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INTERACTIONS MICROORGANISMS - HOSTS - ENVIRONMENT

Course handout for Bachelor's and Master's students in the field of Natural and Life Sciences

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Content

References

Introduction

Within a biocenosis, different types of interactions are observed between individuals of different species (interspecific interactions) or of the same species (intraspecific interactions). Interspecific interactions can be positive (mutualism, commensalism, syntrophism, synergism or protocooperation), neutral (neutralism) or negative (parasitism, amensalism or antagonism, competition, predation).

Positive interactions

Mutualism: an interaction in which both partners benefit from their association, but still can live independently. If the interaction is mandatory, i.e. at least one of the partners involved cannot live without the other, and the organisms are in contact, this is called symbiotic mutualism.

Commensalism*:* long-term interaction between individuals of different species where one partner benefits from the association while the other finds neither advantage nor real disadvantage.

Syntrophisme: a combination of two microorganisms that cooperate for a specific overall metabolic activity and depend on each other for growth and existence.

Proto-cooperation: interaction between organisms of different species in which both benefit without interaction being mandatory.

Neutral interactions

Neutralism: no interaction between two species living in the same biotope.

Negative interactions

Amensalism: an interaction in which one species excretes a substance that inhibits the development of the other.

Parasitism: An interaction between two organisms, one of which (the parasite) lives at the expense of the other (the host) while harming it. A distinction is made between ectoparasites (living on the surface of their hosts) and endoparasites (living inside their hosts).

Competition: competition between organisms for a resource, food, or for the appropriation of a habitat.

Predation: interaction between organisms where one (predator) feeds on the other (prey).

Intraspecific relationships are established between individuals of the same species, forming a population. These are phenomena of cooperation or competition, with the sharing of territory, and sometimes organization into hierarchical societies.

1. Interaction microorganisms – environment

There are three main types of environments: freshwater, marine and terrestrial. Each environment has its own characteristics that make it a habitat for a specific macro and micro flora and fauna.

In all ecosystems (terrestrial and aquatic), the chemical elements that form living beings (plants, animals, microorganisms) are constantly recycled, thus constituting the cycle of matter (biogeochemical cycles); there is a renewal or turnover of the elements (Fig. 1). During their growth and metabolism, microorganisms interact with each other in the cycling of nutrients, such as carbon (C), sulphur (S), nitrogen (N), phosphorus (P), iron (Fe) and manganese (Mn). The elements are oxidized and reduced by microorganisms to meet their metabolic needs. The extent to which microorganisms intervene in the material cycle varies depending on the element considered. In the case of carbon, nitrogen and sulphur, it is crucial, since the absence or even inactivity of microorganisms would lead to a shutdown in the natural supply of nitrogen to the soil and a blockage of the turnover of C, N, S resulting in the accumulation of these elements in organic form unusable by the plants (producers). This leads to the death of the latter which will in turn be the cause of the death of the consuming organisms in turn. In the case of other elements, such as phosphorus, microbial intervention is much more discreet but remains beneficial in plant nutrition, mainly in soils that are poor in it.

In fact, microorganisms break down and transform organic matter into inorganic matter through the different processes of obtaining energy; i.e. aerobic, anaerobic or fermentation respiration. The resulting elements will either be released directly into the atmosphere as gases or reused by other organisms in their nutrition

In addition, all biogeochemical cycles are interlinked, and changes in these nutrients have global implications. Important gaseous components occur in the carbon and nitrogen cycles and to a lesser extent in the sulphur cycles, in contrast to "sedimentary" cycles, such as P and Fe where there are no gaseous components. Thus, soil or aquatic microorganisms can often bind these gaseous forms of carbon and nitrogen.

It should be noted that among all living organisms on our planet, only a few prokaryotic microorganisms (bacteria) are able to fix atmospheric nitrogen and transform it into a form that can be assimilated by plants.

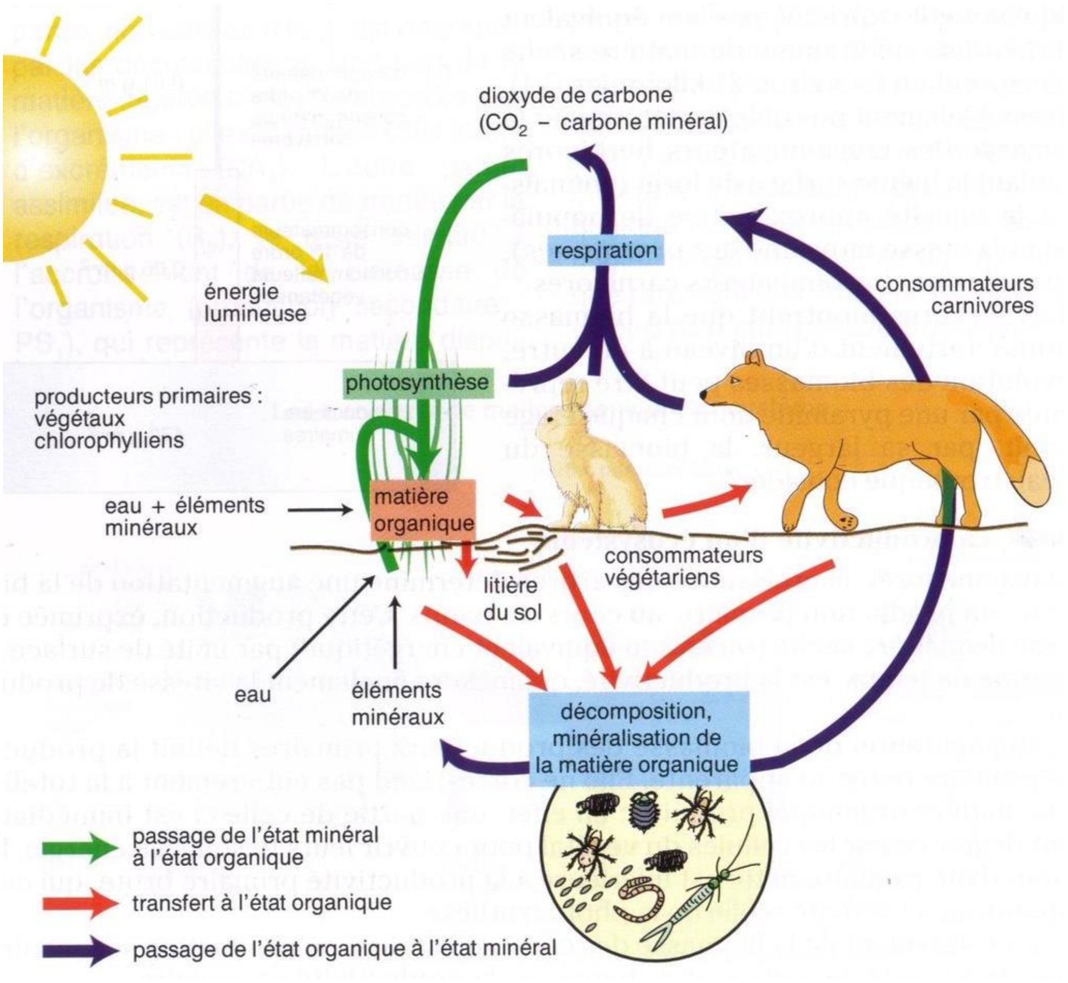

Figure 1. The cycle of matter in an ecosystem.

2. Interactions microorganisms – hosts

2.1. Microorganisms – plants

Different types of microorganisms are associated with plant leaves, stems, flowers, seeds, and roots. The latter are directly or indirectly influenced by the activities of microbial communities which include microorganisms that develop on the surface of the plant or epiphytes and within the plant or endophytes.

a. The rhizosphere

Soil is a living environment in which plants, animals and microorganisms constantly mix their activities and regulate the processes of decomposition of organic matter and nutrient flow through a very complex food web. It develops over long periods of time through complex interactions between base materials (rock, sand, glacial sediment materials, ...), topography, climate, and organisms. This complex biological community contributes to the formation, maintenance and, in some cases, degradation of soils.

Soil microorganisms play two essential roles: on the one hand, they are responsible for many chemical and even physical transformations that take place in soils, contributing to soil structuring, biological fertility (geochemical cycling of nutrients, turnover and mineralization of organic matter), control of certain pathogens and the degradation of certain soil contaminants. On the other hand, they are able to interact with plants in a wide range of interactions from symbiotic mutualism to parasitism.

There is a great diversity of microbial communities in the soil both in terms of taxonomic diversity and functions. Indeed, it is estimated, for example, that one gram of soil contains about 10^{10} to 10^{11} bacteria, including 6000 to 50000 species of bacteria and more than 200 meters of fungal hyphae. Bacteria, actinobacteria and fungi make up the bulk of soil microbial biomass. But, in hydromorphic soils, algae tend to proliferate at the surface and anaerobic bacteria at depth. Viruses are often associated with clays from which they can enter plant roots through wounds.

The rhizosphere (Fig. 2) is the part of the soil surrounding plant roots, it is the habitat of several microorganisms that interact with the plant and influence its growth. The soil microbial population is greatest in the top few centimeters and decreases rapidly with depth. The number and activity of microorganisms are important due to the richness of this area in organic substances released by plant roots. These microorganisms interact with plants either in deleterious or beneficial ways.

Plant-microbial community interactions are bidirectional. Indeed, if the microbial communities in the soil are able to modify the growth of the plant, the latter also has a strong impact on them via rhizodeposition (root exudates). Some of the carbon assimilated during photosynthesis is lost to the soil in the form of rhizodeposits (about 17%), which serve as both nutrients for heterotrophic microbial communities in the soil but also signals in plant-microbial community interactions. This is because plant roots release a wide variety of substrates into their surrounding soil, including ethylene, various alcohols, amino sugars, organic acids, vitamins, nucleotides, polysaccharides, and enzymes. This creates unique environments for soil microorganisms. These environments include (i) the rhizosphere, described by Lorenz Hiltner in 1904, which is represented by the volume of soil around the root influenced by the substrates rejected by the root. (ii) the surface of the plant's root, called the rhizoplane, which also provides a unique environment for microorganisms, such as gaseous, soluble matter, and particles moving from the plant to the soil.

However, the loss of part of the plant's products is more than compensated. Indeed, microorganisms contribute, among other things, to the recycling of elements in the rhizosphere to make them nutrients that can be assimilated by the plant, to the production of plant hormones or to the protection of the plant against pathogens. These microorganisms can be found free-living in the soil or have symbiotic associations with plants.

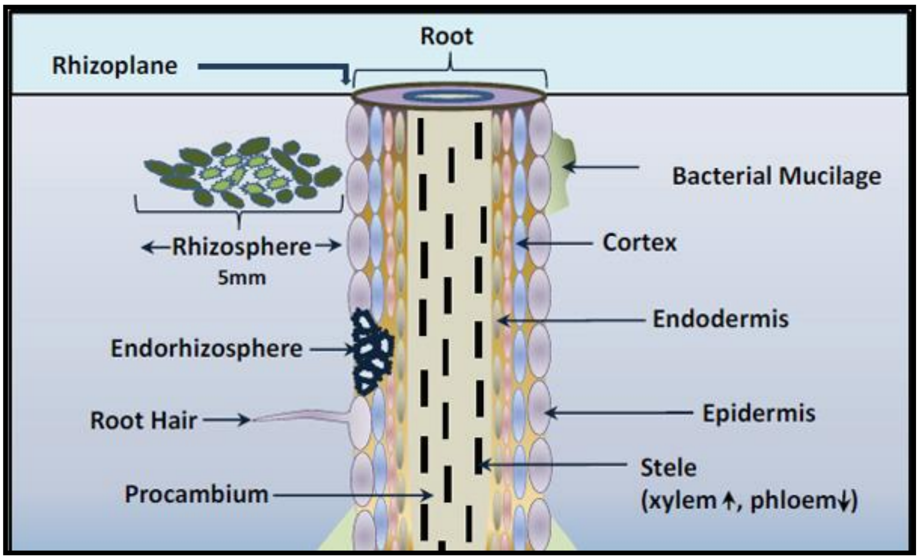

Figure 2. The rhizosphere.

In fact, some bacteria in the rhizosphere are able to enhance plant growth; they are called PGPRs for "Plant Growth Promoting Rhizobacteria". The beneficial effect of PGPR (Fig. 3) can be ensured by direct mechanisms through the stimulation of seed germination. Similarly, the plant's mineral nutrition can be stimulated directly by increasing the mineralization of organic matter; this is called biofertilization. In a more indirect way, mineral nutrition can also be improved via a stimulation of root development called "phytostimulation" by secreting phytohormones such as gibberellin, auxin, cytokinin, ... etc. In addition, plant growth may be affected by a direct effect of some PGPRs on the degradation of certain soil pollutants by "rhizoremediation". PGPRs can protect the plant from the action of certain pathogens, either by competing for nutritional resources by secreting chelating molecules such as siderophores, which trap iron ($Fe^{3+),}$ or by antagonism, by secreting substances that inhibit the development of the pathogen (antibiosis).

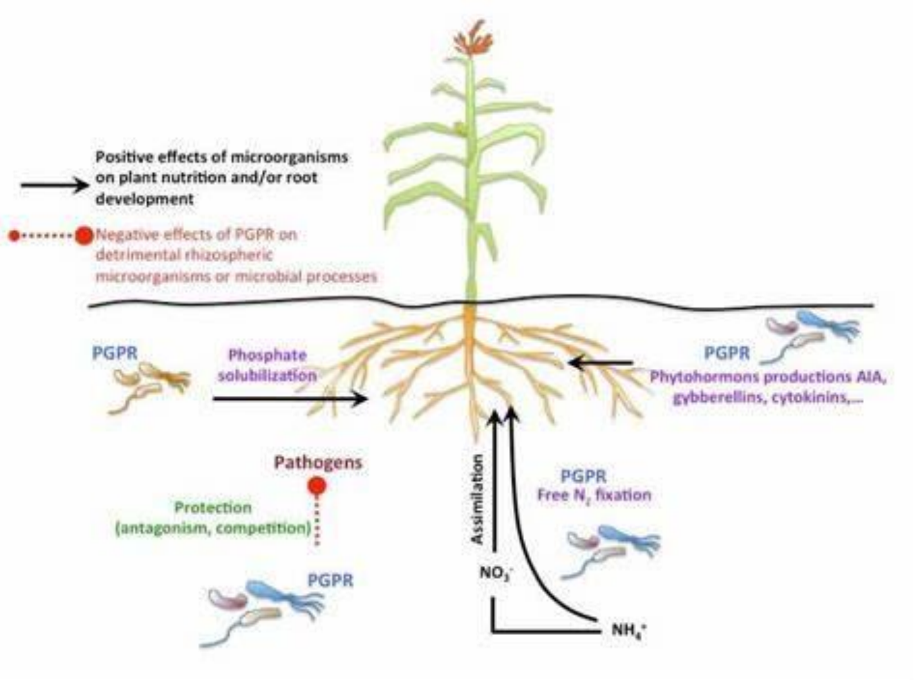

Figure 3. Interactions of PGPRs with plants.

Finally, some PGPRs are able to protect the plant from plant pathogens by conferring a resistance to it called induced systemic resistance (ISR) (Fig. 4).

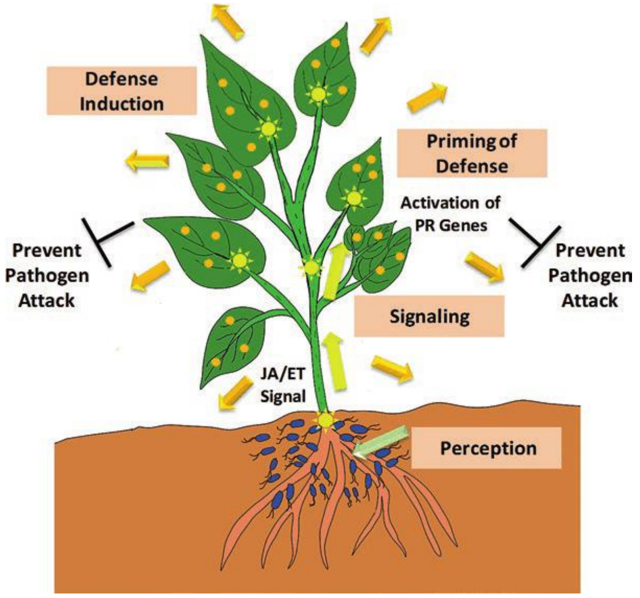

Figure 4. Induced systemic resistance.

(1st Elicitation: PGPRs interact with the host's roots and produce elicitors that are perceived by the plant. 2nd After the recognition of the determinants, a signal is conveyed throughout the plant in order to alert it. 3rd In the event of an attack by a plant pathogen, the plant will be able to respond more effectively to the attack, thus giving it resistance).

PGPRs can be classified into extracellular PGPR (ePGPR) and intracellular PGPR (iPGPR), the former are those of the rhizoplane or those living in the intercellular space of the root cortex, while the latter are generally found inside nodular structures at the level of root cells. Bacteria of the genera *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcous*, *Achromobacter*, *Pseudomonas* et *Serratia* belong to ePGPR. The iPGPR include bacteria of the Rhizobiaceae family namely: *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*, in addition to endophytes and species of the genus *Frankia* fixing nitrogen by forming symbioses with higher plants.

Despite the fact that certain bacteria belonging to PGPR have the capacity to fix atmospheric nitrogen while being free in the soil, however, the yield remains low due to the fact that the enzymatic complex responsible for this fixation “dinitrogenase complex” (Fig. 5) is sensitive to oxygen and therefore requires the bacteria to live anaerobically. Consequently, the energy yield of the bacteria's metabolism will be low while nitrogen fixation remains a highly energy-consuming process. To counter this problem, certain bacteria form symbioses with specific plants through the secretion of signal molecules on both sides of the two organisms. The plant provides essential nutrients and conditions suitable for the metabolism of the bacteria, and the latter provides the nitrogen fixed directly to the plant in order to incorporate it into organic matter.

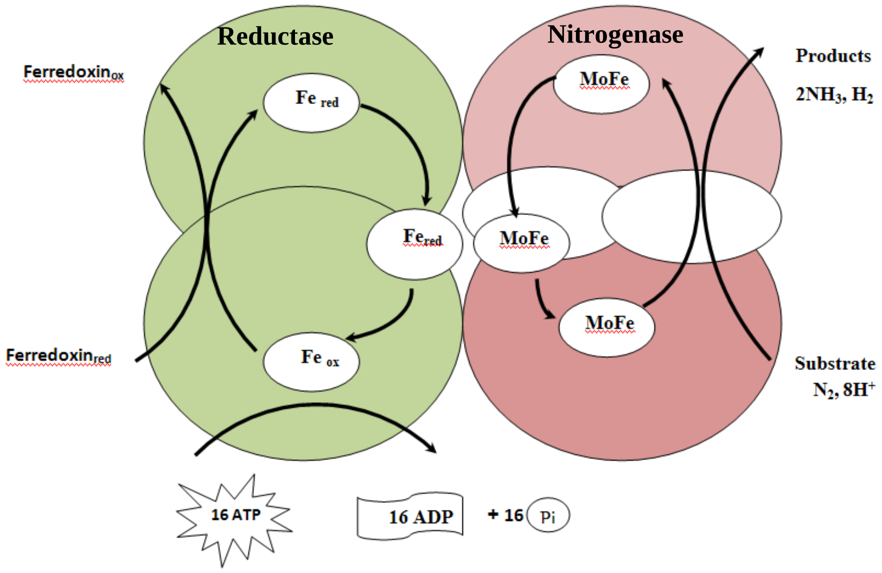

Figure 5. Nitrogen fixation by dinitrogenase complex.

The mutual symbiosis Fabaceae – Rhizobium is one of the most studied plant – microorganism interactions. Bacteria of the Rhizobium genus are Gram-negative bacilli forming nodules on the roots of plants of the Fabaceae family (lentils, soybeans, beans, peas, alfalfa, beans, etc.). The root nodules (Fig. 6) contain bacteria and an oxygen chelating protein “leghemoglobin”, part of which is coded by the genes of the bacteria (heme part) and the other by those of the plants (globin part). Leghemoglobin maintains a low oxygen pressure inside the nodule while allowing the bacteria to live aerobically at the same time promoting the activity of the dinitrogenase complex. An active nodule will have a pink-red color due to leghemoglobin however when it turns yellow this reflects its inactivity.

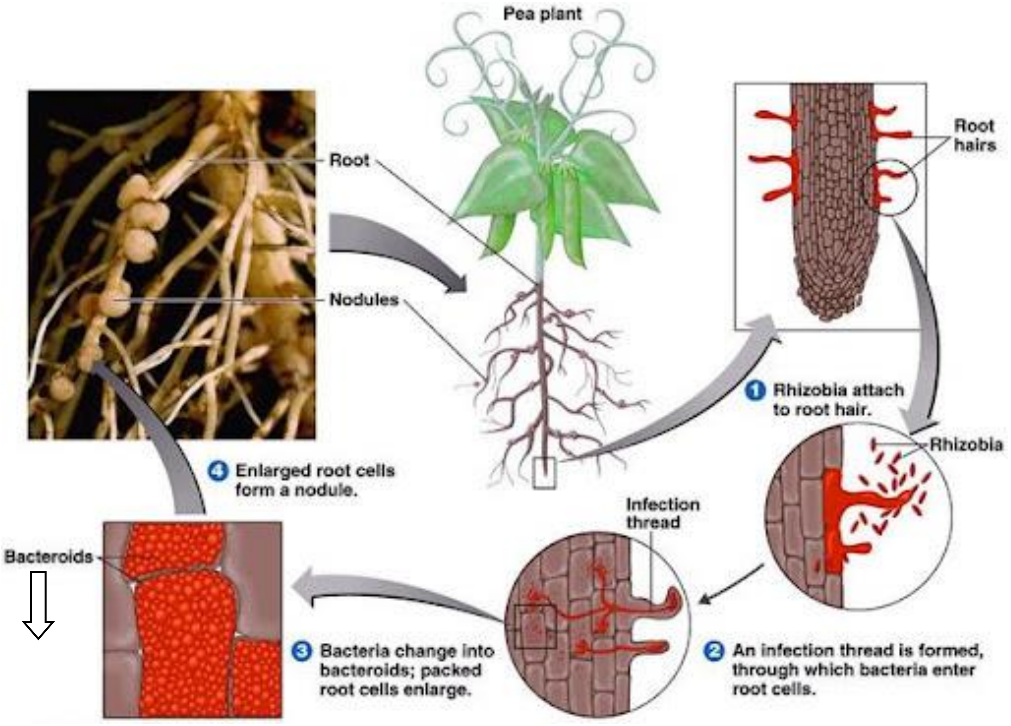

Figure 6. Rhizobium root nodule formation.

Other associations between nitrogen-fixing actinobacteria of the genus *Frankia* with the roots of non-leguminous plants called "actinorhizal plants" (Fig. 7) have been described.

Figure 7. Actinorhize Frankia – Alnus.

Furthermore, fungi of heterogeneous classes and habitats have been shown to function as PGPFs (Plant Growth Promoting Fungi). Molds of the genera *Aspergillus*, *Fusarium, Penicillium, Piriformospora, Phoma,* and *Trichoderma* are the most common PGPF. Studies have demonstrated that the plant-PGPF interaction can have positive effects on the aerial and underground organs of the plant. The most commonly reported effects are significant improvement in germination, seedling vigor, biomass production, root hair development, photosynthetic efficiency, flowering and yield. Some strains have the ability to affect the biochemical composition of the plant. It has also been reported that PGPFs can control several leaf and root pathogens by inducing systemic resistance (ISR) in the host plant. This is due to their ability to enhance nutrient uptake and phytohormone production, as well as reprogram plant gene expression, via differential activation of plant signaling pathways.

One of the most studied and frequently encountered plant-fungus associations is that of mycorrhizae. The roots of about 80 % of all kinds of vascular plants are normally involved in symbiotic associations with mycorrhizae. Mycorrhizal fungi use carbohydrates produced by host plants. In return, they allow increased absorption of nutrients; that is promoting water absorption, mineral nutrition and sometimes secreting hormones or antibiotics. They increase the surface area through which the plant can absorb nutrients, particularly phosphorus, which is not very mobile in the soil. The main mycorrhizal associations are: endomycorrhizas and ectomycorrhizas (Fig. 8).

Endomycorrhizae are found in all families of angiosperms and most gymnosperms (except Pinaceae). The fungal cells penetrate the cortical cells of the plant, intrude and penetrate inside them. The most widespread are Vesicular Arbuscular Mycorrhizae (VAM) belonging to the glomeromycetes. The hyphae of these spores germinate and penetrate the root of the plant forming two types of structures: vesicles and arbuscules. The vesicles are smooth oval bodies that function as storage structures and the arbuscules, tiny structures, are formed inside the plant cells. Nutrients move through the soil through the hyphae of these arbuscules, which gradually break down and release nutrients to the plants.

Ectomycorrhizae are mainly associated with trees in temperate forests; mainly conifers, beeches and oaks. The hyphae infiltrate the roots of the tree between the cells of the outermost layers of the bark, surrounding the cells without penetrating them. An ectomycorrhizal root is characterized by two prominent structures, a fungal sheath completely surrounding the mycorrhizal root and the Hartig net, an intercellular network of hyphae within the root cortex, where the main exchange processes between the symbiotic partners are localized. The fungal sheath (or mantle) also plays a protective role against pathogenic organisms. Associated fungi often produce edible fruiting bodies called caps or carpophores; include, among others, chanterelles (or chanterelles) and boletus. Others, like truffles, never emerge from the ground; they are called “hypogeous”. They are mainly ascomycetes and basidiomycetes. A species of mushroom forms an association with a given species of tree (host specificity), this is why we find, for example: truffles under oaks, porcini mushrooms under pines, etc.

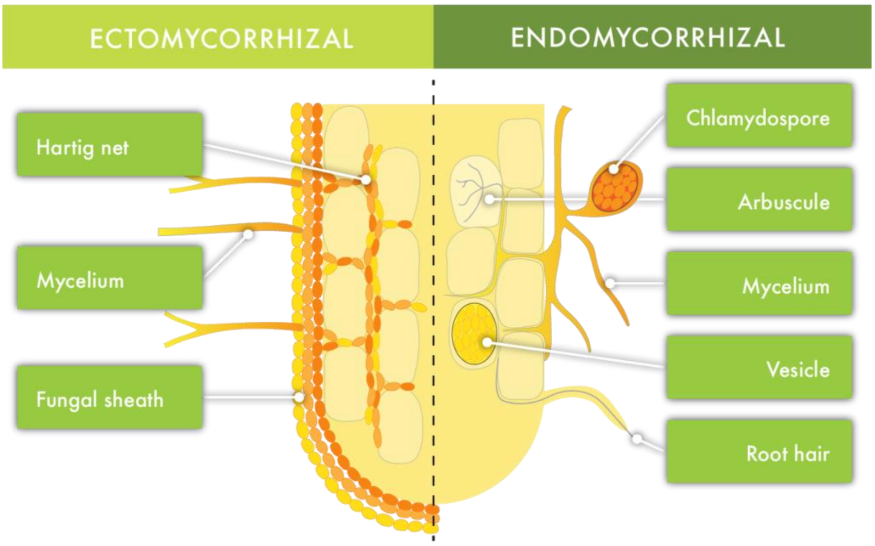

Figure 8. Types of mycorrhizae.

It is important to point out that in certain cases the interactions of microorganisms with each other can promote interaction with the plant, for example a study demonstrated that *Pseudomonas fluorescens*, which is part of the PGPR, would promote endomycorrhization of the roots of the plant *Medicago truncatula*. This example demonstrates that to understand the plant-microbe interaction it is necessary to consider not only the beneficial species but the entire microbial community.

b. The phyllosphere

In addition to the rhizosphere microbiota, a wide variety of microorganisms are found on the aerial part of plants, called the phyllosphere (Fig. 9). The aerial parts of plants are normally colonized by various bacteria, yeasts, fungi, algae and to a lesser extent by protozoa and nematodes. Some species live inside tissues (endophytes), but many are recovered from the surface of healthy plants (epiphytes). These include microorganisms that have complex interactions with the plant at different stages of development. Microbial communities also vary depending on climatic conditions given that they are directly exposed to the sun, variations in humidity, temperature, etc.

Although researches have been conducted on the microbiota of buds and flowers, works have primarily focused on leaves. The habitat found on the surface of the leaves is called phylloplan. Bacteria are the most dominant microorganisms on leaves, often found in average numbers of 10^6 to 10^7 cells/cm^2 (up to 10^8 cells/g) of leaves

Given that the flowers are rich in sugars, they promote the development of some yeasts such as *Candida reukauffi, C. pulcherrima, Torulopsis, Rhodotorula*, etc.

The leaves and stems release organic compounds, and this can lead to massive growth of microorganisms. Microorganisms in the phyllosphere play an important role in protection but can eventually harm the plant.

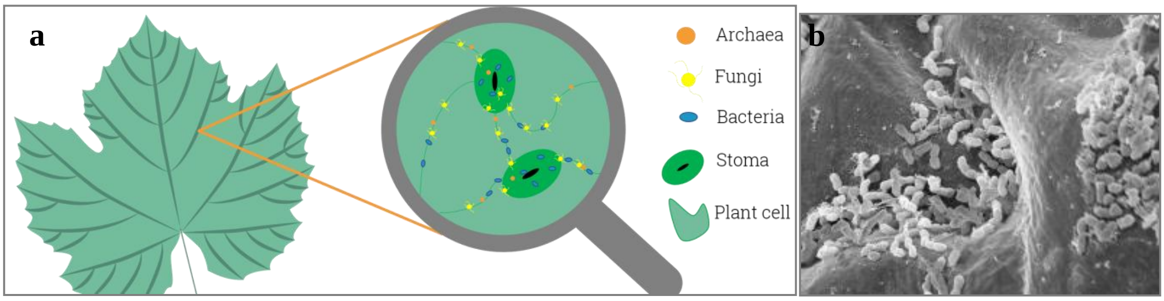

Figure 9. Microorganisms of the phyllosphere. a) schematic representation, b) electron microscope observation of the surface of a plant leaf.

A particularity of the phyllosphere is its abundance of pigmented microbial populations (bacteria and yeasts). Pigmentation gives microbes protection against solar radiation. Some species have structures that allow them to cope with variations in environmental conditions, such as specialized protective walls. In addition, some epiphytes (fungi) produce spores that are transported from one plant to another.

The microorganisms of the phyllosphere interact with each other and with the plant, in fact, some metabolites produced by microorganisms promote the development of others which participate in the protection of the plant by fighting against phytopathogenic agents. Other microorganisms associate with the plant and protect it against other organisms that feed on it. The most described example is that of the association of the mushrooms *Acremonium lolii* and *Acremonium coenophialum* with the herbaceous plants *Lolium perenne* and *Festuca arundinacea* respectively, where these feed on the photosynthates provided by the plant and in return produce alkaloids which are poisons or repellent agents against certain insects, nematodes and herbivorous mammals. Some provide essential nutrients to the plant such as nitrogen, the most studied example is that of the symbiosis between the cyanobacteria of the genus *Anabaena* with the aquatic plant *Azolla* localized on the leaves (unlike *Rhizobium*) where atmospheric nitrogen fixation takes place (Fig. 10). Similarly, other nitrogen-fixing bacteria are found in the phyllosphere of terrestrial plants like conifers.

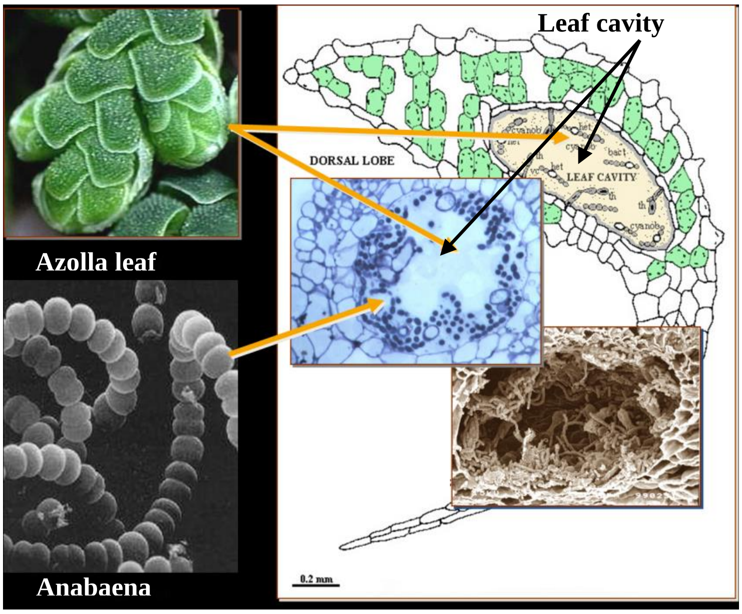

Figure 10. Symbiosis *Anabaena – Azolla*.

c. The phytopathogens

Phytopathogens, whether bacteria, fungi, viruses or protozoa, cause diseases in plants and have significant ecological and economic impacts. For example, downy mildew, which is a fungal disease affecting Solanaceae (potatoes, tomatoes, etc.) and causing necrosis in different parts of the plant, has marked the history of humanity. The first epidemics in Europe, during the 1840s, were the cause of dramatic famines and the death of more than a million people, particularly in Ireland.

Plant pathogens can either cause plant death, disrupt growth or reduce plant yield. They come into contact with the plant in the rhizosphere as well as in the phyllosphere. These microorganisms are either already present in the environment and are attracted to the plant by chemotaxis (attraction or repulsion that a chemical substance exerts on a living cell) or transmitted by a vector such as the air or insects. They enter through wounds or natural openings (e.g. stomata) on the plant and can also release enzymes to degrade plant tissues. Once inside, they will deploy their artillery and release degradative enzymes, toxins and growth regulators disrupting the normal functioning of the organism. Generally, plant soil pathogens produce pectinases, cellulases and hemicellulases causing degeneration of plant structure leading to lesions such as soft rot and many others. Some pathogens produce growth hormones leading to the formation of tumors in the plant, the most described example is that of the Gram-negative bacteria, belonging to the Rhizobiaceae family, *Agrobacterium tumefaciens* responsible for crown gall resulting in a tumor at the site of infection (Fig. 11).

Figure 11. Crown gall.

Other plant pathogens produce toxins that interfere with the plant's normal metabolism. One example is the toxin (tabtoxin) produced by the Gram-negative bacteria *Pseudomonas syringae* pv. tabaci, the agent of cold sore in the tobacco plant *Nicotiana tabacum*; this toxin interferes with the metabolism of methionine in the plant, causing punctate and necrotic spots on the leaves surrounded by a typical chlorotic halo (Fig. 12). Once the plant is weakened and presents the symptoms of the primary infection, it may be subject to secondary infections caused by opportunistic agents, thus causing its death because its defenses are weakened.

Figure 12. *Nicotiana tabacum* leaf affected by cold sore disease.

d. Plant’s defense mechanisms

Like animals, plants have defense mechanisms against pathogens. These can be passive defenses such as protective barriers (cuticle, pectocellulosic wall, lignin) resistant against pests, and preformed chemical substances called phytoanticipins (phenolic compounds, alkaloids, lactones, saponins, cyanogenic glycosides and essential oils) that have antimicrobial action.

Active defenses result in specific signals between the plant and the microbe leading to the synthesis of phytochemicals which will limit infection and even protect the plant against subsequent infections. During a microbial attack, the plant can synthesize molecules called “phytoalexins” which are secondary metabolites, of variable nature depending on the plant, that have antimicrobial properties. In addition, the plant synthesizes PR (pathogenesis related) proteins, such as chitinases, which have the capacity to attack the pathogen by degrading its wall for example. The main signaling pathway leading to the synthesis of PR proteins is that of salicylic acid.

In addition, the plant can react by inducing necrosis of the infected tissue, thus limiting the spread of the infection. This reaction is manifested by the death of the host cell which, before self-destructing, will have emitted alert signals to neighboring cells to create a zone of acquired local resistance (ALR). This is followed by the synthesis of antimicrobial defense molecules with direct or indirect action. These first lines of defense are very effective in confining the pathogen and delaying its invasion into the organism. The propagation of signals and the synthesis of defense molecules can be generalized to the entire plant: this is called systemic acquired resistance (SAR). SAR resembles a permanent state of vigilance which allows the plant to be on alert in the case of a potential attack and to respond promptly to the aggression.

2.2. Microorganisms – Human

At birth human beings are devoid of micro-organisms, however shortly after, they become rapidly colonized by a dense and complex microflora originating from the mother and the surrounding environment. In addition, the human body is formed of approximately 10^{13} cells and hosts approximately 10^{14} microbial cells, mostly bacteria, in its digestive tract, on its skin and mucous membranes. These microbial populations constitute the normal commensal microbial flora. This flora is relatively stable, with specific microbial genera populating various regions of the body during particular periods of an individual's life. This microflora can be resident (commensal) or transient (saprophytic).

The human host and its microbial flora constitute a complex ecosystem whose balance constitutes a remarkable example of reciprocal adaptation. Normal flora influences host anatomy, physiology, pathogen susceptibility, and morbidity.

Microorganisms of the normal flora can help the host, for instance by competing for microenvironments more effectively than pathogens, hindering their establishment or by producing nutrients that the host can use such as vitamin B12 and K and helping in their absorption. However, they can also harm the host by causing dental caries, abscesses, or other infectious diseases; because although most normal microbial organisms are harmless in healthy individuals, they frequently cause disease in weakened hosts. Others can exist as commensals and inhabit the host for long periods without causing any detectable harm or benefit. Viruses and parasites are not considered members of the normal microbial flora by most researchers because they are not commensal and do not help the host.

The commensal flora varies depending on the site, age, environment and state of health of the individual and can be divided into 4 main flora: cutaneous, respiratory, urogenital and digestive (Fig. 13).

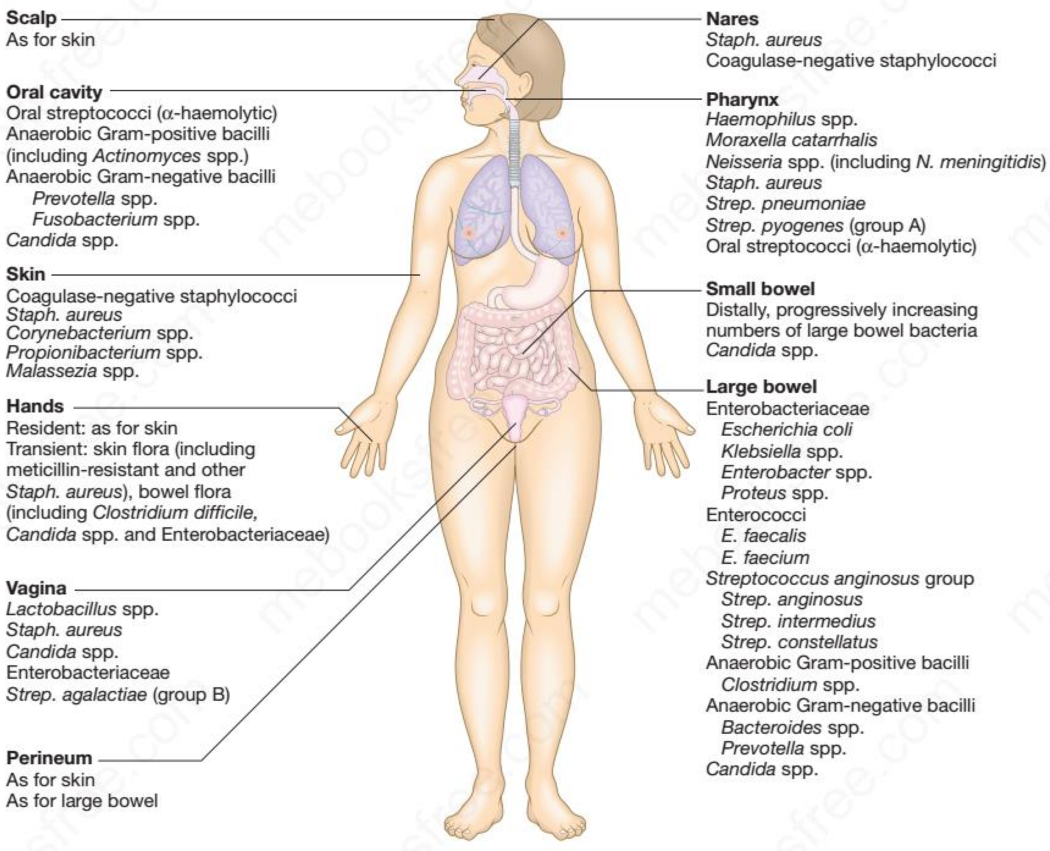

Figure 13. Human commensal flora.

a. Cutaneous flora

An average adult is covered with approximately 2 m^2 of skin. It has been estimated that this surface supports approximately 10^{12} bacteria. The skin surface is the seat of a rich and varied microbial ecosystem which plays an essential role in the balance of the body (Fig. 14). Microorganisms find the nutrients necessary for their development on the skin, whether these are provided by sweat, sebum or cellular debris. Indeed, the secretions of the skin glands are rich in microbial nutrients such as urea, amino acids, salts, lactic acid and lipids. The presence of microorganisms is more marked in certain regions; folds, perineum and hands. Two types of flora are encountered on the skin :

- Permanent or resident flora: made up of species that are established over a long period of time or even permanently on the skin. This resident flora plays an essential role in the physicochemical balance of the skin and constitutes an effective barrier against colonization by exogenous microbes. It is essentially formed by Gram-positive bacteria which are more resistant to the absence of humidity than Gram-negative bacteria; we mainly find Gram positive cocci : *Staphylococcus epidermidis* (face, nostrils, axillary hollows), *S. hominis* (axillary hollow, inguinal hollow and perineum), *S. haemolyticus* (arms, legs, interdigital spaces), Streptococci and Micrococci. Aerobic Gram-positive bacilli: *Corynebacterium* sp. lipophilic (perineum, nostrils, axillary hollows, interdigital spaces). Anaerobic Gram-positive bacilli: *Propionibacterium acnes*, *P. granulosum*, *P. avidum* (regions rich in sebaceous glands; scalp, face, sides of the nose, axillary hollows), mucous membranes. Gram negative bacteria: Cocci (*Neisseria*) and bacilli (*Acinetobacter*, *Proteus*, *Brevibacterium* ...). Fungi: *Malassezia furfur* (yeast also called *Pityrosporum ovale*). Parasites: *Demodex brevis* (mites) and *D. follicularum* (face, near the nose, eyelashes and eyebrows).

- Transient flora: includes species that briefly stay on the skin and which come either from the external environment or from the digestive tract. The unstable nature of the transient flora is the cause of the transmission of pathogenic bacteria, responsible for nosocomial infections. It can be formed by: enterobacteria, *Pseudomonas*, *Staphylococcus aureus*, *Clostridium spores*.

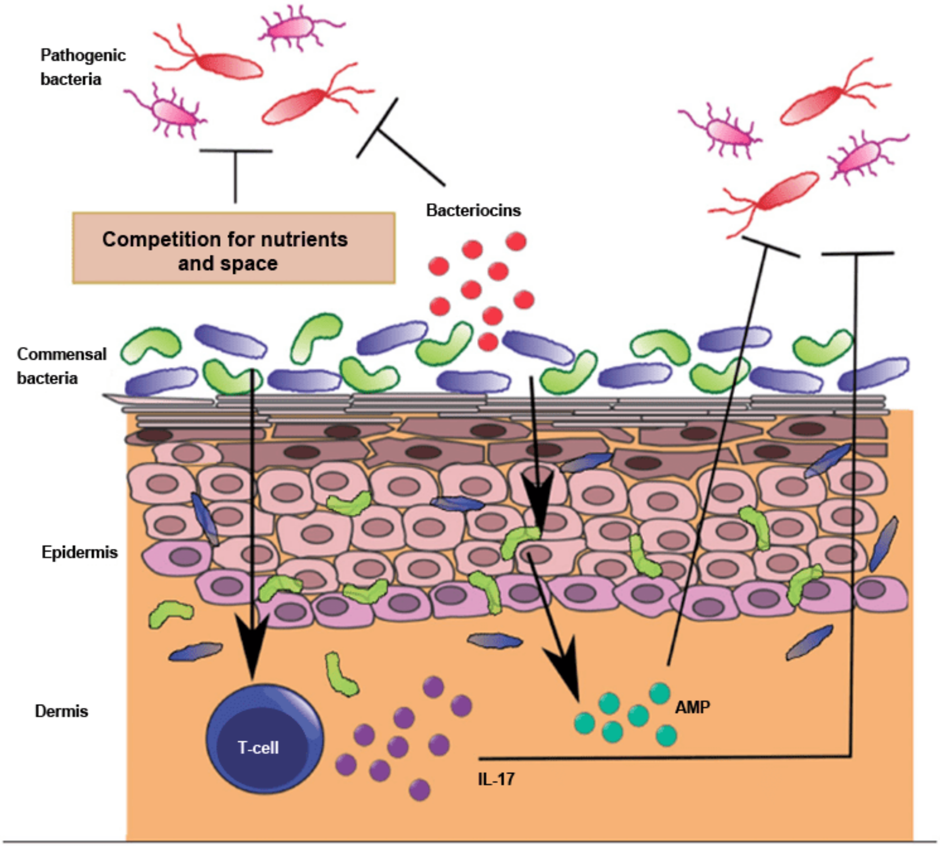

Figure 14. Skin microbiota.

b. Respiratory flora

The airways are divided into upper and lower airways. The upper respiratory tract includes the nose and nasopharynx, oropharynx, and throat, while the lower respiratory tract includes the trachea, bronchi, and lungs.

The flora of the upper respiratory tract is variable and abundant in the nasopharynx (10^8/ml of pharyngeal secretion). It contains many major opportunists: *Staphylococcus aureus* (nasal orifices in particular), Streptococci (including *Streptococcus pneumoniae*), *Haemophilus*, *Neisseria* (possibly *Neisseria meningitidis* whose carriage is transient), *Branhamella catarrhalis,* Anaerobes, corynebacteria and lactobacilli. In the trachea, the flora is minimal and actively fought by mucus, cilia, macrophages, etc. while the lower respiratory tract is sterile.

c. Oral flora

The normal microflora of the oral cavity (mouth) consists of microorganisms that can resist mechanical removal and adhere firmly to surfaces such as gums and teeth. Initially, the microbial flora that establishes itself in the mouth belongs to the genera *Streptococcus, Neisseria, Actinomyces, Veillonella, Lactobacillus* and certain yeasts. These initial microorganisms are obligate aerobes and anaerobes. When the first teeth erupt, anaerobic forms (e.g. *Porphyromonas, Prevotella, Fusobacterium*) dominate because the space between the teeth and gums is anaerobic. Later, *Streptococcus* spp., mainly made up of alpha hemolytic streptococci (*Streptococcus salivarius, S. mitis, S. sanguis, S. milleri,* etc.), settle on the surface of tooth enamel and also attach to epithelial surfaces and colonize saliva. The presence of these bacteria contributes to the possible formation of dental plaque, dental caries, gingivitis (inflammation of gum tissue) and periodontal disease (destruction of tissue and bone). Streptococci also play an important role in the development of endocarditis. The salivary flora reflects the flora of the oral mucosa, and contains a high number of bacteria (10^5-10^6/ml), with a clear predominance of streptococci and particularly *S. salivarius*.

d. Urogenital flora

In the normal urinary tract of men and women, the bladder remains sterile while the urethra is colonized by cocci and facultative aerobic Gram-negative bacilli.

The genital tract (vagina) of an adult woman, due to its large surface area and mucous secretions, has a complex microflora. The vaginal environment is weakly acidic and contains significant amounts of glycogen. *Lactobacillus acidophilus* ferments glycogen to produce lactic acid and maintains acidic conditions. Other microbes such as yeasts (*Torulopsis* and *Candida*), streptococci and *E. coli* may also be present. The role of acidophilic lactobacilli (Döderlein bacillus) is predominant, they are selected by vaginal acidity and constitute competitors against other species by colonizing the vaginal epithelium but also for certain species by producing hydrogen peroxide which inactivates anaerobes and *Gardnerella*.

The vaginal microflora constantly changes between puberty and menopause. Before puberty, a woman's vagina is alkaline and does not produce glycogen, *L. acidophilus* is absent and the flora consists mainly of staphylococci, streptococci, diphtheroids and *E. coli*. After menopause, glycogen production stops, the pH increases and the flora resembles that found before puberty.

In men, the bacteria present in the urethra are *Streptococcus* sp., *Mycobacterium* sp., *E. coli* and *Bacteroides* sp.

e. Digestive flora

The gut microbiota plays a prominent role in host immune function, metabolism, and mediation of the central nervous system

The human gastrointestinal tract, the site of food digestion, includes the stomach, small intestine, and large intestine. The composition of the gastrointestinal flora in humans varies considerably and is somewhat dependent on diet. Indeed, people who consume a considerable amount of meat have higher numbers of highly proteolytic Bacteroides and lower numbers of coliforms and lactic acid bacteria compared to those who consume a vegetarian diet. The digestive flora is the most abundant and the most important. It varies depending on the different parts of the digestive tract (Fig. 15). The bacteria are established from birth to give an endogenous flora which becomes stable from the age of one year. Gut bacteria play an important role in the development of the immune system.

Several studies have demonstrated the influence of the intestinal microbiota on the behavior of hosts. Indeed, mice that received the intestinal bacteria *Lactobaccillus rhamnosus* were less stressed than the others. The γ-aminobutyric acid receptors located in the brain are also more active. Removing the nerve from the gut to the brain removes these features, proving that a signal is being sent by bacteria to the central nervous system. This demonstrates the close mutual interaction existing between the host and its flora.

The stomach is a relatively hostile environment for bacteria. It contains bacteria swallowed with food and bacteria dislodged from the mouth. It has a very poor flora due to its acidity (pH 2). Acidity decreases bacteria counts, which are highest (around 10^3-10^6 organisms/g content) after meals and lowest (often undetectable) after digestion. Some bacteria such as *Helicobacter pylori* can colonize the gastric epithelium and cause ulcers. The small intestine also has poor flora due to peristalsis and the abundance of secretions. The germs present are mainly streptococci, staphylococci and lactobacilli.

The colon flora, on the other hand, is extremely varied and abundant. It includes a large number of bacteria (more than 400 species have been identified); nevertheless, 95 to 99 % belong to anaerobic genera such as *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Peptostreptococcus* and *Clostridium*, followed by enterobacteria (*E. coli*, *Proteus*, *Klebsiella*...), enterococci and Staphylococci. In this highly anaerobic region of the intestine, these genera proliferate, occupy most available niches, and produce metabolic wastes such as acetic, butyric, and lactic acids. Strict anaerobic conditions, physical exclusion, and bacterial waste are factors that inhibit the growth of other bacteria in the large intestine. In addition, this flora is usually stable and limits the establishment of pathogenic species such as *Salmonella*, *Shigella* or *Campylobacter* and the development of potentially dangerous commensal bacteria. In addition, this flora promotes digestion and constitutes to a certain extent an additional contribution to that of food. It synthesizes different vitamins (vitamins K and B9). Species of the genera *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium* play a protective role against mucosal inflammation by inhibiting the production of pro-inflammatory cytokines or by stimulating that of other anti-inflammatory cytokines such as interleukin.

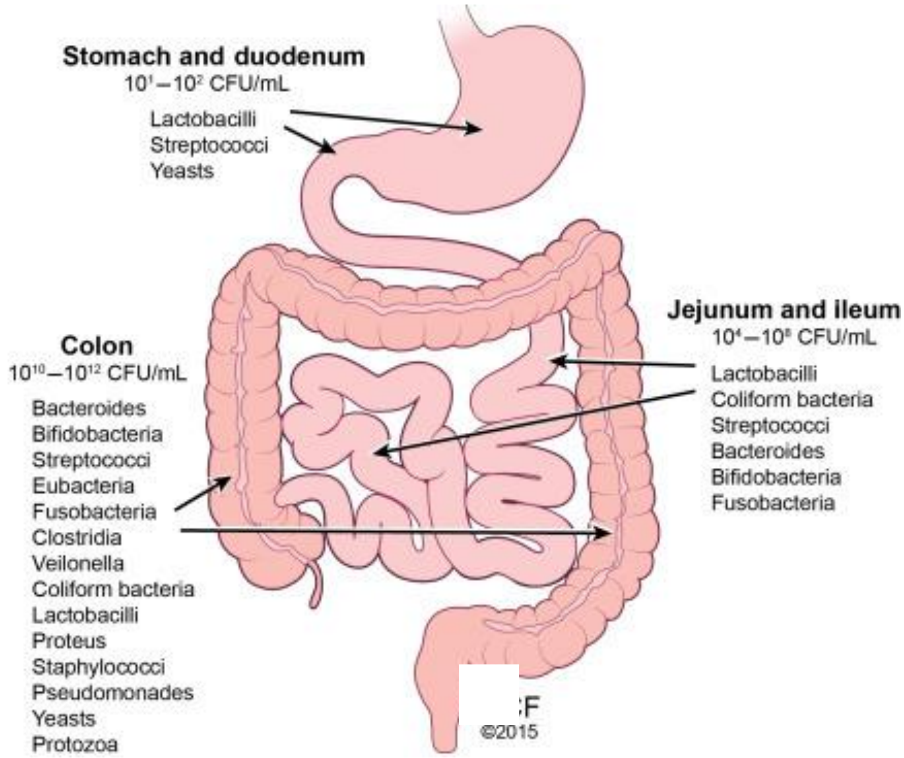

Figure 15. Distribution of the normal human gut flora

Although normal flora can inhibit pathogens, many of its members can cause disease to humans. Anaerobes in the intestinal tract are the main agents of intra-abdominal abscesses and peritonitis. They can also cause problems in the gastrointestinal lumen. In addition, antibiotic treatment may allow certain anaerobic species to become predominant and cause disease. Other intestinal pathological conditions or surgery can cause bacterial overgrowth in the upper small intestine leading to nutrient malabsorption.

Despite their beneficial roles, changes in the gut microbiota composition can lead to many diseases such as cancer, cardiovascular disease, and diabetes. As well, autoimmune diseases were also showed associated with imbalances in the intestinal microbial ecosystem and therapeutic solutions based on the restoration of intestinal biodiversity are being developed.

2.3. Microorganisms – animals

Just like humans, animals are exposed to microorganisms from the moment of birth or hatching. The gastrointestinal tract (GIT) of mammals receives the first inoculum of bacteria from feces and breast milk, while newly hatched chicks receive bacteria from the surface of egg shells. Very little information is available on bacterial colonization of the fish intestine, but it seems logical that the inoculum for the fry comes from the surrounding water. In chickens, the gastrointestinal tract becomes rapidly colonized by bacteria, with maximum density being reached within the first 5 days after hatching. Over the following weeks, the composition of the microflora changes markedly.

By revealing the extraordinary extent of bacterial and archaeal diversity that lives on and within multicellular organisms, recent technological advances in DNA sequencing have challenged this simplistic view. There is growing evidence that these communities of microorganisms, called microbiota, profoundly influence the phenotypes of the host that carries them and vice versa. The intestine of animals presents living conditions conducive to the growth of the microorganisms it hosts; there is indeed an abundance of food (food being digested) and a very low oxygen content (a poison for these microorganisms which are for the most part strictly anaerobic). These communities in turn make it possible to compensate for the enzymatic deficits of the host by digesting part of the food, which allows better extraction of the energy contained in complex food molecules.

Firmicutes, Proteobacteria, and Bacteroidetes are generally the dominant bacteria in the gut microbiome of healthy animals, although they are found at different relative abundances in different individuals and species

The coevolution of animal species and their intestinal microflora have led to a situation in which bacterial fermentation hotspots have been restricted to defined parts of the GIT. In monogastric animals, bacterial fermentation was concentrated in the cecum and colon. These parts of the GIT receive dietary compounds that escape digestion and absorption by the host; therefore, bacteria do not compete with the host when fermenting incoming substrates. However, in the upper GIT there is competition for all the simple sugars and amino acids used by the host, which are also available to the bacteria. In poults, equines, and rodents, the site of intense bacterial fermentation is a well-developed appendix or cecum. In other monogastric animals, such as humans and pigs, the cecal appendix is reduced and bacterial fermentation occurs primarily in the colon.

Mammalian species that have convergently adapted to the same diet, for example the herbivore, have acquired in their intestine identical bacteria belonging to large taxonomic categories. The intestine of herbivores is in fact enriched in bacteria from the Ruminococcaceae and Lachnospiraceae families which produce enzymes necessary for the degradation of complex plant sugars. These enzymes are not encoded by the host genome, so these bacteria play a fundamental role in the assimilation, by the host, of these energy molecules.

Carnivores, for their part, have an intestinal microbiota enriched in certain types of bacteria (such as *Lactobacillus*) involved in the degradation of amino acids, in high concentrations in their diet. Studies on the microbiota of dogs and cats have demonstrated the predominance of Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria.

Although they share large bacterial families, species with the same diet do not harbor the same bacteria. At a more precise taxonomic level, the distribution of bacteria between mammalian intestines is no longer explained by differences in diet, but seems to reflect the phylogenetic position of the species.

a. Herbivores

A herbivore is a vertebrate that feeds on grasses and low plants. In addition, the wall of plant materials is mainly composed of cellulose, hemicelluloses and lignin which ensure the structure and rigidity of plants. However, the carbon energy contained in these plant polymers is difficult to access for primary consumers. Only the synergistic action of a complex assembly of microorganisms makes it possible to hydrolyze dietary fibers and then ferment them.

In herbivorous mammals, different types of fermenters are observed (Fig. 16). A large cecal fermenter in a distal position characterizes small herbivores (lagomorphs, hyraxs, herbivorous rodents, small herbivorous marsupials). For large herbivores, we distinguish the colon fermenter (equines, elephants, orangutans, gorillas) from that of the stomach fermenter (ruminants, camelids, sloths, macropod marsupials).

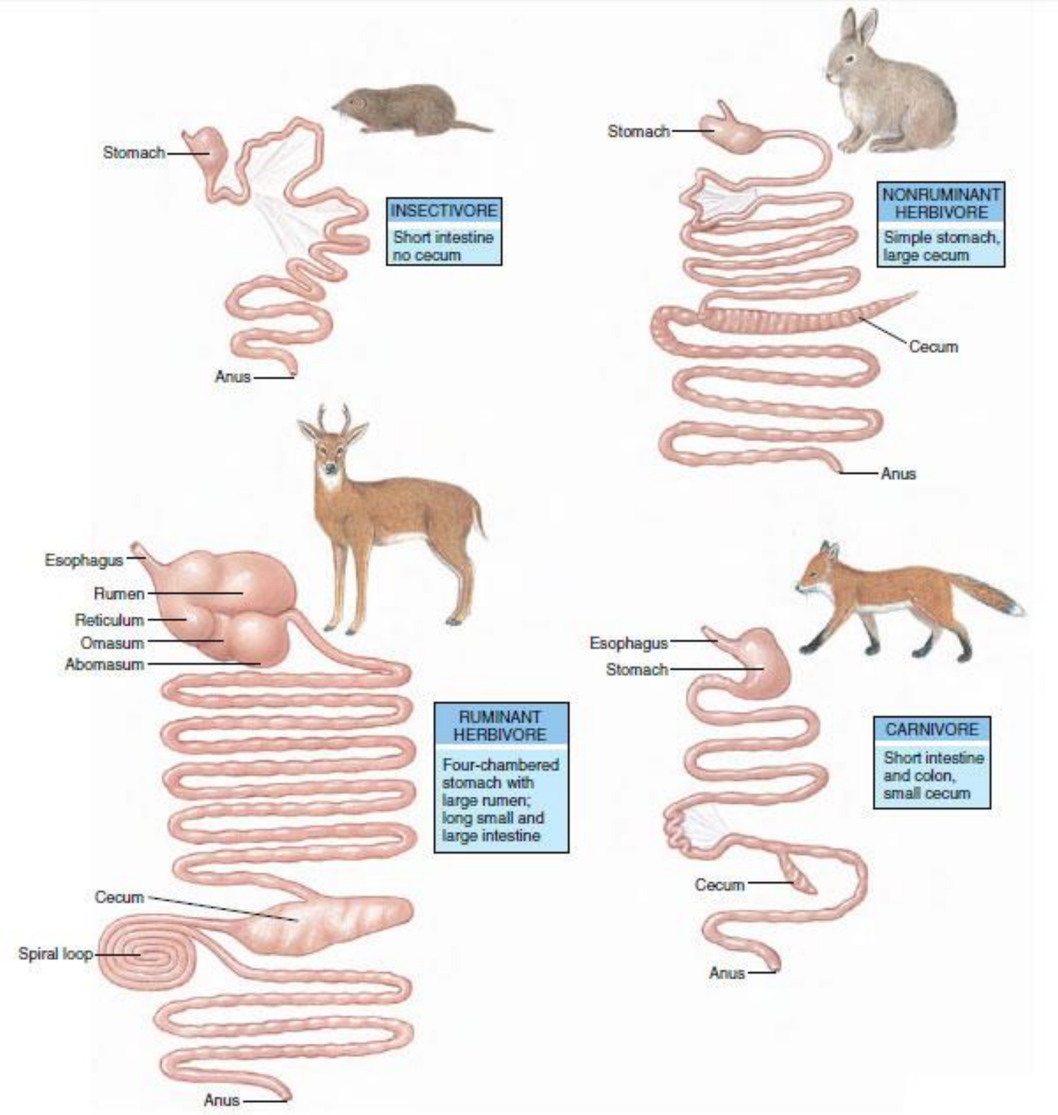

Figure 16. Animal digestive systems.

In a digestive fermenter, a set of mechanical, chemical and biological processes converts the energy contained in food without using oxygen. The particularity of digestive fermenters compared to other digestive organs lies in the density and diversity of the microorganisms present.

Stomach and cecal fermenters are representative of the two main evolutionary strategies encountered in the main herbivorous livestock mammals. The stomach fermenter, that of the domestic cow (*Bos taurus*) is a specific representative, and is based on the cooperative mutualism between the host and its microbiota: the microorganisms use fibrous sources that are not competitive with the host. The disadvantage of this mutualism for the host is the digestion by the microorganisms of carbon sources which could have been accessible to the host's digestion.

In contrast, the cecal fermenter, that of the European rabbit (*Oryctolagus cuniculus*) is a specific representative, and is based on cooperative competitive mutualism between the host and its microbiota. Indeed, the digestive strategy of the rabbit is based on a combined mutualism: the mutualism is said to be competitive because the rabbit, like carnivorous species, maintains privileged access to food by delaying its access to microorganisms; mutualism is said to be cooperative because the microorganisms in the caecum use, like those present in the rumen, non-competitive carbon sources for the rabbit.

In cattle, the digestive strategy is to promote the stay of ingested dietary fibers in the rumen, until achieving a sufficient reduction in particle size, and therefore extensive degradation of the fibers. Additionally, microbial enzymes, unlike mammalian enzymes, can digest cellulose. Under anaerobic conditions, microbes generate volatile fatty acids as fermentation end products. The rumen environment is suitable for microbial fermentations, this allows the host animal to use complex carbohydrates and nitrogen for energy and protein needs. Ruminants supplement microbial activity by regurgitating (rumination), which allows for additional chewing of large food particles. Movement of muscles in the rumen wall allows continuous mixing of rumen contents to maintain digestion by microbes and absorption of volatile fatty acids and other nutrients by the host. Volatile fatty acids, acetate, propionate and butyrate can contribute up to 80 % of the animal's energy needs. In addition, ruminants generally do not need vitamin supplements in their diet, particularly vitamin B which is synthesized by the rumen microflora, often beyond the animal's needs.

In rabbits, conversely, the host's strategy is to take advantage of the rapidly digestible fractions of plants (starch, proteins, etc.) in the small intestine. In the large intestine of the rabbit (caecum and colon) this will allow the finest particles to stay longer in the fermenter, while promoting faster transit of the coarsest particles, evacuated in the feces. Microorganisms, which multiply in the cecum and proximal colon producing absorbable nutrients, are partly ingested via caecotrophy behavior. Caecotrophy is practiced by all Lagomorphs (Rabbits, Hares, etc.) and by some Rodents (Beaver, etc.), consisting of the production of two types of excrement during the same day and systematically ingesting one of both types of excrement (very little modified digestive content) and to systematically reject the other type (real excrement).

Furthermore, the microorganisms that populate digestive fermenters belong to the three domains of life: Bacteria, Archaea and Eucarya. Viruses, which are not considered living beings but biological entities, are also present. The cow's rumen contains 10^{10} to 10^{11} bacteria, 10^{8} to

10^{10} archaea, 10^3 to 10^6 protozoa (ciliates and flagellates), 10^2 to 10^4 fungal zoospores and 10^9 to 10^{10} viral particles per milliliter. The bacterial population of the cecum is largely in the majority and estimated at 10^{10} to 10^{12} bacteria per g of content while the archaea population is estimated at 10^7 per g of content. Regarding the eukaryotic domain, the cecal digestive ecosystem of the rabbit appears to be devoid of anaerobic fungi and yeasts although the presence of “commensal” yeasts has been observed in the cecum (10^6 per g). Protozoa are absent from the cecal ecosystem.

In the rumen, the proportions of Firmicutes and Bacteroidetes are similar (41 vs 47 %); Conversely, in rabbits a large predominance of Firmicutes is observed (92.5 %), Bacteroidetes representing only 3 %. Furthermore, the bacteria present are specific to each host species since no species in common could be found between these two ecosystems. In rabbits, 80 to 90 % of the species housed in the cecum are considered new species never referenced in the databases. Finally, carrying out molecular fingerprints of bacterial communities, “Capillary Electrophoresis Single Strand Conformation Polymorphism”, shows that the diversity of the bacterial community is higher in the rumen than in the cecum.

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