established regulatory mechanisms, while **dashed** arrows mark interactions that remain more speculative. **Looped** arrows indicate positive feedback on cognate AHL synthesis mediated by LasR and RhIR. The underlying circles illustrate the sets of genes directly regulated by each LuxR-type transcription factor.

2. Interactions between microorganisms

Instead, bacteria appear capable of undergoing developmental processes reminiscent of those observed in eukaryotic systems. Second, quorum sensing exerts extensive repressive effects on large sets of genes, suggesting that its primary function may be to activate behaviors conducive to coordinated group living while shutting down processes that are more advantageous for solitary bacteria outside a community structure.

2.3. Quorum quenching

Quorum sensing plays a pivotal role in the global regulation of bacterial physiology, particularly at interfaces where distinct bacterial populations interact or at the host-microbe boundary. Disruption of quorum sensing pathways can confer a competitive advantage in environments where microorganisms vie for limited resources. Likewise, the ability of a host to interfere with bacterial cell-cell communication can hinder colonization by pathogenic species that depend on quorum sensing to coordinate virulence (Figure 11).

Consequently, diverse biological mechanisms have evolved to counteract or inhibit bacterial signaling processes, collectively referred to as quorum quenching.

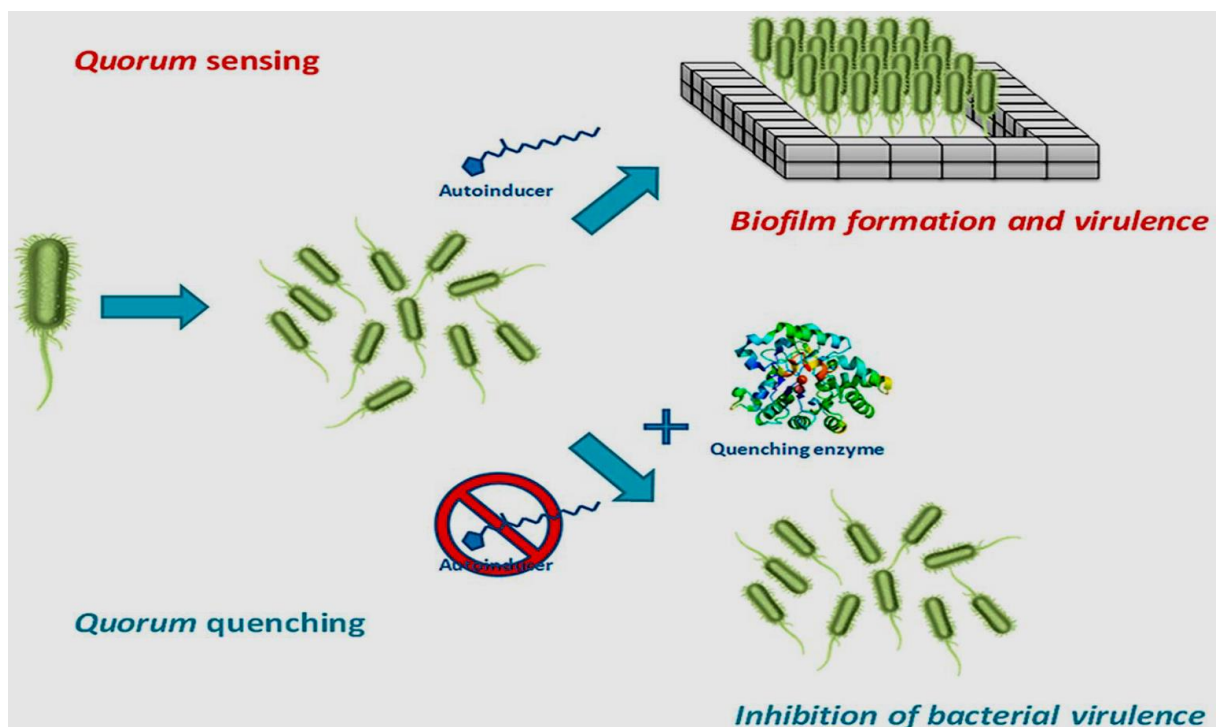


Figure 13: Inhibition of bacterial virulence by Quorum quenching.

2. Interactions between microorganisms

2.4. Interactions and dynamics of microbial populations

2.4.1. Introduction

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.

The growth of microbial populations in nature is dynamic, as the cellular physiology and environment of these populations change. Population dynamics have wide-ranging consequences for ecology and evolution, determining how species interact and which mutations fix.

Understanding these dynamics is also critical for clinical and environmental applications in which we need to promote or inhibit microbial growth.

The focus of microbiology has shifted in the last decade from the study of tractable but simplified laboratory environments to the properties of microbes in their natural environments. Evidence suggests that microbial populations in these environments are highly dynamic: individual taxa can grow 20-fold over the course of a week in the surface ocean or fluctuate fourfold each day in the human gut microbiome.

When interactions are conflictual, they usually give rise to an arms race. Arms races represent one of the fundamental drivers of evolution, because selective pressures that organisms exert on each other remain the sine qua non of evolutionary change.

In a 1973 paper, the American evolutionist Leigh Van Valen gave for this driver of evolution an explanation known as the Red Queen hypothesis, which has become a classic.

2. Interactions between microorganisms

2.4.2. Biodiversity at multiple trophic levels

Global environmental change is driving species loss across multiple trophic groups, with potentially profound impacts on the ecosystem services essential for human well-being. Anticipating the functional consequences of biodiversity decline across these groups remains difficult, as their ecological effects may be either complementary or antagonistic. For example, plant and microbial diversity often act synergistically to enhance nutrient cycling, whereas plant and herbivore diversity exert contrasting influences on biomass stocks.

Evidence indicates that shifts in multitrophic richness and abundance are stronger predictors of ecosystem service outcomes than changes within any single trophic group.

Multitrophic richness showed consistently positive relationships with provisioning, regulating, and cultural services, exceeding the influence of plant richness alone. High levels of species richness across multiple trophic groups were found to be crucial for sustaining overall ecosystem functioning, particularly with regard to regulating and cultural services.

Multitrophic abundance also contributed to ecosystem functioning, although its effects were generally weaker than those exerted by individual trophic groups. Interactions among trophic groups could modulate these effects, with the presence of some groups diminishing the influence of others. For example, high predator abundance partially counteracted the positive contributions of large herbivores to supporting ecosystem services.

Overall, findings highlight the pivotal role of species richness in sustaining ecosystem functioning and underscore the need to account for total biomass abundance, particularly in relation to supporting and provisioning services.

The results demonstrate that preserving high levels of species richness across multiple trophic groups is essential for maintaining a broad suite of ecosystem services. Moreover, the consistency of these patterns across different multifunctionality scenarios suggests limited functional redundancy among trophic groups.

2. Interactions between microorganisms

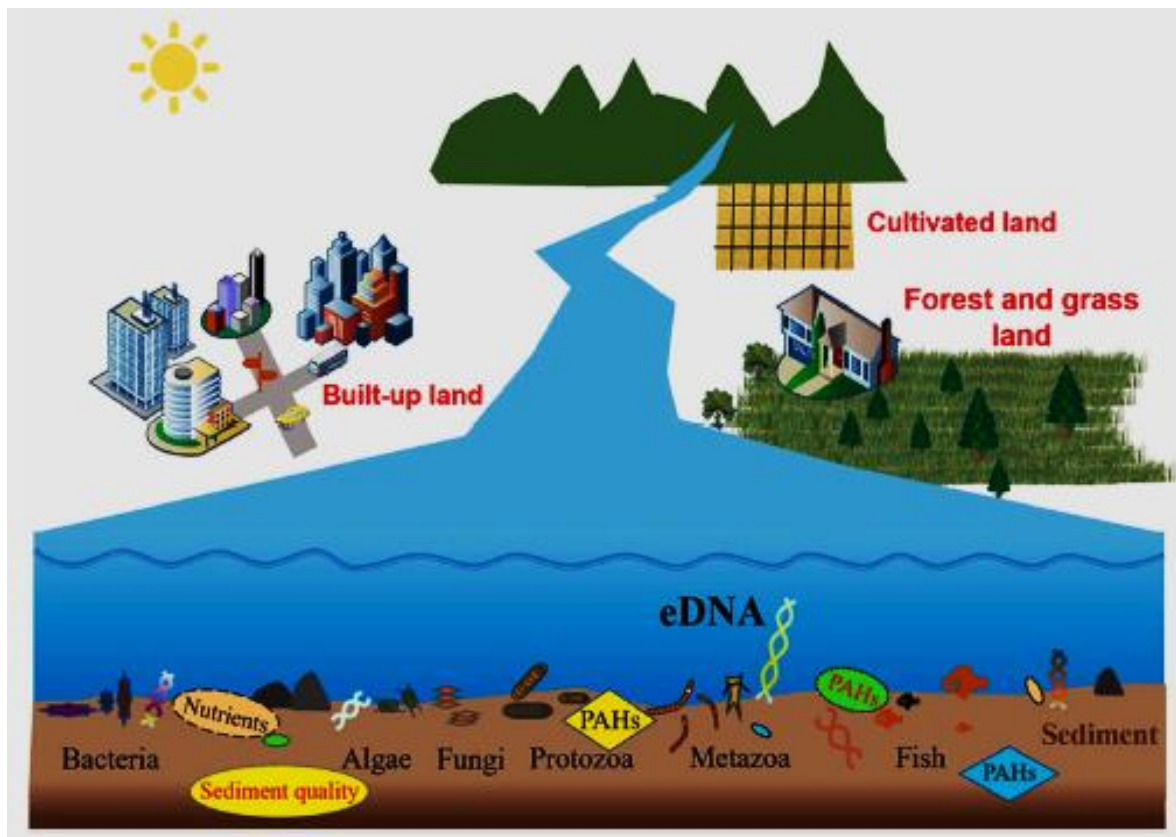


Figure 14. Biodiversity dynamics.

Multitrophic communities were characterized using DNA metabarcoding within a relatively stable riverine sediment compartment in order to investigate biodiversity dynamics.

2.5. Microbial succession

2.5.1. Definition

Research on succession, defined here as the somewhat orderly and predictable manner by which communities change over time following the colonization of a new environment.

Microbial primary succession occurs in a range of natural, host-associated, and engineered environments, which yield unique temporal and spatial contexts for the study of pioneer species.

2. Interactions between microorganisms

2.5.2. Patterns in microbial succession

Defining the appropriate timeframe for examining succession can be difficult given that changes in community composition occur over a wide range of time scales and disturbances can alter or interrupt the successional sequence. Like plants, a variety of biotic and abiotic stressors as well as the frequency of secondary disturbance events will affect the speed with which microbial communities develop.

Moreover, it is uncertain whether microbial ‘climax’ communities actually exist, rendering any endpoint of succession somewhat arbitrary. Therefore, microbial succession can be assessed over very different time scales in different systems.

For example, newly colonized soil environments may undergo successional changes across timeframes of years to decades. In contrast, some systems undergo such rapid rates of change and secondary disturbance frequencies that it is more appropriate to examine shifts in the community on the scale of days.

Table 2. Some of the more important sources of environmental (non-resource) stress that can shape microbial diversity during succession.

Environmental stress	Example of microbial habitat where the stress may be particularly important
Water availability (Ψ) and rapid changes in Ψ	Leaf surface
Shear stress	Water pipes
UV radiation	Exposed rocks
Antibiotics/antimicrobials	Human gut
Temperature (including freezing/thawing)	Surface soils
Salinity	Estuarine sediments
Redox status (particularly O_2)	Sewage bioreactors

2. Interactions between microorganisms

Metal toxicity

Wastewater treatment systems

pH

Fermenting foods

Examining selected studies of microbial succession, we find that biomass accumulation is (not surprisingly) often highest in the earlier stages of succession, tapering off as succession progresses and resources become more limiting. In contrast, the apparent changes in community-level diversity, the accumulation of new taxa over time, are far more variable and there is no single pattern shared by all of the microbial communities.

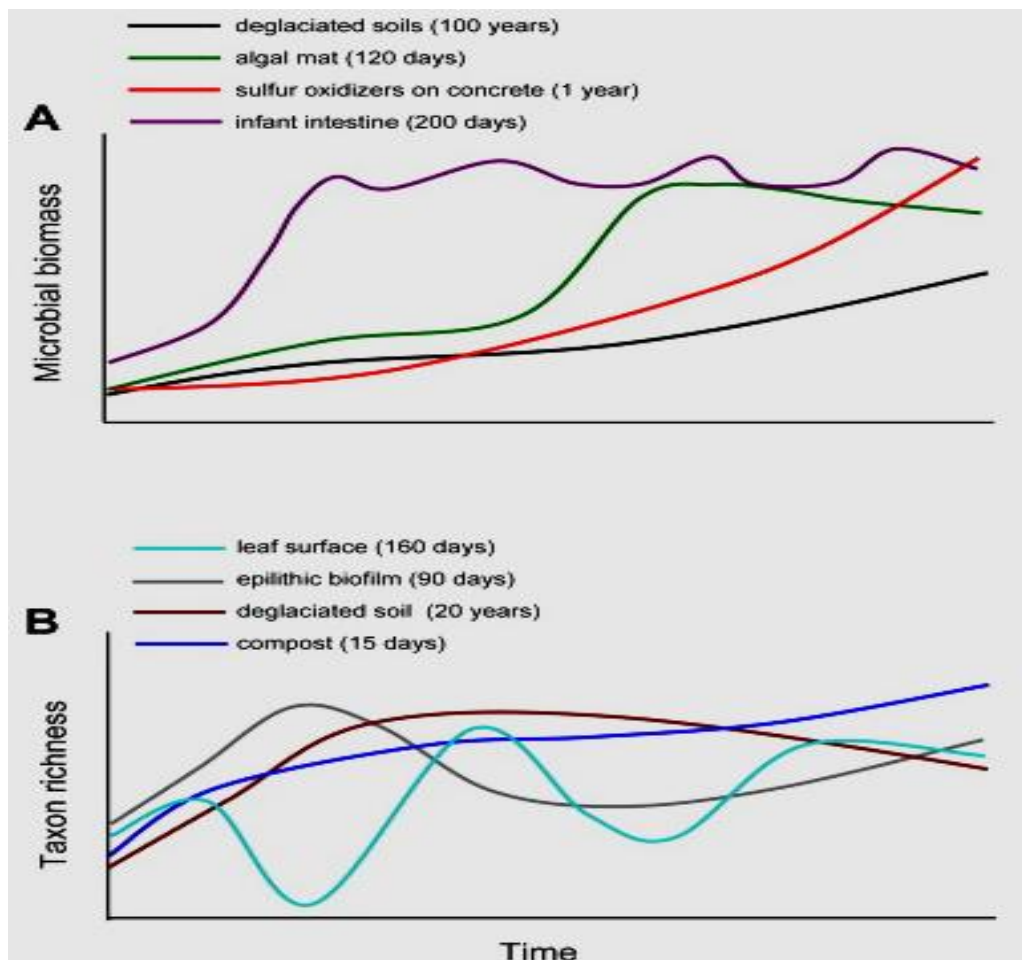


Figure 15. Contrasting successional patterns in some selected microbial environments. Examination of the amount of microbial biomass in a given habitat and changes in taxon richness over time.

2. Interactions between microorganisms

2.5.3. Decomposition of organic matter

Decomposition of organic matter (OM) is considered the most prominent biogeochemical process and directly affects the resilience of ecosystems to climate change. Four key processes are included in the OM decomposition: photo-oxidation, leaching, comminution, and mineralization.

Once decomposition begins, C is lost as CO_2 or sequestered into more recalcitrant carbon difficult to further degradation. As microbial respiration releases carbon dioxide into the atmosphere, microbes act as gatekeepers in the whole process. Microbial activities were found to be the second largest CO_2 emission source in the environment after human activities.

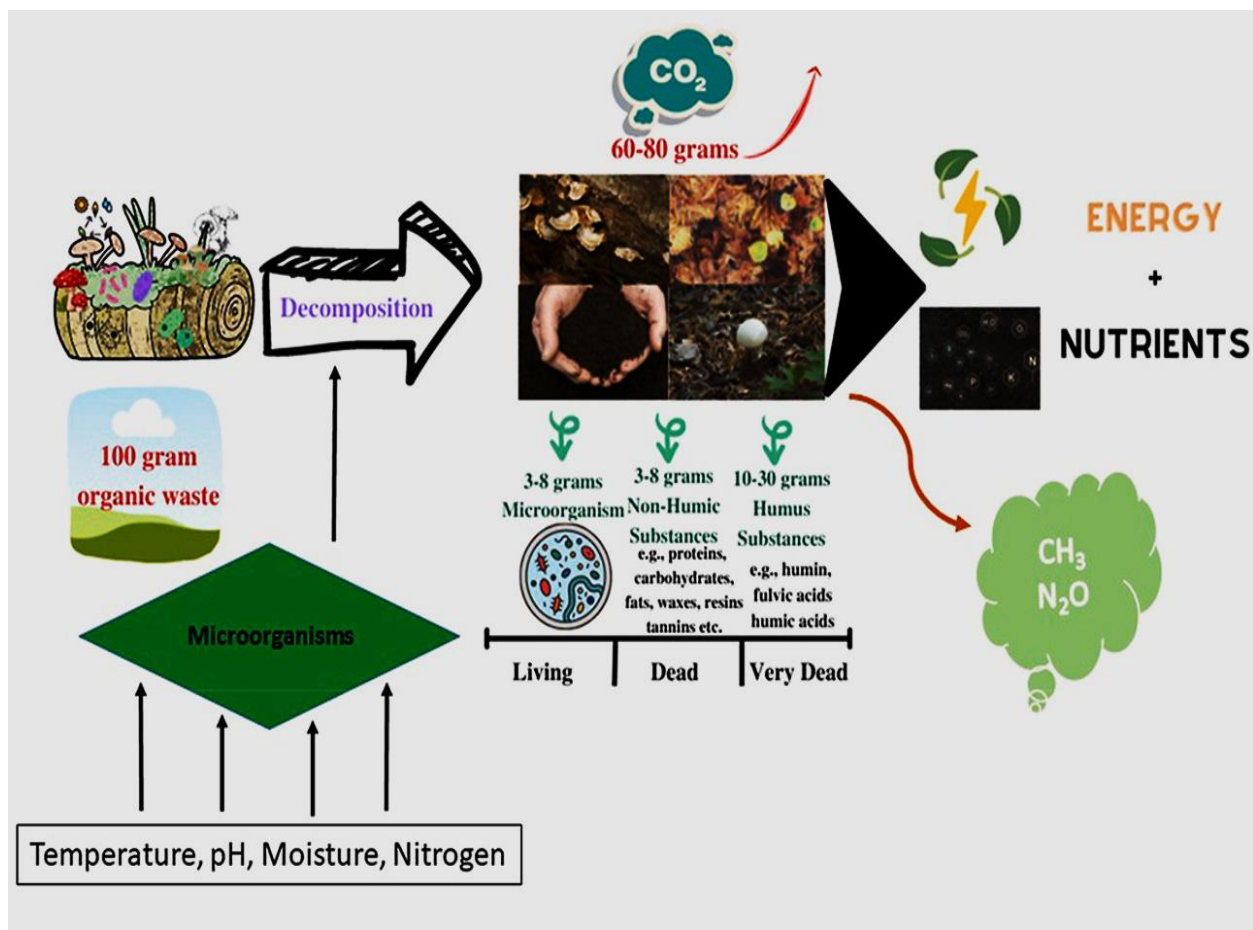


Figure 16. Role of microorganisms in decomposition of organic matter and byproducts.

2. Interactions between microorganisms

Microbial successions involve the sequential dominance of microbial groups with contrasting ecological strategies, including fast-growing r-strategists adapted to nutrient-rich conditions (copiotrophs) and slow-growing K-strategists adapted to nutrient-poor environments (oligotrophs).

Organic matter decomposition relies on the interactions between two functional microbial groups, r-strategists and K-strategists, whose complementary ecological strategies drive the progression of the decomposition process.

A bacterium is defined as oligotrophic if it meets two criteria: (i) the ability to grow in a medium containing low concentrations of organic matter, on the order of 1 to 15 mg C L⁻¹, and (ii) the ability to subsequently grow in a carbon-enriched medium. K-strategist microorganisms are thought to be primarily involved in the degradation of native soil organic matter. Among oligotrophic microorganisms, bacteria belonging to the phylum *Acidobacteria* are frequently observed.

Copiotrophs are microorganisms that utilize labile organic matter and are unable to grow under nutrient-limited conditions. They are therefore expected to dominate environments characterized by high availability of organic matter. Consequently, r-strategist microorganisms are primarily involved in the degradation of fresh organic matter. Among copiotrophic bacteria, the phylum *Bacteroidetes* and the classes *Betaproteobacteria* and *Gammaproteobacteria* are commonly represented.

When litter is labile and nutrient-rich, the decomposers involved are primarily copiotrophic microorganisms, such as opportunistic species. These organisms possess the ability to synthesize hydrolytic enzymes that enable the breakdown of readily accessible carbon within organic matter.

In contrast, when resources are scarce and difficult to access such as recalcitrant, nutrient-poor litter this favors slow biomass growth, mainly by stimulating oligotrophic microorganisms, including ligninolytic fungi. These microorganisms, which appear later during microbial succession, are capable of producing oxidative enzymes that degrade poorly accessible carbon compounds such as lignin and/or secondary metabolites like tannins.

2. Interactions between microorganisms

Within the framework of soil organic matter dynamics, it is generally accepted that r-strategist microorganisms dominate the early stages of fresh organic matter decomposition, whereas K-strategist microorganisms are primarily involved during the later stages.

3. Interactions between microorganisms and higher organisms

3.1. Interactions between microorganisms and plants

3.1.1. Introduction

Plants play a major ecological role as the main primary producers of organic matter in terrestrial ecosystems. Part of this organic material is available for plant-associated microorganisms.

The plant is exposed to high numbers of microorganisms, but most of them probably have little effects on the plant if any. Phytobeneficial microorganisms are much less prevalent comparatively. Cooperating microorganisms predominate over symbiotic ones on plant surfaces or in the rhizosphere.

Plant-microbe interactions, are ranging from mutualism to parasitism, and their implementation of is highly dependent on plant genotype, interactions with other members of the microbial community, and environmental conditions.

Table 3. Microorganisms-plant associations.

Microorganisms	Plant	Type of relationship
N ₂ -fixing bacteria	Legumes	Mutualism
Mycorrhizal fungi	Various	Mutualism
<i>Agrobacterium tumefaciens</i>	Various	Parasitism (Crown gall disease)
<i>Acremonium</i> (fungus)	Grass	Mutualism

3. Interactions between microorganisms and higher organisms

The perception of NFs in the susceptible region of the root stimulates two distinct but interconnected programs simultaneously: bacterial infection at the epidermis, and cell division in the cortex to establish nodules that house rhizobial symbionts.

After being released from infection threads (ITs), the bacteria in organelle-like symbiosomes differentiate into bacteroids and reduce nitrogen gas into ammonia in the low-oxygen environment inside mature nodules. Meanwhile, two or more peripheral vascular bundles surrounding the nodule fixation zone converge toward the nodule apex to facilitate nutrient exchange between the plant and rhizobia.

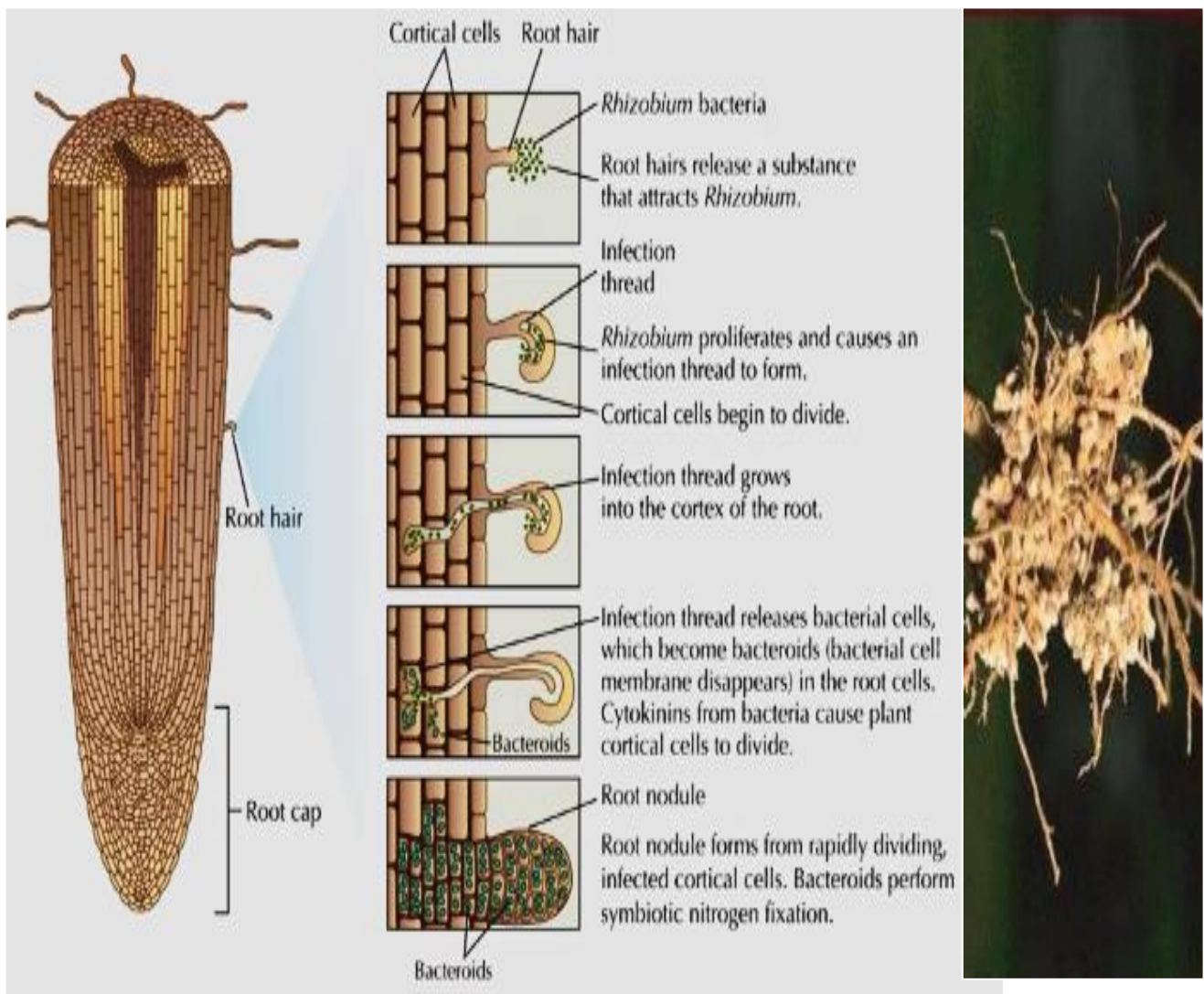


Figure 18. Symbiosis between the host and rhizobia.

3. Interactions between microorganisms and higher organisms

3.1.3. Mycorrhizal symbiosis

The mycorrhizal symbioses implicate plant roots. The fungus provides the plant with minerals taken from the soil (phosphorus and to a lesser extent nitrogen), whereas the plant provides carbon substrates derived from photosynthesis.

The vast majority of plants are mycorrhized in nature. There are several types of mycorrhizae, particularly ectomycorrhizae, arbuscular endomycorrhizae and ectendomycorrhizae.

➤ **Ectomycorrhizal fungi** (Ascomycetes and Basidiomycetes) develop extensively at root tips, forming fungal sheaths over short roots, which is very characteristic and easily visible to the naked eye.

Less than 5 % of plant species are concerned, among which mainly forest species growing in temperate areas. The mycelium grows between root cortical cells but does not penetrate living cells, thus forming an intercellular network called the Hartig net, which is involved in nutrient exchanges between the two partners. The functioning of ectomycorrhizae is under the metabolic and genetic control of both partners.

➤ **Arbuscular endomycorrhizal fungi** do not develop a mantle around the root. The endomycorrhizae are widespread and affect about 90 % of plant species. They are found mainly in herbaceous plants and in some woody species.

The fungus penetrates through plant cell walls and develops arbuscules and vesicles in cortical cells. Ectendomycorrhizae are intermediate between ectomycorrhizae and endomycorrhizae; an external mantle may be produced, but the fungus penetrates into root cells, as coils (arbutoid mycorrhizae; among *Ericaceae*) or very short hyphae (monotropoid mycorrhizae among *Pyrolaceae*).

3. Interactions between microorganisms and higher organisms

The establishment of mycorrhizal symbiosis shows common features with that of bacterial nitrogen-fixing symbioses. Before contact, the mycorrhizal fungus stimulates the production of fine roots to increase the contact sites, secreting hormones including the auxin indole-3-acetic acid (IAA).

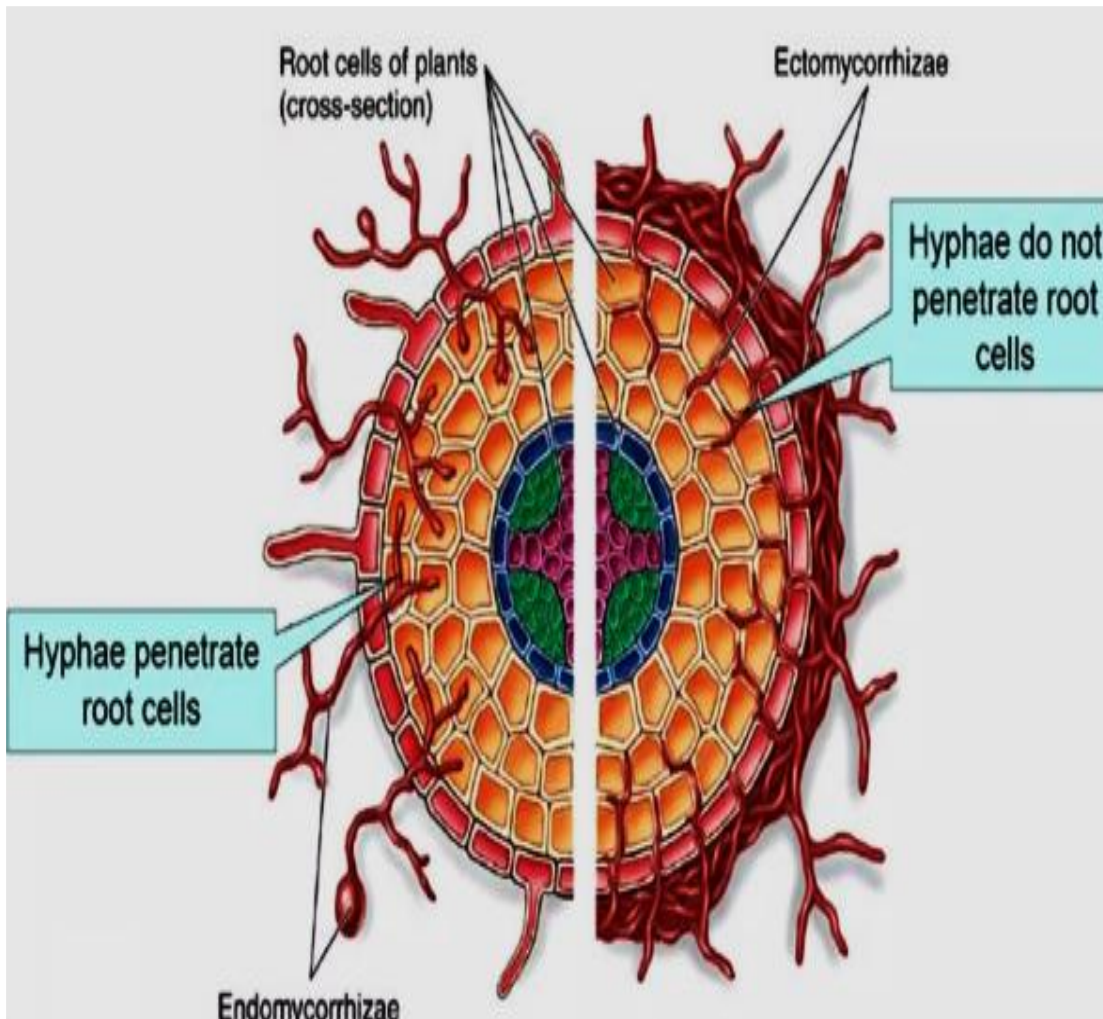


Figure 19. Mycorrhizal symbiosis.

3. Interactions between microorganisms and higher organisms

3.1.4. Colonization process

To colonize plants, a microorganism must not only use the trophic resources they provide (in a context of competition with the rest of the microbial community) but also tolerate abiotic stress conditions (including desiccation and UV in the phyllosphere) as well as toxins and other host defense compounds.

The best adapted microorganisms will be thus favored over the others. This selection process is amplified by the molecular dialogues with the plant, including microbial chemotaxis/chemotropism phenomena and the ability of certain plants to interfere with bacterial quorum sensing.

Infection of the plant is done in several steps. The contact of the plant with spores (constituting the primary inoculum) of fungal pathogens involves several environmental factors including wind, water, insects, and microfauna that vectorize the pathogens, but also the biochemical and mechanical infectious potential of these pathogens.

Production of fungal mucilage consisting of polysaccharides and/ or glycoproteins ensures the adhesion of spores and germ tubes to the plant tissues. Enzymatic hydrolysis of the cuticle surface (*U. necator* on grapevine) or production of small proteins such as hydrophobins Mgp1 (*Magnaporthe grisea* on rice) enhances the adhesion of the propagules to the plant. In most cases, there is no specific site of infection on the surface of the plant.

Fungi penetrate the aerial part of the plant by piercing the cuticle, growing through the anthesis, exploiting wounds (opportunistic fungi), or growing between the stomata guard cells. The fungal germ tube generally forms an appressorium that is simply a swelling of the tip of the hypha (*B. cinerea*) or results from cell differentiation (case of rusts) to puncture through plant cell wall using high physical pressure, cell wall degrading enzymes, or both.

In the case of pathogenic bacteria, penetration occurs most often through natural openings, such as nectaries of flowers (*E. amylovora*), stomata or hydathodes (*X. campestris*) or wounds (*P. syringae*). Their lytic enzymes play an important role in virulence. Quorum sensing is sometimes involved in the infectious process.

3. Interactions between microorganisms and higher organisms

3.1.5. Impact of OGM

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.

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.

Genetically Modified Microorganisms (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.

- **GMMs in Agriculture**

- **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.

3. Interactions between microorganisms and higher organisms

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 icenucleating 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.

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

➤ **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.

➤ **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.

3. Interactions between microorganisms and higher organisms

3.2. Interactions between Microorganisms and Human

3.2.1. Introduction

The human body exists in a state of dynamic equilibrium with microorganisms. Most of the microbes inhabiting the human body benefit from the nutrients and protective habitat it provides.

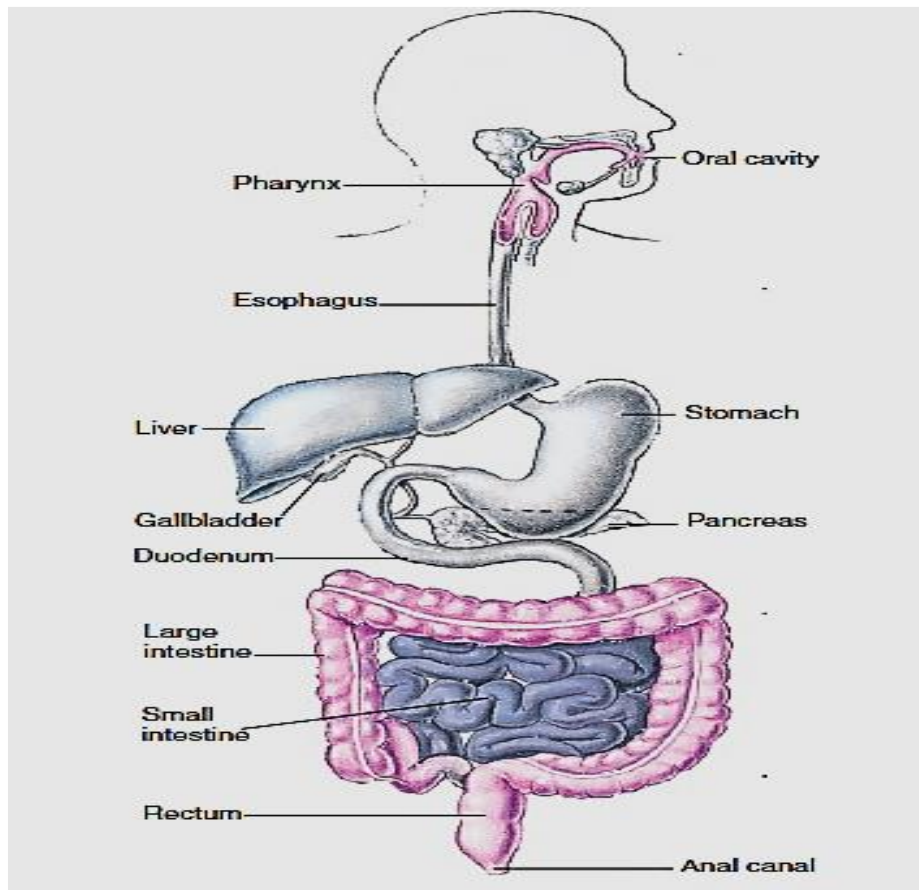


Figure 20. Distribution of flora.

3.2.2. Flora of the Gastrointestinal Tract

The gastrointestinal (GI) tract receives, moves, digests, and absorbs food; it also removes waste. It encompasses the oral cavity, esophagus, stomach, small intestine, large intestine, rectum, and anus. The only areas that harbor appreciable permanent flora are the oral cavity, large intestine, and rectum.

3. Interactions between microorganisms and higher organisms

Table 4. Flora of the Gastrointestinal Tract.

Stomach and duodenum	<ul style="list-style-type: none">• Harbor very low numbers of microorganisms: < 10³ cells per gram of contents• Mainly lactobacilli and streptococci• Acid, bile, and pancreatic secretions suppress most ingested microbes• Phasic propulsive motor activity impedes stable colonization of the lumen (also true for the small intestine)
Jejunum and ileum	<ul style="list-style-type: none">• Numbers progressively increase from 10⁴ in the jejunum to 10⁷ cells per gram of contents in the distal ileum
Large intestine	<ul style="list-style-type: none">• Heavily populated by anaerobes: up to 10¹² cells per gram of luminal contents

3.2.2.1. Flora of the oral cavity

The oral cavity has a unique flora that is among the most diverse and abundant of the body. The most common residents are aerobic *Streptococcus* species (*S. sanguis*, *S. salivarius* and *S. mitis*) that colonize the smooth superficial epithelial surfaces. Two species, *S. mutans* and *S. sanguis*, make a major contribution to dental caries.

Once the teeth have erupted, an anaerobic habitat is established in the gingival crevice that favors the colonization of anaerobic bacteria that can be involved in dental caries and periodontal infections.

3. Interactions between microorganisms and higher organisms

3.2.2.2. Flora of the Large Intestine

The flora of the intestinal tract has complex and profound interactions with the host. The large intestine (cecum and colon) and the rectum harbor a huge population of microbes (10^8 – 10^{11} per gram of feces).

Because of the state of the intestinal environment, the predominant fecal flora are strictly anaerobic bacteria (*Bacteroides*, *Bifidobacterium*, *Fusobacterium*, and *Clostridium*). Coliforms such as *Escherichia coli*, *Enterobacter*, and *Citrobacter* are present in smaller numbers.

3.2.2.3 Probiotics

The concept of probiotics has been proposed by the Russian Nobel Prize winner Elie Metchnikoff. It is defined as any living microorganism which, when ingested at a certain amount, exerts beneficial effects beyond basic nutritional functions.

The probiotic microorganisms most frequently cited are lactobacilli and bifidobacteria, and the reported effects range from changing the intestinal ecology to the stimulation of the immune system or the decrease of the risk of cancer.

➤ Lactic acid bacteria: Traditionally used for the preservation of food products due to their fermentative abilities, lactic acid bacteria also contribute to improving the organoleptic qualities of foods, such as flavor and texture. Species from the genus *Lactobacillus* represent the largest group of live microorganisms consumed through the diet.

➤ Genus *Bifidobacterium*: Although long considered as lactic acid bacteria, bifidobacteria are phylogenetically distinct from them. They are used in the agri-food industry for both the production and preservation of food products. Bifidobacteria are also part of the human intestinal microbiota and are among the first bacterial species to colonize the gut of breastfed newborns.

➤ Genus *Bacillus*: Although better known for its pathogenic species than for those with probiotic properties, the genus *Bacillus* nonetheless presents significant potential in the latter context. These bacteria are capable of producing endospores, which confer enhanced

3. Interactions between microorganisms and higher organisms

resistance to environmental conditions. As a result, neither storage conditions nor pH variations within the gastrointestinal tract are likely to compromise the viability or effectiveness of *Bacillus* species during use.

➤ Yeasts-Genus *Saccharomyces*: Brewer's yeast or baker's yeast, *Saccharomyces cerevisiae*, is one of the oldest yeasts used in the production of alcoholic beverages such as beer, as well as in baking. A closely related species, *Saccharomyces boulardii*, is used as a probiotic.

❖ Selection of probiotic candidates

Beyond safety, the selection of a probiotic strain is driven primarily by its potential to confer a health benefit for humans. It is commonly considered that, for food applications, probiotics need to survive until they reach the part of the GI tract where they exert their intended effect.

For example, to be active in the colon, probiotics must resist salivary enzymes, stomach acid, small intestinal secretions of bile and enzymes as well as the pH changes and chemical milieu of other foods and beverages they will encounter during their passage along the GI tract.

In addition, they need to compete with the resident microbiota. Finally, a selected strain has to fulfil a number of technological requirements, such as culturability on a large scale, genetic stability and maintaining viability in a food product or supplement.

- **Safety criteria**

➤ Taxonomic identification is an important step in the development of new potentially probiotic strains. Each must be identified using reliable molecular techniques and compared to an up-to-date nomenclature.

3. Interactions between microorganisms and higher organisms

- The origin of the strain is another piece of information to verify: it should be of human origin for those intended for human use and preferably from the same site as the target to ensure maximum effectiveness.
- Probiotic strains must also be free of adverse effects and safe for human health. They should be evaluated through clinical trials in both animals and humans.

- **Functional criteria**

- Probiotic strains must retain their viability up to their site of action. Before reaching the intestinal tract, they must primarily withstand the acidic environment of the stomach and the bile secreted into the duodenum. The level of tolerance to these conditions varies between strains.
- Adhesion to the mucosal surface is another essential property that probiotic strains must exhibit. This adhesion enhances the retention time at the site of action and facilitates close contact between the bacteria and epithelial cells.

Therefore, a probiotic strain with a high adhesion rate may potentially stimulate the immune system and prevent pathogen colonization through competitive exclusion.

- **Technological criteria**

Probiotic strains must withstand the various stages of production of the final probiotic product and regain their full effectiveness upon use, without contamination or alteration of their properties.

3. Interactions between microorganisms and higher organisms

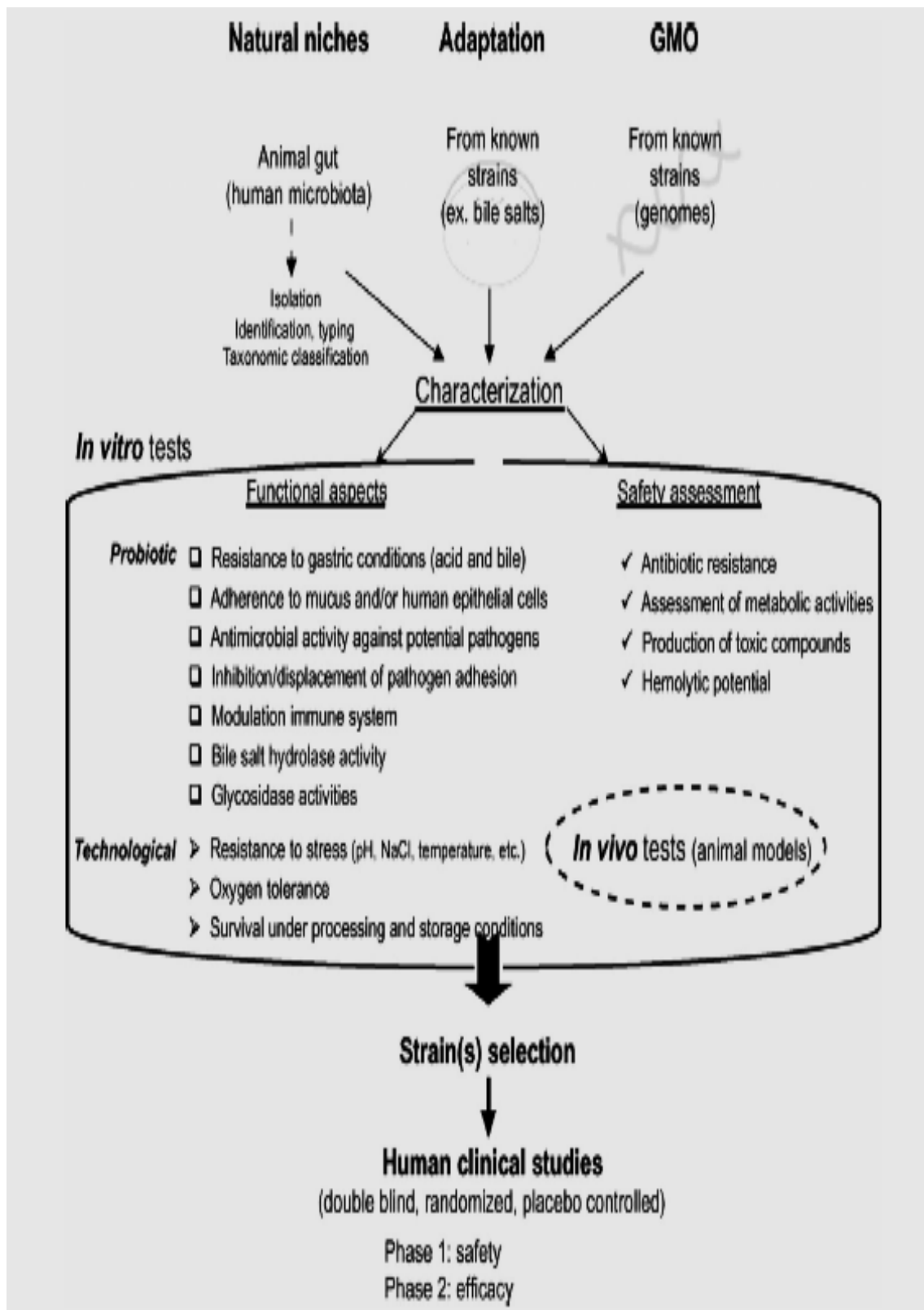


Figure 21. Procedure for the isolation and characterization of novel strains with putative probiotic status.

3. Interactions between microorganisms and higher organisms

❖ Impact of probiotics on the gastrointestinal tract

Many micro-organisms ferment lactose, the sugar found in milk and products made from milk. Although infants rely on lactose, which contributes as much as 10% of the energy in breast milk, many populations around the world have a high proportion of adults who are unable to digest this sugar.

In humans, and in fact in all mammals, expression of the enzyme lactase is down-regulated in adulthood with the exception of some population groups.

Lactose intolerance is a condition in which the colonic fermentation of undigested lactose results in gastrointestinal effects such as abdominal pain, bloating, borborygmi or laxation.

There is evidence that the live bacteria of yogurt are able to compensate for the lack of endogenous lactase in the human gut by digesting lactose.

The typical measure of improved lactose digestion is a reduction in breath hydrogen excretion (breath hydrogen is usually raised when undigested carbohydrate reaches the colon and is fermented). This improved digestibility reduces the symptoms related to lactose intolerance in some lactose malabsorbers.

3.2.3. Flora of the skin

The skin is the largest and most accessible of all organs. Its major layers are the epidermis, an outer layer of dead cells continually being sloughed off and replaced, and the dermis, which lies atop the subcutaneous layer of tissue.

3. Interactions between microorganisms and higher organisms

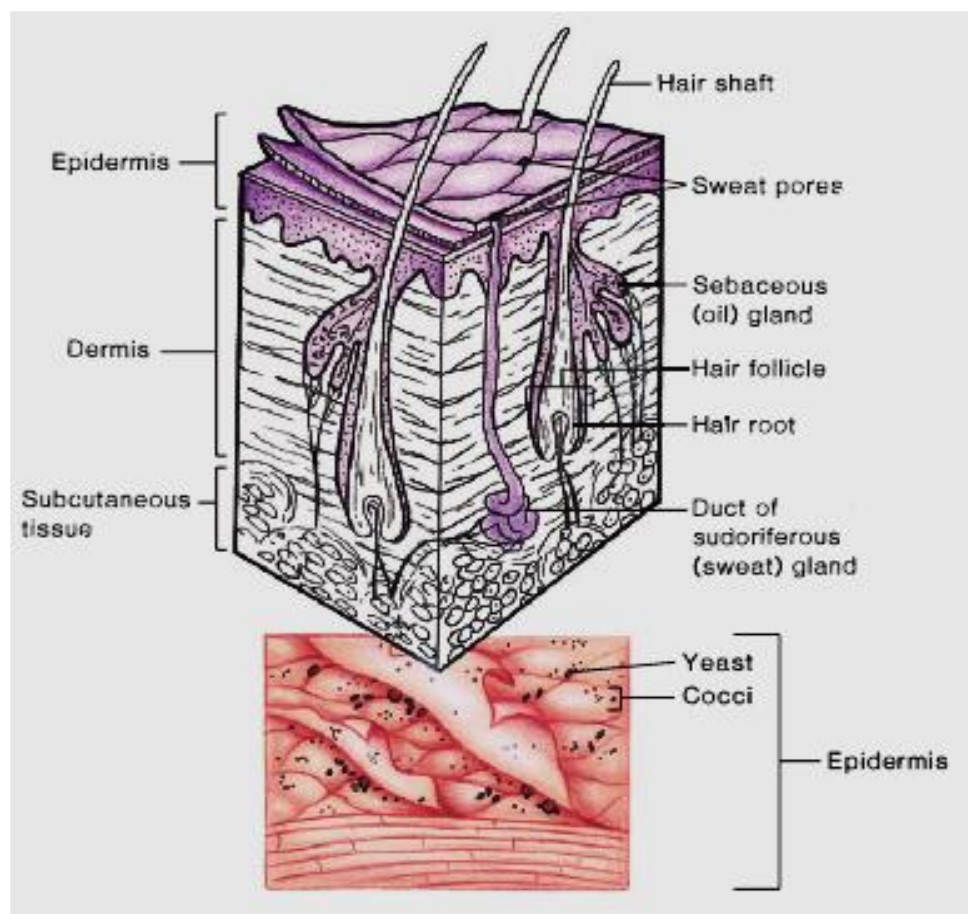


Figure 22. The landscape of the skin.

Ordinarily, there are two cutaneous microbes population.

➤ **The transient** population which is acquired by routine contact. The transient flora includes any microbe a person has picked up, often species that do not ordinarily live on the body, and it is greatly influenced by the hygiene of the individual.

➤ The **resident** population lives and multiplies in deeper layers of the epidermis and in glands and follicles, its composition is more stable than is the transient flora. The normal flora consists primarily of bacteria (notably *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*) and yeasts.

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3.2.4. Flora of the Respiratory Tract

The first microorganisms to colonize the upper respiratory tract (nasal passages and pharynx) are predominantly oral streptococci. *Staphylococcus aureus* preferentially resides in the nasal entrance, nasal vestibule, and anterior nasopharynx, and *Neisseria* species take up residence in the mucous membranes of the nasopharynx behind the soft palate.

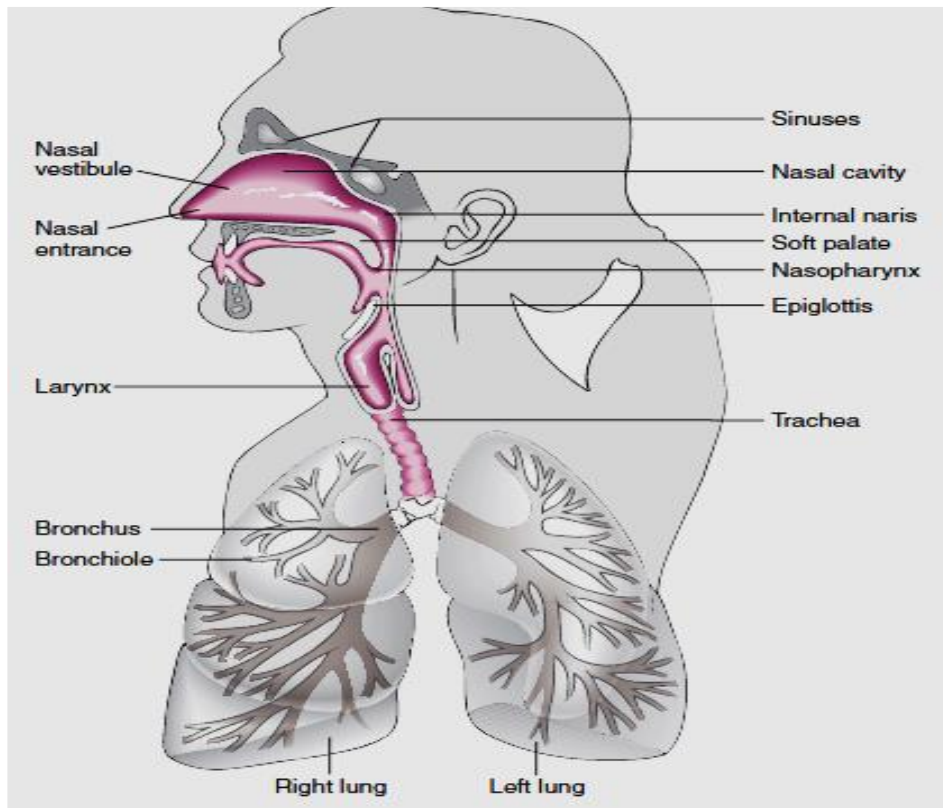


Figure 23. Colonized regions of the respiratory tract.

3.2.5. Flora of the Genitourinary Tract

The regions of the genitourinary tract that harbor microflora are the vagina and outer opening of the urethra in females and the anterior urethra in males.

Because the urethra in women is so short it can form a passage for bacteria to the bladder and lead to urinary tract infections. The principal residents of the urethra are nonhemolytic streptococci, staphylococci, corynebacteria, and occasionally, coliforms.

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3.3. Interactions between microorganisms and animals

3.3.1. Introduction

Animals, whether vertebrate or invertebrate, live in environments (air, water, soil) constantly populated by microorganisms consisting mainly of bacteria, fungi, protozoa, and viruses. Such cohabitation usually leads to more or less intimate associations between microorganisms and animals.

Spatial proximity, contact time, and the degree of dependence between the interacting partners influence the type and evolution of these associations.

Microorganisms use animals as habitats in which they live and from which they retrieved nutrients necessary for their multiplication. They colonize the outlying areas, such as the surface of the skin or mucous membranes, and can penetrate the tissues and organs of host animals.

Table 5. Microorganisms-animal associations.

Microorganisms	Animal	Type of relationship
Anaerobic bacteria	Ruminants	Mutualism
Flagellated protozoans	Termites	Mutualism
Sulphur-oxidising bacteria	<i>Riftia</i> (marine tube worm)	Mutualism
Luminescent bacteria	Fish, molluscs	Mutualism
Bacteria, yeasts	Honey guide (bird)	Mutualism
Fungus	Leaf cutter ants	Protocooperation
Resident bacteria of skin, large intestine, etc	Humans	Commensalism

3. Interactions between microorganisms and higher organisms

3.3.2. Symbiosis between the squid *Euprymna scolopes* and *Vibrio fischeri*

Another exemplary mutual interaction of the marine world is observed in the association between the squid *Euprymna scolopes* and the *alphaproteobacterium* *Vibrio fischeri*. This squid that lives mainly on the seabed is exposed to predation when swimming back to the surface at night.

To escape predators, *E. scolopes* emits a ventral light that serves as camouflage concealing its silhouette against the light of the stars. The emission of light by the squid results from the phenomenon of bioluminescence produced by *V. fischeri* (formerly *Photobacterium fischeri*) located in a specialized light organ (photophore) of the host.

In the photophore, *V. fischeri* can reach high densities in the order of 10^{10} cells per ml, and the light is emitted at a certain bacterial density (or quorum perceived by homoserine lactone-like molecules). At daybreak, almost all (~90 %) bacteria are expelled into the environment. During the day, the remaining bacterial population (~10 %) in the light organ is multiplied in order to reach again the maximum density required to produce luminescence at night.

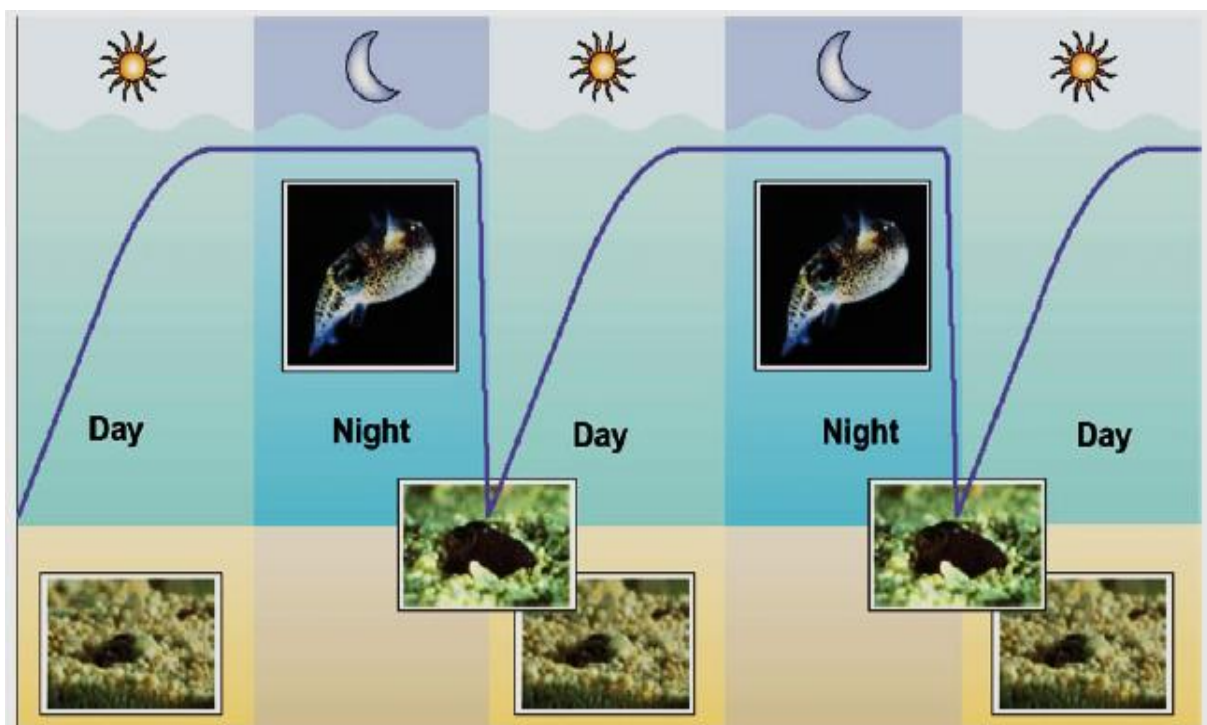


Figure 24. Symbiosis between the squid *E. scolopes* and *V. fischeri*.

3. Interactions between microorganisms and higher organisms

3.3.3. Symbiosis between The giant worm *Riftia pachyptila* and *Proteobacteria*

At the bottom of the deepest oceans, around geothermal vents, live enormous (two metres or more) tube worms belonging to the genus *Riftia*. These lack any sort of digestive system, but instead contain in their body cavity a tissue known as the trophosome.

This comprises vascular tissue plus cells packed with endosymbiotic bacteria belonging to the *Proteobacteria*. These are able to generate ATP and NADPH by the oxidation of hydrogen sulphide generated by volcanic activity and fix carbon dioxide via the Calvin cycle, providing the worm with a supply of organic nutrients. Hydrogen sulphide is transported to the trophosome from the worm's gill plume by a form of haemoglobin present in its blood.

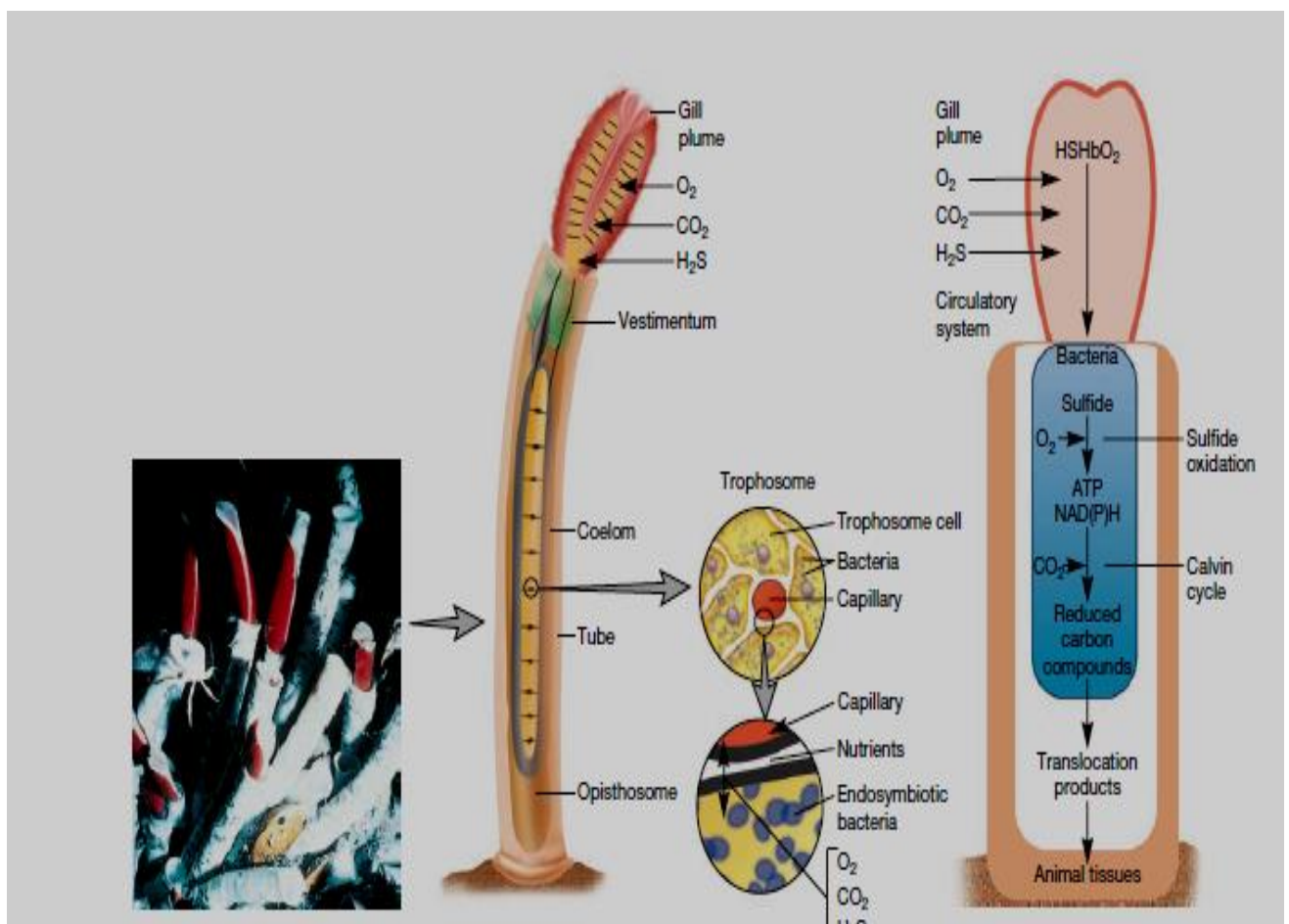


Figure 25. The Tube Worm-Bacterial Relationship.

3. Interactions between microorganisms and higher organisms

3.3.4. Microbe-Vertebrate Mutualism

In vertebrates, the mutualistic associations between the indigenous microbiota of the intestine and the animals are clearly established in the carbon metabolism of ruminants.

The microbiota of the intestinal tract is structured in trophic network that plays an essential role in the degradation of polysaccharides such as cellulose and lignin of plant material ingested by the hosts. From this mutualistic association, ruminants and draws energy as carbohydrates, while microbes are protected in a germ-free biotope and are supplied with nutrients.

3.3.5. Insect Nutritional Symbioses

The example of aphids (*Homoptera, Insecta*) and their symbiotic bacteria is one of the best described. These insect pests attack almost all plant species feeding off their phloem rich in carbohydrates but low in amino acids.

This imbalance is compensated by a mutualistic association with symbiotic bacteria *Buchnera*, which are housed in specific organs of the host, bacteriomes, or bacteriocytes where they play nutritional functions. *Buchnera* genomes contain multiple copies of genes that encode the biosynthesis of essential amino acids such as tryptophan and appropriate gene regulators.

The intracellular localization of the bacteria facilitates the transfer of microbial compounds produced to the host. In return, the host provides the bacteria with energy for growth in an environment free of competitors.

3.4. Notions of reservoirs (soil, plants and water)

3.4.1. Definition of the reservoir

A reservoir is defined as a habitat in which biological agents persist, accumulate, and multiply. These agents can proliferate in a wide range of environments. Reservoirs may occur in natural ecosystems, including soil, freshwater and marine environments, and plants. In addition, reservoirs can be associated with living hosts, such as humans and animals, where

3. Interactions between microorganisms and higher organisms

biological agents may be found on external surfaces or within internal compartments, including the skin, respiratory tract, saliva, blood, and animal integuments (e.g., wool).

3.4.2. Soil

Soil represents the largest reservoir of microorganisms on Earth. Since the colonization of terrestrial environments by plants originating from marine ecosystems, numerous symbiotic associations have evolved between plants and soil microorganisms, including bacteria and fungi.

These symbioses play a crucial role in promoting plant growth by enhancing nutrient acquisition. In such interactions, the colonization of plant roots by soil microorganisms relies on a complex molecular dialogue between both partners, ultimately leading to specific plant morphogenetic responses, as exemplified by symbioses involved in biological nitrogen fixation.

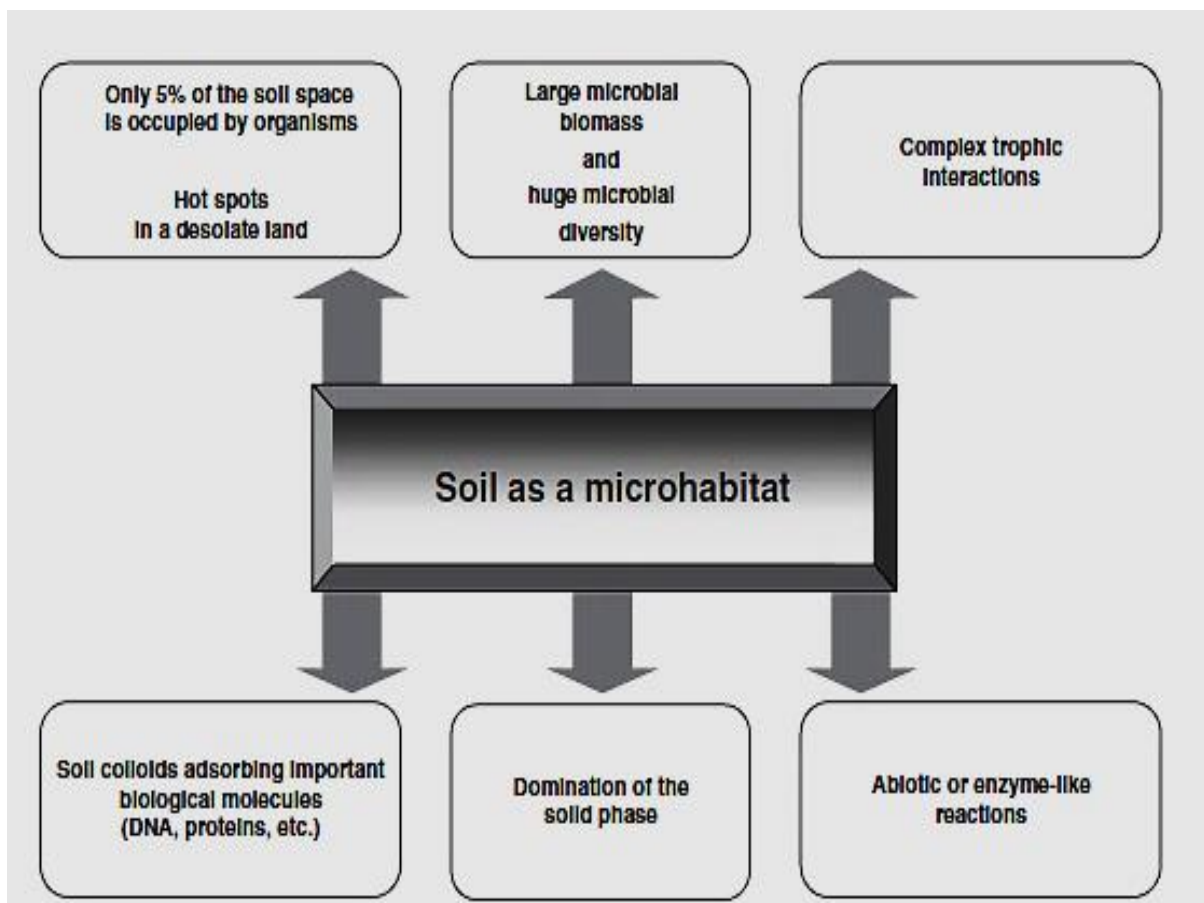


Figure 26. Schematic representation of the main characteristics of soil as a microhabitat.

3. Interactions between microorganisms and higher organisms

As a result, soil contains an immense diversity of microorganisms organized into complex ecosystems, whose contributions to plant growth, development, and health are increasingly emphasized in the scientific literature.

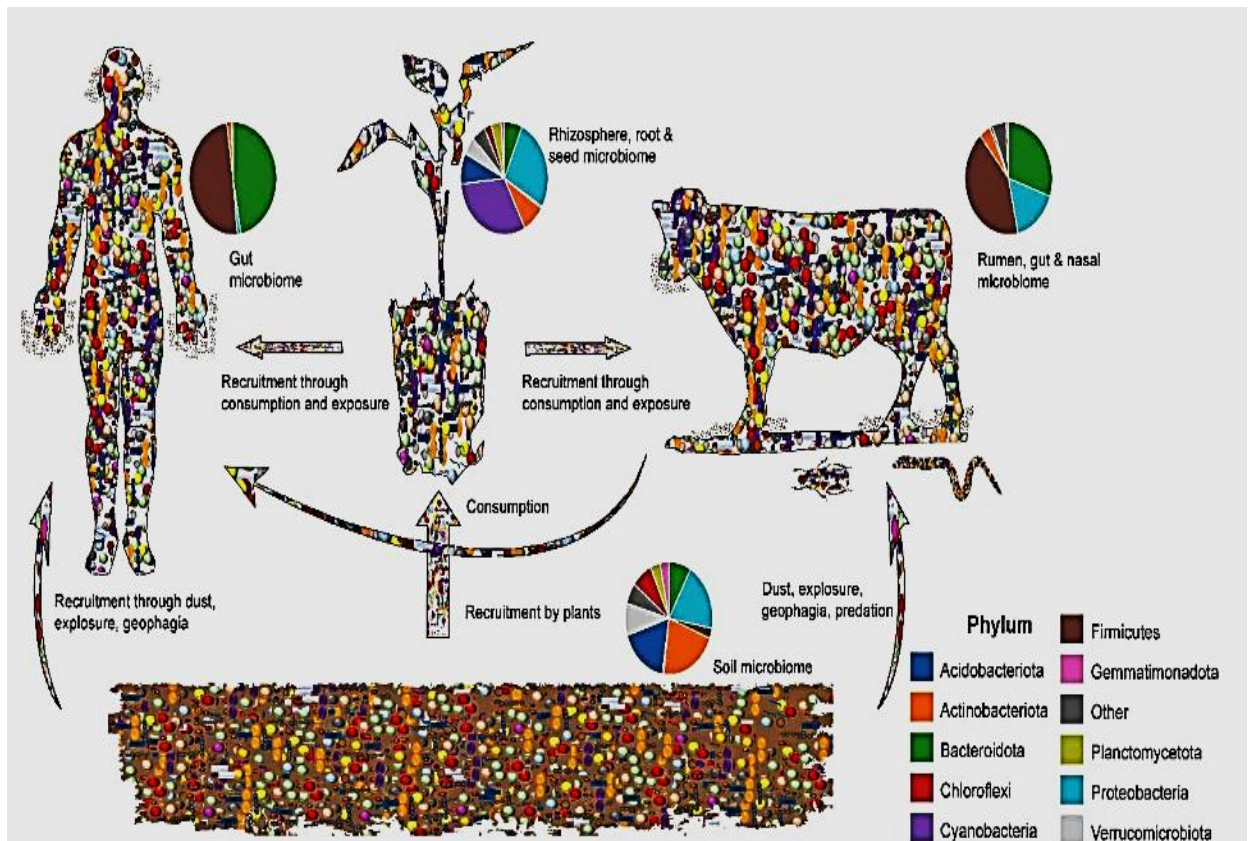


Figure 27. Soil microbiomes.

3.4.3. Plants

Plants evolve in environments that are rich in microorganisms originating from multiple sources, including soil, air, water, and insects, all of which can come into contact with plant tissues.

Consequently, each plant interacts with a highly diverse microbial environment comprising thousands of species belonging to bacteria, archaea, fungi, and protists.

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In addition, viruses, including bacteriophages, may also constitute components of these microbial communities following the infection of living cells.

Among this extensive microbial diversity, certain microorganisms are capable of establishing direct interactions with plants and may colonize plant surfaces or internal tissues, thereby forming what is referred to as the plant microbiota.

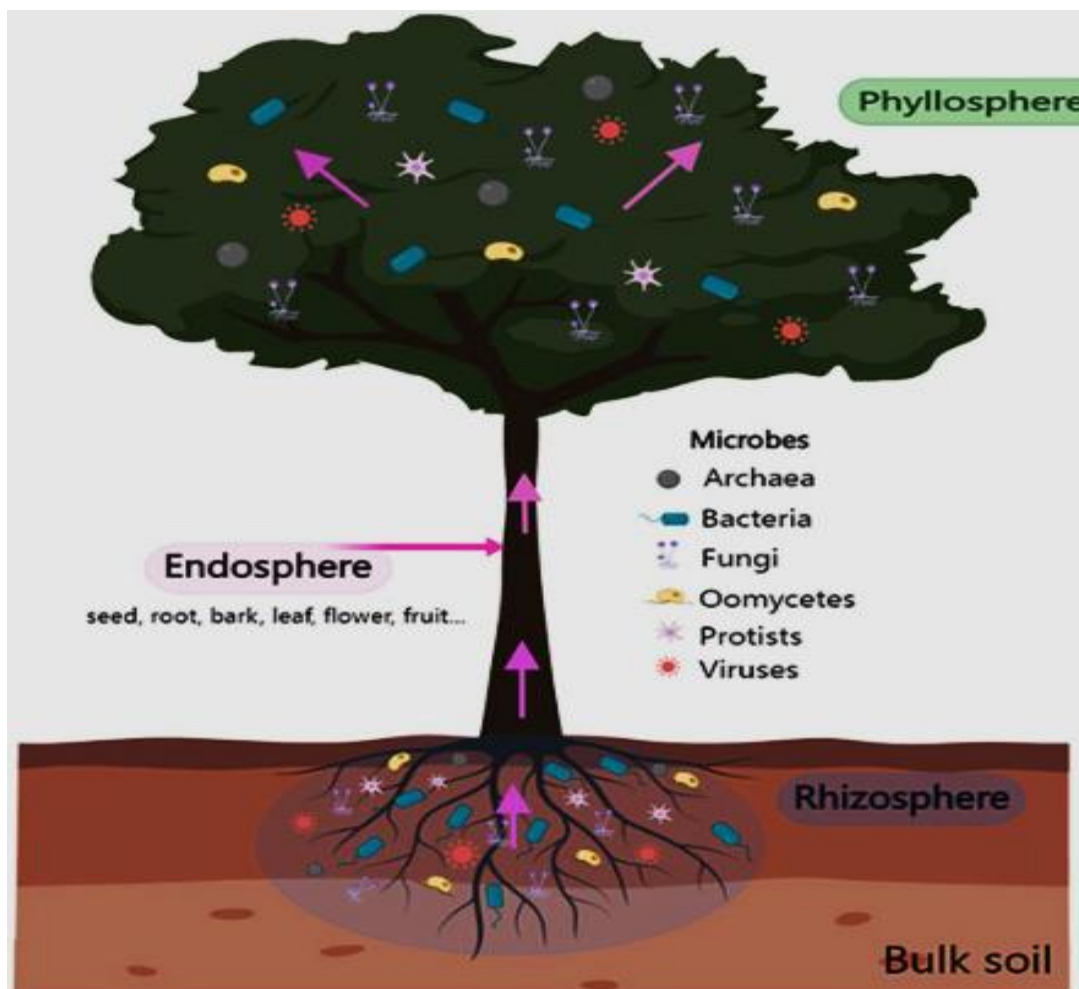


Figure 28. Plant microbiota.

3. Interactions between microorganisms and higher organisms

3.4.4. Water

Regardless of its origin marine or continental, water constitutes both a vital resource, supporting food production, recreation, and industrial and agricultural activities, and an environmental and heritage framework, providing habitats and sustaining a wide range of human uses and activities.

Within these environments, microbial communities are highly diverse and include viruses, bacteria, protozoa, unicellular algae, and microscopic fungi. Through complex interactions among themselves and with their surrounding environment, these microorganisms play a fundamental role in ecosystem functioning.

In aquatic environments, pathogenic microorganisms, may constitute an integral component of the natural microbial community. This is notably the case for *Legionella*, *Vibrio* spp., and amoebae, which are indigenous to natural ecosystems. In other instances, pathogenic microorganisms may be introduced into aquatic environments through the release of fecal matter from infected hosts or via contamination by human or animal wastewaters, representing allochthonous pathogens in these ecosystems.

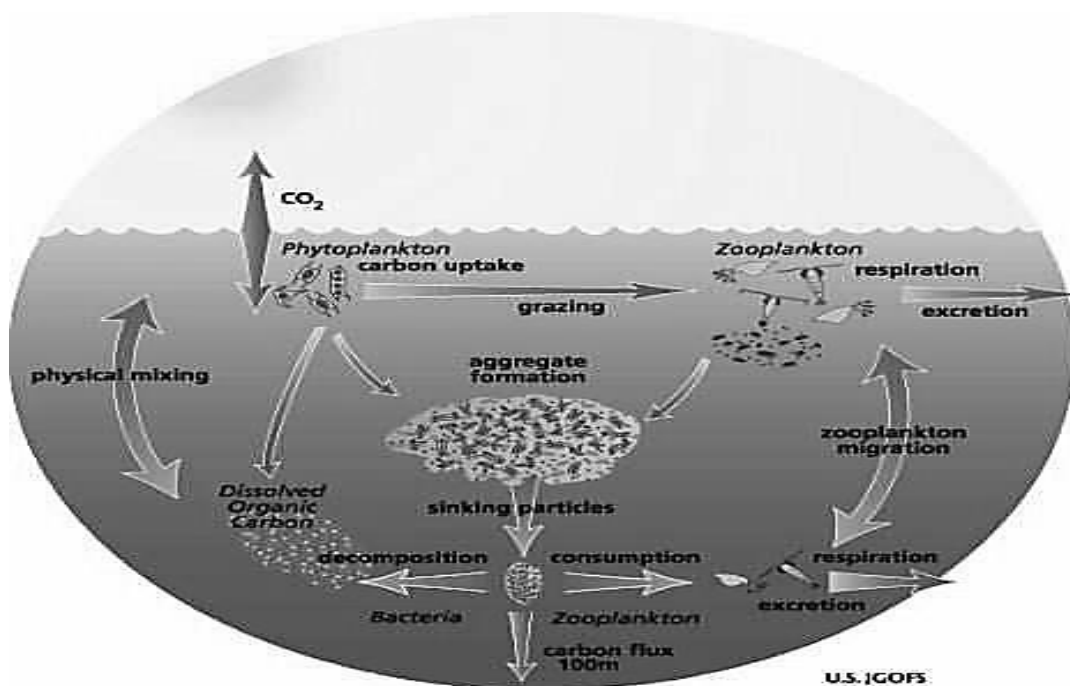


Figure 29. Role of Microbes in the marine environment.

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